Franz-Xaver Reichl
Michael Schwenk
*Editors*

# Regulatory Toxicology

Springer Reference

# Regulatory Toxicology

Franz-Xaver Reichl • Michael Schwenk
Editors

# Regulatory Toxicology

With 144 Figures and 105 Tables

Springer Reference

*Editors*
Franz-Xaver Reichl
LMU Munich, Munich, Germany

Michael Schwenk
Formerly Medical School
Hannover, Germany

ISBN 978-3-642-35373-4 ISBN 978-3-642-35374-1 (eBook)
ISBN 978-3-642-35375-8 (print and electronic bundle)
DOI 10.1007/ 978-3-642-35374-1
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2014932168



Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

# Foreword

Regulatory toxicology requires knowledge of aspects related to a number of disciplines of natural and social sciences, including chemistry, biology, bioinformatics, toxicology, epidemiology, exposure assessment and nutrition, as well as sociology, psychology and communication, to name a few. It builds a bridge between science and decision-making. In fact, risk analysis, the centrepiece of regulatory toxicology, is often seen as an art, in addition to its being a science. This explains why standard textbooks on regulatory toxicology are rare.

Ten years after the publication of the first standard work on regulatory toxicology in German, an updated, expanded version is now at hand in English language. This major publication addresses questions covering various aspects of risk assessment and risk management in general, paying attention to a number of fields including health protection, occupational health, environmental health and consumer protection. Basic principles are outlined, new developments described, and scientific, social and philosophical questions discussed at length. In times of an increased understanding that risk assessment and management need to be conducted in full transparency and with full involvement of all stakeholders, issues of risk perception and risk communication are addressed.

This breadth of information, complemented by a listing of the newest guidance values, makes this book a standard reference to those involved in the field of regulatory toxicology. It is of interest to risk scientists of various backgrounds, to policymakers and their advisors, and also to informed consumers.

A welcome and timely publication, indeed.

Madged Younes, WHO, Geneva, Switzerland
Herman Autrup, IUTOX-president, Aarhus, Denmark

# About the Editors

**Univ.-Prof. Dr. Dr. Franz-Xaver Reichl** Department of Operative/Restorative Dentistry, Periodontology and Pedodontics, LMU Munich and Walther-Straub-Institute of Pharmacology and Toxicology, LMU Munich, Nussbaumstr. 26, Germany

Univ.-Prof. Dr. Dr. Franz-Xaver Reichl studied microbiology and medicine in Munich, Germany. He then qualified as a toxicologist/pharmacologist and works at the Ludwig Maximilains University of Munich (LMU) as a dental-toxicologist at the Dental Clinic and at the Walther-Straub-Institute of Pharmacology and Toxicology at the LMU. His research was mainly related to molecular, cellular, and analytical (dental) toxicology. F.-X. Reichl consulted health agencies in toxicological risk management and is member of toxicological/pharmacological committees. He is head of the "International Advisory Board for the Biocompatibility/Toxicology of Dental Materials" at the LMU.

**Prof. Dr. Michael Schwenk** Formerly Medical School, Hannover, Germany

Prof. Dr. Michael Schwenk studied Biochemistry and Medicine in Tüebingen, Germany. He then qualified as a toxicologist/pharmacologist; worked in universities, industry, and research institutions; and later became head of the environmental health department of a state health agency. His research was mainly related to molecular and cellular toxicology, but he was also involved in human trials with volunteers and in epidemiological studies on exposure–response relationships. M. Schwenk consulted health agencies in toxicological risk management and is member of regulatory committees.

# Contents

## Volume 1

**Part I The "Modus Operandi" of Regulatory Toxicology** . . . . . **1**

**Aims and Mission of Regulatory Toxicology** . . . . . 3
Helmut Greim

**Working Areas of Regulatory Toxicology** . . . . . 21
Michael Schwenk and H. Paul A. Illing

**National and International Collaboration in Regulatory Toxicology** . . . . . 31
Klaus E. Appel

**The Regulatory Process in Toxicology** . . . . . 49
Dietrich Henschler and Wolfgang Dekant

**Quality Assurance in Regulatory Toxicology** . . . . . 57
Werner Lilienblum and Stephen Harston

**Toxicological Risk Assessment** . . . . . 65
Maged Younes

**Part II Approaches Used** . . . . . **73**

**Characterization of Physicochemical Parameters in Toxicology** . . . . . 75
Mathias Locher

**Examination of Acute and Chronic Toxicity** . . . . . 81
Karl Georg Heimann and Kevin Doughty

**Examination of Organ Toxicity** . . . . . 89
Wolfgang Kaufmann and Matt C. Jacobsen

**Specific Toxicity Tests for Neurotoxicity, Immunotoxicity, Allergy, Irritation, Reprotoxicity, and Carcinogenicity** . . . . . 99
Eckhard von Keutz

**Toxicity Testing In Vitro. Regulatory Aspects** . . . . . 107
Eckhard von Keutz

**New and Future Toxicological Assays and Their Regulation** . . . . . 115
Horst Spielmann

**Computer-Based Prediction Models in Regulatory Toxicology** . . . . . 123
Thomas Steger-Hartmann and Scott Boyer

**Metabolism Tests in Regulatory Toxicology** . . . . . 133
Gert Ulrich Kuerzel and Christine Mauriac

**Toxicokinetic Tests** . . . . . 141
Jürgen Pauluhn

**Toxicodynamic Tests** . . . . . 161
Dieter Schrenk

**Omics in Toxicology** . . . . . 173
Heidrun Ellinger-Ziegelbauer and Hans-Juergen Ahr

**International Regulation of Toxicological Test Systems** . . . . . 181
Horst Spielmann

**Epidemiological Methods in Regulatory Toxicology** . . . . . 191
Ulrich Ranft and Gregory A. Wellenius

**Studies in Volunteers and Its Regulation** . . . . . 207
Klaus Mörike

**Statistical Evaluation Methods in Toxicology** . . . . . 213
Ludwig A. Hothorn

**Dose-Response Analysis, Identification of Threshold Levels for Chemicals** . . . . . 225
Hans-Karl Heim and Peter Mayer

**Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds** . . . . . 237
Lutz Edler

**Probabilistic Methods in Toxicology** . . . . . 253
Odile Mekel and Rainer Fehr

**Toxicodynamic Models** . . . . . 261
Lutz Edler and Angelika Tritscher

**Toxicokinetic Models** . . . . . 269
Johannes Georg Filser

**Exposure Analysis for Indoor Contaminants** . . . . . 277
Gerhard Volland

**Exposure Scenarios in Toxicology** . . . . . 289
Gerhard Heinemeyer

**Quality Criteria for Primary Literature in Toxicology** . . . . . 305
Klaus Schneider, Agnieszka Kinsner-Ovaskainen, and Martin Hassauer

**Data-Mining in Toxicology** . . . . . 311
Inge Mangelsdorf

**Principles of Analytical Chemistry for Toxicology** . . . . . 321
Jürgen Durner and David C. Watts

**Benchmark Dose in Regulatory Toxicology** . . . . . 359
Lutz Edler

**Uncertainty Analysis in Exposure Assessment. Relevance for Regulatory Toxicology** . . . . . 377
Michael Schümann, Özkaynak Haluk, and Alexandre Zenié

**Part III Paradigms used in Risk Evaluation . . . . . 395**

**Do Carcinogens Have a Threshold Dose? Pro and Contra** . . . . . 397
Bernd Kaina, Adam D. Thomas, and Jan G. Hengstler

**Single Compounds Versus Combination Effects in Toxicology** . . . . . 407
Thomas R. H. Büch, Eva A. M. Schäfer, John H. Duffus, and Thomas Gudermann

**Biomolecules Versus Smaller Chemicals in Toxicology** . . . . . 413
Manfred Ruthsatz, Carlo Chiavaroli, and Emmanuelle Voisin

**Sensitive Humans Versus Average Persons in Toxicology** . . . . . 423
Alexander Eckhardt

**Extrapolation Factors and Safety Factors in Toxicology** . . . . . 431
Rainer Konietzka, Klaus Schneider, and Leonard Ritter

**Background Exposure Versus Additional Exposure in Human Biomonitoring** . . . . . 439
Hans-Günter Neumann

**Adverse Effects Versus Non-adverse Effects in Toxicology** . . . . . 449
Norbert Englert and Robert L. Maynard

**Health-Based Threshold ADI Versus MOS in Toxicology** . . . . . 457
Ursula Gundert-Remy

**Precaution Principle Versus Danger Prevention in Toxicology** . . . . . 463
Ludwig Müller and Neill H. Stacey

**Hygienic Versus Toxicological Approaches in Regulation** . . . . . 469
Roland Suchenwirth

**Protected Property and Protection Level in Regulatory Toxicology** . . . . . 475
Bernhard Liebl and Ines Liebl

**Ethical Issues in Science. Focus on Regulatory Toxicology** . . . . . 481
Beate Henrikus and Wolfgang Eisenmenger

## Volume 2

**Part IV Risk Assessment and Evaluation . . . . . 491**

**Current Role of the Risk Concept in Regulatory Toxicology** . . . . . 493
Rolf Hertel, Michael Schwenk, and H. Paul A. Illing

**Risk Cycles in Toxicology** . . . . . 501
H. Paul A. Illing and Michael Schwenk

**Risk Minimization in Drug Development. Regulatory Aspects** . . . . . 509
Elke Roehrdanz and Klaus Olejniczak

**Importance of Physical-Chemical Properties for Toxicological Risk Assessment** . . . . . 521
Hans-Uwe Wolf and Michael Schwenk

**Importance of Intrinsic Toxic Properties for Risk Assessment** . . . . . 525
Hermann Kappus and Michael Schwenk

**Importance of Metabolism. Mechanistic Considerations Relevant for Toxicological Regulation** . . . . . 533
Franz Oesch and Jan G. Hengstler

**Dose–Response Relationship and Extrapolation in Toxicology. Mechanistic and Statistical Considerations** . . . . . 547
Werner K. Lutz, Roman W. Lutz, David W. Gaylor, and Rory B. Conolly

**Importance of Exposure Level for Risk Toxicological Assessment** . . . . 569
Hans Drexler and Anuradha Shukla

**Risk Characterization in Regulatory Toxicology** . . . . . 577
Maged Younes

**Risk Evaluation in Regulatory Toxicology** . . . . . 583
Maged Younes

**Risk Comparison in Toxicology** . . . . . 589
Franz-Xaver Reichl

**Risk-Benefit Considerations in Toxicology** . . . . . 601
Rolf Hertel, Michael Schwenk, and H. Paul A. Illing

**Toxicological Risk Assessment in Different Jurisdictions** . . . . . 607
Dietrich Henschler and Wolfgang Dekant

**Part V Risk Management and Risk Communication . . . . . 615**

**Purpose of Risk Management in Regulatory Toxicology** . . . . . 617
Rolf Hertel, Michael Schwenk, and H. Paul A. Illing

**Assessment of Limit Values in Regulatory Toxicology** . . . . . 627
Hermann H. Dieter

**Limit Value Setting in Different Areas of Regulatory Toxicology** . . . . 649
Klaus-Michael Wollin and Paul Illing

**Registration and Approval in Regulatory Toxicology** . . . . . 661
Thomas Wallenhorst

**Health Hazards Classification and Labeling** . . . . . 677
Herbert Desel, Pieter Brekelmans, and Ronald de Groot

**Human Biomonitoring. Its Importance in Toxicological Regulation** . . . . . 685
Michael Wilhelm

**Restrictions and Prohibitions as Tools in Regulatory Toxicology** . . . . 703
Ullrich Kleeberg, Hans Jochen Kittel, and Alfred Georg Hildebrandt

**Observance of Susceptible Population Groups in Regulatory Toxicology** . . . . . 719
Ursula Gundert-Remy

**Toxicological Report** . . . . . 727
Thomas Gudermann, Harald Mückter, John H. Duffus, and Hermann M. Bolt

**Risk Management in Toxicological Disasters** . . . . . 739
Kai Kehe and John H. Duffus

**Institutionalized Participation in Regulatory Toxicology** . . . . . 747
Andreas D. Kappos

**Risk Communication** . . . . . 757
Werner Lilienblum and Marianne Lilienblum

**Dealing with Diseases That Have Been Attributed to Chemical Exposures** . . . . . 767
Thomas Zilker

**Reach (and CLP). Its Role in Regulatory Toxicology** . . . . . 779
Walter Aulmann and Nathan Pechacek

**Bridging. The Regulation of Toxic Mixtures** . . . . . 797
Walter Aulmann and Nathan Pechacek

**Risk Assessment of Food Additives** . . . . . 803
Rainer Gürtler

**Risk Assessment of Food Components with Botanical Origin** . . . . . 813
Birgit Dusemund

**Risk Assessment of Novel Food and Genetically Modified Food and Feed** . . . . . 827
Annette Pöting, Marianna Schauzu, Birgit Niemann, and Regina Schumann

**Notification of Cosmetic Products and Dangerous Mixtures in Regulatory Toxicology** . . . . . 849
Herbert Desel, Pieter Brekelmans, and Ronald de Groot

**Chemical and Biological Weapons and Their Regulation** . . . . . 855
Horst Thiermann, Kai Kehe, Julia Riehm, and Lothar Zöller

**Part VI Addendum: Tables and Lists** . . . . . **869**

**Checklist: Toxicological Risk Assessment in Practice** . . . . . 871
Michael Schwenk and H. Paul A. Illing

**Limit Values and Guideline Values in Regulatory Toxicology** . . . . . 875
Karin Heine and Alexander Eckhardt

**Appendix** . . . . . **899**

**Regulatory Toxicology: Glossary** . . . . . 901
Franz-Xaver Reichl and Gisela Degen

**Index** . . . . . 929

# Contributors

**Hans-Juergen Ahr** Investigational Toxicology, Bayer Pharma AG, Wuppertal, Germany

**Klaus E. Appel** Federal Institute for Risk Assessment, Berlin, Germany

**Walter Aulmann** Ecolab Deutschland GmbH, Monheim, Germany

**Hermann M. Bolt** Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany

**Scott Boyer** Global Head of Molecular and Investigational Toxicology, Global Safety Assessment, AstraZeneca R&D, Mölndal, Sweden

**Pieter Brekelmans** National Poisons Information Center (NVIC), University Medical Center Utrecht, Utrecht, GA, The Netherlands

**Thomas R. H. Büch** Rudolf-Boehm Institute of Pharmacology and Toxicology, Clinical Pharmacology, University of Leipzig, Leipzig, Germany

**Carlo Chiavaroli** Voisin Consulting Life Sciences, Lausanne, Switzerland

**Rory B. Conolly** Integrated Systems Toxicology Division, NHEERL/ORD, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

**Ronald de Groot** National Poisons Information Center (NVIC), University Medical Center Utrecht, Utrecht, GA, The Netherlands

**Gisela Degen** Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany

**Wolfgang Dekant** Institute for Toxicology, University of Würzburg, Würzburg, Germany

**Herbert Desel** GIZ-Nord Poisons Center, University Medical Center Göttingen - Georg-August-Universität, Göttingen, Lower Saxony, Germany

**Hermann H. Dieter** Department of Toxicology of Drinking-Water, Federal Environment Agency of Germany/Umweltbundesamt (UBA), Dessau-Roßlau, Germany

**Kevin Doughty** Product Stewardship Manager, Bayer CropScience, Registration Department, Monheim, Germany

**Hans Drexler** Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, FAU, Erlangen, Germany

**John H. Duffus** The Edinburgh Centre of Toxicology, Edinburgh, Scotland, UK

**Jürgen Durner** Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University of Munich, Munich, Germany

**Birgit Dusemund** Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany

**Alexander Eckhardt** Toxicology of Drinking Water and Swimming Pool Water, Federal Environment Agency, Bad Elster, Germany

**Lutz Edler** Department of Biostatistics –C060, German Cancer Research Center (DKFZ), Heidelberg, Germany

**Wolfgang Eisenmenger** Ethics Committee Ludwig-Maximilians-University Munich, Munich, Germany

**Heidrun Ellinger-Ziegelbauer** Investigational Toxicology, Bayer Pharma AG, Wuppertal, Germany

**Norbert Englert** Formerly: Federal Environmental Agency, Berlin, Germany

**Rainer Fehr** Department of Public Health, University of Bielefeld, Bielefeld, Germany

**Johannes Georg Filser** Institute for Toxicology and Environmental Hygiene, Technical University of Munich, Munich, Germany

**David W. Gaylor** Formerly: National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA

**Helmut Greim** Institute for Toxicology and Environmental Hygiene, Technical University Munich, Freising-Weihenstephan, Bavaria, Germany

**Thomas Gudermann** Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität München, Munich, Germany

**Ursula Gundert-Remy** Institute for Clinical Pharmacology and Toxicology/ Institut für Klinische Pharmakologie und Toxikologie, Charité Universitätsmedizin Berlin, Berlin, Germany

**Rainer Gürtler** Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany

**Özkaynak Haluk** U.S. Environmental Protection Agency (EPA), Durham, NC, USA

**Stephen Harston** Hofheim am Taunus, Germany

**Martin Hassauer** Research and Advisory Institute for Hazardous Substances, FoBiG, Freiburg, Germany

**Hans-Karl Heim** Federal Institute for Drugs and Medical Devices, Bonn, Germany

**Karl Georg Heimann** Expert Regulatory Toxicology, Köln, Germany

**Karin Heine** Research and Advisory Institute of Hazardous Substances, FoBiG - Forschungs- und Beratungsinstitut Gefahrstoffe GmbH, Freiburg, Germany

**Gerhard Heinemeyer** Federal Institute for Risk Assessment, Berlin, Germany

**Jan G. Hengstler** Leibniz Research Centre for Working Environment and Human Factors at the Technical University of Dortmund (IfADo), Dortmund, Germany

**Beate Henrikus** Ethics Committee Ludwig-Maximilians-University Munich, Munich, Germany

**Dietrich Henschler** Institute for Toxicology, University of Würzburg, Würzburg, Germany

**Rolf Hertel** Formerly Federal Institute of Risk Assessment, Berlin, Germany

**Alfred Georg Hildebrandt** (former) Head of Federal Institute of Drugs and Medicinal Products, Bonn, Germany

**Ludwig A. Hothorn** Leibniz University Hannover, Hannover, Germany

**H. Paul A. Illing** Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK

**Matt C. Jacobsen** AstraZeneca, R&D Innovative Medicines, Global Safety Assessment, Macclesfield, Cheshire, UK

**Bernd Kaina** Department of Toxicology, University Medical Center, Mainz, Germany

**Andreas D. Kappos** Former Head of Department of Health and Environment, Hamburg Health Authority, Frankfurt, Germany

**Hermann Kappus** Formerly MAK-Commission, Freising-Weihenstephan, Germany

**Wolfgang Kaufmann** Merck KGaA, Non-Clinical Safety, Global Pathology and Reproduction Toxicology, Darmstadt, Germany

**Kai Kehe** Bundeswehr Medical Academy, Military Medical Research and Development, Munich, Germany

**Agnieszka Kinsner-Ovaskainen** European Commission DG Joint Research Centre, Institute for Health and Consumer Protection, Ispra (Varese), Italy

**Hans Jochen Kittel** Institute for European Projects/Institut für Europaprojekte e.V, Berlin, Germany

**Ullrich Kleeberg** Berlin Medical Association – Berliner Medizinische Gesellschaft, Berlin, Germany

**Rainer Konietzka** Federal Environment Agency, Dessau-Roßlau, Germany

**Gert Ulrich Kuerzel** Sanofi-Aventis Deutschland GmbH, Industriepark Hoechst, Frankfurt am Main, Germany

**Bernhard Liebl** Bavarian Health and Food Safety Agency, Oberschleissheim, Germany

**Ines Liebl** Bavarian State Ministry for the Environment and Public Health, Munich, Germany

**Marianne Lilienblum** Faculty of Social and Behavioural Sciences, University of Amsterdam, Amsterdam, The Netherlands

**Werner Lilienblum** Formerly: Governmental Institute of Public Health, Hannover, Germany

**Mathias Locher** Covagen, Zurich, Schlieren, Switzerland

**Roman W. Lutz** Formerly: Seminar for Statistics, Swiss Federal Institute of Technology, Zürich, Switzerland

**Werner K. Lutz** Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg, Germany

**Inge Mangelsdorf** Department Chemical Risk Assessment, Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany

**Christine Mauriac** Sanofi R&D, Chilly-Mazarin, France

**Peter Mayer** Federal Institute for Drugs and Medical Devices, Bonn, Germany

**Robert L. Maynard** Bideford, Devon, UK

**Odile Mekel** Department of Prevention and Innovation, Innovation in Health, NRW Centre for Health, Bielefeld, Germany

**Klaus Mörike** Division of Clinical Pharmacology, Eberhard Karls University Tübingen, Tübingen, Germany

**Harald Mückter** Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität München, Munich, Germany

**Ludwig Müller** State Health Administration, unit 44 (Pharmaceutics, Toxicology, Genetic Engeneering), Bremen, Germany

**Hans-Günter Neumann** Department of Toxicology, University of Würzburg, Würzburg, Germany

**Birgit Niemann** Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany

**Franz Oesch** Oesch-Tox Toxicological Consulting and Expert Opinions GmbH&Co.KG, Wackernheim, Germany

**Klaus Olejniczak** Non-clinical Regulatory Consultant, Berlin, Germany

**Jürgen Pauluhn** Experimental Toxicology, Bayer HealthCare, Wuppertal, Germany

**Nathan Pechacek** Ecolab Inc, St. Paul, MN, USA

**Annette Pöting** Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany

**Ulrich Ranft** IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

**Franz-Xaver Reichl** LMU Munich, Munich, Germany

**Julia Riehm** Bundeswehr Institute of Microbiology, Munich, Germany

**Leonard Ritter** School of Environmental Sciences, University of Guelph, Bovey Bldg, Canada

**Elke Roehrdanz** Federal Institute for Drugs and Medical Devices, Bonn, Germany

**Manfred Ruthsatz** Nestlé Health Science, Vevey, Switzerland

**Eva A. M. Schäfer** Walther-Straub Institute of Pharmacology and Toxicology, Ludwig-Maximilian University Munich, Munich, Germany

**Marianna Schauzu** Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany

**Klaus Schneider** Research and Advisory Institute for Hazardous Substances, FoBiG, Freiburg, Germany

**Dieter Schrenk** Food Chemistry and Toxicology, Technical University of Kaiserslautern, Kaiserslautern, Germany

**Michael Schümann** Department of Health and Consumer Protection, Hamburg, Germany

**Regina Schumann** Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany

**Michael Schwenk** Formerly Medical School, Hannover, Germany

**Anuradha Shukla** Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, FAU, Erlangen, Germany

**Horst Spielmann** Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany

**Neill H. Stacey** Southern Cross Pharma Pty Ltd, Malua Bay, NSW, Australia

**Thomas Steger-Hartmann** Head of Investigational Toxicology, Global Early Development, Bayer Healthcare, Berlin, Germany

**Roland Suchenwirth** Head of Department of Environmental Medicine, Governmental Institute of Public Health of Lower Saxony, Hannover, Germany

**Horst Thiermann** Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany

**Adam D. Thomas** Department of Toxicology, University Medical Center, Mainz, Germany

**Angelika Tritscher** Department of Food Safety and Zoonoses, World Health Organization, Geneva 27, Switzerland

**Emmanuelle Voisin** Voisin Consulting Life Sciences, Boulogne, France

**Gerhard Volland** Schwäbisch Gmünd, Germany

**Eckhard von Keutz** Bayer HealthCare, Global Early Development, Wuppertal, Germany

**Thomas Wallenhorst** Sanitized AG, Burgdorf, Switzerland

**David C. Watts** School of Dentistry and Photon Science Institute, University of Manchester, Manchester, UK

**Gregory A. Wellenius** Department of Epidemiology, Brown University, Providence, RI, USA

**Michael Wilhelm** Department of Hygiene, Social and Environmental Medicine, Ruhr-University Bochum, Bochum, Germany

**Hans-Uwe Wolf** Department of Pharmacology and Toxicology, University of Ulm, Ulm, Germany

**Klaus-Michael Wollin** Lower Saxony Governmental Institute of Public Health, Hannover, Germany

**Maged Younes** Former Director, Food Safety and Zoonoses, World Health Organization (WHO), Mies, Switzerland

**Alexandre Zenié** Joint Research Centre, European Commission, Ispra, Italy

**Thomas Zilker** Formerly head of the Department for Clinical Toxicology, Medizinische Klinik Klinikum rechts der Isar Technical University München, Munich, Germany

**Lothar Zöller** Bundeswehr Institute of Microbiology, Munich, Germany

# Introduction

"All things are poisonous, there is nothing that is not poisonous; it is the dose that makes the poison." This saying was coined 500 years ago by Paracelsus (Phillipus Aureolus Theophrastus Bombastus von Hohenheim, 1493–1541). It still serves as a maxim for toxicological risk assessment, although what constitutes a poison (or harmful chemical or biological agent) has changed dramatically.

New chemical entities are synthesized in increasing numbers and new uses are found for existing chemical entities. These new chemicals and new uses mean that chemicals have to be tested for their toxic properties. Only then can it be decided whether the intended applications pose a toxicological risk to humans or the environment.

Regulatory toxicology has become increasingly complex and fragmented in recent years, and the number of regulated areas continues to increase. New computer-based methods help to make predictions of structure–activity relationships more reliable and effective. At the same time, new cell biological and molecular biological methods are introduced into toxicology, partly to replace animal experiments and partly to augment them. The actual significance of some of these tests for risk assessment may be unclear at first, but becomes clear with experience. Finally, an increasing number of risk extrapolation models are evaluated and used.

Nevertheless, one can consider regulatory toxicology as a uniform discipline, for it pursues a common goal, to protect human health and the environment, and uses a specific methodology for testing and evaluating. Thus, regulatory toxicologists in industry, government, universities, and other institutions have a common basis for action, even though the interest of each of the institutions may differ.

The present International edition is based on a book that was published in German by Springer in 2004. Toxicologists from the various working areas considered it necessary to collect all the aspects of regulatory toxicology in a single book. The present edition was thoroughly revised and updated by the authors and their international coauthors. This English edition makes the book and its chapters accessible to toxicologists worldwide. It is hoped that this will contribute to an improvement of the understanding between countries and between regulated areas. It is for professional toxicologists, but also should be of interest to other professionals who are involved in the protection of the environment and human health.

Opinions expressed are those of the individual authors and do not necessarily represent the views of their institutions.

The editors have sought to bring consistency to the diversity of opinions concerning toxicological risk assessment. We thank all the authors for their valuable input. We also thank Britta Müller, Susanne Friedrichsen and their colleagues Barbara Wolf, Ingrid Fischer and Ariane Israel at Springer for their excellent continuing support during the writing of this book. Finally, we thank Dr. Paul Illing for his help in translating the linking text.

F.-X. Reichl, Munich
M. Schwenk, Tübingen

# Part I

# The "Modus Operandi" of Regulatory Toxicology

Toxicology is the science of poisons and toxic effects. Regulatory toxicology is a subdiscipline concerned with the question of how man and the environment can be protected from toxic effects. Much regulatory toxicology is aimed toward making appropriate regulations and setting suitable standards. This difficult task requires a high degree of scientific understanding across a number of disciplines associated with the chemical, biological, and medical aspects of toxicology as well as an interest in practical implementation. Usually there is a logical division of labor between industry, government, and research institutions. Unfortunately, sometimes there is still a lack of institutional contacts between the different use sectors (e.g., drugs, personal care products, food, and industrial chemicals). Undoubtedly, a good basic education in toxicology coupled with effective cross-sector continuing professional development is the best guarantee for consistency in working practices.

**Aims and Institutions**

The aim of regulatory toxicology is the protection of human health and the environment from the hazards of chemicals, including drugs. Although the collection and evaluation of data must proceed according to scientific criteria, setting up the legislative background also requires political and legal input and depends on the psychological background and sociological attitudes to risk of the relevant population. Historically, different ministries and departments have made regulations independently of one another. Cross-departmental collaboration between the toxicologists in different institutions is improving this situation. Supranational harmonization means that, in recent years, much of the critical legislation is based on international agreements.

**Procedures and Standards**

A regulatory process defines the target populations that must be protected and the way(s) in which protection can be undertaken, identifies possible exposure scenarios, assesses toxicological hazards, and describes data gaps that need filling. Based on all available information, a first risk estimation can be made. The next step is risk evaluation. It incorporates nonscientific arguments, such as sociological or psychological or economic criteria. The aim is to define either what exposure level constitutes the maximum "acceptable" or "tolerable" risk (i.e., a standard) or

to decide that a particular exposure level (and hence risk) is acceptable. Process quality and outcome quality should be assured and examined at several levels. The professional independence of the toxicologists involved is an important quality factor.

# Aims and Mission of Regulatory Toxicology

Helmut Greim

## Contents

Introduction ..... 4
Components of Risk Assessment ..... 5
Hazard Identification ..... 5
Dose Response and Toxic Potency ..... 6
Exposure Assessment ..... 7
Risk Assessment ..... 9
Additional Information to Evaluate Human Relevance of Experimental Data ..... 10
Toxicokinetics ..... 10
Mode and/or Mechanism of Action ..... 11
Evaluation of Uncertainties ..... 12
Evaluation of Mixtures ..... 13
Classification of Carcinogens ..... 14
The Precautionary Principle ..... 15
The TTC Concept ..... 16
Regulations for Specific Chemical Classes ..... 16
REACH ..... 17
Summary ..... 19
Recommended Reading ..... 20

**Abstract**

The aim of regulatory toxicology is to control production, use, and deposition of dangerous materials to prevent adverse effects on human health and the environment. This requires sufficient information on the hazardous properties of a chemical compound, their relevance to man and of human and environmental exposure, which is a prerequisite for appropriate risk assessment and the decision whether regulatory consequences are warranted. The three elements of risk

H. Greim
Institute for Toxicology and Environmental Hygiene, Technical University Munich, Freising-Weihenstephan, Bavaria, Germany
e-mail: helmut.greim@lrz.tum.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_25, 

assessment of chemical substances are hazard identification, evaluation of dose–response relationship, and exposure assessment. The risk assessment process requires differentiation between reversible and irreversible effects, i.e., identification of a NOAEL and/or a LOAEL for the most critical effect of the substance, or, in the latter case, estimation of the risk of an exposure. The margin of exposure (MOE) or margin of safety (MOS) can be calculated by comparing the NOAEL with the human exposure. In general, up to now the risk of genotoxic carcinogens at a certain exposure concentration is estimated by linear extrapolation of the dose–response curve. Additional information for the evaluation of the human relevance of experimental data may be available from toxicokinetics or the mode and/or mechanism of action. By setting, for example, limit values like ADI or DNELs or implementing risk management measures, the uncertainties of the database on chemical substances have to be taken into consideration. Another challenge is the evaluation of mixtures. The systems for classification used by various national and international institutions are summarized. The use of the so-called precautionary principle and of the "Threshold of Toxicological Concern" (TTC) concept for risk assessment purposes is explained. In addition, the regulations for specific chemical classes like drugs or pesticides are completed by a short description of the EU REACH regulation for chemical substances.

## Introduction

Regulatory toxicology uses the information on the hazard and risks of human and environmental exposure of agents for their regulation. This requires understanding of the relevant regulations in this area as well as a basic understanding of the principles of toxicology. The latter is of specific importance because regulations are based on precise numbers like the ADI (acceptable daily intake) or cutoff levels for the labelling of hazardous chemicals, whereas toxicology as a biological and experimental discipline does not provide such precise data. A NOAEL (no observed adverse effect level) is a number obtained from animal experiments using different doses. Its preciseness depends on the number of animals used at each dose and the difference to the LOAEL (lowest observed adverse effect level), which might be two- or tenfold. Thus, expert judgment is required to correctly interpret the reliability of the data on which the ADI value is based and to decide on the need of regulatory consequences if the ADI is exceeded. Moreover, as implied by REACH regulations, the socioeconomic consequences including the availability of alternatives and their own hazardous properties need to be considered. Although there are guidelines and regulations for almost all aspects of risk assessment, risk management, and regulations, it is the quality of hazard identification and risk assessment for human and the environmental exposure, which determine the regulatory consequences. Consequently toxicology has to provide sufficiently defendable data for hazard identification and risk assessment and provide information, which are applicable for regulations as decided by the risk manager. On the other side, the risk managers have to understand

uncertainties of the risk assessment process and that a slight exceedance of an ADI or the RCR (risk control ratio) of 1 does not per se pose a non-tolerable risk.

Since toxicology is the basis of all regulations to protect human health, a profound understanding of toxicology is essential for regulatory toxicology.

To characterize the risk of a given or potential exposure, the adverse effects of chemicals have to be understood and by evaluating the dose response to identify at what exposure a chemical will produce adverse effects.

It is obvious from this that risk characterization comprises the three elements:

- Hazard identification, i.e., a description of the agent's toxic potential
- Evaluation of the dose response, including information on the concentration above which the agent induces toxic effects to identify the no observable adverse effect level (NOAEL)
- Exposure assessment to understand the concentration of the agent in the relevant medium, time, and routes of human exposure

A stepwise procedure provides information on the reactivity of the tested compound, its absorption and distribution in the organism, and possibly on critical targets. This allows the decision whether the database is appropriate for further testing by repeated dose studies in animals for 28 and 90 days, which depending on their outcome and intended use of the chemical are followed by a 6-month or lifetime study to evaluate potential effects upon long-term exposure including carcinogenicity.

Information on toxicokinetics, mechanisms, or mode of action allows evaluation of the relevance of the findings to humans and an appropriate risk assessment for a given or potential human exposure. For regulatory purposes acceptable exposure limits can be defined or in case of non-threshold mechanisms like genotoxic carcinogens, the definition of risk at a given exposure.

## Components of Risk Assessment

### Hazard Identification

Chemicals induce either local or systemic effects such as embryotoxicity, hepatotoxicity, and neurotoxicity after absorption from the gastrointestinal tract, through the skin, or via the lungs. Depending on exposure concentration and time of exposure, acute or chronic effects may result. Acute intoxication usually occurs in response to large doses of the parent compound less due to its metabolites. Acids or bases are directly acting agents which cause local irritation or corrosion at the site of exposure. Chronic effects are seen after repeated exposure during which time the chemical reaches critical concentrations at the target organ, and the result is persistent accumulated damage. Some chemicals, such as the widely banned 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), accumulate in tissues esp. body fat because they are lipophilic and are not well metabolized. In humans the half-life of excretion of TCCD is about 8 years. To ensure safe use by the consumers or the specific conditions in the workplace, the toxicological profiles of each chemical, either preexisting or newly

developed, need to be evaluated. Such evaluation may take different forms for new and existing chemicals. In the case of newly developed drugs, pesticides, or new chemicals, a stepwise procedure is used starting from simple in vitro and in vivo short-term tests. Depending on the hazardous potential of the agent, studies can be extended to evaluate long-term effects by repeated dose studies, toxicokinetics, and toxic mode of action. For existing chemicals, the available information can be collected, and a risk assessment based on exposure data, knowledge of the dose–response relationship, and the mode of action can be performed.

Reactivity, solubility, and metabolism of the chemical or its metabolites determine the target organ of the critical effects. Irritation or corrosion may occur when the chemical comes into contact with the skin or mucous membranes of the eye, the gastrointestinal tract, or the respiratory system. Distribution and metabolism of the chemical can result in various systemic effects upon interaction at targets in the critical organ, e.g., liver, kidney, and the central and peripheral nervous system. Histopathological and biochemical changes have been the major parameters used to detect organ toxicity. Increasing availability of sensitive methods in analytical chemistry and molecular-biological approaches including toxicokinetics and the various "omics" has significantly improved the understanding of the mechanisms by which cellular and subcellular functions are impaired and how the cells are responding to toxic insults. This results in a better understanding of toxic mechanisms, species differences, and the consequences of exposures at high and low concentrations over different times.

## Dose Response and Toxic Potency

Intensity of toxic effects is dose dependent, which implies a dose–response relationship and a dose which is without an adverse effect. Animal or human exposure is usually defined as the dose, e.g., in mg of the chemical/kg body weight/day. This daily dose may result from oral, inhalation, or dermal exposure or as a sum thereof. The external dose leads to a specific internal dose, which depends on the amount absorbed via the different routes. Absorption rates via the different routes can vary significantly, although oral and inhalation exposure usually lead to the highest internal dose. For example, about 50 % of cadmium in tobacco smoke is absorbed in the lung, whereas cadmium absorption from the gastrointestinal tract is about 10 %. Ultimately, it is the dose which reaches the cellular target over a given time period that results in the toxicological response. No toxic effects will be seen at doses up to the no observed adverse effect level (NOAEL), which is the starting point to derive values of acceptable exposures for consumers (acceptable daily intake: ADI) or at the workplace (occupational exposure limits: OELs). The dose–response curve may be expressed using a variety of mathematical formulas. Using the linear form of the dose–response relationship, the curve is sigmoid in shape and varies in slope from chemical to chemical. Thus, if the curve is shallow, a doubling of the dose results in a small increase of effects, whereas effects increase several-fold when the slope is steep (see Fig. 1). The log of the dose is plotted on the

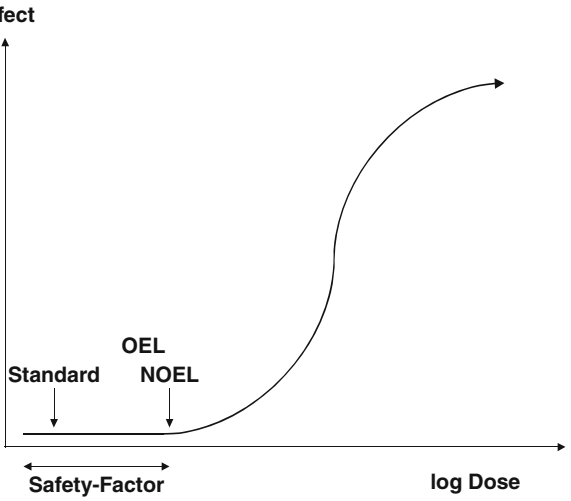



**Fig. 1** Dose–response curve showing the log of the dose on the X-axis and percentage response (Effect) on the Y-axis. The figure illustrates the location of regulatory values such as the NOEL, occupational exposure levels (OELs) or environmental standards such as acceptable daily intake (ADI). Note that a doubling of dose in the *lower or upper part* of the S-shaped curve results in small increases of effects, whereas it is much more prominent in the steep part

abscissa (X-axis) and increases toward the right. The location of the curve on the abscissa is a measure of the potency of the chemical.

## Exposure Assessment

According to the general principle of toxicology as expressed by Paracelsus (1493–1541), "In all things there is a poison, and there is nothing without a poison. It depends only upon the dose whether a poison is poison or not" or in short "the dose makes the poison"; the consequences of human or environmental exposures depend on the amount and duration to which these individuals or populations are exposed. Thus, exposure assessment or prediction of exposure is an ultimate requirement for risk assessment and to decide on the need for regulations.

Exposure defines the amount of a chemical to which a population or individuals are exposed via inhalation, oral, and dermal routes and is commonly defined by mg of the chemical/kg body weight per day.

Since occupational exposure is regular and repetitive, it can easily be measured in the air of the workplace and/or by use of personal monitoring equipment. Exposure of the general population is more difficult to assess. It usually is a combination of the presence of the compounds in indoor/outdoor air, drinking water, food, or use of products that contain the chemical. Moreover, frequency, duration and site of exposure, concentration, and weight of substance in the products need to be considered. Children represent a special case of exposure. For example, they may be exposed to chemicals that are released from toys during mouthing or via skin contact. Exposure can be modelled based on data such as information on frequency of mouthing, migration rates of the specific compound

from the toy during mouthing, and absorption rates from the oral cavity and gastrointestinal tract. The rate of absorption through the skin will also influence the body burden of the chemical. Use of these parameters to assess exposure is plagued by many uncertainties, which often lead to overestimation of the actual exposure. This external exposure may not necessarily correlate with internal exposure. Biomonitoring of the compound or its reaction products in the exposed individuals provides the most reliable estimate of internal exposure. However, dose–response curves usually provide a correlation between external dose and effects. Therefore, risk assessment of an internal exposure either requires knowledge of the dose response of internal exposure versus adverse effects or information to which extent external and internal doses correlate. The estimation of exposure is more complicated when mixtures of chemicals are the source of exposure.

Ultimately, it is the dose, which reaches the cellular target over a given time period, that results in the toxicological response. Thus, the toxic potency of a chemical is the product of the interrelated external, internal, and target doses, which results from the multiple pathways and routes of exposure to a single chemical (aggregate exposure). In the case of existing chemicals, an appropriately designed program to measure the chemical in the different media will provide the necessary information.

The measurement of external dose is either done on collected samples, i.e., food samples, or by direct measurement, i.e., air. In case collected samples are used, representative sampling and appropriate storage conditions as well as accurate and reproducible measurement techniques are essential. This also applies to biomonitoring programs.

In the case of new chemicals, such data are not available and cannot be provided so that modelling of exposure is the only option.

In the EU Technical Guidance Document on Risk Assessment Part I (ECB 2003), the following core principles for human exposure assessment for new and existing chemicals and biocides are listed:

- Exposure assessments should be based upon sound scientific methodologies. The basis for conclusions and assumptions should be made clear and be supportable and any arguments developed in a transparent manner.
- The exposure assessment should describe the exposure scenarios of key populations undertaking defined activities. Such scenarios that are representative of the exposure of a particular (sub)population should, where possible, be described using both reasonable worst-case and typical exposures. The reasonable worst-case prediction should also consider upper estimates of the extreme use and reasonably foreseeable other uses. However, the exposure estimate should not be grossly exaggerated as a result of using maximum values that are correlated with each other. Exposure as a result of accidents or from abuse shall not be addressed.
- Actual exposure measurements, provided they are reliable and representative for the scenario under scrutiny, are preferred to estimates of exposure derived from either analogous data or from the use of exposure models.

- Exposure estimates should be developed by collecting all necessary information (including that obtained from analogous situations or from models), evaluating the information (in terms of its quality, reliability, etc.), thus enabling reasoned estimates of exposure to be derived. These estimates should preferably be supported by a description of any uncertainties relevant to the estimate.
- In carrying out the exposure assessment, the risk reduction/control measures that are already in place should be taken into account. Consideration should be given to the possibility that, for one or more of the defined populations, risk reduction/control measures which are required or appropriate in one use scenario may not be required or appropriate in another (i.e., there might be subpopulations legitimately using different patterns of control which could lead to different exposure levels).

Biomonitoring (see chapters "► Background Exposure Versus Additional Exposure in Human Biomonitoring" and "► Human Biomonitoring. Its Importance in Toxicological Regulation" in this book) is the best tool to determine internal exposure. It allows to measure:

- The amount of a chemical taken into the organism by all routes (aggregate exposure)
- The metabolic fate of the chemical, its persistence in the organism, its rate of elimination, and by that the total body burden at the time of measurement
- The amount of the chemical and/or metabolites that reach the target organs

## Risk Assessment

The risk assessment process requires differentiation between reversible and irreversible effects. The dose–response curves for chemicals that induce reversible effects display a region below which no effects can be observed. The highest dose at which no adverse effects are seen is called the "no observable adverse effects level" (NOAEL). The point at which adverse effects become observable is called the "lowest observable effect level" (LOAEL). It is to be noted that threshold is not the equivalent of an NOAEL, since it describes a concentration or exposure where the slope of the dose–response curves changes.

If damage is not repaired and/or eliminated, the effect persists and may accumulate upon repeated exposure. In such cases a NOAEL cannot be determined and every exposure is related to a defined risk. Reversibility depends on the regenerative and repair capacity of cells, subcellular structures, and macromolecules during and after exposure. Epithelial cells of the intestinal tract or the liver have a high regenerating capacity and rapidly replace damaged cells by increased cell replication. The highly specialized cells of the nervous system have lost this capacity during natal and postnatal development. Consequently damaged nerve cells are not or slowly replaced, at least in the adult.

For chemicals, which induce reversible effects, the NOAEL of the most sensitive endpoint is determined and compared with the human exposure to describe the margin of exposure (MOE) (or margin of safety: MOS). If the

NOAEL is derived from animal experiments, a MOE of 100 or greater is desirable, which comprises a factor of 10 for interspecies differences and another factor of 10 for intraspecies differences. A MOE of at least 10 is sufficient if the NOEL is derived from human data.

The covalent binding of genotoxic mutagens and carcinogens to DNA has been considered as an irreversible event. Since the dose response of mutations parallels that of DNA adducts and is seen at higher exposures, the DNA adducts are considered as an indicator for exposure rather than for effects such as mutations. Moreover, there is increasing knowledge about DNA-repair mechanisms, the role of tumor-suppressor genes, apoptosis, and the level of background mutation rates; the assumption that even genotoxic effects exhibit a threshold becomes increasingly plausible (see Greim and Albertini 2012). However, at least so far, the general agreement remains that the potency of genotoxic carcinogens increases with increasing dose and that a NOAEL cannot be identified. As a consequence any exposure is associated with a certain risk, and the risk at a given exposure needs to be estimated by linear extrapolation from the dose–response data obtained from experimental studies in animals or from data obtained from studies in humans.

## Additional Information to Evaluate Human Relevance of Experimental Data

### Toxicokinetics

A chemical may enter the body via the gastrointestinal tract, the lung, or the skin. The amount absorbed depends on the concentration in the different media like food, air, and on physical-chemical parameters such as solubility in water and fat, stability, and the route of exposure. Toxicokinetics describe absorption, distribution, metabolism, and elimination (ADME) of a chemical in humans or experimental animals. Of specific importance for interpretation of animal studies and for extrapolation between species is the comparative information on the exposure and the dose that reaches the critical target.

Upon inhalation or skin penetration, the compound directly enters the cardiovascular system and distributes into the organs. When absorbed from the gastrointestinal tract, the chemical enters the liver via the portal vein and to a much less extent is taken up directly into the cardiovascular system. The epithelial cells of the gut wall and the liver present a large capacity for metabolizing chemicals so that a compound may be extensively metabolized by this "first-pass effect" before entering the (cardiovascular) systemic circulation. Larger molecules, e.g., the glucuronosyl conjugates, can be excreted via the biliary system into the duodenum where the conjugates may be hydrolyzed so that the original compound is reabsorbed and reenters the liver. This process is defined as *enterohepatic circulation*. Inhalation or dermal exposure to a chemical and intravenous or intraperitoneal injection may result in different effects than after oral exposure because of the "first-pass effect."

After entering the cardiovascular system, the chemical or its metabolites distribute to the organs where they can accumulate in organs such as fat or bones or are further metabolized. Reactive metabolites will interact with tissue components and may induce cellular damage. This "tissue dose," i.e., the concentration of a chemical or its metabolite at the critical target over a given time, is an important factor that helps to understand the correlation between internal exposure and external (environmental) exposure in relation to toxicity. By comparing tissue doses in different species at similar exposures, it also helps us to understand species differences in the sensitivity to chemicals as well as interindividual variations.

The chemical or its more water-soluble metabolites are primarily excreted via the kidneys or the biliary system. Volatile compounds may be exhaled. The great variety of processes observed during absorption, metabolism, distribution, and excretion cannot be predicted by modelling or by in vitro experiments without confirmatory data from animals and man.

## Mode and/or Mechanism of Action

There are many mechanisms by which chemicals or other stressors like heat or radiation can lead to toxicity. Knowledge of the modes or mechanisms by which a chemical induces toxicity are essential to understand species specificities, species differences, sensitive populations, or the interpretation of data regarding threshold or non-threshold effects. They also help to evaluate the relevance of the toxic effects to humans when the data are derived from experimental animals. Whereas the toxic mechanism is often not known in detail, modes of action, which can be described in a less restrictive manner, are helpful in the risk assessment process as well. They may be differentiated as follows:

*Physiological changes* are modifications to the physiology and/or response of cells, tissues, and organs. These include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal estrogens and/or androgens, and changes in immune surveillance.

*Functional changes* include alterations in cellular signalling pathways that manage critical cellular processes such as modified activities for enzymes involved in the metabolism of chemicals such as dose-dependent alterations in phase I and phase II enzyme activities; depletion of cofactors and their regenerative capacity; alterations in the expression of genes that regulate key functions of the cell, e.g., DNA repair; cell cycle progression; posttranslational modifications of proteins; regulatory factors that determine rate of apoptosis; secretion of factors related to the stimulation of DNA replication; and transcription or gap-junction-mediated intercellular communication.

*Molecular changes* include reversibility or irreversibility of changes in cellular structures at the molecular level, including genotoxicity. These may be formation of

DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy, and changes in DNA methylation patterns.

Mechanistic information is most relevant for the evaluation, classification, and regulation of all hazardous and at a given exposure to risky chemicals including carcinogens. For example, if the carcinogenic effect is induced by a specific mechanism that does not involve direct genotoxicity, such as hormonal deregulation, immune suppression, and cytotoxicity, the detailed search for the underlying mode of action may allow identification of a NOAEL. This can also be considered for materials, such as poorly soluble fibers, dusts, and particles, which induce persistent inflammatory reactions as a result of their long-term physical presence that ultimately lead to cancer.

## Evaluation of Uncertainties

When discussing regulations of specific chemicals, one has to be aware that the data used to set a DNEL or to determine the carcinogenic risk of the exposure to a certain chemical always include uncertainties, with the consequence that the risk of a certain exposure may be over- or underestimated. For example, the NOAEL may not be a real NOAEL for statistical reasons in that too few animals have been used in the specific experiment. Or the NOAEL is rather conservative because the next higher dose, which determines the LOAEL of a weak adverse effect, is tenfold higher. Usually this uncertainty is covered by deliberately applying assessment factors that build in a margin of error so as to be protective of the population from risks. In case of DNELs, the uncertainty factor of 100 covers the uncertainties of inter- and intraindividual differences unless toxicodynamic and/or toxicokinetic information allows its reduction. Whereas the experts, who have performed the risk assessment, are usually aware of uncertainties, the risk manager tends to use the numbers as such, with the consequence that any exposure even slightly higher than the DNEL is not considered to be acceptable.

Acknowledging these uncertainties with data, the distribution of uncertainties may be defined by statistical approaches to characterize and weigh the different assumptions from various components (including dose response, emissions, concentrations, exposure, valuation). This will improve understanding of the reliability of the available information, how risk may vary in a population and thereby allowing better mean estimates of risk, and of the magnitude of risk for different individuals.

There is an ongoing discussion about the risk of chemicals in toys, which can be mouthed by children. Obviously, the resulting exposure is determined by the migration rates of the chemical from the material and the daily mouthing time. Since there is no standard procedure to determine migration rates and the data on mouthing time published in the scientific literature differ by a factor of almost 10, the database for regulatory consequences is rather uncertain. This is why the Scientific Committee on Toxicology, Ecotoxicology, and the Environment in its opinion on diethylhexylphthalate in teethers only concluded that the resulting

exposure is only about 20-fold below the NOAEL instead of the usually applied factor of 100. Based on this information, the European Commission restricted the use of diethylhexylphthalate in teethers **and** in all toys, which can be mouthed by children. With this the Commission applied the precautionary principle, because such toys usually contain lower concentrations of diethylhexylphthalate and mouthing times will be less than for teethers.

Methods for the evaluation of unquantified uncertainties are described by EFSA (2006) and ECHA (2008).

## Evaluation of Mixtures

Humans and their environments are exposed to a wide variety of substances. The potential adverse effects of the interactions between those substances when present simultaneously in a mixture have been analyzed in several reviews and documentations. Most recently the available scientific literature has been analyzed by the nonfood Scientific Committees of the European Commission. The following conclusions have been reached (see SCCS/SCHER/SCENIHR (2012):

1. Under certain conditions, chemicals will act jointly in a way that the overall level of toxicity is affected.
2. Chemicals with common modes of action will act jointly to produce combination effects that are larger than the effects of each mixture component applied singly. These effects can be described by dose/concentration addition.
3. For chemicals with different modes of action (independently acting), no robust evidence is available that exposure to a mixture of such substances is of health or environmental concern if the individual chemicals are present at or below their zero-effect levels.
4. Interactions (including antagonism, potentiation, and synergies) usually occur at medium- or high-dose levels (relative to the lowest-effect levels). At low exposure levels, they are either unlikely to occur or are toxicologically insignificant.
5. In view of the almost infinite number of possible combinations of chemicals to which humans and environmental species are exposed, some form of initial filter to allow a focus on mixtures of potential concern is necessary. Several criteria for such screening are offered.
6. With regard to the assessment of chemical mixtures, a major knowledge gap at the present time is the lack of exposure information and the rather limited number of chemicals for which there is sufficient information on their mode of action. Currently, there is neither an agreed inventory of mode of actions nor a defined set of criteria on how to characterize or predict a mode of action for data-poor chemicals.
7. If no mode of action information is available, the dose/concentration addition method should be preferred over the independent action approach. Prediction of possible interaction requires expert judgment and hence needs to be considered on a case-by-case basis.

## Classification of Carcinogens

The systems for classification of carcinogens used by various national or international institutions were developed in the 1970s. Classification is based on qualitative criteria and reflects essentially the weight of evidence available from animal studies and epidemiology. Classification is usually based on the certainty with which a carcinogenic potential for a chemical can be established. Generally three categories, the definitions of which slightly differ, are used:

- Human carcinogens
- Animal carcinogens, reasonably anticipated to be human carcinogens
- Not classifiable because of inadequate data

For classification, mode of action and potency of a compound are either not taken into account or at best used as supporting arguments. The advancing knowledge of reaction mechanisms and the different potencies of carcinogens may lead to a reevaluation of the traditional concepts.

The International Agency for Research of Cancer and the OECD propose to use data on the carcinogenic mechanism and potency in decision making. The SCOEL (Scientific Committee for Occupational Exposure Limits) of the General Directorate Employment of the European Union applies information on carcinogenic mechanisms and potency as criteria for a revised classification. The US Environmental Protection Agency (EPA) and a committee of the German Research Foundation (Deutsche Forschungsgemeinschaft) recommended consideration of mode of action and have published modified concepts for classification. These activities in part originate from the recognition that one can distinguish between mechanisms of carcinogenicity caused by non-genotoxic and genotoxic carcinogens. Thus, it is possible to identify a NOAEL for non-genotoxic carcinogens, provided there is sufficient information on the primarily non-genotoxic mechanism. The American Conference of Governmental Industrial Hygienists (ACGIH) uses a concept, which considers carcinogenic potency for classification since 1995.

To determine the potency of genotoxic carcinogens and cancer risk at a given exposure, a linear or sublinear extrapolation from the high-dose effects observed in animals to the usually lower human exposure is requested by regulatory agencies. The European Food and Safety Authority (EFSA 2005) recommends to avoid this extrapolation because of the inherent uncertainties. Instead, the margin of exposure (MOE) between a benchmark dose and the T25 calculated from a carcinogenicity study in animals and human exposure should be determined. A MOE of 10,000 and more is of minor concern. The advantage is that neither a debatable extrapolation from high to low doses needs to be performed nor are hypothetical cancer cases calculated.

As indicated above the REACH uses the C&L criteria of the Globally Harmonized System (GHS), which is exclusively hazard based. This leads to classification of CMR (carcinogenic, mutagenic, reprotoxic) compounds without considering whether the test conditions of the animal experiments are relevant for humans nor whether there is human exposure which may result in a risk. Since classification in

CMR categories 1A or 1B has consequences for consumer exposure, this may lead to scientifically non-justified restrictions. Although industry can submit a proposal for authorization, the severe consequences of a toxicologically and socioeconomically not justified C&L can be avoided if the C&L process would become risk based.

## The Precautionary Principle

According to Article 191 of the Treaty on the Functioning of the European Union (EU), the precautionary principle aims to assure a higher level of environmental protection through preventative decision taking in the case of risk. However, in practice, the scope of this principle is far wider and also covers consumer policy, European legislation concerning food and human, and animal and plant health. It is a measure to enable rapid response in the case of a possible danger to human, animal, or plant health or to protect the environment. In particular, where scientific data do not permit a complete evaluation of the risk, this principle may, for example, be used to stop distribution or order withdrawal from the market of products likely to be hazardous.

Since this description allows various interpretations, a more precise definition is given in the Communication from the Commission of 2 February 2000 on the precautionary principle. There it is outlined that the precautionary principle may be invoked when a phenomenon, product, or process may have a dangerous effect, identified by a scientific and objective evaluation, if this evaluation does not allow the risk to be determined with sufficient certainty. The Commission specifically stresses that the precautionary principle may only be invoked in the event of a potential risk and that it can never justify arbitrary decisions. To allow its application, three preliminary conditions should be met:

- The fullest possible scientific evaluation, the determination, as far as possible, of the degree of scientific uncertainty
- A risk evaluation and an evaluation of the potential consequences of inaction
- The participation of all interested parties in the study of precautionary measures, once the results of the scientific evaluation and/or the risk evaluation are available

In addition, the following general principles of risk management remain applicable when the precautionary principle is invoked:

- Proportionality between the measures taken and the chosen level of protection
- Nondiscrimination in application of the measures
- Consistency of the measures with similar measures already taken in similar situations or using similar approaches
- Examination of the benefits and costs of action or lack of action
- Review of the measures in the light of scientific developments

Other states use slightly different definitions. For example, the Canada definition is as follows:

> The precautionary principle is an approach to risk management that has been developed in circumstances of scientific uncertainty, reflecting the need to take prudent action in the face of potentially serious risk without having to await the completion of further scientific research.

Canada refers to the definition of the precautionary principle of the Rio Conference on Environment and Development (Principle #15 of the June 1992, Declaration), which reads:

> In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.

Although these definitions seem to be contradictive, they generally indicate that the precautionary principle should be applied in cases of potentially *serious* risks without having to wait for a complete risk assessment. This implies that the principle is only applicable in case of a severe risk in case it cannot be sufficiently defined at present.

## The TTC Concept

The Threshold of Toxicological Concern (TTC) is a concept to establish a level of exposure for chemicals, regardless of their chemical-specific toxicity data, below which there is no appreciable risk to human health. The concept is based on knowledge of the chemical structure for evaluating structural alerts, the amount of a specific chemical in a product, and the daily human exposure. So far the TTC concept is applied for chemicals in food. It is defined as a nominal oral dose which poses no or negligible risk to human health after a daily lifetime exposure. At a mean dietary intake below the level of the TTC, toxicology safety testing is not necessary or warranted. By that the TTC concept can contribute to a reduction in the use of animals for safety tests. The TTC concept may also represent an appropriate tool to evaluate or prioritize the need for toxicological testing. There is ongoing discussion on its general applicability for safety assessment of substances that are present at low levels in consumer products such as cosmetics or for impurities or degradation products. For the recent evaluation of the general applicability of the TTC concept by the nonfood Scientific Committees of the European Commission, see SCCS/SCHER/SCENIHR (2012).

## Regulations for Specific Chemical Classes

Jurisdictions and regulatory agencies around the world have established a variety of guidelines for risk assessment and permissible exposure standards for chemicals in the workplace, the home, and the general environment. Regulatory decision making depends upon the estimation of health risks from chemical exposure.

Health risks of chemicals designed for specific applications, e.g., consumer products, drugs, or pesticides, must be assessed when people are exposed in the many types of environment in which people can be found. Therefore, all elements of risk assessment such as hazard identification, dose response, exposure, and the risk have to be thoroughly evaluated.

Data requirements for *new and existing chemicals* usually depend on annual production rate and the extent of human exposure. When there is considerable exposure, regulatory requirements demand an extensive toxicological evaluation of the potential adverse effects of the specific chemical and the likelihood of their expression under the conditions of use or exposure and the definition of the MOE or the health risk under defined conditions of exposure.

For *drugs* special emphasis must be placed on efficacy, therapeutic index, potential side effects, and the effects of overdosage.

For *pesticides* the relative impacts of the chemical on the target versus on people is a critical requirement. Thus, the NOEL for people must be established, and an acceptable daily intake (ADI) must be determined because of the possibility of contamination of food and other consumer products with the pesticide, and the margin of safety needs to be established.

Exposures to *chemicals at the workplace* are, accordingly to law, controlled by the Occupational Safety and Health Administration (OSHA) in the United States and by the Chemicals Law Act in Europe. Various governmental and nongovernmental institutions are involved in setting occupational exposure standards. Since the institutions publish the complete toxicologically relevant information and a justification for the proposed limit value, these documentations are valuable sources for the toxicological database of the compounds.

Table 1 provides references to the institutions which publish documents on the toxicological data of chemicals.

## REACH

In 1992 the European Commission estimated that about 100,000 chemicals are in use. They are produced in quantities ranging from less than one ton to several million tons per year. Except drugs and pesticides, data requirement for existing or new chemicals has not been regulated. Although it is the responsibility of the producer and downstream user to release safe products, there are high-volume products with a relatively small database. Several programs have been launched to obtain knowledge at least for compounds with high annual production rates. In the USA, EPA has initiated a HVP program. In an international cooperation, the OECD has launched the ICCA program, which evaluates and documents the available information on environmental and human health hazards and risks for about 1,000 chemicals. In Europe, Risk Assessment Reports under the Existing Chemical Program of about 150 compounds are being produced.

REACH (Registration, Evaluation, and Authorization of Chemicals) of the European Union is to identify substances of hazardous properties and to evaluate the risks of human and environmental exposure. The regulation became effective by 2008. It is the responsibility of the producer or downstream user to provide the necessary information to the European Chemical Agency (ECHA). The extent of toxicological information largely depends on the annual production rate of a chemical. As long as there is no indication of a specific risk, the chemicals will

**Table 1** International institutions that publish documentations on chemicals

| |
|---|
| ACGIH (American Conference of Governmental Industrial Hygienists) http://www.acgih.org/TLV/ [last date of access: 03.03.2013] |
| ATSDR (The Agency for Toxic Substances and Disease Registry) http://www.atsdr.cdc.gov/ [last date of access: 03.03.2013] |
| BUA – Advisory Committee on Existing Chemicals (of the GDCh, the German Chemical Society http://www.gdch.de/publikationen/weitere-publikationen.html [last date of access: 03.03.2013] |
| The Canadian Centre for Occupational Health and Safety http://www.ccohs.ca/ [last date of access: 03.03.2013] |
| Dutch Expert Committee on Occupational Standards (DECOS) http://www.gezondheidsraad.nl/en/publications/healthy-working-conditions/results [last date of access: 03.03.2013] |
| Environmental Protection Agency (EPA) http://www.epa.gov/ [last date of access: 03.03.2013] |
| European Centre for Ecotoxicology and Toxicology of Chemicals http://www.ecetoc.org/ [last date of access: 03.03.2013] |
| HSE (UK Health and Safety Executive) http://www.hse.gov.uk/ [last date of access: 03.03.2013] |
| International Agency for the Research of Cancer http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php [last date of access: 03.03.2013] |
| International Programme on Chemical Safety http://www.inchem.org/ [last date of access: 03.03.2013] |
| MAK Commission (German Research Foundation) http://onlinelibrary.wiley.com/book/10.1002/3527600418/topics [last date of access: 03.03.2013] |
| NIOSH: http://www.cdc.gov/niosh/homepage.html [last date of access: 03.03.2013] |
| The Nordic Expert Group: http://www.nordicexpertgroup.org//[last date of access: 03.03.2013] |
| OSHA: http://www.osha.gov/ [last date of access: 03.03.2013] |
| SCOEL – EC Scientific Committee on Occupational Exposure Limits http://ec.europa.eu/social/main.jsp?catId=148&langId=en&intPageId=684 [last date of access: 03.03.2013] |

be registered for the intended use. Special attention will be paid to carcinogens, mutagens, and reproductive toxicants (CMR compounds) as well as to chemicals, which show bioaccumulation, persistence, and toxicity (BPT compounds) in the environment. According to the regulation, the extent of information to be submitted depends on the amount produced or imported annually, and requirements are highest for compounds of >1,000 t/a, less for <100 t/a, and lowest for 10–100 t/a chemicals.

Member states can propose classification and labelling of chemicals and restrictions, and the proposals are evaluated by the Risk Assessment Committee (RAC) of ECHA. The consequence of a CMR classification in category 1A or 1B is a ban for consumer exposure. In such cases industry can apply for authorization for a specific use by providing evidence that there are no alternatives and the risk of consumer exposure is low. Proposals for restrictions presented by member states also need to demonstrate that there are no less toxic and economically acceptable alternatives, and in case the chemical is further used, the risk of consumers is not tolerable. Both the proposals for authorizations and restrictions are evaluated by the RAC

and the Socio-economic Committee (SEAC) of ECHA. The latter committee performs a cost-benefit analysis for the restriction or authorization of the chemical and the alternatives.

## Summary

The aim of regulatory toxicology is to control production, use, and deposition of dangerous materials in order to prevent adverse effects on human health and the environment. This requires sufficient information on the hazardous properties of a compound, their relevance to man and of human and environmental exposure, which is a prerequisite for appropriate risk assessment and the decision whether regulatory consequences are warranted.

The sensitivity of analytical chemistry has advanced to the point where infinitesimally small amounts of chemicals can be detected and identified in the various media of human environment. Since the dose makes the poison, not the mere presence of a chemical needs regulatory consequences.

In most countries regulatory agencies take the responsibility to identify hazardous material and after careful risk assessment propose regulations. Due to the specific requirements, specific agencies for compounds like drugs, pesticides, or chemicals used in consumer products have been installed. Mostly expert panels of independent scientist assist these agencies. Usually the socioeconomic consequences of a regulation are analyzed for a cost-benefit evaluation of the restriction or ban of a compound and of the available alternatives. To prevent non-tolerable risks to man and the environment from the use of compounds, levels of tolerable concentrations or exposures such as the ADI or DNELs can be established and implemented. Their implementation and compliance need to be controlled. In case tolerable levels are exceeded, the uncertainties involved in risk assessment and the derivation of tolerable levels of exposure need to be considered to evaluate whether exposures slightly exceeding such levels require measure for improvement.

There is an array of testing procedures to determine the hazardous properties such as acute, subchronic, and chronic toxicity, irritation and phototoxicity, sensitization and photosensitization, genotoxicity, carcinogenicity, or toxicity to reproduction. Information on the toxicokinetics and mechanisms of the toxic effects improve the relevance of the findings for man. More recent methodologies like toxicogenomics or high-throughput testing of agents for a single endpoint will become increasingly available and may improve hazard identification and aid in the identification of common mechanisms of multiple agents.

The public and the scientific community expect that regulatory toxicology is science based and that the proposed regulations rely on an appropriate evaluation of the intrinsic properties of an agent (hazard identification) and of the risk of a defined human and environmental exposure. Thus, the prerequisite of regulatory toxicology consequences is an understanding of the principles of toxicology, the uncertainties of hazard assessment and risk assessment, and by that identification of adverse and no adverse effect levels and exposure assessment. The precautionary principle

should be applied only in case of insufficient information for a final regulatory decision, not to justify elimination of exposure to any non-wanted chemicals.

Therefore, any regulatory decisions need to be based on an appropriate risk assessment of the possible human or environmental exposure. This can be retrospective in case of existing chemicals or prospective for newly developed compounds.

## Recommended Reading

EC SCCS/SCHER/SCENIHR (2011) Toxicity and Assessment of Chemical Mixtures. http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_150.pdf. Accessed 3 Mar 2013

EC SCCS/SCHER/SCENIHR (2012) Use of the Threshold of Toxicological Concern (TTC) Approach for Human Safety Assessment of Chemical Substances with focus on cosmetics and consumer products. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_092.pdf. Accessed 3 Mar 2013

ECB (2003) Technical Guidance Documents, European Chemicals Bureau. http://ihcp.jrc.ec.europa.eu/our_activities/public-health/risk_assessment_of_Biocides/doc/tgd. Accessed 3 Mar 2013

ECHA (2008) Guidance on information requirements and chemical safety assessment. Chapter R.19: uncertainty analysis. European Chemicals Agency (ECHA). Helsinki, Finland

EFSA (European Food Safety Authority) (2005). Opinion of the scientific committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic. http://www.efsa.europa.eu/en/science/sc_commitee/sc_opinions/1201.html. Accessed 3 Mar 2013

EFSA (2006) Guidance of the scientific committee on a request from EFSA related to uncertainties in dietary exposure assessment. EFSA J 438:1–54

Greim H, Albertini R (eds) (2012) The cellular response to the genotoxic insult. The question of threshold for genotoxic carcinogens. RSC, Cambridge, UK

Greim H, Snyder R (eds) (2008) Toxicology and risk assessment. A comprehensive introduction. Wiley, Chichester/Hoboken

Opinions of Scientific Committees of DG SANCO (2012). http://ec.europa.eu/health/scientific_committees/all_opinions/index_en.htm. Accessed 3 Mar 2013

US EPA (2007) Assessing Health risks from Pesticides (Pesticides: Topical and Chemical Fact Sheets). http://www.epa.gov/pesticides/factsheets/index.htm. Accessed 3 Mar 2013

# Working Areas of Regulatory Toxicology

Michael Schwenk and H. Paul A. Illing

## Contents

Institutions ..... 22
Authorities ..... 23
Industry ..... 23
Universities and Other Basic Research Institutions ..... 24
Contract Research Organizations ..... 25
Advocacy (Lobbying) ..... 25
Expert Witness/Court and Public Enquiry Advisor Work ..... 25
Scientific Societies and Journals ..... 25
Chemical Properties ..... 26
Regulated Areas and Legislature ..... 27
Regulations Concerning Marketing ..... 27
Ambient Media ..... 27
Understanding Regulations ..... 28
Alarm Systems ..... 28
Working Methods ..... 29
Recommended Reading ..... 30

**Abstract**

As in other technical fields, there is increasing diversification in the toxicological risk assessments undertaken by, or on behalf of regulatory agencies. This is reflected in the many ways in which regulatory toxicology (health and environmental risk assessment) work areas can be divided. These include by

M. Schwenk (✉)
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

H.P.A. Illing
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK
e-mail: paul@sherwood37.demon.co.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_112, 

end use, by institution, by chemical properties, and by working methods. Although coordination is essential, different institutions sometimes make regulatory decisions independently of one another. Consistency of decision involves harmonizing; thus, cross-border cooperation of toxicologists and other regulatory affairs specialists is essential.

## Institutions

Regulatory toxicologists do not operate in a vacuum. There is an objective and there are societal, legal, and philosophical contexts that underlie the scientific decision-making processes of regulatory toxicology. Setting these contexts involves other professionals and nonprofessional groups, such as citizen action committees, lobbying groups, trade associations, and legislators (politicians and lawyers). Understanding and explaining these contexts and how they operate is the role of psychologists and sociologists. Further information on this aspect of regulatory toxicology is beyond the scope of this chapter but can be found in, for example, Illing and Marrs (2009) and Illing (2009).

The expertise for undertaking regulatory risk assessments comes from toxicologists, epidemiologists and exposure specialists, and, in some cases, economists concerned with risk-benefit assessments. These may be found working in government authorities, industry, contract research organizations, and academia (Fig. 1). Each of these institutions has extended international communications networks (both to regional, e.g., European, and international [UN and OECD] bodies). Despite some competition, there is also a constructive cooperation between the institutions.

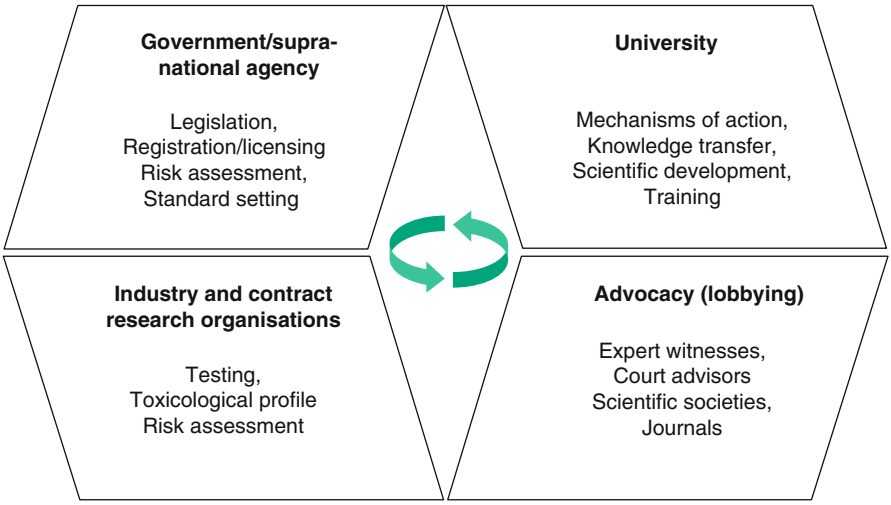



**Fig. 1** Institutions

## Authorities

Toxicologists, (including clinical toxicologists) and other related specialists such as epidemiologists, occupational hygienists, exposure specialists and policy makers in government (and supranational, including EU) agencies advise the authorities on various levels such as local administrations, ministries, and the government. Toxicologists are involved in the generation and monitoring of test method standards, audit procedures, and standards, registrations, and licensing procedures. Since they have to consider long-term unwanted aspects on the population and environment, they largely work on the basis of conservative risk assessments and, when dealing with environmental issues, the "precautionary principle." They use their toxicological and ecotoxicological expertise to estimate specific risks (in a risk assessment) and, when the risk is not sufficiently low to constitute an acceptable risk, they may then join with others in undertaking a risk-benefit analysis in order to determine a "tolerable risk" based on trading the usefulness of a substance with the necessity of protection.

While it may be developed by individual scientists and regulatory specialists, acceptance of the relevant conceptual underpinning for this work is usually very slow and obtained via authoritative national and international bodies. Test methods and audit systems ("Good Laboratory/Clinical/Manufacturing Practice guidelines") are also developed through authoritative international bodies. Of particular importance are the OECD (Organisation for Economic Cooperation and Development), the ICH (International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use), the EU Scientific Committees, and academic bodies such as the US National Academy of Sciences, the UK Royal Society, and the DFG (Deutsche Forschungsgemeinschaft). The regulation of different sectors may be a) by sector defined by end use: pharmaceuticals, veterinary medicines, medical devices, food (including additives and contaminants), animal feed, plant protection products, and biocides; b) by environmental compartment: water quality; indoor, outdoor, or workplace air quality; soil contamination; or c) reserve schemes for chemicals or radiation. These sectors can involve different agencies and the agencies may be largely independent of each other (Fig. 2). Here, more networking is required to allow for better harmonization.

## Industry

Toxicologists and regulatory affairs specialists in industry have the responsibility to ensure that products placed on the market have a satisfactory risk/benefit ratio. This is of particular interest for quality conscious companies. Toxicologists in industry may commission contract research organizations (CROs) to undertake standard tests to protocols described by the authorities, or they may undertake testing "in house." Studies for regulatory purposes rely largely on internationally standardized protocols for determining the toxic potential of individual substances. These studies usually seek to identify pathological and clinical-chemical endpoints and a dose–response in

| *Authority* | *Toxicological Responsibility* |
|---|---|
| US EPA/EU National Authorities (Environment Agencies) | toxicology of drinking water |
| EMEA (EU) /FDA (US) | pharmaceutical toxicology |
| Individual national or sub-national investigators (e.g. Police) | forensic toxicology |
| US Defense and Homeland Security/ EU National Defence and Interior Departments | toxicology of agents associated with warfare/terrorism |
| US OHSA/EU National bodies | workplace toxicology |
| US FDA and Dept Agric/ EU EFSA | food toxicology |

**Fig. 2** Examples of toxicology-associated agencies and fields

animals. Investigative studies using structure-activity relationships and/or in vitro methods may be conducted in order to better understand the potential toxicity. These results form the basis for the initial hazard assessment for a newly developed chemical. Exposure assessment is also conducted to see if there is a sufficient margin of exposure for the intended use. If specific risks have to be further clarified, additional experimental work related to, for example, toxicokinetics and mechanisms of action may be performed. Such nonstandard tests often require very specific methodologies and may be performed in cooperation with partners from universities or from contract research institutions. Where possible, the standardized regulatory testing is subjected to an audit process, Good Laboratory Practice, supervised by the relevant national authorities. The tests are conducted to standardized protocols, and the results evaluated using standard procedures. This is the main information source for the authorities, who make a regulatory decision about the registration and categorization of the compound.

Once a substance has been placed on the market, either for a specific use or more generally, there is a need for monitoring for unidentified toxic effects ("unknown unknowns"). For drugs this is called "pharmacovigilance." Through this process it is possible to check if the risk management procedures (either for the specific chemical or use or more generally) are adequate or, if not, to reassess and reevaluate the risks.

## Universities and Other Basic Research Institutions

Toxicologists at universities and basic research mainly aim at understanding toxicological mechanisms at the cellular level. They often use investigation techniques which are not subject to standardization but provide new methodological approaches and scientific knowledge. In this context, they develop novel methods that are suited to better predict toxic effects. Epidemiologists and experts in exposure modelling and measurement also contribute to the sciences underpinning risk analysis. All of these specialists must encourage cooperation with neighboring scientific disciplines and networking with regional and national partners. They often act as experts in regulatory committees. Finally, they play a central role in the education of young academics.

When there is a need for risk-benefit analysis, there is a need to environmental economists. Integrating their role with that of the other participants in the risk (or risk-benefit) evaluation is still at an early stage, and there is therefore much scope for academic research in this field.

Of increasing importance is the need for an understanding of the psychological and sociological aspects of the process of risk analysis (risk assessment and risk management) and of how the public perceives risks. It is essential that the public (as a whole) has confidence in the regulators and a key need is an understanding of how public and regulatory understanding can be merged. Psychologists and sociologists working on aspects of risk perception offer insights into this process, and their contribution should not be disregarded.

### Contract Research Organizations

CROs are often specialists in specific tests or evaluations, in which they are highly experienced. In these niches, they are likely to be more efficient and more economical than other institutions.

### Advocacy (Lobbying)

Advocacy groups (such as Greenpeace, Friends of the Earth, anti-vivisectionists, trade associations) are essentially aimed at trying to persuade regulators, either directly or through persuading public opinion, that their views concerning issues should be preferred in place of those accepted by or about to be accepted by the regulator.

### Expert Witness/Court and Public Enquiry Advisor Work

Generally this work is carried out by the individual rather than by a type of institution. The focus of this type of specialist is in defined problem fields, such as advising in litigation or in criminal prosecutions concerning causes of damage or in Public Enquiries into incidents/accidents. The expert witness prepares expert statements containing toxicity profiles set against information on specific incidents (and the requirements of the legislation) in order to indicate to the parties and, if it comes to Court, the Court the relevant facts and their implications. The Public enquiry expert advisor advises the presiding officer (usually a Judge) on the scientific facts and their implications for the enquiry.

## Scientific Societies and Journals

The toxicological scientific societies are self-administered organizations of toxicologists from the different working areas. They have the main aim to promote

the toxicological sciences. Scientific questions concerning how toxic agents work are traditionally the main focus of these societies.

Risk is a statistical concept that relies on toxicological data to define the hazard on one hand and statistics (probability) to define the likelihood of the event occurring or of the exposure resulting in harm. Traditionally, scientists in universities and university-associated research units are research-oriented and not much interested in the principles and issues associated with the risk evaluation part of the regulatory process. These issues involve nonscientific aspects of risk (such as attitudes to risk and risk perception) and nonscientific aspects may prevail.

As a political process is involved, there is room for contributions from the social sciences (sociological and psychological aspects of risk, notably the influence of risk perception on risk evaluation). The ability to obtain a compromise may have a greater role in toxic risk regulation than scientific exactness. Hence the ability to influence regulatory decisions is becoming increasingly important as an activity in which chemical and toxicological societies participate. It also provides a platform for the participation of science in international regulatory spheres and sometimes opens the door to highly interesting new ideas for research.

So it is not surprising that many scientific societies are increasingly engaging in issues of regulatory toxicology at the national and international level. They provide a forum in which basic scientists, risk analysts, and toxicologists can freely exchange ideas, without the restrictions, which they might have within their institution.

As a consequence of the recognition of this wider role for experts in regulatory toxicology, risk assessment and risk evaluation are increasingly important parts of the training of toxicologists. This is being encouraged by the scientific societies. In parallel, articles on topics involving regulatory toxicology are increasingly found in the scientific journals. This trend has been early recognized and promoted by the "International Society of Regulatory Toxicology and Pharmacology" and its journal and the foundation and development of journals in the field of risk analysis that accept articles on toxicological aspects of risk analysis.

## Chemical Properties

The chemist is usually most interested in the chemical properties of a substance and will therefore find it logical to classify toxic substances according to their chemical properties. Thus, one can distinguish between the regulation of inorganic chemicals (e.g., metal toxicology), organic chemicals (many industrial chemicals), and natural products (e.g., toxins, genetically engineered products – these are a subgroup of organic molecules, usually of high complexity). A more far-reaching differentiation can be based on functional groups (nitrosamine regulation) or the chemical backbone (dioxin regulation). Finally, it may be crucial for the toxicological assessment whether one deals with a pure substance or a mixture (combination effects such as inhibition or synergism) and whether these are dissolved or in particulate form (e.g., dust).

The effect researcher, who may typically be a biologist or physician, is more interested in biological and medical effects. He/she accordingly arranges groups of substances with the same effect, such as allergens, irritants, initiators, promoters, endocrine disruptors, cytochrome inductors, and neurotoxic or hepatotoxic substances.

The attention of toxicologists in the event of toxicological emergencies is focused on the harmful effects and the causing substances (e.g., dioxins after the accident at Seveso). The legal regulation then follows mainly the pattern of the regulated areas.

## Regulated Areas and Legislature

It is not unusual that different levels of protection are defined for different purposes. The two principal criteria are the "broadly acceptable" criterion and the "intolerable" criterion. There may be a range of circumstances between these two criteria where a risk-benefit analysis indicates that a risk is "tolerable." Thus, for a pharmaceutical with a high positive effect (e.g., a "lifesaving" drug), it may be acceptable to take into account a certain level of unwanted effects that would be unacceptable for a treatment for a minor effect such as headache. This means that a risk-benefit analysis is applied. In the case of regulation of persistent environmental pollutants (e.g., dioxins) in the human body, one has to accept that it will take years before reduction measures, such as minimization of exposure, achieve visible success. These are circumstances where it might be appropriate to apply the "precautionary principle" and minimize exposure.

### Regulations Concerning Marketing

When marketing a chemical there is a clearly identifiable supplier. Regulations are made according to the use to which the substance is put, with a reserve scheme for those chemicals and uses not subject to more specific legislation. Regulated uses include pharmaceuticals, cosmetics, biocides, flame retardants, food additives, industrial chemicals, radiochemicals, solvents, or chemical weapons. Regulations concerned with ambient media are more difficult to enforce as there may be no clearly identifiable source and/or they have no identifiable supplier. They are regulated by medium (air, water, soil) where it occurs.

### Ambient Media

Among the regulated media are water, soil, ambient air, indoor air, workplace, food, consumer products, and human body fluids. The example of "water" can demonstrate, in how many subareas regulations of chemicals are effective: drinking water, mineral water, bottled water, water for baby food, water for injection, pool water, river water, bathing water, wastewater, surface water, groundwater, etc. A clear demarcation between regulated uses and regulated media is not always possible.

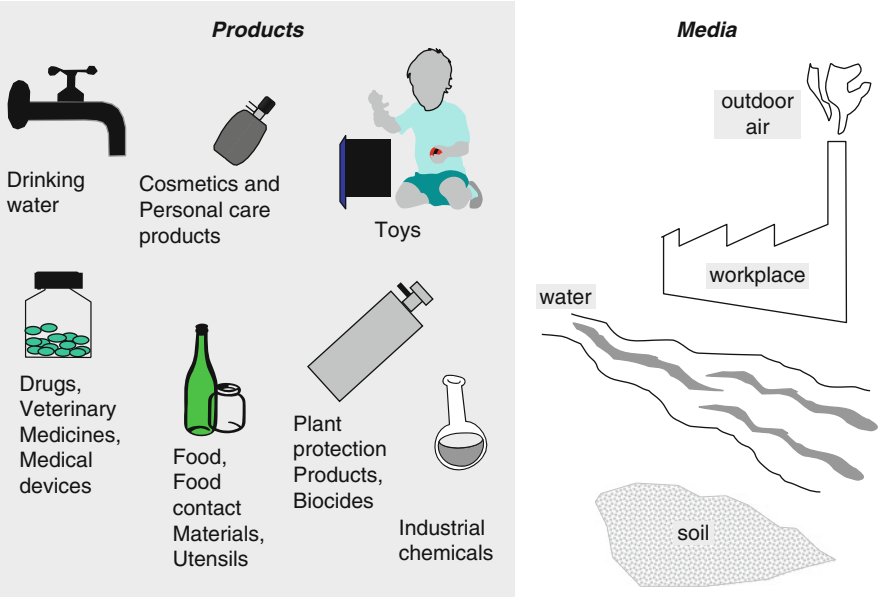



**Fig. 3** Regulated products and media (examples)

## Understanding Regulations

Often, there are detailed technical specifications, guidelines, and limit values associated with legislation and administrative measures associated with the control of toxic chemicals (Fig. 3). The relevant laws and regulations usually describe the levels of protection required and provide guidance on the technical rules and procedures that were applied in order to generate a guideline or a limit value. Knowledge about the background of the respective regulations and about the state of discussion among experts in the relevant area is a prerequisite for appropriate work by the regulatory toxicologists. Regulations are often updated in order to take into account new developments and insights to protect the population and environment. Much of this work is becoming international in nature. For an individual toxicologist, it is no longer possible to keep an overview of the entire width of all areas either nationally or internationally. Therefore, a division of labour is essential. But it is just as important to have an exchange between the fields and to encourage harmonization, provided that it does not impose a "drag" on the implementation of new procedures.

## Alarm Systems

There are three types of risk: "known knowns" (identifiable and quantifiable risks), "known unknowns" (identifiable but unquantifiable risks), and "unknown unknowns" (risks that have not yet been identified). There are also accidents and failures to

adhere to risk reduction measures. Even a good regulation for the protection of workers, consumers, and the public and good management systems may not completely exclude the possibility of a toxicological accident or an unforeseen situation. This is, for example, the case, when an unforeseen rare immunological sensitivity is triggered by a compound in few individuals or when a substance is applied the wrong way. To detect such incidents, many countries have a monitoring requirement. For medicines, one such scheme is known as "pharmacovigilance," and physicians are expected to report suspicions of "side effects." The collected information is analyzed by toxicologists, who thus gain insight into the role of specific substances in incidents and can change the risk management measures (greater supervision, e.g., by restricting prescribers and outlets, improved regulation).

## Working Methods

Based on toxicological data, the regulatory toxicologist considers the safety requirements for the particular use and then estimates under what conditions and to what extent the population, including pre-defined groups at extra risk, may be exposed to a substance, ideally without incurring any ill health. For this task, he/she requires special knowledge and experience in the interpretation of toxicological findings, the regulatory standards, the legal framework, and the implementation process. Specifically, in-depth knowledge of the common working methods, shown in the figure, is required (Fig. 4).

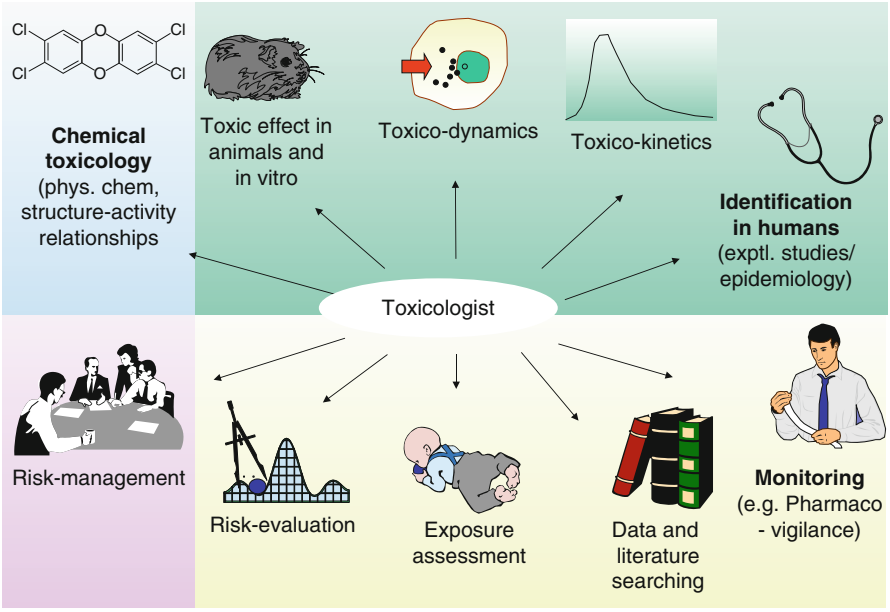



**Fig. 4** Work areas in regulatory toxicology

In addition to that methodological experience, the regulatory toxicologist should have some technical creativity that helps to find acceptable solutions for unsolvable problems and should exhibit a high communicative competence. The latter is required, because the regulatory toxicologist must sometimes explain unpleasant findings or defend unpopular decisions in his institution or in public. In conflict situations, he must be able to defend the ethics of toxicology, explain safety standards, and discuss technical feasibility.

As in all professions, there is a hierarchy concerning the professional status of toxicologists. The experimental toxicologist can publish in esteemed journals and thus contribute to global knowledge and ensure its status among peers. The regulatory toxicologist will remain more anonymous, since his written work will normally be used by commissions, who will incorporate it in statements or in laws. This gives little scientific credit, but a great deal of satisfaction due to the practical importance of his/her work.

## Recommended Reading

Chengelis CP, Holson JF, Gad SC (2001) Regulatory toxicology. Taylor & Francis, New York

Ettlin RA, Dybing E, Eistrup C, Forster R, Harpur ES, Hodel CM, Kobel W, Neslon D, Pratt I, Stemplewski H, Virgo D (2001) Careers in toxicology in Europe. Arch Toxicol 75:251–261

Illing HPA (2009) The role of veterinary pharmacovigilance in risk analysis and the influence of risk perception. In: Woodward KN (ed) Veterinary pharmacovigilance – adverse reactions to veterinary medicinal products. Wiley-Blackwell, Chichester, pp 691–708

Illing HPA, Marrs TC (2009) Regulatory toxicology. In: Ballantyne B, Marrs TC, Syverssen T (eds) General and applied toxicology, 3rd edn. Wiley, Chichester, pp 2527–2553

Schwenk M, Werner M, Younes M (2002) Regulatory toxicology. Toxicol Lett 126:145–153

Stober R (1998) Key environmental laws. Verlag Neue WirtschaftsBriefe, Herne

Wexler P, Gad SC (1998) Encyclopedia of toxicology. Academic, San Diego

# National and International Collaboration in Regulatory Toxicology

Klaus E. Appel

## Contents

Global Chemical Production ..... 32
Milestones in International Chemical Safety ..... 33
International Program on Chemical Safety (IPCS) ..... 33
United Nations Conference for Environment and Development (UNCED) ..... 34
International Forum on Chemical Safety (IFCS) ..... 34
Interorganization Program for the Sound Management of Chemicals (IOMC) ..... 35
World Summit on Sustainable Development (WSSD) ..... 35
The Strategic Approach to International Chemicals Management (SAICM) ..... 35
International Agreements ..... 36
Rotterdam Convention: Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade ..... 36
Stockholm Convention: Convention on Persistent Organic Pollutants (POPs) ..... 36
Basel Convention: Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal ..... 37
Minamata Convention on Mercury ..... 37
ILO Convention Concerning Safety in the Use of Chemicals at Work ..... 37
Chemicals Weapons Convention (CWC): Convention on the Prohibition of the Development, Production, Stockpiling, and Use of Chemical Weapons and on Their Destruction ..... 38
Vienna Convention for the Protection of the Ozone Layer and Montreal Protocol on Substances That Deplete the Ozone Layer ..... 38
International Organizations ..... 38
World Health Organization (WHO) ..... 38
United Nations Environment Program (UNEP) ..... 39
Food and Agriculture Organization of the United Nations (FAO) ..... 39
International Labor Office (ILO) ..... 39
United Nations Industrial Development Organization (UNIDO) ..... 39
Organization for Economic Cooperation and Development (OECD) ..... 40
World Bank ..... 40

K.E. Appel
Federal Institute for Risk Assessment, Berlin, Germany
e-mail: Klaus-Erich.Appel@bfr.bund.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_26, 

United Nations Development Program (UNDP) ..... 40
Chemical Safety in the European Union (EU) ..... 41
European Chemicals Agency (ECHA) ..... 41
European Chemicals Bureau (ECB) ..... 41
European Food Safety Agency (EFSA) ..... 42
National Chemical Safety ..... 42
Federal Institute for Risk Assessment (BfR) ..... 43
Federal Office for Consumer Protection and Food Safety (BVL) ..... 43
Federal Institute for Occupational Safety and Health (BAuA) ..... 44
German Federal Environment Agency (UBA) ..... 44
Julius Kühn Institute (JKI): German Federal Research Institute for Cultivated Plants .... 45
German Federal Institute for Drugs and Medical Devices (BfArM) ..... 45
References ..... 46
Resources ..... 46

**Abstract**

Achieving the world's social and economic objectives is not possible without the use of significant quantities of chemicals, including industrial chemicals, pesticides, and pharmaceuticals. Comprehensive and cost-effective use of these substances while maintaining high health and safety standards is, in principle, possible and has been shown in a number of cases. However, it is still a long way until these standards are implemented worldwide following the principles of sustainable development with the goal of a better quality of life of all people. A number of international bodies and authorities promote the sound management of chemicals at national and international level, some of which are described in this chapter.

## Global Chemical Production

Production, trade, and consumption of chemicals are of enormous economic importance. Chemicals valued at US$ 1,500 billion were produced worldwide in 1998. This corresponds to 7 % of global income and 9 % of international trade. Although 80 % of all chemicals are produced in only 16 countries, chemicals are used in all countries worldwide. Economic indicators point to a significant increase in chemical production and use in the decades to come. Most of this production is still expected to take place in OECD countries (see Fig. 1). However, a shift to developing countries takes place in parallel: Developing countries are experiencing a disproportionate increase in the production and use of chemicals.

Today, approximately 100,000 chemicals are available on the market, and many new substances are added each year. In addition, thousands of chemicals of natural origin exist. Taking into account that people can potentially come into contact with all of these chemicals, the resulting need for information concerning related health and environmental risks is enormous. Considering costs and time necessary to

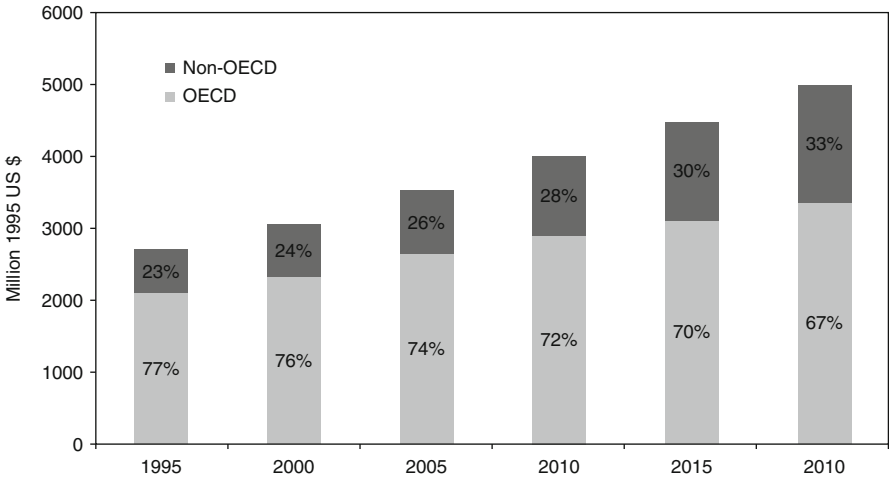



**Fig. 1** Chemicals manufacturing industry production worldwide - an OECD prognosis for 1995–2020 (cited from: Environmental Outlook for the Chemicals Industry, OECD 2001)

collect relevant toxicological information, it is only normal that the international community shares the task of data collection, especially since no country alone would probably be able, to cope with this challenge alone.

## Milestones in International Chemical Safety

Chemical safety dates back to the beginning of the twentieth century. At the time, few chemicals of known risks were regulated, e.g., international recommendations existed concerning the safe handling of white phosphorus in the production of matches. In fact, little to nothing was known about the risks of the majority of the chemicals produced and used at that time, and accordingly, these chemicals were not regulated. It was only decades later that industrialized countries begun evaluating and classifying some chemicals in order to inform the process of establishing safety measures. In addition, countries started to evaluate not only the risks associated with the acute health effects but also chronic, genetic, environmental, and other effects that may be caused by handling the chemicals (Somogyi et al. 1999).

### International Program on Chemical Safety (IPCS)

In 1972, the United Nations Conference on the Human Environment was held in Stockholm, Sweden. At this conference, among other things, countries asked for

an international chemicals safety program to serve as an early warning system to prevent disease burden associated with chemicals by undertaking risk evaluations of chemicals by applying internationally harmonized methodologies. As a result of the Stockholm Conference, the IPCS was established in 1980. IPCS is a joint venture of the UNEP, ILO, and WHO. The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer-review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

## United Nations Conference for Environment and Development (UNCED)

In 1992, UNCED introduced sustainable development as the guiding principle of national and international environmental policy (United Nations 1992). The principles for effective international chemical safety and the sound management of chemicals were presented in Chapter 19 of Agenda 21, the program of action for the twenty-first century, adopted by more than 170 countries. Chapter 19 contains objectives for the environmentally sound management of toxic chemicals including the prevention of illegal international traffic in toxic and dangerous products. The program areas for chemical safety in Chapter 19 of Agenda 21 include (i) international assessment of chemical risks, (ii) harmonization of classification and labeling of chemicals, (iii) information exchange on toxic chemicals and chemical risks, (iv) establishment of risk reduction programs, (v) strengthening of national capabilities and capacities for management of chemicals, and (vi) prevention of illegal international traffic in toxic and dangerous products.

## International Forum on Chemical Safety (IFCS)

In 1994, the IFCS was established in response to the request by governments at UNCED. IFCS provided an open, transparent, and inclusive forum for discussing issues of common interest and also new and emerging issues in the area of sound management for governments, intergovernmental organizations, and nongovernmental organizations, including from the private sector. The IFCS facilitated consensus building on issues and actions addressing chemicals safety and adopted recommendations for governments and intergovernmental organizations, including the Bahia Declaration on chemical safety in 2000. By its efforts, IFCS made an important contribution to the implementation of the Strategic Approach to International Chemicals Management (SAICM). With the adoption of SAICM, the existing of IFCS has practically ended (see below).

## Interorganization Program for the Sound Management of Chemicals (IOMC)

The IOMC was established in 1995 following recommendations made by UNCED in 1992. IOMC's role is to promote coordination of policies and activities of chemical programs of international organizations, pursued jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment. FAO, ILO, UNEP, UNIDO, WHO, and OECD initially signed a Memorandum of Understanding; UNITAR joined the IOMC in 1997, the World Bank in 2010, and UNDP in 2012. The IOMC works on subjects related to those elaborated in Chapter 19 of Agenda 21 (see above) and now covered by the SAICM (Strategic Approach to International Chemicals Management) Global Plan of Action (see below).

## World Summit on Sustainable Development (WSSD)

The WSSD in Johannesburg, South Africa, was held 10 years after the UNCED Conference in Rio de Janeiro and 30 years after the first United Nations Conference on the Human Environment in Stockholm Environmental Conference. Chapter 22 of the action plan adopted in Johannesburg is addressing chemical safety renewing the commitments of Agenda 21 of UNCED for the environmentally sound use of chemicals for the purpose of sustainable development, as well as the protection of human health and the environment. WSSD targeted that by 2020, chemicals are used and produced in ways that minimize significant adverse effects on human health and the environment, taking into account the precautionary principle.

## The Strategic Approach to International Chemicals Management (SAICM)

SAICM is a policy framework to promote chemical safety around the world. SAICM has as its overall objective the achievement of the sound management of chemicals throughout their life cycle so that, by 2020 meeting the WSSD goal (see above), chemicals are produced and used in ways that minimize significant adverse impacts on human health and the environment.

SAICM is distinguished by its comprehensive scope: ambitious "2020" goal for sound chemicals management, multi-stakeholder and multi-sectoral character, endorsement at the highest political levels, emphasis on chemical safety as a sustainable issue, provision for resource mobilization, and formal endorsement or recognition by the governing bodies of key intergovernmental organizations. SAICM comprises the Dubai Declaration on International Chemicals Management, expressing high-level political commitment to SAICM, and an Overarching Policy

Strategy which sets out its scope, needs, objectives, financial considerations underlying principles, and approaches and implementation and review arrangements. Objectives are grouped under five themes: risk reduction, knowledge and information, governance, capacity building and technical cooperation, and illegal international traffic. The Declaration and Strategy are accompanied by a Global Plan of Action that serves as a working tool and guidance document to support implementation of SAICM and other relevant international instruments and initiatives. Activities in the plan are to be implemented, as appropriate, by stakeholders, according to their applicability.

## International Agreements

Intense debates in the 1980s and 1990s led to the beginning of the adoption of a number of important conventions related to chemical safety.

### Rotterdam Convention: Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade

The Rotterdam Convention prevents unwanted trade of chemicals through the application of the legally binding prior informed consent (PIC) procedure. PIC regulates the exchange of information and the shared responsibility of export and import country in the international trade of certain hazardous chemicals in order to protect human health and the environment from potential harm. The convention deals with chemicals that are banned or severely restricted in some countries (particularly in industrialized regions) but that are still exported to other countries (particularly in developing regions).

### Stockholm Convention: Convention on Persistent Organic Pollutants (POPs)

The Stockholm Convention is a global treaty to protect human health and the environment from highly dangerous, long-lasting chemicals by restricting and ultimately eliminating their production, use, trade, release, and storage. Twenty-one compounds are covered by the convention, including pesticides, industrial chemicals, and unwanted combustion by-products. Once released into the environment, POPs remain intact for exceptionally long periods of time (many years), become widely distributed throughout the environment as a result of natural processes involving soil, water, and, most notably, air. POPs are found at higher levels in the food chain, they accumulate in the fatty tissue of living organisms including humans, and are toxic to both humans and wildlife.

## Basel Convention: Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal

The Basel Convention was adopted in 1989 to protect human health and the environment against the adverse effects resulting from the generation, management, transboundary movements, and disposal of hazardous and other wastes. It was created as a result of tighter environmental regulations in industrial countries in the 1980s which had led to the trade of toxic waste from developed to developing countries where the wastes were dumped indiscriminately, spilled accidentally, or managed improperly, causing severe health and environmental problems.

The convention regulates the transboundary movements of hazardous and other wastes and obliges its countries to ensure that these wastes are managed and disposed of in an environmentally sound manner. Countries are also required to minimize transboundary movements to the extent consistent with the environmentally sound and efficient management of such wastes and treating and disposing of wastes as close as possible to their place of generation. The export of hazardous waste to non-contracting countries is prohibited and permitted only in exceptional circumstances. The export to the Antarctic is generally prohibited.

## Minamata Convention on Mercury

The Minamata Convention on Mercury was named after a city in Japan where serious health damage occurred as a result of mercury pollution. Adopted in January 2013, the convention provides controls and reductions across a range of products, processes, and industries where mercury is used, released, or emitted. These range from medical equipment such as thermometers and energy-saving light bulbs to mercury-emitting activities such as mining, cement, and energy production. Governments have agreed on a range of mercury-containing products to be banned by 2020. In addition, governments agreed to draw up strategies to reduce the amount of mercury used by small-scale miners. Furthermore, the treaty aims at controlling mercury emissions and releases from, for example, coal-fired power stations, industrial boilers, smelters, waste incineration, and cement clinker facilities.

## ILO Convention Concerning Safety in the Use of Chemicals at Work

The convention from 1990 and its accompanying recommendation aim to improve safety and health in the use of chemicals at work, which includes the production, the handling, the storage, and the transport of chemicals as well as the disposal and treatment of waste chemicals, the release of chemicals resulting from work

activities, and the maintenance, repair, and cleaning of equipment and containers of chemicals. In addition, it allocates specific responsibilities to suppliers and exporting states.

### Chemicals Weapons Convention (CWC): Convention on the Prohibition of the Development, Production, Stockpiling, and Use of Chemical Weapons and on Their Destruction

This agreement of 1993 aims to eliminate an entire category of weapons of mass destruction by prohibiting the development, production, acquisition, stockpiling, retention, transfer, or use of chemical weapons by countries. Countries have agreed to chemically disarm by destroying any stockpiles of chemical weapons they may hold and any facilities which produced them, as well as any chemical weapons they abandoned on the territory of other countries in the past. A unique feature of the CWC is its incorporation of the "challenge inspection," whereby any State Party in doubt about another State Party's compliance can request to send an inspection team.

### Vienna Convention for the Protection of the Ozone Layer and Montreal Protocol on Substances That Deplete the Ozone Layer

The Vienna Convention (1985) and the Montreal Protocol (1987) aim to protect environment and thus human health against detrimental effects of human activity which change or could change the ozone layer. Concrete objectives set out in the Vienna Convention are specified in the Montreal Protocol which is to eliminate the production and use of almost 100 substances that damage the ozone layer, including freons and halons and the production and use of methyl bromide. Substances that are very stable in the atmosphere allowing them to reach and destroy the ozone layer which protects the Earth from the UV radiation.

## International Organizations

### World Health Organization (WHO)

Through the International Program on Chemical Safety (IPCS), WHO works to establish the scientific basis for the sound management of chemicals and to strengthen national capabilities and capacities for chemical safety. Main activity areas include the evaluation of priority chemicals and risk assessment, the prevention and treatment of poisonings, and the health aspects of chemical incidents and emergencies. Jointly with FAO, WHO provides the Secretariat of the Joint FAO/WHO expert meeting on pesticide residues (JMPR) and the Joint FAO/WHO

Expert Committee for food additives, veterinary drugs, and contaminants. The International Agency for Research on Cancer (IARC) of WHO evaluates the cancer risks of chemicals which are published in the IARC Monographs.

## United Nations Environment Program (UNEP)

UNEP provides leadership and encourages partnership in caring for the environment by inspiring, informing, and enabling nations and peoples to improve their quality of life without compromising that of future generations. The UNEP Chemicals program is the focal point of UNEP activities on chemicals and provides assistance to countries in risk assessment and reduction of hazardous substances. In addition, UNEP is providing the secretariats for SAICM, the Basel and Stockholm Conventions, as well as the Rotterdam Convention jointly with FAO (see above).

## Food and Agriculture Organization of the United Nations (FAO)

FAO was founded to improve the food situation and to increase agricultural productivity. Chemical safety at FAO is focusing on plant protection products and chemicals in food. FAO has developed an international code of conduct on the distribution and use of pesticides. Other activities are dealing with the disposal of obsolete and unwanted pesticides, particularly in Africa. Together with the UNEP, FAO provides the Secretariat for the Rotterdam Convention and, together with the WHO, the Secretariats for JMPR and JECFA (WHO see also).

## International Labor Office (ILO)

Chemical safety forms part of ILO's mandate to improve occupational safety and health. In addition the Chemicals Convention mentioned above, ILO Conventions and recommendations in the field of chemical safety are dealing, for example, with the prevention of major industrial accidents, asbestos, the working environment (air pollution, noise, and vibration), and occupational cancer. ILO's main areas of activity are the development and implementation of their conventions and recommendations, including the development, promotion, and distribution of guidelines and technical standards.

## United Nations Industrial Development Organization (UNIDO)

UNIDO promotes and accelerates sustainable industrial development in developing countries and in countries with economies in transition. UNIDO promotes the development of Clean Production Centers and develops and promotes risk

minimization strategies for the production of agricultural chemicals as well as the transfer of safe and environmentally friendly technologies and industrial processes.

## Organization for Economic Cooperation and Development (OECD)

OECD is a non-UN international governmental organization to promote policies to improve the economic and social well-being of people around the world. The OECD provides a forum in which governments can work together to share experiences and seek solutions to common issues. Chemical safety activities aim to identify, prevent, and mitigate chemical-related environmental and health risks, to prevent unnecessary trade barriers, to optimize national resources for chemical safety, as well as to integrate economics and chemical safety policy. OECD programs are dealing with chemicals safety issues, including the testing and test guidelines and assessment of chemicals, good laboratory practice and compliance monitoring, pesticides, pollutant release and transfer register (PRTR), risk management and chemical accidents, emission scenarios, and harmonization of regulatory oversight in biotechnology.

## World Bank

In its environmental strategy, the World Bank lays out its ambition to support "green, clean, resilient" paths for developing countries, as they pursue poverty reduction and development in an increasingly fragile environment. The environment strategy recognizes that while there has been notable progress in reducing global poverty, there has been significantly less progress in managing the environment sustainably, and while developing countries will still need rapid growth to reduce poverty over the next decade, the global environment has reached a critical state that could undermine livelihoods, productivity, and global stability. World Bank's objectives in the environment strategy are to improve the quality of life in countries and to protect people's health from environmental risks and pollution to reduce the disease burden. Among others, particular emphasis is given in the strategy to reduction of exposure to toxic substances.

## United Nations Development Program (UNDP)

UNDP promotes the sound management of chemicals and waste as an important aspect of their work to reduce global poverty and achieve the Millennium Development Goals (MDGs). UNDP addresses unsustainable management approaches, as well as unsustainable consumption and production patterns, including poor design and material choices. These issues are considered to be the root causes for resource depletion, waste generation and pollution, impeding sustainable human development. UNDP advocates for the integration of sound chemicals management

priorities into national environmental and poverty reduction planning frameworks and helps countries access resources to improve their chemical and waste regimes.

## Chemical Safety in the European Union (EU)

Previously, decision-making concerning marketing of chemicals took place at the national level. Today, nearly all these decisions take place at the level of the EU. Therefore, legislation of chemicals is largely harmonized in the EU, e.g., through the requirement that National legislation must be in accordance with EU law resulting in a uniform level of protection in all Member States. Concerning toxicological testing of substances, there are, in addition to the EU harmonized methods, supranational test strategies such as the test guidelines provided by OECD (Munn and Hansen 2002).

A number of EU regulatory institutions have been established.

## European Chemicals Agency (ECHA)

Established 1 June 2007 in Helsinki, Finland, ECHA regulates the technical, scientific, and administrative aspects for the registration, evaluation, authorization, and restriction of chemical substances in the EU following uniform procedures.

ECHA is central to the European REACH Regulation (Registration, Evaluation, and Authorization of Chemicals) by being the recipient and the agency that verifies the registration documents submitted by manufacturers and importers.

In collaboration with agencies in Member States, ECHA develops statements concerning the risks associated with the substances themselves as well as concerning the socioeconomic consequences associated with related risk mitigation measures (prohibition, restrictions, approvals). A network of agencies in EU Member States has been established for the implementation and monitoring of chemical safety activities within the EU. It maintains a central database and develops guidance material to assist businesses. With the acceptance of all EU Member States, ECHA defines the toxicological and ecotoxicological investigations to be carried out to describe possible dangerous properties. An appeal may be brought to Board of Appeal against decisions of the agency.

## European Chemicals Bureau (ECB)

The ECB in Ispra (Italy) is the central reference for toxicological information on new and old substances within the EU. It provides scientific and technical support for the development, implementation, and monitoring concerning EU regulation, especially related to toxic chemicals. It fulfills the legal requirement to classify and label chemicals based on their hazardous and toxicological properties. It assesses the risks of industrial chemicals. Furthermore, the ECB contributes to the

development and harmonization of test methods within the EU. It notifies about new substances, authorizes and evaluates biocides, and facilitates information exchange for the import and export of hazardous substances. The ECB's main partners are the corresponding scientific institutions in Member States and Norway.

## European Food Safety Agency (EFSA)

EFSA's role is to provide independent scientific risk assessment advice directly or indirectly of concern to food safety and consumer protection. All stages of food production and supply are covered, and scientific assessments can be made for each stage, starting from animal feed safety, primary production, to distribution to consumers (from stable to table). Related animal and plant health issues are covered as well. EFSA's main costumer is the EC Commission which in turn can also address scientific requests of the European Parliament and Member States directly and initiate risk assessments on its own. EFSA's scientific advice is provided through scientists in a number of scientific panels. A Scientific Committee coordinates the work ensuring coherence of the scientific advice produced by the various panels.

The scientific panels are composed of independent experts of different subject areas (Table 1).

The Advisory Forum is at the heart of EFSA's collaborative approach to working with EU Members States. Its members are representatives of national food safety authorities and use the forum to advise EFSA on scientific matters, its work program, and priorities and to address emerging risk issues as early as possible.

## National Chemical Safety

The space of this chapter does not allow to describe the situation in different nations. Therefore, the example of just one country, in this case Germany, will be described.

**Table 1** EFSA's scientific panels

| |
|---|
| Scientific Committee (SC) |
| Additives and products or substances used in animal feed (FEEDAP) |
| Animal health and welfare (AHAW) |
| Biological hazards (BIOHAZ), including BSE-TSE-related risks |
| Contaminants in the food chain (CONTAM) |
| Dietetic products, nutrition, and allergies (NDA) |
| Food additives and nutrient sources added to food (ANS) |
| Food contact materials, enzymes, flavorings, and processing aids (CEF) |
| Genetically modified organisms (GMO) |
| Plant health (PLH) |
| Plant protection products and their residues (PPR) |

## Federal Institute for Risk Assessment (BfR)

BfR is the German scientific authority responsible for the development of risk assessment reports and expert opinions concerning food safety and consumer protection. The development of risk assessment reports and expert opinions is based on international scientific criteria, e.g., the process is transparent open and comprehensible for stakeholders, including the public, the scientific community, as well as other interested groups. In general, the results are published by maintaining the confidentiality of private data. In addition to and based on the evaluation of risks, BfR develops recommendations concerning actions to be taken to manage/mitigate the risks aimed at improving food safety and consumer protection.

In particular, the role of the BfR is to evaluate possible health risks of food and feed as well as of dietetic products and novel foods with regard, e.g., to natural ingredients, food additives, and contaminants. Another role of the BfR is to assess the risks of industrial chemicals, plant protection and biocidal products with regard to human safety. It is then, for example, the responsibility of the BVL to authorize/register plant protection products, while it is the BAuA that is in charge of registration/authorization of biocidal products. Furthermore, BfR provides scientific advice to the federal ministries and the BVL, and it cooperates with a number of scientific institutions and organizations in other nations with EFSA being one main partner. Finally, BfR initiates and conducts scientific research in relation to the assessments it undertakes regarding food safety and consumer protection.

## Federal Office for Consumer Protection and Food Safety (BVL)

BVL has a number of responsibilities concerning risk management. It acts, for example, as the national focal point for the European rapid alert system in the food and feed sectors (RASFF) as well as in certain sectors of product safety. RASFF warns about dangerous food and feed. In addition, BVL is responsible for the management of crises in the food and feed sectors. One aim is to make a proper risk communication and management of risks before they turn into a crisis. Among other aspects BVL's role is to warn other relevant competent authorities in Germany as early as possible about products that might cause a risk to the consumers. Furthermore, BVL provides support to the federal states to oversee the market concerning food, tobacco products, cosmetics, and any commodities as well as feed, e.g., be ensuring that food surveillance is been undertaken in a harmonized way in all federal states. For that purpose, BVL hosts the European and national reference laboratory for measuring residues in food and acts as the national contact point for monitoring and measurements. Data obtained from food monitoring campaigns are sent by the federal states to the BVL for analyses, documentation, and reporting.

In addition, BVL is the responsible agency for the registration/authorization of plant protection products. The decisions of the BVL with regard to plant protection

products are based on the scientific input provided by other competent authorities, e.g., BfR, UBA, and JKI. Finally, BVL is the responsible agency for the registration/authorization of veterinary drugs.

## Federal Institute for Occupational Safety and Health (BAuA)

BAuA is a governmental research institution within the Federal Ministry of Labor and Social Affairs. Its role is to monitor and analyze the situation with regard to occupational safety and health. BAuA advices the Federal Ministry on all matters related to occupational safety, occupational health, and workplace design. The agency provides recommendations on medical care, surveillance, and the control of occupational diseases related to working conditions. In addition, BAuA assesses possible health risks due to the exposure of chemicals at the workplace, establishes occupational exposure limits, and develops protection strategies for handling hazardous substances. BAuA is the federal authority for chemicals based on the chemical law, and it manages the national office for the implementation of the REACH regulations. It coordinates the national tasks concerning these regulations and cooperates with ECHA. According to REACH, producers and importers are required to notify new chemicals before they can enter the market. Data and information need to be submitted to BAuA on the physical-chemical, toxicological, and ecotoxicological properties, classification, and labeling as well as on the safe handling of these substances. BAuA reviews the data in collaboration with other national agencies such as BfR and UBA and shares it with ECHA at the EU level.

Furthermore, BAuA is the responsible agency for the authorization/registration of biocidal products.

## German Federal Environment Agency (UBA)

UBA advises the German Federal Ministry for Environment, Nature Conservation, and Nuclear Safety on environment and health issues, particularly in the field of air pollution, noise pollution, waste and water management, soil conservation, and environmental chemicals. UBA's main role is to provide the scientific and technical knowledge for the drafting of legislation aimed at protecting human health and the environment, especially concerning the control, restriction, and ban of environmentally hazardous substances and preparations as well as genetically modified organisms. In particular, UBA has a role in the ecotoxicological evaluation of different types of chemicals. In collaboration with other federal agencies (e.g., BAuA and BfR), UBA is involved in the evaluation of, e.g., pesticides, biocides, and genetically modified organisms in relation to a number of various laws, including the Washing and Cleaning Agents Act, Act on water pollutants, Plant Protection Act, Biocides Act, Federal Communicable Diseases Act, and the Genetic Engineering Act. In particular, the UBA is responsible for the evaluation of

chemicals hazardous to the aquatic environment and their storage and transportation (classification of these chemicals).

Regulations concerning the sound management of chemicals and genetic engineering are essentially determined by the laws of the European Union.

## Julius Kühn Institute (JKI): German Federal Research Institute for Cultivated Plants

JKI activities are in the field of plant health and nature. Its role is given by the Plant Protection Act. As the Federal Research Institute for Cultivated Plants, the JKI is dealing with the efficiency, efficacy, and benefits of pesticides. In addition, JKI has a role in the diagnoses of plant diseases, including the identification of harmful organisms and pathways and routes of infection. Methods are being developed for the detection and characterization of viruses, bacteria, and other pathogens.

JKI studies the effects of pesticides on organisms, especially on organisms present/living near crops and in adjacent water bodies (ecotoxicology); it studies the impact of climate change on pests and pest management strategies; and it contributes to the further development of integrated pest management. The latter includes the development of eco-friendly methods of plant protection, the study of natural pest resistance of crops, and the promotion of production of crops with high natural pest resistance.

Additional mandatory tasks include evaluations for the registration and use of genetically modified organisms (GMO Act).

## German Federal Institute for Drugs and Medical Devices (BfArM)

The BfArM is an independent federal authority in the Federal Ministry of Health with the aim of preventing health risks by continuous improvement in the safety of medicinal products and by risk monitoring of medical devices as well as by monitoring the legal traffic in controlled substances. Main activities focus on the authorization and registration of medicinal products on the basis of the German Medicines Act. This includes the assessment of the efficacy, safety, and pharmaceutical quality of these products on the basis of pharmaceutical, pharmacological-toxicological, and clinical studies. The license of medicinal products is limited to 5 years. Renewals are granted upon application and after new evaluation. In addition, the BfArM has a role in the registration of pharmaceuticals in the European Union with EMA (European Medicines Agency) being responsible for the evaluation of medicinal products.

After marketing, the use of medicinal products might present rare adverse drug reactions which had not been observed during clinical trials. BfArM collects and assesses reports of adverse drug reactions and decides whether the information for the corresponding drugs needs to be revised. In case where the risks of medicinal products outbalance their benefits, BfArM withdraws the license of these products.

In such cases, BfArM shares the information with agencies of the European Union and the World Health Organization (WHO).

On the basis of the Narcotics Act (BtMG), the Federal Opium Agency of BfArM issues licenses for the trade in narcotics. In addition, it controls the production and/or importation of narcotic and psychotropic substances.

The term "medical devices" refers to a wide range of products, including products for diagnosis, prevention, monitoring, treatment, or alleviation of disease and injury and handicaps as well as products for the replacement or modification of the anatomy such as pacemakers, X-ray, radiotherapy, and surgical instruments as well as in vitro diagnostic medical devices, prostheses, artificial teeth, etc. Health risks related to these products need to be reported to the BfArM by manufacturers, operators, and users. In turn, the role of the BfArM is to provide recommendations for risk mitigation.

## References

Munn SJ, Hansen BG (2002) EU risk assessment: science and policy. Toxicology 181–182:281–285

Somogyi A, Gori GB, Appel KE (1999) Regulatory toxicology. In: Marquardt H, Schäfer SG, McClellan R, Welsch F (eds) Toxicology. Academic, San Diego

United Nations (1992) Agenda 21: programme of action for sustainable development. United Nations, New York. ISBN 92-1-100509-4

## Resources

Basel Convention. http://www.basel.int. Last date of access: 03 Mar 2013

BfR. www.bfr.bund.de/. Last date of access 03 Mar 2013

BVL. www.bvl.bund.de/. Last date of access 03 Mar 2013

Chemical Weapons Convention. http://www.opcw.org. Last date of access 03 Mar 2013

ECHA. echa.europa.eu/. Last date of access 03 Mar 2013

EFSA. www.efsa.europa.eu/de/. Last date of access 03 Mar 2013

Environmental Outlook for the Chemical Industry. http://www.oecd.org/ehs. Last date of access 03 Mar 2013

FAO. http://www.fao.org. Last date of access 03 Mar 2013

ILO Convention on Chemical Safety. http://www.ilo.org/safework. Last date of access 03 Mar 2013

ILO. http://www.ilo.org. Last date of access 03 Mar 2013

Inter/Organization Programme for the Sound Management of Chemicals (IOMC). http://www.iomc.ch. Last date of access 03 Mar 2013

International Forum on Chemical Safety (IFCS). http://www.ifcs.ch. Last date of access 03 Mar 2013

International Programme on Chemical Safety (IPCS). http://www.who.int/pcs. Last date of access 03 Mar 2013

Minamata Convention. http://unep.org/hazardoussubstances/Mercury/Negotiations/INC5. Last date of access 03 Mar 2013

OECD. http://www.oecd.org. Last date of access 03 Mar 2013

Rotterdam Convention. http://www.pic.int. Last date of access 03 Mar 2013

Stockholm Convention. http://www.pops.int. Last date of access 03 Mar 2013
Strategic Approach to International Chemicals Management (SAICM). http://www.saicm.org/. Last date of access 03 Mar 2013
UBA. www.umweltbundesamt.de/. Last date of access 03 Mar 2013
UNDP. http://www.undp.org/. Last date of access 03 Mar 2013
UNEP. http://www.unep.org. Last date of access 03 Mar 2013
UNIDO. http://www.unido.org. Last date of access 03 Mar 2013
UNITAR. http://www.unitar.org. Last date of access 03 Mar 2013
Viennea Convention und Montreal Protokoll. http://www.vienna.convention.at/. Last date of access 03 Mar 2013
WHO. http://www.who.int. Last date of access 03 Mar 2013
World Bank. http://www.worldbank.org/. Last date of access 03 Mar 2013
World Summit on Sustainable Development (WSSD; Johannesburg Summit). http://www.johannesburgsummit.org. Last date of access: 03 Mar 2013

# The Regulatory Process in Toxicology

Dietrich Henschler and Wolfgang Dekant

## Contents

Possibilities of Regulation ..... 50
Threshold Limit Values (TLV) ..... 50
Stock-Taking ..... 51
Profiles of Demand ..... 52
Procedural Steps ..... 52
Historic Developments ..... 54
Types of Organization ..... 55
Recommended Reading ..... 55

**Abstract**

The regulation to avoid or reduce potential health and *environmental risks* due to chemicals or physical factors in Germany, the European Union, and worldwide carries extremely heterogeneous features. Fundamental differences are encountered not only with regard to institutional responsibilities but also – and in particular – to nomenclature(s); definition of aims of protection; types of organization; scientific basis and extent of justification, implementation, and controls; as well as the legal status. The situation is even more complicated by interfering mandates. The system suffers from a crisis of credibility. However, recent efforts towards harmonization gain pace.

D. Henschler (✉) • W. Dekant
Institute for Toxicology, University of Würzburg, Würzburg, Germany
e-mail: popa-henning@toxi.uni-wuerzburg.de; schraut@toxi.uni-wuerzburg.de; dekant@toxi.uni-wuerzburg.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_27, 

## Possibilities of Regulation

On principle, there are four organizational types of regulation: *banning of production, sale, and uses of toxic materials*; *restrictions on use; mandatory use of protective procedures to avoid/reduce hazardous exposure(s);* and the introduction of *health-based environmental threshold limit values*. Banning of production is only realized for materials with very high hazard potential. As voluntary *withdrawals from the market* by producers, bans regarding production or import (e.g., 2-naphthylamine, PCBs, pentachlorophenol) may be reduced in their effectiveness by imports due to globalization and removal of trade barriers. Bans are also excluded in case of materials which cannot be waived due to technical reasons, are formed by transformation processes in the environment, or have natural sources (such as heavy metals). In these situations, more and more preference has been given to the development and introduction of *alternative compounds* which are designed to avoid undesirable properties such as high stability in the environment. While intelligently designed alternatives may have significant advantages such as reduced potential for specific toxicities, complete toxicological data and experience from practical use of such alternatives are often not available; thus, other potential risks may be present. Another domain is *restrictions in practical applications* – a field of activities more for administrators than for toxicologists. *Protective measures in loco* (exposure prevention by personal protective equipment or using closed processes) are mostly dealt with by specialists in occupational toxicology. The most important protective instrument is the establishment and application of *threshold limit values (TLVs)*. They constitute the most frequently used method of health-based protection. Therefore, the following description will focus on such limit values.

## Threshold Limit Values (TLV)

Threshold limit values (and environmental standards) are maximum permissible concentrations of chemicals (and physical stressors such as electromagnetic radiation) in specified environmental compartments, in specific tissues of organisms, or in excretion products. They are presented in the form of definitive figures, expressed as mass/volume, mass/mass, volume/volume, or doses in the form of mass/time. In case of *physical stressors* (radiation, noise, heat, pressure), physical quantities are valid accordingly. Such *official limit values* are established in laws, enactments, or regulations. They are either to be adhered to or function as recommendations. *Nonofficial limit values* are established by private institutions in the form of recommendations, which may or may not be taken over in legal technical rules (e.g., *MAK values*) (DFG = German Research Association, VDI = Professional Organization of Engineers, DIN = Administration for Technical Norms).

## Stock-Taking

According to a systematic analysis performed by the Expert Council for Environmental Questions in Germany, there are more than 150 types of limit values in Germany alone. Chronologically, these were first developed for pharmaceuticals. The first dose limit for a pharmaceutical was introduced by the official German Pharmacopoeia (second edition in 1882) in the form of a maximum single or daily dose. The first limit values for workplace exposures to chemicals were introduced in 1886 (K.B. Lehmann). The numbers of limit values for chemicals in occupational or environmental settings were steadily increasing since 1960 with an exponential tendency, often enforced by increasing public pressure. Today, app. 20 % of the derived limits each account for victuals and soil, ca. 10 % each for air and water, and less than 10 % each for chemicals, noise, and radioactivity. Human health is the predominant aim for the protective measures and presents 93 %, followed by general protection of environment (19 %), plants (16 %), and animals (14 %) (in part repetitive counting). Regarding the legal status, 50 % each are introduced as official and nonofficial standards. At least 30 different nomenclatures are in use (see Table 1).

The authorization for the organization of work to be performed to justify a derived value varies widely, from multidisciplinary recruited commissions or committees, down to the desk of a single clerk of an agency. This confusing complexity is, in its major proportion, due to the historical development: different academic disciplines picked up, mostly incidentally, a problem and made use of their categories of reasoning and evaluation, thus paving the way for a great variety

**Table 1** Designations of threshold values as used in 154 German systems of regulation of hazardous materials (according to SRU = Council of Experts of Environmental questions, 1996)

| | |
|---|---|
| Environmental values | Unhesitating values |
| Tolerance values | Maximum values |
| Maximum tolerance values | Precarium values |
| Scrutiny values | Background values |
| Encumbrance values | Input values |
| Hazard suspicion values | Target values |
| Interference values | Acceptance values |
| Intervention values | Adjusting values |
| Action values | Coordination values |
| Occasion values | Damaging values |
| Restoration values | Threshold values |
| Alarm values | Preliminary values |
| Release values | Hesitation values |
| Release threshold | Environmental standards |
| Orientation values | Toxicity values |
| Scruple values | |

of experience and competence. Since approximately two decades, increasing criticism of status and further development is arising, mainly driven by the interest of industry and jurisdiction to achieve reliability for planning and legal status. The lack of clear-cut targeting and rules of procedure induced activities to improve harmonization, standardization, and simplification. As a result, useful and intentionally calibrated criteria have been elaborated (SRU = Council of Environmental Questions 1996); a new commission for risk evaluation has been charged with establishing and handling uniform rules.

## Profiles of Demand

Regulatory processes are understood as political decisions – ideally in the form of consensus – based on scientific assessment of potential risks, under adequate participation of societal groups. The substantial elements of demand are:

1. *Participation of the public* before and in the course of procedures
2. *Complete transparency of all steps of procedure*, e.g., publish intentions and timing
3. An essential element of transparency is to be seen in the obligation of a detailed *justification* of
4. *All scientific evaluations and proposals for regulations and decisions* in the form of detailed documents which should be available to everybody
5. Concerned *societal groups should be involved* in the discussions for the preparations of decisions
6. Accomplished decisions, particularly regarding the level of a standard, need to be enforced by validated analytical methodology to warrant *compliance*

A new element has been introduced later: obligation of *continuous reevaluation in predetermined intervals*, taking into consideration new scientific data and eventually changes in sociopolitical principles.

## Procedural Steps

The profiles of demand require the integrated cooperation of expertise of different scientific domains, making the process of regulation a multidisciplinary task. The evaluations to be performed require working elements of different groups of experts. This necessitates a *sequential procedure* of defined steps, which allows for recourses from one step to each other. A model of sequential steps is presented in Fig. 1.

The process starts with the determination of *objects of protection* (targets) (human beings, plants, soil, etc.) and with *aims of protection* (e.g., complete elimination or gradual reduction of risk). Right and duty of making proposals is not restricted to governmental institutions but open to everybody. The decision about the aim(s) of protection is bound to the duty of detailed justification. This is followed by a *scientific analysis*, including a risk evaluation mostly based on published data on toxicological information or results of targeted toxicity test. Normally, a *proposal for a standard* is elaborated by the group of scientists who

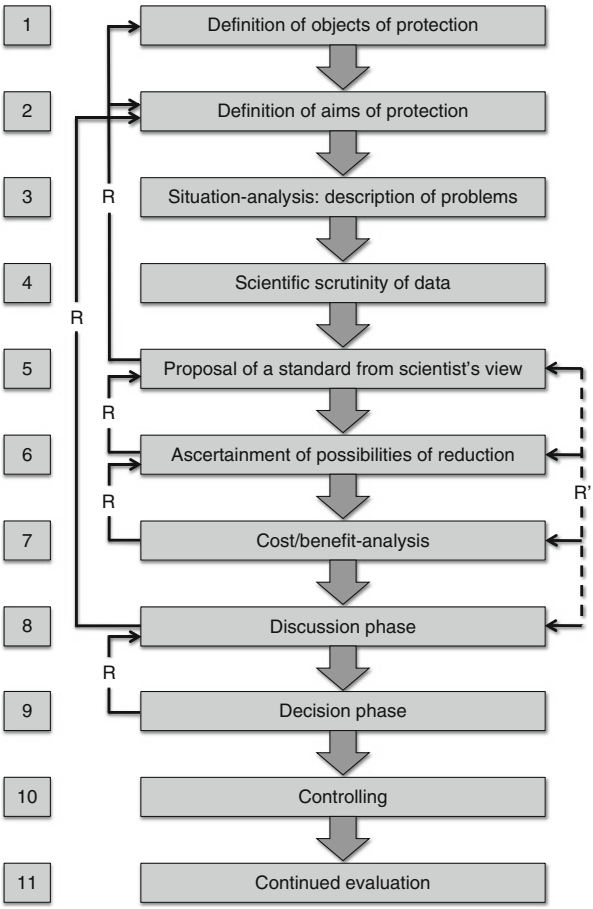



**Fig. 1** Scheme of sequential progress in the form of an ideal model of steps in the regulatory process (R, R′ = checkback; SRU, 1996)

evaluated the data as a result of the critical evaluation of all data for which a detailed justification is mandatory, including the identification of gaps of knowledge. This step is followed by the ascertainment of *possible technical reduction of risk(s)* (often called "status of technology"), as well as the elaboration of a *benefit/risk analysis* and a *cost analysis*, both steps involving experts in engineering and economy. Again, these proposals have to be justified in detail.

After these basic steps have been accomplished, a *discussion phase* tries to set a starting point for a solution, may be in the form of several alternatives. Participants are societal groups (producers, users, employers); for checkback questions, scientists who participated in the foregoing steps should be available. The guidance of the discussions should be handled by those responsible for the (final) decision-making (governmental and/or nongovernmental). They should prepare, in the following *decision phase*, the finalized version of the standard proposal, including the detailed justification, and put through the final decision. The same group of participating experts shall also prepare the operational steps of *control of*

*compliance* to the standard and for a *continued reevaluation* in predetermined intervals, taking into account new developments in data production and interpretation. For this purpose, a new standard necessitates the provision of suitable analytical methodology according to internationally accepted rules.

## Historic Developments

The classical form of organization of the process of regulation is the *commission of experts*. This has a long tradition in Germany, particularly by the DFG (German Research Association) who, according to their statutes, provides recommendations for health-related issues. Since 1952, DFG has established so-called Senate Commissions in different domains of regulations (occupational toxicants preparing MAK values = maximum tolerable concentrations, plant-protecting chemicals, foodstuffs, cancer research, etc.). The MAK Commission has held a pilot function for many other commissions. For ambient air pollution regulations, numerous commissions have been established and are still active in the VDI (Union of German Engineers). In addition, governmental agencies – from federal down to community level – have established their own committees for giving advice in environmental problems or setting standards of their own. Some are working permanently, some ad hoc only; the latter ones suffer, in some cases, from a lack of consistency and continuity.

*Membership* in these commissions of scientists in general, and of toxicologists in particular, should be based on independency in their professional activities and reasoning. There is a legal basis for proving the evidence of independency in the form of official rules of administration: new members of a commission have to declare by signature that they do not hold contracts with industry, share holding included. In this context, there remains an open issue of membership of professionals in industry: on one hand, they may contribute a high amount of special knowledge and competence, and they may contribute to the process by submitting valuable data (sometimes unpublished) and by specific experience. One way out of this conflicting situation may be seen in having them participate by seat but not by vote. But this certainly is not satisfactory to everybody. The agencies should create clear regulations referring to this sensitive point, now and forever.

Finally, there remains one important question to be solved: Who should participate in which sector of the regulatory process and who should take which part of responsibility? Two models are in operation: (1) Unitarian, every member of the commission participates in all steps of the procedure, participates in voting, and thus carries full responsibility. (2) Separatistic, the activities in the scientific analysis, discussion, and decision are strictly separated from each other, which means everybody participates just in that sector where he/she is professionally competent and thus takes responsibility just in that part. The separation shall avoid influences upon the scientific evaluation and decision by members of

interested societal groups. Further development indicates preference of the separatistic model. However, the lawmakers in Germany have not yet taken decision towards a clear and comprehensive regulation of this issue.

## Types of Organization

Similar processes as those described above for Germany have been developed on the level of the European Union and internationally. However, within the different legislative contexts, the involvement of scientific expert committees varies between the sole responsibility of the advisory group regarding limit values developed to an advisory role after the value has been defined by a regulatory authority.

For example, panels of the *European Food Safety Authority (EFSA)* and Scientific Committees of the European Commission with specific legislative mandates develop tolerable limits for food additives, food contact materials, food contaminants, or cosmetic ingredients based on scientific principles for health risk assessment and carry the sole responsibility for the process. In contrast, in the Registration, Evaluation, Authorization of Chemicals (REACH) process, the manufacturer or importer of a chemical (registrant) is responsible for performing risk assessment and for developing tolerable exposure following specific and detailed guidance outlined in REACH regulations. The Agency (ECHA – European Chemicals Agency) interacts with the registrant and can require specific information to address issues identified in the derived exposures and potential uncertainties in the evaluation. However, due to resource constraints, it is expected that only a limited number of the submitted registration dossiers will be evaluated in great detail.

In addition, a significant role of scientists employed by regulatory agencies (governments) in risk assessment is also frequently observed. In many cases, scientific advisory boards have the role to provide comments to the developed documents regarding risk assessment. For example, in the USA, many regulatory decision documents regarding chemical safety are drafted by regulatory agencies, and conclusions are presented to a scientific advisory board and the general public requesting comments on the conclusions.

## Recommended Reading

German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Kommission) The MAK Collection for Occupational Health and Safety available online at: http://onlinelibrary.wiley.com/book/10.1002/3527600418

The Scientific Committee on Occupational Exposure Limits (SCOEL) (2009) Methodology for the derivation of occupational exposure limits, version 6

US-EPA (2005) Guidelines for carcinogen risk assessment, EPA/630/P-03/001F. US Environmental Protection Agency, Washington, DC

# Quality Assurance in Regulatory Toxicology

Werner Lilienblum and Stephen Harston

## Contents

Quality Must Be Defined in Advance ..... 58
Quality of Data ..... 58
Quality Management (QM) and Quality Assurance Systems (QA Systems) ..... 59
First Strategy: Traceability and Transparency of Studies ..... 59
Second Strategy: Reproducibility and Comparability of Data ..... 59
Good Laboratory Practice (GLP) and Other "GxP" Systems ..... 60
Accreditation and Certification ..... 61
"Codes of Conduct" and Quality Assurance ..... 62
Recommended Reading ..... 62
Resources ..... 63
Scientific Societies for Research and Quality Assurance ..... 63

**Abstract**

Reliable data are essential for the assessment and evaluation of the toxicological characteristics of chemical substances and of exposure levels for man and the environment. Data reliability is closely linked with the exclusion or minimization of errors and mistakes in the generation of data. These objectives can be reached by the implementation of appropriate Quality Assurance (QA) systems. An important part of such systems is Quality Management (QM). The major characteristics and differences of the more important quality assurance systems are presented in this chapter.

W. Lilienblum (✉)
Formerly: Governmental Institute of Public Health, Hannover, Germany
e-mail: Werner.Lilienblum@t-online.de

S. Harston
Hofheim am Taunus, Germany
e-mail: HarstonSJ@web.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_28, © Springer-Verlag Berlin Heidelberg 2014

## Quality Must Be Defined in Advance

The quality of a finished product or of a service function at the end of a value creation chain is determined by a number of factors – the basic elements of quality first described by Kaoru Ishikawa (1968) and linked by him into a "fishbone" or "cause-and-effect" diagram. These factors include management, environment, methods, machines, materials, measurement, and – last but not least – people. "Quality" in this context is so defined that any activity, performance, or technical product should meet specific, predefined requirements and characteristics on completion. In order to reach and maintain such quality requirements, specific prerequisites and boundary conditions on the road to the finished product must be defined in advance. These will include quality criteria and quality control procedures applicable not only to the end product but also for all critical initial parameters and intermediate steps. In the case of reproducible or frequently repeated activities, such prerequisites and boundary conditions are often defined in (official) *Standards, Guidelines, or Directives*. This applies to the majority of physical, chemical, and biological-medicinal measurement systems and to methods for the generation of data relating to chemical substances and their actions.

## Quality of Data

Relevant data are required to assess and evaluate the toxicological characteristics of chemical substances or of exposure levels. The quality of the available data is of decisive importance and thus has to be carefully considered during the evaluation process. Good quality means not only that the data provide an important or significant contribution in the sense of providing new insights or filling a previous gap of knowledge but also that the data is reliable, in the sense that both the probability of errors occurring and the extent of any which may occur are as small as possible.

Practically every measurement (no matter how accurate) or other form of experimental or epidemiological data collection carries some risk of random or systematic errors, which then result in a deviation from the "true" value (which is – in general – not known). An important aim of any institution generating such data must thus be to implement appropriate general conditions and control procedures so that there is a high probability that the data obtained approach the "true" value and can be confirmed – either by repeating the process or by some other method. Given a certain process or method, the probability of approaching the "true" value can thus only be improved by systematically eliminating all known sources of error and – gradually – identifying and eliminating unexpected or previously unimaginable sources of error. *Data quality* in terms of *reliability* thus depends on the systematic elimination of sources of error. This necessitates a Quality Management approach with a suitable Quality Assurance system.

## Quality Management (QM) and Quality Assurance Systems (QA Systems)

The aim of Quality Management is firstly to ensure that errors in on-going processes are excluded so far as possible. As part of a continuous learning and improvement process, any remaining errors should be identified, documented, and avoided in the future. This can be achieved by the choice and implementation of a QA system with appropriate boundary conditions, methods, and controls.

The aim of every QA system is to generate *credibility* and *confidence* in the reliability of the data internally and externally – that is within the institute, with direct clients, and with all others who may be interested in the data concerned. In practice, two different strategies can be identified, neither of which alone is sufficient but which supplement each other in various QA systems with varying degrees of emphasis on individual features.

## First Strategy: Traceability and Transparency of Studies

Experimental data are usually generated in the course of studies and projects. Many such studies cannot – either on ethical grounds or because of the workload involved – be easily repeated should any doubt about the reliability of the data arise. Examples of such studies are long-term experimental studies in animals (often with large numbers of animals), studies in human beings, and field studies with crop protection agents. Any attempt to reconstruct such studies shortly or long after the event requires extensive and detailed recording of all initial conditions, methods, working steps, and the results obtained. In such cases, an extensive *documentation and archiving system* is required, such as that particularly described in the *Good Laboratory Practice* (*GLP*) system. The workload for the testing facilities involved with such systems is significant, also for relatively small or short-term studies.

## Second Strategy: Reproducibility and Comparability of Data

Many studies to determine – for example – physicochemical properties of substances such as melting or boiling point or the presence of substances in whole organisms or in other matrices can be fairly easily and quickly repeated under the same experimental conditions or can be easily checked by other means. This applies to the majority of chemical-analytical and many other physicochemical determinations. The comparability of the results can then be assured – at least in theory – by working back to *international SI units*. This has increased requirements regarding technical expertise, calibrations, and comparison measurements (e.g., participation in interlaboratory tests) for data validation and quality management procedures in the laboratories concerned. However, the documentation effort is then

reduced and more flexible. Quality assurance systems of this type include *accreditation* and – for products and services – *certification*.

## Good Laboratory Practice (GLP) and Other "GxP" Systems

Some quality assurance systems are required in relevant laws and regulations and thus fall under legal controls, for example, those for ***Good Laboratory Practice*** (***GLP***). The first GLP regulations were issued by the US Food and Drug Administration (FDA) in the late 1970s after irregularities were discovered in the planning, conduct, and reporting of animal safety studies submitted in the registration dossiers for medicinal products (FDA 1978). Similar regulations were subsequently issued by the US Environmental Protection Agency (EPA) covering studies conducted with agrochemicals and other chemical substances (EPA 1983). The need to comply with these regulations acted as a nontariff barrier to international trade in such substances, which led the Organisation for Economic Co-operation and Development (OECD) to develop internationally harmonized "Testing Guidelines" and "Principles of GLP" which were then recommended for worldwide use to ensure the *Mutual Acceptance of Data* (*MAD*) generated according to the Testing Guidelines and Principles (OECD 1981). The GLP Principles were recommended for use within the European Communities in 1987.

The Principles of GLP (and the Testing Guidelines) are reviewed on an ad hoc basis by OECD Expert Groups and – where appropriate – revised to reflect best scientific practices. The last revision of the GLP Principles took place in 1995–1996, and the Revised Principles were formally adopted by the OECD in 1997. The Revised Principles were adopted in the European Communities in 1999 and are now binding within all Member States (in Germany, e.g., as Annex 1 to the Chemicals Law (Chemikaliengesetz)). At the time of writing (2012), the European Regulations and Directives relating to biocides, chemical substances, cosmetics, detergents, feeding stuffs, foodstuffs, medicinal products, novel foods, REACH, and veterinary products all require that at least some of the test data required for the registration or regulatory approval of such products for use within the European Union be generated in compliance with the Principles of GLP or with equivalent standards (EC website 2012).

The OECD has also developed standards for governments to monitor the compliance of testing facilities with the GLP Principles (first adopted 1983, first revision 1989, second revision 1995), and these documents have also been implemented by the individual Member States within the European Union (Directive 88/320, now replaced by Directive 2004/9 of March 2004). In addition, the OECD has sponsored the preparation and publication of a series of "Consensus Documents" providing further comments and explanations on certain specific items of the GLP Principles (including quality assurance, laboratory supplies, field studies, short-term studies, computerized systems, full listing available on OECD website).

These documents have no legal force but are – in practice – regarded as "state of the art" and are widely used by testing facilities and by compliance monitoring authorities. Although common rules and standards exist, there may be differences in their interpretation, application, and enforcement between countries and even between monitoring authorities in the same country. For instance, whereas Seiler (2005) describes the implementation and application of the GLP Principles from a more "European" point of view, the same GLP Principles may be in part differently interpreted and applied in the United States and even between the two monitoring authorities US FDA and US EPA (Weinberg 2003). Moreover, despite some differences, both monographs may be used when implementing GLP in a test facility or as additional sources for managing GLP in practice.

*Good Manufacturing Practice* (*GMP*) is a QA system (also first developed in theUnited States) to control the manufacture of medicines and medical devices. The application and monitoring of GMP requirements is also largely harmonized, within Europe initially (1989) as "Guidelines to Good Manufacturing Practice," subsequently by Commission Directives 91/356 (June 1991) and 91/412 (July 1991).

*Good Clinical Practice* (*GCP*) provides a quality assurance system for planning, conducting, and reporting clinical studies carried out – for example – to provide data in support of applications for marketing authorizations for medicinal products. The requirements were first developed by an expert working group of the "International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use" (ICH 1996) and subsequently adopted by the regulatory bodies in the European Union, Japan and the USA. Among other issues, the GCPs require that clinical studies be planned and carried out according to the ethical standards described in the World Medical Association "Declaration of Helsinki." Further information on GCP can be found, for example, on the website of the European Medicines Agency (2012).

## Accreditation and Certification

Most *accreditation* and c*ertification* systems are based on voluntary participation and are not governed by legal requirements. However, the use of such systems is often a prerequisite before a facility or laboratory may conduct studies the results of which are to be used in legally controlled activities. This applies – for example – to laboratories performing analyses for the control of foodstuffs, the monitoring of ambient air or drinking water quality, or measurements to be used as part of health and safety requirements in the working environment. Both systems give high priority to the use of appropriate quality management procedures.

*Accreditation* is a system to monitor and approve the competencies of testing laboratories and their Quality Management systems. The organizations issuing such approvals are – themselves – monitored and accredited by the so-called Accreditation Bodies, as laid down in the International Standards Organization (ISO) Standards

17011 and – when appropriate – 17020–17025. For instance, in Germany, the Accreditation Body (since January 2010) is the Deutsche Akkreditierungsstelle (DAkkS) (previously known as the Deutsche Akkreditierungsrat).

*Certification* relates to the quality of products and/or service functions in the sense of a guarantee that certain defined characteristics are provided by the product or function. Appropriate certification and the establishment of a quality management system according to ISO Standard 13485 is – for example – a precondition for the use of the CE Mark on certain types of products to be placed on the market within the European Economic Area (EEA).

## "Codes of Conduct" and Quality Assurance

A number of scientific societies and professional associations (e.g., those for medical practitioners or for pharmacists) have developed codes of conduct which are binding on their members. These Codices contain certain elements which help toward a quality assurance but are – usually – directed to ensuring a responsible and ethical behavior in professional activities. Such elements, for example, a requirement for scientific honesty, are important but cannot – alone – be regarded as a quality assurance system.

The concept of "Safeguarding Good Scientific Practice" has been developed by some major institutes for basic research in response to spectacular cases of scientific misbehavior and/or fraud. For example, the German Research Foundation (Deutsche Forschungsgemeinschaft 1998) has issued proposals for "Safeguarding Good Scientific Practice" with some 16 detailed recommendations and suggested their use in scientific institutions, particularly those in academia. Among the more important recommendations in the sense of a quality assurance are those related to organizational structure of working groups and the need for complete documentation and long-term archiving of important primary data; however, it is unclear in how far these recommendations have been followed by the institutions concerned.

## Recommended Reading

EEC (1999) Commission Directive 1999/11/EU of 8 March 1999 adopting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC. Published in Official Journal of the European Communities, 23 March 1999, No L 77, pp 8–21

OECD (1995) Council Decision amending the Annexes to the Council Decision-Recommendation on Compliance with Principles of Good Laboratory Practice. C(95)8 of March 1995. With Annex I (Revised guides for compliance monitoring procedures for GLP) and Annex II (Revised guidance for the conduct of laboratory inspections and study audits)

Seiler JP (2005) Good laboratory practice, 2nd edn. Springer, Berlin/Heidelberg

Weinberg S (ed) (2003) Good laboratory practice, 3rd edn. Marcel Dekker, New York/Basel

## Resources

Deutsche Forschungsgemeinschaft (1998): Sicherung guter wissenschaftlicher Praxis/ Safeguarding Good Scientific Practice. http://www.dfg.de/download/pdf/dfg_im_profil/reden_stellungnamen/download/empfehlung_wiss_praxis_0198.pdf

European Commission Web-Site (2012) On good laboratory practice (only available in English). http://ec.europa.eu/enterprise/sectors/chemicals/documents/specific-chemicals/laboratory-practice/index_en.htm. Accessed Oct 2012

European Medicines Agency (EMA) Web-Site (2012) On good-clinical-practice. http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000072.jsp&mid=WC0b01ac05800268ad. Accessed Oct 2012

FDA (1978) Food and drug administration (USA): Nonclinical laboratory studies – good laboratory practice regulations, federal register, 43(247), 59986–60025

International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). "Harmonised tripartite guideline: E6: Guideline for Good Clinical Practice" (2006) Adopted in Europe in July 1996, in Japan in March 1997 and in the USA in May 1997. Current version available at www.ich.org/products/guidelines/efficacy. Accessed Oct 2012

Organisation for Economic Co-operation and Development (OECD) Web-Site (2012) On good laboratory practice. http://www.oecd.org/chemicalsafety/testingofchemicals/goodlaboratory-practiceglp.htm. Accessed Oct 2012

United States Environmental Protection Agency (US-EPA) FIFRA: Good Laboratory Practice Standards: Final Rule. US Code of Federal Regulations, Chapter 40, Part 160. First published in Federal Register, November 1983. Current version available at www.ecfr.gov or http://www.epa.gov/compliance/monitoring/programs/fifra/glp.html. Both accessed Oct 2012

## Scientific Societies for Research and Quality Assurance

DGGF (Deutsche Gesellschaft für Gute Forschungspraxis). www.dggf.de
JSQA (Japan Society of Quality Assurance). www.jsqa.com
RQA (The Research Quality Association). www.therqa.com
SoFAQ (Societé Francaise d'Assurance de la Qualité). www.sofaq.fr
SQA (Society of Quality Assurance). www.sqa.org

# Toxicological Risk Assessment

Maged Younes

## Contents

Introduction and Definitions ..... 66
Definition of Risk Analysis ..... 66
Why Risk Analysis? ..... 66
Steps in Risk Regulation ..... 66
Risk Assessment ..... 67
Risk Evaluation ..... 67
Risk Management ..... 67
The Process of Risk Assessment ..... 67
Hazard Identification: Assessing the Potential to Cause Harm ..... 68
Dose-Response Assessment: The Relation Between Exposure and Effect ..... 69
Exposure Assessment ..... 69
Risk Characterization: The Synthesis of Risk Information ..... 69
The Need for Harmonization ..... 70
Recommended Reading ..... 70

**Abstract**

Risk analysis encompasses the scientific review and evaluation of all relevant scientific data on the toxicity of, and the exposure to, a certain compound or mixture. To enable a systematic analysis of the different types of information needed, various risk analysis paradigms have been developed. Among these, the scheme developed in 1983 by the US National Academy of Sciences (NAS) has been the most widely utilized. Risk analysis provides the scientific basis for regulatory actions within the context of risk management.

M. Younes
Former Director, Food Safety and Zoonoses, World Health Organization (WHO), Mies, Switzerland
e-mail: magedyounes@gmail.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_29, 

## Introduction and Definitions

### Definition of Risk Analysis

The term "risk analysis" is not used in a uniform manner. In some instances, the term is considered to have the same meaning as "risk assessment," while some institutions, as is the case with the Codex Alimentarius Commission, employ the term to describe the broader concept of risk regulations, encompassing risk assessment, management, and communication. For others, risk analysis is seen as the mathematical analysis and quantification of risks. Given these differences in using the term risk analysis, a clear, uniform definition cannot be given. For the purposes of this chapter, risk analysis will be described as the broader process encompassing the scientific assessment, management, and communication of risks.

### Why Risk Analysis?

The toxicity of a given substance can be defined as its ability to harm living organisms. This is an inherent characteristic of any compound and will only be expressed as a function of the dose as described already by Paracelsus. Thus, any compound can be toxic if a certain threshold of exposure is surpassed. This is the reason why a distinction between "toxic" and "nontoxic" or "harmful" and "safe" substances makes no sense. In fact, the toxicity of a given substance cannot be defined without reference to the administered/absorbed amount (dose); the route through which the exposure and distribution of the substance take place (e.g., by inhalation, ingestion, dermal absorption); the level, frequency, and duration of exposure; the type and grade of the damage caused; and the lag time required to illicit the toxic effect.

It is only once the potential to cause harm and the probability of a damage are known that options to reduce/eliminate potential harm can be assessed and regulatory action be taken (risk management). Such measures need to consider other factors besides the scientific evaluation of risks, for example, socioeconomic impacts and the risk-benefit relation. The aim of risk management is to avoid risk or, if this is not possible, to reduce it as far as achievable. The basis for meaningful risk management decisions remains, however, a thorough characterization and evaluation of scientific data on toxicity and exposure: Risk assessment.

## Steps in Risk Regulation

In the scheme of the German Risk Commission (Deutsche Risikokommission), risk regulation encompasses the whole societal process of dealing with risks. Ideally, the process should cover three areas of risk analysis: risk assessment, risk evaluation, and risk management.

### Risk Assessment

Risk assessment is the process of identifying and quantifying the potential harm due to a certain exposure to a substance (risk). Normally, it targets individuals, but there are several instances in which population risk is assessed. To accomplish this task, knowledge about toxicity and exposure, but also information on the dose-response (or exposure-effect) relation, and target populations including vulnerable groups is required (see below).

### Risk Evaluation

Risk evaluation bridges risk assessment and risk management. It encompasses a value judgment of the risk posed by the substance under consideration. Questions addressed here include whether or not the risk is higher than seen with other comparable compounds, what the risk-benefit ratio is, and if there are any protective measures that can be taken to reduce the risk. In addition, social, cultural, and political factors may also be considered. The outcome of this process is a recommendation for risk management.

### Risk Management

Risk management is the decision process during which the results of the risk assessment are used to develop and analyze options for avoiding or minimizing risks of exposure to a given substance, taking into consideration political, social, cultural, economic, and technical aspects. The aim of this process is to define the best possible and feasible action(s). Risk assessment and management are distinct, though closely related, interactive processes: while risk assessment is a scientific, technical discipline, risk management is a sociopolitical decision-making process. Newer models of risk analysis have endeavored to develop a closer interlink between the two processes (see below).

## The Process of Risk Assessment

Scientific information needed to conduct risk assessment includes qualitative and quantitative data on the toxicity of the agent in question, on the dose-response relation, as well as on the exposure. Various paradigms have been developed to facilitate a systematic analysis of such complex data and, consequently, to allow for the development of a comprehensive estimation of potential risks. The most commonly used scheme worldwide is the one developed by the US National academy of Sciences (NAS) in 1983. It is currently in use by many regulatory agencies, though some variations of it are also applied, and more modern

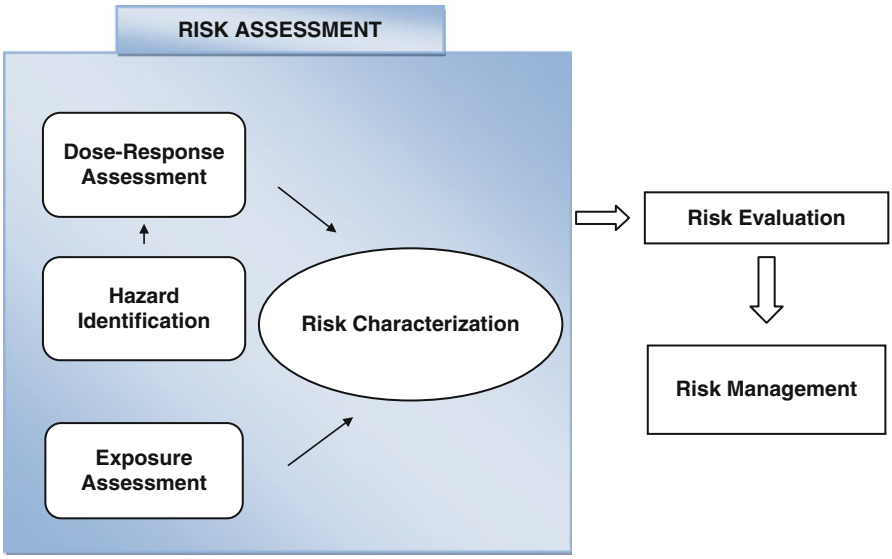



**Fig. 1** Schematic presentation of the risk analysis process: Following risk assessment, with its four components, risk evaluation is conducted to allow for consideration of additional factors, such as socio-economic impacts, before risk management decisions are taken

approaches have expanded on it to provide a better link between the processes of risk assessment, management, and communication. The NAS model divides the process of risk assessment into 4 distinct steps (Fig. 1).

## Hazard Identification: Assessing the Potential to Cause Harm

Hazard identification is the step during which all relevant data are analyzed that provide information to assess the inherent potential of an agent to exert harmful effects. These data can stem from toxicological studies, including by alternative methods to animal experiments, and also from epidemiological or human volunteer studies.

It is worth noting that the terms "hazard" and "risk" are often used synonymously. This is incorrect. The term "hazard" describes the "potential to harm," that is, the principal ability of a given substance to exert a toxic effect (which, logically, will only occur at a certain exposure level). Hazard is therefore an inherent characteristic of the agent in question. "Risk," by way of contrast, describes the probability that a harmful effect will, in fact, occur. Risk is the actual or potential danger posed by an existing or an expected exposure.

## Dose-Response Assessment: The Relation Between Exposure and Effect

In the course of this step, a quantitative estimation of toxic effects, be it the severity of an observed outcome, such as the level of liver damage as evidenced by an increase in blood levels of liver-specific enzymes, or the frequency of occurrence of a yes-or-no outcome, such as cancer or even death, at different exposure levels is conducted. This allows for a characterization of potential toxic outcomes as a function of exposure or dose.

## Exposure Assessment

Exposure assessment encompasses the qualitative and/or quantitative determination of the level and frequency of exposure, potentially the lag time between subsequent exposures, the exposure media (air, drinking water, soil, recreational water, food), as well as the exposure route(s) (inhalation, ingestion, dermal absorption).

## Risk Characterization: The Synthesis of Risk Information

The last step in risk assessment is risk characterization (see also chapter "► Risk Characterization in Regulatory Toxicology"), which is a synthesis of all evaluated data and information. Strengths and weaknesses of the database must be clearly identified, methods and criteria of all evaluations described, and the results of the evaluation of all data outlined. The outcome of risk characterization is the basis for developing strategies to avoid or, if this is not possible, to minimize the risk (risk management). Vulnerable groups, which are at particular risk due to higher exposure levels and/or an enhanced susceptibility must be characterized in order for risk management decisions and actions to take their particular situation(s) into consideration.

The scheme described is a conceptual framework which should help in organizing all scientific data in a manner that allows a sequential, logical analysis. Other models/schemes have been developed, but the NAS paradigm is the most widely used till now. Individual steps of the process are more exhaustively described in other parts of this book.

Recent advances have been made to better link risk assessment with risk management. The US National Research Council recommended in 2009 that risk analysis should be divided in three phases. The first phase should cover problem formulation and scoping in order to better identify data needs and target risk assessment. The second phase should encompass the planning (stage 1) and conduct (stage 2) of risk assessment, pretty much following the NAS paradigm, but with an

additional stage 3 to confirm the utility of the assessment. In this latter stage, questions to address include if the assessment had the attributes called for in the planning, if the assessment provides sufficient information to discriminate among risk management options, and if the assessment has been sufficiently peer-reviewed. Only then phase 3, risk management, actions can be evaluated and decided upon.

## The Need for Harmonization

Despite the fact that the scientific data used for risk assessment purposes by different institutions are mostly identical for the same compound, they are often analyzed and treated differently and may result in different outcomes. For example, carcinogenic risk is characterized in the USA through a calculation of an exposure corresponding to a theoretical tumor incidence. In this context, dose extrapolation is conducted via different methods to very low levels, often below analytical detection limits. In this manner, exposures leading to a tumor incidence of, for example, 1 in 100,000 or 1 in 1,000,000 are calculated. Such methodologies are seldom used in Europe. Still, it is possible to compare the results of risk assessments conducted in different ways and to use performed data analysis to a certain degree, as long as the methodology, including all assumptions and uncertainties, is clearly outlined. It should be noted that there are recommendations to unify risk assessments for carcinogens and noncarcinogens, for example, in the 2009 report of the NRC.

At the international level, efforts are underway to harmonize, though not to standardize, risk assessment methods. In this context, the aim is to promote the understanding of different approaches to risk assessment, so that the results of such assessments conducted by a different institution can be understood by other institutions and eventually adapted to their specific needs. Thus, risk assessments can be utilized universally.

Risk assessment and the subsequent risk evaluation are the basis for regulatory decisions to manage risks. Regulatory measures are obviously different in different areas of regulation: In the case of pharmaceuticals, for example, the risk related to treatment must be put in relation to its therapeutic value. In the case of chemicals, it is important to estimate the potential direct exposure of workers in all areas (production, use, storage, and transport) and consumers, as well as the indirect exposure through various environmental media in order to reach regulatory decisions that would, indeed, eliminate or reduce to a minimum the exposure of the respective groups of the population.

## Recommended Reading

European Commission, Environment Directorate General (2007) REACH in Brief. http://ec.europa.eu/environment/chemicals/reach/pdf/2007_02_reach_in_brief.pdf

National Research Council (1983) Risk assessment in the federal government: managing the process. National Academy Press, Washington, DC
National Research Council (1993) Issues in risk. National Academy Press, Washington, DC
National Research Council (2009) Science and decisions: advancing risk assessment. National Academy Press, Washington, DC
World Health Organization (1999) Principles of the assessment of risks to human health from exposure to chemicals. Environmental Health Criteria 210. WHO, Geneva
Younes M, Meek ME, Hertel RF, Gibb H, Schaum J (1999) Risk assessment and management. In: Herzstein JA, Bunn WB, Fleming LE, Harrington JM, Jeyaratnam J, Gardner IR (eds) International occupational and environmental medicine. Mosby, St. Louis, p 62

# Part II

# Approaches Used

A very broad range of approaches are used in regulatory toxicology. Animal testing is still the main focus of the experimental investigations. The interpretation of animal trials requires experience in pathology and clinical chemistry. In vitro tests, which are often aimed at reducing the need for animal testing, provide important information on mechanisms of action. Many test methods are internationally harmonized. Recently, high-throughput procedures have dramatically increased the extent of testing and, hence, the flood of data. In human studies, signs and symptoms are important. They provide information on the affected organs in humans and evidence for the type of dose-response relationship. Wherever possible, the data should be analyzed statistically. The regulatory toxicologist must have sufficient experimental experience to enable him or her to judge the value of the individual techniques and the statistical significance and biological importance of the results. Fortunately, the toxicologist today has rapid access to large toxicological data banks and original literature to help in setting the findings into their context.

**Toxicological Tests**

As a preliminary to toxicity l testing, the physicochemical properties of the compound should be identified. They help in identifying and characterizing the acute and chronic toxicity of a substance, the affected organs, and the dose-response relationship. Studies on the mechanism of action, toxicodynamics, and toxicokinetics complete the picture. Novel in vitro tests are introduced at high speed, though, in some cases, their value for regulatory purposes is still debated. It is expected that the continuously improving techniques of genomics and proteomics, combined with the evaluation by bioinformatics techniques, will provide fundamentally new information and could even bring about a paradigm shift in toxicology.

**Data Acquisition in Humans**

Usually animal experiments or tests with human cell lines serve as a surrogate for possible effects in humans. But in some cases, notably in drug development, meaningful human studies are unavoidable. Two main methods are available: In human experimental studies, volunteers are exposed under controlled conditions in order to investigate tolerability, kinetics, or effectiveness of a substance.

In epidemiological studies and clinical trials, population groups are investigated to find out possible "side effects" (toxicity). Appropriate ethical principles and privacy must be observed.

**Toxicostatistics and Toxicological Models**

Statistics play an important role in the evaluation process of all quantifiable tests in toxicology. When extrapolating from test dose levels to very low dose levels, the choice of the statistical model can have a significant influence on the value of the acceptable exposure. Modern probabilistic methods are suitable when quantifying risks in population groups. Theoretically, toxicodynamic and toxicokinetic models permit the estimation of concentrations of a harmful substance at the site of biological action and contribute to an understanding of the mechanism of action.

**Estimation of External Exposure**

One can differentiate between external exposure (concentration in air, food, soil, skin surface) and internal exposure (concentration in blood, target organ). External exposure calculations are based on theoretical distribution models. These are often subject to considerable uncertainties. Therefore, in the case of a chemical incident, the concentration of the harmful chemicals in the affected medium (air, food, water, etc.) should be measured as soon as possible with suitable analytical chemical procedures. When the external exposure is known, the internal exposure can be estimated.

**Use of Toxicological Data**

Risk characterization should be built on all available experimental and chemical substance information. However, not all published studies are of equal validity or equal quality. Therefore, it is important to understand quality criteria for both the primary scientific literature and test reports. In recent years, data search has been greatly facilitated by the easy access to large national and international toxicological databases. Search and valuation of literature now belong to the core activities of regulatory toxicologists.

# Characterization of Physicochemical Parameters in Toxicology

Mathias Locher

## Contents

Physicochemical Properties and Bioavailability ..... 75
Identification, Content and stability of the Test Article ..... 77
Methods ..... 77
Inhalative Toxicology ..... 78
Impurities and Content ..... 78
Guidelines ..... 80
References ..... 80

**Abstract**

Toxicological tests are only meaningful and interpretable, when the chemical composition and the physicochemical properties of the applied substances are known. These properties determine to a large extent the behaviour of a substance in the environment and in the living organism. Thus knowledge of physicochemical properties is important for the development of therapeutic drugs as well as for the risk assessment of all chemicals.

## Physicochemical Properties and Bioavailability

Physicochemical properties, like solubility, coefficient of distribution (octanol/water pH 7.4), combined with particle size, or crystal structure usually influence and correlate with Absorption, Distribution, Metabolism and Excretion (ADME) of drugs (Krämer and Wunderli-Allenspach 2001). In depth knowledge of these basic physicochemical characteristics of a drug substance are important for

M. Locher
Covagen, Zurich, Schlieren, Switzerland
e-mail: mathias.locher@covagen.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_30, 

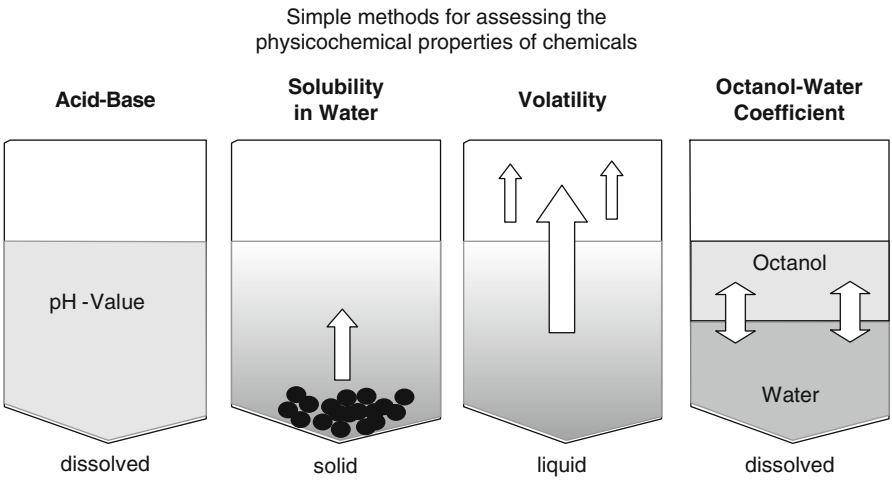



**Fig. 1** Simple methods for assessing the physicochemical properties of chemicals

the characterization of chemicals as well as for the development of innovative and ideal drug formulations and to optimize bioavailability of drugs. Figure 1 shows some simple methods to study physicochemical properties.

With regard to physicochemical properties, solubility and coefficient of distribution of the drug substance are mainly of interest for the toxicologist. The paramount of a toxicological investigation is to show toxicity to be able to estimate the risk associated with human (or environmental) exposure to the substance. Therefore, the maximal dose, given in a toxicity test, and the resulting systemic exposure (measured in plasma or serum), should be as high as possible.

The systemic exposure is a delicate mixture of a series of events. The extent of absorption, extent of distribution, extent of metabolism and extent of excretion. While of course drug molecules can be substrates of cellular transport systems (not discussed here), absorption, distribution and accessibility for metabolism are influenced by the physicochemical properties like solubility and coefficient of distribution of the drug molecules. For the absorption of an orally administered encapsulated powder or an administered suspension the drug substance has to be solubilized in advance in the gastro-intestinal tract. This sometimes rate-limiting step could be circumvented by the intravenous route, but for the practicability of a daily administration and for the convenience of the patients an oral formulation for a therapeutic agent is highly preferable. Therefore – if possible – the oral administration of a solution to the animal species (e.g., rat) is not only the most simple approach, an oral solution usually guarantees high plasma concentrations (*Cmax*) and high systemic exposure (*AUC*).

Usually solubility is tested in a series of organic solvents. For the toxicologist solubility in DMSO is important because this solvent is used to solubilize molecules to be tested in the Ames-Test. Of course in addition substantial knowledge should be available on the solubility of the test compound in buffer systems used for i.v. or

oral administration to animals (e.g., rodents, dogs, monkeys). In pharmaceutical industry the close interaction of the toxicologist with the chemist and pharmacist sets the basis of a straight forward risk assessment process.

To ensure the reliability of a toxicological study, the quantitative determination of the test article in the test solution is a must. Usually specific HPLC based techniques like HPLC-UV or HPLC-MS are used for small molecule drugs.

For biotherapeutics like monoclonal antibodies (mAbs) or protein replacement therapies partly other measurements are of importance compared to small molecules (see also chapter "▶ Biomolecules Versus Smaller Chemicals in Toxicology") (Swami and Shahiwala 2013). Proteins usually are not given via the oral route. The main administration routes for proteins in toxicology are either the intravenous or the subcutaneous route. Solubility and stability of the protein is of great importance especially to prepare highly concentrated subcutaneous formulations (>100 mg/ml) for mAb toxicity testing. Aggregation of the protein has to be prevented, therefore a series of buffer systems should have been tested before starting the toxicity testing of proteins. Quantitative determination of the protein concentration in the test solution as well as the exposure in plasma/serum are still done by immuno assay techniques.

## Identification, Content and stability of the Test Article

The test article tested in vitro or in vivo toxicology studies should be comparable to the test article used for clinical studies in humans. Therefore, information on the identity, the content and the storage as well the benchtop stability of the test article are important information for the toxicologist and should be available before testing.

Testing the identity primarily tells the toxicologist if the test article has still the quality required for testing. As mentioned above it is important to measure the concentration in the test solution to calculate the exact dose administered and to exclude that there is precipitation or adsorption of the test article to glass or plastic vessels used for the preparation of the test solution which could invalidate the toxicological study.

## Methods

Methods to assess the identity of the test article should be able to discriminate the test article from structural similar molecules i.e., the methods should be specific (e.g., IR-spectroscopy and mass spectroscopy). To identify a test article only by a single HPLC method is not acceptable. A second chromatographic method using a different separation or detection technique is necessary to ensure the identity of a molecule.

If the test article is an enantiomer, the method used to describe the identity of the test article should be able to discriminate between the enantiomers.

Compared to small molecule, the biotherapeutics (mAbs and other proteins) are complex molecules and only in rare cases exist as a single unique molecule. Proteins produced by expression in mammalian cells or bacteria usually exist in different isoforms and their glycosylation pattern usually varies. These isoforms may have different pharmacokinetics, binding affinity and bioactivity (European Medicines Agency 2007). Independent of the complexity of the protein therapeutics, the test article tested in toxicology studies has to meet the specifications (melting temperature, SDS-page, molecular weight, glycosylation pattern, binding, bioactivity) of the material produced for human use.

Immunogenicity is a very special event following the administration of proteins to animals or humans. The resulting anti-drug antibodies (ADAs) may influence not only bioactivity, but also clearance of the protein. Therefore, it is necessary to asses immunogenicity using respective assays that not only measure ADAs as such but can also discriminate between neutralizing and non-neutralizing ADAs, which is important for the further toxicological testing strategy (Jefferis 2011).

## Inhalative Toxicology

Inhalative toxicological studies ask for high analytical demands. The test article will be delivered as gas, aerosol or powder that is aspirated by the animals. Therefore, in addition to the usual analytical control of the test article the particle size of the aerosol or powder as well as their homogeneous distribution within the experimental system has to be assessed routinely (see chapter "► Toxicokinetic Tests" in this book).

## Impurities and Content

The test article already used in early safety studies (safety pharmacology and short term toxicology) should be comparable to the test article later tested later in clinical studies and will reach market approval. Therefore, it is important that the content of the test article and the impurity profile – the specifications – of the early available drug substance meet the specifications of the drug product marketed later. At the beginning of drug development process test article specifications should not be too tight on the other hand a high level of impurities may negatively influence results of toxicology studies e.g., the Ames-Test. Specifications of the test article batches tested in safety relevant studies have to be listed in the regulatory documents and have to be compared with the specification of the batches used in clinical trials (Fig. 2).

The impurity profile is an important characteristic data set for the drug substance as well as the drug product and is related to the synthesis/production process of the test article. A major change in the impurity profile of a marketed product e.g., because of a process change requires a new safety assessment that may include preclinical and clinical studies (Fig. 3).

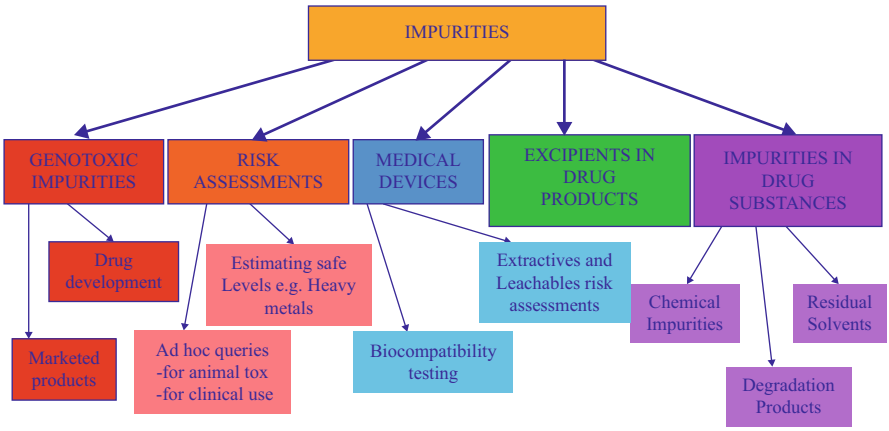



**Fig. 2** Impurities and their importance in drug development

**Fig. 3** Safety Assessment - it's all about risk assessment

For biotherapeutics aggregates, viral contaminations and host cell proteins are a major source for impurities. Therefore, a harvest and down stream process (purification process) has to be established using methods to separate the impurities from the products and with special procedures for virus inactivation.

Today, for small molecules HPLC-MS/MS is the method of choice to quantify the test article and the impurities. For protein drugs, immuno assays (ELISA) are usually used to quantify the test article and chromatographic, electrophoretic or PCR methods are used to quantify impurities. But protein drugs have to be characterized further. In addition to the content the bioactivity of the test article measured in a validated cell based assay is usually required to characterize the test article and to ensure the comparability of test article used in preclinical and clinical drug development with the marketed product.

## Guidelines

For drug development, the International Conference on Harmonization (ICH) has published a series of guidelines for all aspects and phases (e.g., Quality, Efficacy, Safety) of drug development (http://www.ich.org/products/guidelines.html).

Information on requirements to assess the quality (e.g., stability, impurities, specifications, analytical validation) can be found in the quality guidelines Q1 – Q11. All regulatory requirements with regards to drug safety are summarized in the safety guidelines S1 – S10. A special notice has to be given to the guideline S6. This guideline is only valid for the safety assessment of biotherapeutics.

It is well known that toxicological as well as safety pharmacological studies have to be performed according to the regulations of GLP. Therefore, the analytical methods used to characterize the test article have to be validated and the analysis has to be performed accordingly. If analytical investigations are not performed accordingly this has to be described and explained. In the US, GLP regulations are described in the "21 CFR 58 – Good Laboratory Practice Regulations".

## References

European Medicines Agency (2007) Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins

Jefferis R (2011) Aggregation, immune complexes and immunogenicity. MAbs 3(6):503–504. doi: 10.4161/mabs.3.6.17611. Epub 2011 Nov 1

Krämer SD, Wunderli-Allenspach H (2001) Physicochemical properties in pharmacokinetic lead optimization. Farmaco 56(1–2):145–148

Swami R, Shahiwala A (2013) Impact of physiochemical properties on pharmacokinetics of protein therapeutics. Eur J Drug Metab Pharmacokinet

# Examination of Acute and Chronic Toxicity

Karl Georg Heimann and Kevin Doughty

## Contents

Determination of Acute and Chronic Toxicity ..... 82
Aim and Study Protocol ..... 82
Units of Investigation ..... 82
Marginal Conditions ..... 82
Acute Toxicity ..... 83
Acute Toxic Class Method ..... 84
Long-Term Studies ..... 85
Responsibilities ..... 85
Subacute Studies (28 day) ..... 86
Performance Ratio ..... 86
Dose Range Finding ..... 86
Reversibility ..... 87
Subchronic and Chronic Studies ..... 87
Definition of NOAELs ..... 87
Recommended Reading ..... 88
Resources ..... 88

**Abstract**

Determination of the toxicological profile is necessarily related to the toxicity of a substance considering risk potential/risk estimation for human. Essential relevance includes the dose level as well as the application period. Studies considering acute, subacute, subchronic, and chronic intake are the basic

K.G. Heimann (✉)
Expert Regulatory Toxicology, Köln, Germany
e-mail: heimann.kg-g@gmx.de

K. Doughty
Product Stewardship Manager, Bayer CropScience, Registration Department, Monheim, Germany
e-mail: kevin.doughty@bayer.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_31, 

which can be enlarged by specified studies. In general the results of those studies are relevant to set characteristic (LD 50/LC50 values, MOS, AOEL, ADI).

## Determination of Acute and Chronic Toxicity

### Aim and Study Protocol

Toxicity studies have to be performed with new substances (toxicity unknown) or known substances where production or use of precomponents has changed which can lead to a different toxicological profile in contrast to the existing data, i.e., different spectrum of precomponents, adjustment, or rate of enantiomers/isomers. Furthermore, they are indicated for substances or structure-related compounds where a medical indication is known. For most of the substances (i.e., human, veterinary pharmaceuticals, plant protection, hygiene, pesticidal substances), such investigations are requested by law/regulation (law of pharmaceuticals, plant protection, chemicals, pesticides). Before starting a study, a harmonized study protocol (standard) has to be performed according to national as well as international guidelines (i.e., OECD, WHO, EU) which schedules exactly the aim – subdivided in units of investigation – as well as all marginal conditions of the investigation in detail. Aim and marginal conditions have to be standardized by standard operating procedures (SOPs) to reduce as much as possible the error rate and to guarantee the transparency of the procedures and the resulting facts (good laboratory practice, GLP). The basic study types are shown schematically in Fig. 1.

### Units of Investigation

The following units of investigation have to be compiled: body weight, relevant organ weights (absolute/relative), food/water intake, behavior, clinical-chemical parameters in blood/urine, and macroscopic (visible) as well as microscopic (histopathological) evaluation of the organs. Which parameters have to be measured in detail is related to the target. The minimum is recorded in the guidelines mentioned before. The results have to be proofed then according to accepted statistical methods related to their biological/toxicological relevance.

### Marginal Conditions

The following marginal conditions have to be recorded: selection of a professionally accepted species including the strain, starting weight/age, food/water quality and source, quality of substance (purity grade, homogeneity, stability in the application medium), temperature, air humidity/exchange/pressure and duration of lightning in the stall, size of cages/inhalation chambers, litter, hygiene management, use of validated methods and appropriate material, as well as follow-up of

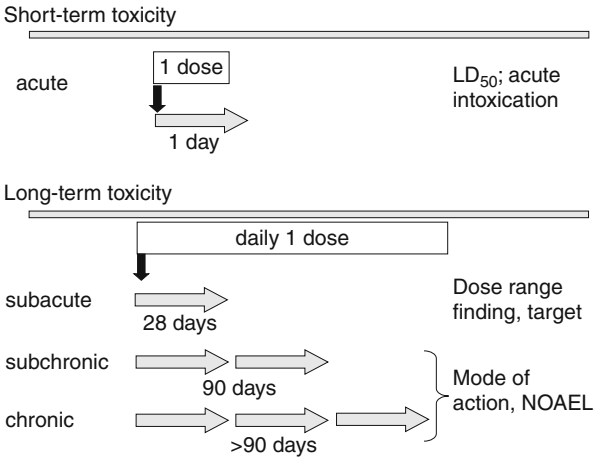



**Fig. 1** Type and aim of the studies (schematic)

historical control data. The results have to be listed in the report as single values and deposit as raw data – signed and dated – in an archive certified by GLP. Study protocol, test procedure, as well as the transfer of the results to the final report have to be proofed and certified by an independent quality assurance unit. According to animal welfare regulations, animal studies which are not requested by authorities are subject to approval; the others are notifiable only.

## Acute Toxicity

The aim of acute toxicity evaluation is the compilation of the action profile after application of relatively high doses within 24 h. The main application routes are oral, dermal, as well as inhalative which are more or less related to the routes of human exposure. There are further application routes of high impact which are used especially in medicine or as special studies (i.e., crossover study). The further process is comparable. The single oral application is carried out as a bolus application (gavage/capsule) using 3–5 animals/dose/sex. The dermal application is performed moistened or as a pasty related to the physical condition (liquid/solid). The skin is treated occlusively covered over 24 h. Via inhalation dust/liquid aerosols as well as gas or vapors are used related to the physical conditions of the test substance. To determine the hazard/risk potential, the "nose-only" system is preferred where the animals are exposed exclusively via the respiratory tract (nose/mouth) over 4 h. This guarantees the explicit correlation of the effects. Considering special indications (indoor hygiene, pest control, and others), the "whole body" exposure in an inhalation chamber is used which reflects the reality. As a disadvantage, no differentiation between inhalative and dermal intake can be made. After the single application, a 14-day post-application observation period follows which has to be enlarged if symptoms still continue. During this period progressing symptoms are protocolled related to intensity and

**Table 1** Dose response (calculated according to Litchfield and Wilcoxon)

| Dose (mg/kg/b.w.) | Toxicol. results[a] | Duration of signs | | |
|---|---|---|---|---|
| Rat – male | | | | |
| 50,0 | 0/0/5 | – | – | 0 |
| 100,0 | 0/0/5 | – | – | 0 |
| 150,0 | 0/5/5 | 52 m–2 day | – | 0 |
| 170,0 | 0/5/5 | 2 h–2 day | – | 0 |
| 200,0 | 3/5/5 | 2 h 15 m–1day | 3 h 45 m–1 day | 60 |
| 300,0 | 1/10/10 | 2 h 30 m–2 day | 5 h 30 m | 10 |
| 1000,0 | 5/5/5 | 9 m–1 day | 48 m–1 day | 100 |
| LD50 > 170 < 200 mg/kg Kgw. (mg/kg b.w.) | | | | |
| Rat – female | | | | |
| 50,0 | 0/0/5 | – | – | 0 |
| 100,0 | 0/2/5 | 2 h 45 m–1 day | – | 0 |
| 150,0 | 2/5/5 | 51 m–2 day | 5 h 45 m–1 day | 40 |
| 170,0 | 0/2/5 | 3 h 45 m–1 day | – | 0 |
| 200,0 | 4/5/5 | 2 h 15 m–1 day | 3 h 30 m–1 day | 80 |
| 300,0 | 4/10/10 | 29 m–1 day | 2 h–1 day | 40 |
| 1000,0 | 5/5/5 | 13 m–4 h 15 m | 2 h 15 m–4 h 15 m | 100 |
| LD 50 > 150 < 200 mq/kq Kqw. (mq/kq b.w.) | | | | |

[a]1. Number. = No. of dead animals
2. Number. = No. of animals with symptoms
3. Number. = No. of animals used

start/end time in minutes/hours/days. Time of death of dead/moribund killed animals is protocolled in addition. Thus, conclusions can be made about symptoms and death related to the latency. Short latency periods hint to a substance, longer-lasting periods to an accumulated impact. At the end of the observation period, surviving animals are anesthetized, dissected, and assessed macroscopically (anatomic-pathological; dead animals directly). As further parameter, body weight has to be determined before treatment, weekly post-application, and before section. Based on the results (type, intensity, duration of symptoms, body weight gain), the acute toxic profile of a substance can be gained. Based on the number of dead animals/group, the LD 50 (lethal dose 50 %, i.e., dose where 50 % of the animals die) can be calculated according to Litchfield and Wilcoxon (Table 1), for example.

## Acute Toxic Class Method

At present the Acute Toxic Class Method (OECD-guideline 423, Fig. 2) is used. Having in mind animal welfare, this method allows the determination of the LD 50 using clearly reduced numbers of animals. Instead of numerical LD 50/LC 50 values, ranges of toxicity are tested which are related to given national/international classification systems (WHO, EU, USA). Thus, LD 50/LC 50 values represent key numbers of a possible acute intoxication.

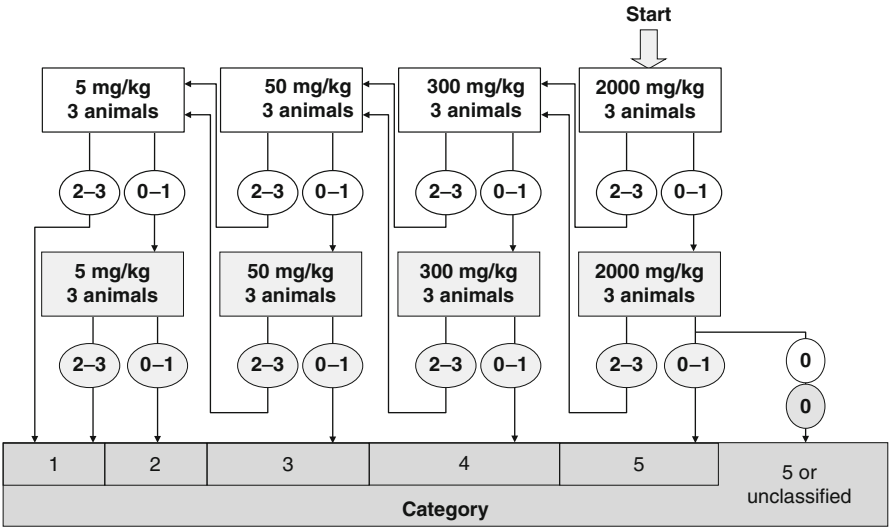



**Fig. 2** Acute Toxic Class Method: Rectangular frames show dose and number of animals used (one sex, mostly females), round frames show the number of ill/dead animals for every step

## Long-Term Studies

The aim of the chronic toxicity studies is the compilation of the profile of a substance after repeated intake of low doses over a longer time period. Three categories can be differentiated: subacute (28 day), subchronic (90 day), as well as chronic (> 90 day until 52 weeks and more) in two different mammalian species (e.g., rat/dog). The examination of two clearly different mammalian species reduces false-negative findings according to a species specificity but increases the percentage of false-positive findings. Anyhow, not all relevant effects/findings can be detected adequately in one species considering sensitivity for man (e.g., teratogenicity of thalidomide). All repeated application studies comprise in general four dose groups (control-, low-, medium-, as well as highest-dose group) whose number can be increased if necessary. The dose range should be spread widely and derived in an algorithmic manner. Ideally effects of the low dose are comparable to the control; the medium dose shows slight aberrant and the highest-dose pronounced effects.

## Responsibilities

The functions of the responsible persons can be specified as follows: The survey of all data which are investigated on the live animal (body weight gain, food/water intake, behavior, organ weights (absolute/relative)) section as well as macroscopic

(anatomic-pathological, dead animals) findings of the organs is due to the toxicologist (study director). Investigations of blood/urine parameters and enzymes/hormones in the related organs like liver, adrenals, or gonads if requested rest to the clinical chemist. Fixation of organs, production and coloring of the organ slides, and evaluation and assessment of the slides are under the responsibility of the pathologist. In conclusion the toxicologist, as study director, reports all data received (tabulary or in detail) as a comprehensive written report using accepted statistical methods (e.g., *U*-test according to Whitney and Mann). The report includes a discussion and assessment of the results in coordination with the clinical chemist as well as the pathologist. Design and content of the report (minimum requirement) are provided already as a base set by the abovementioned guidelines. These reports have to be seen legally as a document and signed (legally binding confirmation) by the study director (toxicologist) and the director of the institute. In addition the study director has to sign the declaration of compliance with the "Good Laboratory Practice (GLP)" which has to be stated in a written manner by an independent quality assurance unit too.

## Subacute Studies (28 day)

The subacute study serves at first to receive first information of targets in an early state. For this, activities – claimed as marginal conditions – have to be performed.

## Performance Ratio

Besides the investigation of the profile according to the basic requirement (guideline), a high performance ratio has to be pursued especially related to animal welfare. Based on the fact that standard investigations need only vanishing small amounts of the material, further parameters can be determined like investigation of immunotoxicity on thymus, spleen, lymph nodes, Peyer-plaques, phase I and II enzymes in the liver, or a liver foci test as a short-term carcinogenicity test. In addition they give much more information of the profile and hints on what one has to look for in the longer-lasting studies (subchronic/chronic) to avoid additional doses or even repetition of the whole study.

## Dose Range Finding

A further important function of the subacute study type is the dose range finding for the subsequent studies (subchronic/chronic). Based on the large spread of the dose range, one can identify tentative NOELs (no observed effect levels = effectless doses) as well as clear, nontoxic/low toxic doses to determine the profile. In doubt the dose range has to be enlarged.

### Reversibility

The reversibility of findings can be studied by adding satellite groups/dose group. The animals of the satellite groups are not treated with the test substance after the application period, whereas all other activities continue over a variable time period in general over 4 weeks. (Non-)recovery of organ functions can be detected as hint to (non-)intact repair mechanisms or adaptation capacities of the organs. Related to the target, the proof of reversibility can be useful also in subacute dermal as well as inhalation studies.

## Subchronic and Chronic Studies

Based on the results of the subacute studies, the performance of subchronic/chronic studies is more or less comparable to that of the subacute studies. The basic difference is the treatment period (subchronic 13 weeks, 10–20 animals/dose/sex); chronic from 26 weeks onwards to 52 weeks or 105 weeks as carcinogenicity studies on rats and mice (50–70 animals/group/sex). For non-rodents (e.g., dog) four animals/dose/group/sex are used. The toxicological profile provides much more information and can be relativized to single parameters by adaption processes (restitution of small deviations considering blood/urine/enzyme parameters, retardation of body weight). The longer-lasting treatment period allows to detect effects which need a certain latency period to develop, for example, tumors or secondary effects based on primary impact (idiosyncrasy, late reaction, proliferation of tissue due to permanent irritation). Those effects complete the toxicological profile after repeated application. A typical course (data collection) of a combined chronic carcinogenicity study in rats is shown in Table 2.

### Definition of NOAELs

Besides the determination of the toxicological profile, chronic studies are used to define a NOAEL (no observed adverse effect level). Considering the definition of NOAELs, there is a prominent influence of the dose range finding on the effect range (profile) as well as the nontoxic range. At the moment it is still under discussion how the term “adverse” has to be interpreted. Are temporarily marginal increases of enzyme activities of phase I and II in the liver to be seen as “adverse,” or do they reflect the present physiological reaction of the organ? Which percentage of cholinesterase activity inhibition due to phosphoric acid ester application is to be declared as a toxicological relevant effect and in consequence to be seen as “adverse”? Effects can show a statistical significance without having any biological/toxicological relevance and therefore are not an “adverse” effect. In general the NOAELs of subacute, subchronic, and chronic studies serve as deduction of threshold limit values like ADI (acceptable daily intake), AOEL (acceptable operator exposure level), and others, which are considered for the health risk assessment

**Table 2** Combined chronic carcinogenicity study in rats: performance of units of investigation (occurrence and time schedule)

| | |
|---|---|
| Obtain finding of animals | Twice daily, once daily, on weekend/holiday |
| Detailed investigations of clinical findings | Once weekly |
| Functional observation battery | Week 53 |
| Determination of: | |
| Body weight | Weekly until week 13, then every 2 weeks |
| Food intake | Weekly until week 13, then every 4 weeks |
| Water intake | Every 4 weeks |
| Feeding period | 7 days/week |
| Absolute feeding period[a] | > 730 days |
| Ophthalmological investigations | Start, week 1, 2, 53, 104 (end) |
| Clinical-chemical investigations: | |
| Hematology | Week 27, 54, 79, 105 |
| Clinical chemistry | Week 27, 54, 79, 105 |
| Investigations in urine | Week 27, 54, 79, 105 |
| Investigation of calcium/phosphorus | 1 and 2 year(s) after start of treatment in bones (optional) |
| Organ weights | 1 and 2 year(s) after start of treatment |
| Interim/end section | 1 and 2 year(s) after start of treatment |
| Anatomic/histopathological investigations | 1 and 2 year(s) after start of treatment (including number of tumors and incidences) |

[a]Number of days which are used to calculate the food intake

and – in consequence – used in regulation to set "cutoffs." Toxicological relevant effects of other studies like teratogenicity, genotoxicity, or carcinogenicity studies lead to an increase of the safety factor (SF), which has to be included. The treatment period has to be seen in analogy to the exposure/pollution whether it is seasonal (subacute/subchronic) or perennial or lifelong.

## Recommended Reading

Diener W, Siccha L, Mischke U, Kayser D, Schlede E (1994) The biometric evaluation of the acute-toxic-class method (oral). Arch Toxicol 68(10). Springer Verlag, Heidelberg

Niesink RJM, de Vries J, Hollinger MA (1996) Toxicology: principles and applications. CRC Press, Boca Raton

## Resources

OECD Guidelines for Testing of Chemicals (1981 ff), OECD Publication Service, Paris, Web address: www.oecd.org

# Examination of Organ Toxicity

Wolfgang Kaufmann and Matt C. Jacobsen

## Contents

Basic Principles for the Examination of Organ Toxicity ..... 90
Gross Pathology ..... 90
Histopathology ..... 90
Diagnostic Approach, Procedures, and Considerations ..... 92
Clinical Pathology Parameters ..... 93
Results of a Well-Concerted Combination of Anatomical and Clinical Pathology Data Analyses ..... 94
International Activities on Harmonization in the Use of Diagnostic Terms ..... 96
Summary and Future View ..... 97
Recommended Reading ..... 97
Resources ..... 98

**Abstract**

As a result of their anatomy and physiology, the organs and tissues of animals and man may show different morphological responses and sensitivity to xenobiotics. Toxic responses can manifest systemically (e.g., the immune system) or may produce specific toxic effects in a single organ system (skin) or single organ (liver). Organ toxicity may therefore result from a direct and primary effect on a target organ or as a result of secondary effects in organs and tissues that have a physiological dependence on the primary target. The assessment

W. Kaufmann (✉)
Merck KGaA, Non-Clinical Safety, Global Pathology and Reproduction Toxicology, Darmstadt, Germany
e-mail: Wolfgang.kaufmann@merckgroup.com

M.C. Jacobsen
AstraZeneca, R&D Innovative Medicines, Global Safety Assessment, Macclesfield, Cheshire, UK
e-mail: matt.jacobsen@astrazeneca.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_32, 

of organ toxicity must therefore take into account the complex physiological interdependence of tissues and organs within the body.

## Basic Principles for the Examination of Organ Toxicity

Toxicological alterations of the morphology and structure of organs and tissues are detected by anatomical pathology examination following single or multiple doses of a drug or chemical administered to a laboratory species. During the in-life phase and at the end of an animal study, clinical pathology parameters (e.g., hematology and urinalysis) are assessed and provide important biomarkers for functional metabolic disturbances and maybe the first indicators for potential organ toxicity (e.g., liver or kidney). Both anatomical and clinical pathology disciplines offer a broad diagnostic repertoire to analyze organ toxicity in a well-considered, stepwise, so-called tiered approach concept.

## Gross Pathology

After the in-life phase of an animal experiment, all study animals are necropsied according to a standardized, systematic procedure. As a rule, the study design is based on the most recent effective guidelines (e.g., OECD guideline for the testing of chemicals or US EPA Health Effects Test Guidelines OPPTS); however, the study design should always be adapted if the mode of action of a chemical is already known. All observations and macroscopic lesions identified at necropsy are recorded in a validated electronic data system for each single study animal. A standard study protocol lists a wide range of tissues and organs that have to be removed for further histopathology examination. Some selected organs (e.g., liver, kidneys, adrenal glands) are weighed. Organ weights are often sensitive indicators of a treatment-related effect and may provide initial information on potential target organs during or shortly after necropsy (e.g., *increased liver or thyroid gland weights* are often recorded for compounds that act as enzyme inducers). Macroscopic observations during necropsy also have the potential to alert the toxicologist to possible target organs (e.g., *yellow-brown or clay-like discoloration of the liver* is indicative of a degenerative change, and the finding of a *mass* could turn out to be a chronic inflammatory process, an abscess, or a malignant tumor). The careful consideration of organ weights and macroscopic findings are an essential part of the detection of organ toxicity in experimental animal studies (Fig. 1).

## Histopathology

The histopathological examination (by light microscopy) of a diverse range of organs and tissues by a well-trained toxicological pathologist is one of the most

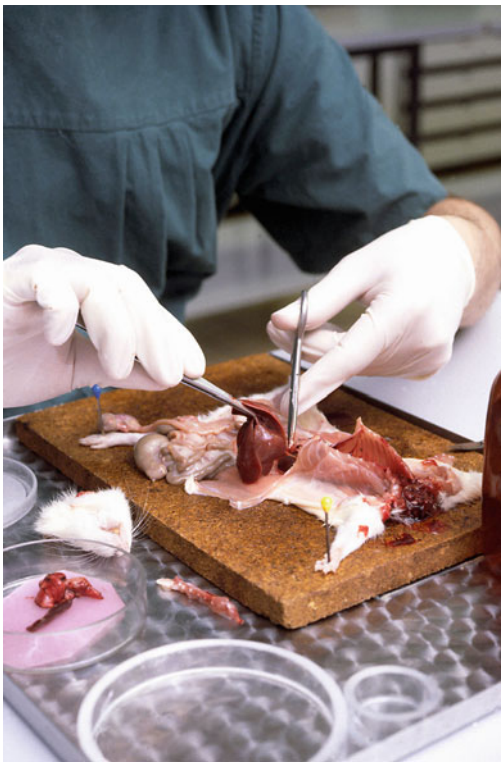


**Fig. 1** Necropsy of laboratory animals with organ and tissue collection. The figure shows the removal of the liver from the abdominal cavity of a Wistar rat

important and time-consuming elements in the assessment of organ toxicity. The minimum scope of examination is dictated by the various guidelines already mentioned above. In principle, organs and tissues are selected that are considered highly relevant determinants of basic metabolic and detoxification processes (e.g., the liver), organs that act as portals of entry for drugs/chemicals and may have been in direct contact with the test compound (gastrointestinal tract, lungs, skin), and organs that are crucial for excretion (e.g., kidneys, urinary bladder, and biliary system in the liver). Furthermore, representative samples are also examined from the immune and hematopoietic systems (two lymph nodes, one close to the site of exposure, thymus, spleen, bone marrow), the skeletal system (bone, joints, skeletal muscle), and the nervous system (various coronal sections from the brain including the cerebrum and cerebellum, two to three cross and longitudinal sections from the spinal cord, and one peripheral nerve). The cardiovascular system (arteries, veins) is examined as a constituent of many of the routine organ samples but specifically in one or more targeted sections from the heart and one section of the aorta. The reproductive system (including the testes, epididymides, prostate, accessory sexual glands and ovaries, oviducts, uterus, vagina, and mammary gland) is also included in the organs and tissues examined (Fig. 2).

**Fig. 2** Histopathology. Scope of examination for one single test animal in a carcinogenicity study (*left* paraffin blocks, *right* histological slides)

A thorough histopathological examination is essential as toxicological changes can manifest microscopically in the absence of alterations to organ weights and clinical pathology parameters.

## Diagnostic Approach, Procedures, and Considerations

As a minimum, all organ and tissue alterations from the high-dose and concurrent control group are recorded systematically in a validated data entry system to produce a pathology finding incidence table. The pathologist chooses an appropriate morphological diagnosis for the lesion observed and may describe the findings in more detail in the narrative pathology report. The pathologist must also grade the severity of findings where appropriate in order to help establish the presence of a dose–response. The grading system used by the pathologist will take into account the type of study (duration of exposure) and the nature of findings observed. The pathologist relies on his knowledge of the spontaneous pathology of the test species used to help differentiate spontaneous from treatment-related findings. A thorough understanding of spontaneous lesions in the animal strain used also helps the pathologist to ascribe adversity to any treatment-related lesions present.

There has been an ongoing debate as to whether the study pathologist should have knowledge of which animals are treated and which animals are controls to guarantee a more *objective examination*. However, the approach of *blind reading* is

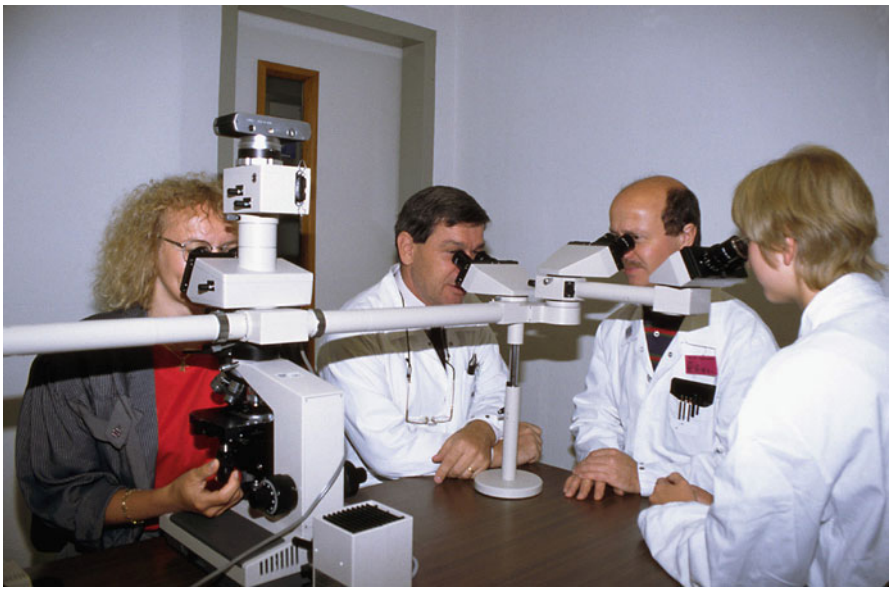


**Fig. 3** Evaluation of histological slides by light microscopy. Pathologists during an internal review of histopathological findings at a multi-headed microscope

not recommended by toxicological pathologists or their societies for the initial histopathology examination. The risk of introducing "bias" or the potential to overlook or to misinterpret minor treatment-related variations in the morphology of organs is high, and blind reading should therefore not be performed. However, blind reading of histological slides is often used at a later stage during slide evaluation – e.g., to find a no-observed-effect level (NOEL) for a specific organ toxicity and to allow the study pathologist to consistently identify a subtle or borderline lesion. "Blinding" slides with codes can also be performed if there are different opinions on the interpretation of a lesion between pathologists and the lesion is being considered by a pathology working group (PWG). A PWG is a formal and well-documented process to resolve different opinions on the diagnosis and relationship to treatment of pathology findings from a toxicology study by an independent panel of expert pathologists, which also includes the study and peer-reviewing pathologist. These experts undertake a "blind reading" so as not to be biased (Fig. 3).

## Clinical Pathology Parameters

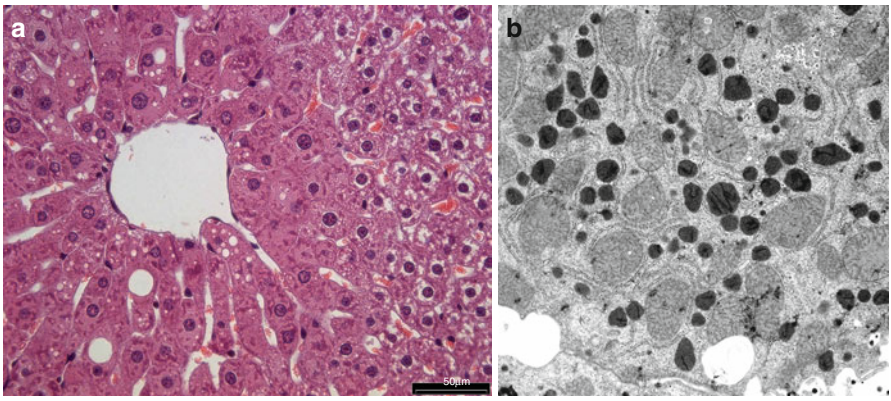
In addition to the analysis of anatomical pathology data after completion of an animal study, the analysis of the clinical pathology data will add significant value for the detection of organ toxicity. For clinical pathology, blood and urine samples

are taken during the in-life phase of a study at scheduled time points and, as a minimum, at least once before the final sacrifice of the test animals. Diverse hematology parameters are measured and calculated (e.g., number of red and white blood cells, hemoglobin concentration, mean corpuscular volume, coagulation tests, and differential blood count). As these tests are highly automated, the results can provide an initial assessment of such things as anemia and inflammation prior to the histopathology results. Blood sample analysis also includes clinical chemistry parameters (e.g., enzymes, biochemical analytes in plasma, like transaminases, urea, creatinine, serum protein levels) that may indicate organ toxicity in the liver or the kidneys. As a rule, the selection of the standard minimum panel of clinical pathology parameters in animal experiments follows guideline recommendation and aims to detect major metabolic impacts of a potential toxic compound. If the mode of action of a test item is known, clinical pathology tests may be specifically designed and additional parameters analyzed (e.g., hormones). The clinical pathology data are another important component and, together with the gross pathology, organ weights, and histopathology data analysis, allow the detection of specific organ toxicity with a high degree of certainty.

## Results of a Well-Concerted Combination of Anatomical and Clinical Pathology Data Analyses

For the majority of cases, standard approaches like the analysis of hematoxylin and eosin-stained histological slides by light microscopy are sufficient to detect organ toxicity. However, there are also exceptions where more sophisticated methods are required. A liver cell swelling (*centrilobular hypertrophy of hepatocytes*) noted by light microscope may indicate a degenerative or an adaptive response of the liver parenchyma (Fig. 4a). A degenerative process that leads to liver cell death is much more critical than an adaptive process, where the morphological change is based on a physiological and fully reversible response of the liver tissue. There are a number of different chemicals, so-called enzyme inducers, that produce an adaptive liver cell swelling by a proliferation of the smooth endoplasmic reticulum (SER). A proliferation of the SER is the morphological correlate for an induction of the cytochrome P450 enzyme superfamily (CYP) and major enzymes in the metabolism of xenobiotics (toxic chemicals and drugs). Morphologically, a definitive diagnosis can be made by using electron microscopy of the liver, and clinical biochemistry allows a diagnosis by the analysis of specific enzymes (e.g., ethoxyresorufin-*O*-deethylase and pentoxyresorufin-*O*-dependentylase).

Both methods are also appropriate approaches to identify another group of substances that also induce a centrilobular hypertrophy of hepatocytes, e.g., peroxisome proliferators. The latter result in an accumulation of specific intracytoplasmic cell organelles, the peroxisomes, which play a significant physiological role in lipid metabolism. Peroxisomes can be selectively stained by cytochemical or immunohistochemical methods and can be morphologically quantified. Results from the latter techniques correlate well with a significant increase of the cyanide-insensitive




c

Table
Quantitative measurements of the peroxisomal enzyme activity in B6C3F1 mice

| Dose groups | Males | Females |
|---|---|---|
| Control | 4.03±0.91[a] | 5.28±0.41 |
| 500 ppm | 6.05±1.42** | 5.91±0.82 |
| 1500 ppm | 9.31±2.00*** | 8.88±0.74*** |
| 4000 ppm | 23.14±5.33*** | 22.05±1.60*** |
| 8000 ppm | 43.37±2.18*** | 43.90±1.52*** |

The data show the activity of the cyanide-insensitive palmitoyl-CoA –oxidation. Results are presented as milliunits (mU) per milligram protein as group mean ± standard deviation

*p<0.05
**p<0.02
***p<0.002

**Fig. 4** Example of a successful contemporary approach using three different methods to analyze organ-specific lesions and to correlate structure and function: (**a**) Centrilobular liver cell hypertrophy (*arrows*) is detected in a histological slide by light microscopy examination (**b**) The liver cell hypertrophy is characterized by electron microscopy examination as proliferation of specific cell organelles in the cytoplasm, the peroxisomes (here stained as black rounded bodies) (**c**) The clinical pathology examination of the cyanide-insensitive palmitoyl-CoA oxidation in liver homogenates resulted in a statistically significant functional increase and shows a clear dose–response relationship

palmitoyl-CoA oxidation that can be analyzed from liver homogenates taken during necropsy (Fig. 4b, c (table)).

These examples show the complementary use of clinical pathology and anatomical pathology approaches to assure the accurate diagnosis and interpretation of certain types of organ toxicity and to contribute to the understanding of the mode of action of xenobiotics.

## International Activities on Harmonization in the Use of Diagnostic Terms

For many years, the major scientific societies of toxicological pathology in Europe (European Society of Toxicologic Pathology [ESTP] and British Society of Toxicological Pathology [BSTP]) and the United States of America (Society of Toxicologic Pathology [STP]) have worked on harmonizing the nomenclature and diagnostic criteria used in toxicology studies. These activities were guided by the need for pathologists globally to use the same pathomorphological diagnostic criteria in the description of findings from toxicity studies using drugs and chemicals. For example, a *hyperplasia of the mammary gland* should be always differentiated from an *adenoma of the mammary gland* using the same diagnostic criteria regardless of geographical location. As pharmaceutical and chemical companies become more globalized, preclinical safety and toxicology studies for a single compound may be performed in research facilities in different geographical regions. As a result of these trends, it was considered extremely important to come to a common understanding in the use of diagnostic criteria. Initially, the primary focus was given to proliferative lesions in rodents as these findings were easier to harmonize among the international community of toxicological pathologists. As a result working groups of toxicological pathologists from Europe and America published a series of *International Classification of Rodent Tumours* for rats and mice between 1992 and 2001. The subsequent use of these published criteria significantly helped to harmonize the diagnosis of tumors in rodent oncogenicity studies. However, there will always be borderline lesions and growth patterns of tumors where harmonized criteria do not fit the lesion and the pathologist has to make their own informed judgement based on experience.

On the basis of an initiative of the ESTP and the US STP, a further important step forward was started in 2005. In conjunction with the German-based Registry of Industrial Toxicology Animal-data (RITA), a collaborative process of review, update, and harmonization of existing diagnostic nomenclature, documents, and databases of rodents was initiated. The BSTP and the Japanese Society of Toxicologic Pathology (JSTP) joined this process in 2006. This project, known as INHAND (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) project, includes efforts to harmonize nomenclature not only for proliferative lesions but also for nonproliferative lesions at a level that gains international acceptance. Up to 2012, INHAND nomenclature has been published for the respiratory (2009), hepatobiliary (2010), urinary, nervous system, male reproductive system and the mammary gland (2012). The complete set of organ systems is in preparation for publication until end of 2014. The INHAND nomenclature is also available electronically at the goRENI webpage and is presented at the international annual meetings of the societies of toxicological pathology to discuss the practical use of these harmonized diagnostic criteria.

## Summary and Future View

Organ toxicity is the result of physiological dysfunction and structural alteration. Clinical pathology and histopathological examination are complementary approaches that underpin the detection and characterization of organ toxicity. Despite the many advances in molecular biology (genomics, metabolomics), the use of routine clinical pathology measurements and histopathological examination of hematoxylin and eosin-stained tissue sections are unlikely to be replaced as a first-tier approach for detecting organ toxicity in animal toxicology studies. The latter techniques can be complemented by more sophisticated examination using electron microscopy, immunohistochemistry, and molecular pathology approaches, once the target organs have been identified, and may help to identify the mode of action of the toxicity in question. In vitro assays are now commonly used in an attempt to predict the toxic effects of chemicals or drugs but are not able to mimic the complex metabolic pathways present in vivo. As a result, they often fail to predict the diverse toxicities and specific toxicological profile that a test compound can show in different laboratory species, including man.

## Recommended Reading

Creasy D, Bube A, De Rijk E, Kandori H, Kuwahara M, Masson R, Nolte T, Reams R, Regan K, Rehm S, Rogerson P, Withney K (2012) Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. Toxicol Pathol 40:40S–121S

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, Bube A (2012) Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol 40(4 Suppl):14S–86S

Greaves P (2012) Histopathology of preclinical toxicity studies: interpretation and relevance in drug safety evaluation, 4th edn. Academic/Elsevier, Amsterdam

Haschek WM, Rousseaux CG, Wallig MA (2009) Fundamentals of toxicologic pathology, 2nd edn. Elsevier, Amsterdam

Kaufmann W, Bolon B, Bradley A, Butt M, Czasch S, Garman RH, George C, Gröters S, Krinke G, Little P, McKay J, Narama I, Rao D, Shibutani M, Sills R (2012) Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicol Pathol 40(4 Suppl):87S–157S

Mann P, Vahle J, Keenan CM, Baker JF, Bradley AE, Goodman DG, Harada T, Herbert R, Kaufmann W, Kellner R, Nolte T, Rittinghausen S, Tanaka T (2012) International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. Toxicol Pathol 40:7S–13S

Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P, Wöhrmann T (2009) Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol Pathol 37(7 Suppl):5S–73S

Rudmann D, Cardiff R, Chouinard L, Goodman D, Küttler K, Marxfeld H, Molinolo A, Treumann S, Yoshizawa K (2012) Proliferative and nonproliferative lesions of the rat and mouse mammary, zymbal's, preputial, and clitoral glands. Toxicol Pathol 40(6 Suppl):7S–39S.

Thoolen B, Maronpot RR, Harada T, Nyska A, Rousseaux C, Nolte T, Malarkey DE, Kaufmann W, Küttler K, Deschl U, Nakae D, Gregson R, Vinlove MP, Brix AE, Singh B, Belpoggi F, Ward M (2010) Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. Toxicol Pathol 38(7 Suppl):5S–81S

## Resources

European Society of Toxicologic Pathology (ESTP). http://www.eurotoxpath.org/. Accessed 08 March 2013

goRENI – The standard reference for nomenclature and diagnostic criteria in toxicologic pathology. http://www.goreni.org/. Accessed 08 March 2013

OECD Guidelines for the Testing of Chemicals. http://www.oecd.org/env/chemicalsafetyandbiosafety/testingofchemicals/. Accessed 08 March 2013

Registry of Industrial Toxicology Animal-data (RITA). http://reni.item.fraunhofer.de/reni/public/rita/index.php. Accessed 08 March 2013

Society for Toxicologic Pathology (STP). http://www.toxpath.org/. Accessed 08 March 2013

US EPA Health Effects Test Guideline OPPTS. www.epa.gov/oppts/pubs/frs/home/guidelin.htm. Accessed 08 March 2013

# Specific Toxicity Tests for Neurotoxicity, Immunotoxicity, Allergy, Irritation, Reprotoxicity, and Carcinogenicity

Eckhard von Keutz

## Contents

Reproductive Toxicity Testing ..... 99
Genotoxicity Testing ..... 100
Carcinogenicity Testing ..... 102
Neurotoxicity Testing ..... 104
Immunotoxicity Testing ..... 105
Resources: Test Methods ..... 106

**Abstract**

The specific test methods used in toxicology classically include tests for reproductive toxicity, genotoxicity, and carcinogenicity. There are now also other test methods such as testing for possible immunotoxic or neurotoxic properties of a substance. Special, usually internationally applicable, test guidelines form the regulatory basis for the test methods, which apply to chemicals, crop protection products, and medicinal products.

## Reproductive Toxicity Testing

The importance of reproduction toxicology as part of the assessment of safety gained sad notoriety in the wake of the thalidomide (Contergan) tragedy. At that time, routine testing for possible teratogenic properties of a substance was not yet established. This was because such a possibility was not expected on the basis of the scientific knowledge back then. We now know that chemical substances are fundamentally capable of causing damage in all stages of reproduction. The maturation of gametes can be disturbed in women or men, for example. But the release of

E. von Keutz
Bayer HealthCare, Global Early Development, Wuppertal, Germany
e-mail: Eckhard.vonkeutz@bayer.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_33, 

mature gametes can also be disturbed, as can fertilization; cell division; egg implantation in the uterus; intrauterine development, i.e., the development of organs during the embryonic phase and fetal maturation; and development after birth (postnatal phase). In order to clarify whether and at what point in time reproductive effects can occur, the treatments must be carried out during specific periods of time. A distinction is therefore made between testing for effects on male or female fertility (from spermatogenesis/follicle maturation to implantation), testing for effects on intrauterine development (during organogenesis), and testing for effects on peri- and postnatal development (from fetal development to the end of lactation).

Testing for possible ***teratogenic/embryotoxic properties*** is now mandatory for all substance classes. The procedures for carrying out this testing are described in publications including the OECD Guideline for Testing of Chemicals No. 414 ("Teratogenicity") and in ICH Guideline S5A ("Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products"). Testing is based on the principle that two different animal species (usually the rat and the rabbit) are exposed to the substance to be tested during the most sensitive stage of pregnancy. Immediately before the natural end of the pregnancy, the fetuses are delivered by cesarean section and then examined for external and internal malformations. Rats are treated from day 6–15 and rabbits from day 6–18 of pregnancy. The most important organs are formed during these periods. Any teratogenic/embryotoxic potential a substance may have should therefore have its greatest impact under this treatment regimen and should therefore also be detectable with the greatest degree of reliability.

***One- or two-generation studies*** in rats, conducted for chemicals and crop protection products, represent additional test methods that investigate the effects of a substance on male or female fertility and on progeny. The methods are described in the OECD Guidelines for Testing of Chemicals No. 415 ("One-Generation Reproduction Toxicity Study") and 416 ("Two-Generation Reproduction Toxicity Study"). For medicinal products, the legislation stipulates testing for ***effects on male or female fertility*** in the rat (ICH Guideline S5 (R2)) and ***testing for peri- or postnatal toxicity***, also in the rat (ICH Guideline S5 (R2)), in addition to testing for teratogenic or embryotoxic properties.

Study objectives and common study types:

- Testing for embryotoxic or teratogenic properties in the rat and rabbit
- Testing for effects on male or female fertility in the rat
- Testing for peri- or postnatal toxicity in the rat
- One- or two-generation studies in the rat

## Genotoxicity Testing

Testing for genotoxic properties (effects on the cell's genetic material) is now mandatory for most substances. A multitude of test methods (in vitro and in vivo) are available for this, and these can be used to detect a variety of harmful effects.

More specifically, these include gene or point mutations, structural chromosome changes (chromosomal aberrations), and changes in chromosome number (changes in the DNA). Mutations are significant for humans in a number of respects. On the one hand, mutations in somatic cells can pave the way for cancer, and they therefore have a direct effect on the individual concerned. Germline mutations, on the other hand, can lead to prenatal death or to malformations ("hereditary diseases") in offspring. They therefore have effects on subsequent generations.

Because ***point mutations*** are not visible under the microscope, the induction of these mutations is detected indirectly via their effects. These consist mainly of protein changes, with enzyme functions often being used as a means of detection. Bacteria are eminently suited to this task, as they enable individual mutants to be detected among millions of cells with the aid of selection media. Probably the best-known mutagenicity test of all is the Ames test, which will be described briefly here as an example of a method for detecting point mutations. The object of the test is a defective (mutant) strain of *Salmonella typhimurium* that is no longer capable of synthesizing histidine. The mutated bacteria have the ability to revert to the normal wild type under the influence of mutagens. A concentration-dependent increase to the point of histidine independence is considered as evidence of point mutagenicity. Because of the simplicity of the method, the Ames test is a suitable screening test for many substances. It is a quick and sensitive assay. A high degree of mathematical correlation has been shown in some cases by comparison of the mutagenic effects of substances in the Ames test and their carcinogenic effects in animal studies.

In vitro tests to detect ***chromosome or ploidy mutations***, known as "cytogenetic studies," can generally be performed with all primary or permanent cell lines possessing a relatively constant set of chromosomes. Chinese hamster, mouse, and rat cells are used most often, although human lymphocytes are also used. In vivo studies are usually conducted in small mammals, and the assay in Chinese hamster bone marrow can be mentioned as an example of the basic procedure. The test substance is administered to the animal which is then sacrificed after exposure of the organism to the substance for a period of time. Shortly before sacrifice, cell division is arrested at metaphase by administration of a spindle inhibitor. The bone marrow is removed from the sacrificed animal and examined under the microscope for chromosomal changes.

Another option for testing consists of investigating whether a substance has caused ***DNA damage***. This involves determining whether the cell has initiated an enzymatic repair process with a view to removing the defective part of the DNA and replacing it with a resynthesized part. This process, known as "unscheduled DNA synthesis" to distinguish it from the "normal" synthesis that occurs during replication, is the basis for the UDS test, which is performed with mammalian cells under in vitro and in vivo conditions.

Detailed descriptions of the test methods can be found in the OECD Guidelines (OECD Guidelines for Testing of Chemicals Nos. 471–486). For medicinal products, the corresponding information can be found in ICH Guideline S2 (R1).

Study objectives and common study types:

- Point mutagenicity testing (e.g., Ames test in *Salmonella typhimurium* strains)
- Testing for chromosomal aberrations
  - In vitro, e.g., cytogenetic studies in Chinese hamster cells
  - In vivo, e.g., tests using hamster bone marrow or micronucleus test in the mouse
- Testing for DNA damage (e.g., unscheduled DNA synthesis in vitro and in vivo)

It is not possible to assess the possible mutagenic risk of a substance on the basis of just one test. Instead, the different ways in which damage can be caused must be addressed and studied on the basis of specific end points. This can only be done within the context of a testing strategy in which the test systems must be considered hierarchically. A combination of a bacterial test (e.g., Ames test using various strains of *Salmonella typhimurium*), an in vitro test in mammalian cells (e.g., cytogenetic studies in Chinese hamster ovary cells), and an in vivo study (e.g., micronucleus test in the mouse) represents a standard battery of tests. If a substance has been tested sufficiently for mutagenic properties without any evidence of mutagenic potential being found, it can be assumed that the risk to humans is negligibly small. The risk assessment in the presence of positive findings in lower organisms which cannot be confirmed using relevant methods in mammalian organisms is more complex. As a general rule, for the assessment of any potential risk a substance might pose to humans, the significance of the method increases the more the test system corresponds to the conditions in mammals. If in vivo studies in mammals yield positive findings, this must be seen as a clear indication of the possibility of mutagenic effects in humans. Because positive findings must also always be viewed in the context of a risk of cancer, they are of predictive significance in the assessment of the possible carcinogenic potential of a substance. Herein may lie the true value of these tests that are so quick and easy to perform. Positive findings in genotoxicity studies must always be taken seriously and require careful further investigation. The question of what risk must be deduced from the results of genotoxicity studies can be answered only after an assessment of all the studies. The ultimate classification and evaluation of the findings must take place within the overall context of the risk/benefit assessment.

## Carcinogenicity Testing

One of the most complex toxicological tests is the testing of a substance for possible tumorigenic (carcinogenic) properties. The tests are usually performed in two rodent species, specifically rats and mice, and more rarely in hamsters. Ideally, the substance to be tested should be metabolized similarly in the animal species used to the way it is metabolized in humans. The study duration is generally 24 months in rats and 21–24 months in mice and hamsters, depending

on the animal strain used, and thus covers the majority of the life expectancy of the study animals. This mimics almost in fast motion the lifelong exposure to a substance in humans. The way the substance to be tested is administered is guided by the conditions in humans (administration in the feed, in the drinking water, by gavage, by inhalation, etc.). The testing includes three-dose groups and an untreated control group. Fifty animals of each sex are generally used per dose level. The doses are selected in such a way that there are clear intervals between them. The highest dose should be close to the maximum tolerated dose (MTD). If this dose were exceeded, the animals would die from the effects of the substance before it was possible for cancer to develop in the first place. Administering the maximum tolerated dose makes the carcinogenicity study particularly sensitive. This is also necessary because it is only ever possible to study the substance in a limited number of animals, although the risk needs to be assessed for a large number of exposed people. The exact procedure for conducting carcinogenicity studies is described in the OECD Guidelines (OECD Guidelines for Testing of Chemicals Nos. 451/453).

A few particularities need to be taken into account in the testing of medicinal products for possible tumorigenic properties. The option exists, for example, to replace the long-term study in mice with a meaningful short-term test. Various transgenic animal models can be used for this. Which model is the most suitable must be decided depending on the substance and the particular parameter(s) being studied. Ideally, this should be done in close consultation with the competent authorities. In the case of medicinal products, the dose is selected taking into account pharmaceutical considerations, with a key role being played by the comparison of human/animal exposure on the basis of the achieved/achievable plasma concentrations. Information on conducting carcinogenicity studies for medicinal products can be found in ICH Guidelines S1A, S1B, and S1C (R2). It should be mentioned that for medicinal products analyses are ongoing in order to explore new and eventually better ways to predict a carcinogenic potential. The results of these analyses hold promise in driving to support modifications to current carcinogenicity testing guidelines while maintaining patient safety, accelerating patient access, and significantly reducing animal testing.

Basic structure of carcinogenicity studies:

- Two rodent species (rat and mouse or hamster)
- Three-dose groups and an untreated control group
- High numbers of animals (50 animals of each sex per dose level)
- Lifelong treatment (24 months)
- Highest possible dosages (maximum tolerated dose = MTD or exposure calculations, if relevant)

The crucial evidence in respect of the outcome of carcinogenicity studies is provided by the necropsy of the study animals and the subsequent histopathological examination. There are no hard and fast rules on how the results of carcinogenicity

studies, whether positive or negative, should be evaluated. An assessment of the risk can be performed only on the basis of well-planned and conducted studies and by a committee of experienced top specialists.

## Neurotoxicity Testing

Specific tests for neurotoxic effects are required for crop protection products in particular. The range of tests covers three main elements: the "functional observational battery" (FOB), motor activity (MA), and neuropathology.

In the ***functional observational battery (FOB)***, a series of noninvasive tests is performed which can be used to detect and quantify behavioral abnormalities and neurological effects in the study animals (usually rats). The initial focus is on close observation. Even the tiniest changes in posture, appearance, and movement are noted, with a distinction being made, for example, between observation of the animal in its own cage, during handling, and outside the cage (on a free surface). In addition, a range of responses (including the approach, touch, noise, and tail-pinch responses) and reflexes (including pupil response, righting reflex, and grip strength) are assessed. The basic requirement for the reproducibility of these tests is the standardization of the test conditions. This includes, for example, all the animals in a study always being assessed by the same investigator. For ***motor activity (MA)*** testing, the animals are placed in chambers equipped with infrared light barriers and their movements observed closely. The automated recording of findings enables even the tiniest changes in the motor activity of the study animals to be recorded. Possible substance effects can result in an increase or decrease in motor activity. Testing for neurotoxic effects includes a thorough ***neuropathological examination.*** For this purpose, different localizations of the central and peripheral nervous system are prepared at the end of the study using specific techniques and examined and assessed in respect of morphological effects. A detailed description of the test methods can be found in publications including the OECD Guideline for Testing of Chemicals No. 424 and the EPA Health Effects Test Guidelines (OPPTS870.6200).

Key elements of neurotoxicity testing:

- Functional observational battery (FOB)
- Motor activity (MA)
- Neuropathology

Possible neurotoxic properties of a substance can have particularly serious consequences for the developing organism. It is therefore not surprising that particular importance is attached to this aspect in connection with specific tests, the main focus of interest being the recording of behavioral changes and neurological deficits in progeny/young animals.

## Immunotoxicity Testing

Testing substances for possible immunotoxic properties has taken on much greater prominence in recent years and is now established in numerous test guidelines.

Some immunotoxicological tests have been an established component of toxicological testing for many years. This applies to investigations of the possible skin-sensitizing/allergic potential of a substance, for example. The ***Buehler test*** and the ***Magnusson and Kligman maximization test*** represent typical methods for detecting these kinds of reactions. Both tests are conducted in guinea pigs, with a distinction being made between adjuvant (maximization) and non-adjuvant (Buehler) tests. Freund's adjuvant is administered additionally to boost any immune response induced by the test substance in the test concerned. The principle of the test consists of the animals, after initial exposure to the substance ("induction") and after a waiting period (generally 14 days) has elapsed, being confronted with the substance a second time ("challenge"). The responses which then occur are used to assess whether the substance has skin-sensitizing potential or not. A detailed description of these tests can be found in the OECD Guideline for Testing of Chemicals No. 406 and in the EPA Health Effects Test Guidelines (OPPTS870.2600). A more recent test is represented by what is known as the ***local lymph node assay***, which is described in the OECD Guideline for Testing of Chemicals No. 429. For medicinal products, the aspect of skin sensitization plays a role with dermal dosage forms in particular. Testing is required specifically in the European "Note for Guidance on Non-Clinical Local Tolerance Testing of Medicinal Products" (CPMP/SWP/2145/00).

Some of the parameters studied in standard toxicological testing can themselves provide information on immune system involvement. These include hematological parameters (white blood cell count, differential blood count), clinical chemistry parameters (protein electrophoresis and albumin/globulin ratio), organ weights (spleen, thymus, lymph nodes), and especially histopathological examination of the spleen, lymph nodes, Peyer's patches, thymus, and bone marrow. Specific tests are now also available in addition to these standard parameters. These are functional tests such as the ***plaque assay***, which involves immunizing the test animals against sheep red blood cells a few days before necropsy and measuring the resulting immune response on the day of necropsy, or more in-depth cytofluorometric analyses of ***lymphocyte subpopulations*** in the spleen and blood using FACS. If any of these tests yield evidence of immunotoxicity, the range of tests must be expanded ("tier approach"). In such cases, consideration should be given to performing a ***host resistance (HR) assay***, for example, in which the treated animals are infected with bacteria or viruses, and any impairment of immunity by the substance is measured.

The exact procedure for testing for immunotoxic properties of a substance is described in various test guidelines. Specific reference should be made here to the EPA Health Effects Guideline, "Immunotoxicity" (OPPTS 870.7800), and the ICH Guideline S8 (Immunotoxicity study for human pharmaceuticals).

## Resources: Test Methods

EMA (2013) http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000083.jsp&mid=WC0b01ac0580027548
EPA (2013) http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm
FDA (2013) http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065014.htm
ICH (2013) http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000432.jsp&mid=WC0b01ac05800296c2
OECD (2013) http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm

# Toxicity Testing In Vitro. Regulatory Aspects

Eckhard von Keutz

## Contents

Definition, Objective, and Purpose of In Vitro Testing ..... 107
Tests with Liver Cell Cultures (Biotransformation and Cytotoxicity) ..... 109
Tests with Mouse Fibroblasts (Phototoxicity) ..... 110
Tests with Isolated Ion Channels (Cardiotoxicity, ECG Changes) ..... 111
Tests with Mammalian Cell Cultures (Genotoxicity) ..... 111
Tests with Incubated Chicken Eggs (Various Parameters) ..... 112
Human Embryonic Stem (hES) Cells for Use in Toxicity Testing, e.g., Early Development Toxicity Testing ..... 112
Possibilities and Limitations of In Vitro Toxicity Testing ..... 112
References ..... 113

**Abstract**

In vitro testing in toxicology was limited for a long time to testing for possible genotoxic properties of a substance. Cell or tissue culture methods are now used for the early toxicological assessment of new substances within the context of screening tests and for mechanistic investigations. In this connection, in vitro methods represent a valuable adjunct to animal studies, without being able to replace animal studies completely at the present time.

## Definition, Objective, and Purpose of In Vitro Testing

"In vitro" tests are known colloquially as "test tube experiments," i.e., they are performed outside the living organism. According to this definition, in vitro testing encompasses tests with isolated organs or tissues, cells, cell organelles, receptors, or ion channels.

E. von Keutz
Bayer HealthCare, Global Early Development, Wuppertal, Germany
e-mail: Eckhard.vonkeutz@bayer.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_35, 

The culture of cells from different organs is the in vitro method most commonly used in toxicology, with a distinction being made between ***primary cell cultures*** and permanent ***cell lines.*** Primary cell cultures are prepared from freshly isolated cells obtained during a necropsy or from surgical specimens, for example. Permanent cell lines, such as tumor cell lines, are obtained from cells which have been transformed spontaneously or in a targeted manner such that they can be passaged without limit and kept in stock. They can now be acquired easily from cell banks. Another source for human cells is stem cells. **Adult stem cells** are isolated from donor fetal or adult tissue(s), whereas **pluripotent stem cells** can be isolated from the inner cell mass of the blastocyst, as with **embryonic stem (ES)** cells, or through nuclear reprogramming, as with induced pluripotent stem (iPS) cells. Which culture systems should be used must be decided individually, depending on the specific parameter(s) being studied.

With regard to their aims, in vitro tests can be viewed in two ways: firstly, as a method of screening and, secondly, in the context of ***mechanistic evaluation***. Whereas pharmacological research has been making use of cell and tissue cultures for a long time in the efficacy testing of new medicinal products, the use of such systems in toxicology was previously limited to just a few areas of investigation. In vitro methods now represent a vital tool in the early toxicological characterization of new substances, however. This can be attributed, using pharmaceutical research as an example, to the significant increase in efficiency (higher output) and the resulting need to subject potential candidates for development to toxicological assessment at an early stage and to support optimization. It is clear that, because of their low throughput and the large amounts of substance required, conventional toxicological methods, and animal studies in particular, are not able to meet the requirements placed on screening. In vitro methods, on the other hand, have low substance requirements; they can be performed quickly and they are cheap. The relatively simple in vitro systems are often overstretched when it comes to generating data on a substance about which little or no previous information exists, however. This kind of information can usually be provided only by methods with a high level of complexity. This naturally cannot be achieved with in vitro methods. The particular value of in vitro methods therefore lies in the investigation of questions arising on the basis of specific evidence from an animal study. In this connection, early screening must always be seen as "***screening via knowledge***," i.e., based on previous information. Once a data pool generated under in vivo conditions is available, this can be examined in detail with the aid of cell or tissue culture methods. Another particular benefit of in vitro methods lies in the fact that human material (such as surgical specimens) can be used, and the basis for the risk assessment can thus be improved. In vitro methods can therefore represent a useful adjunct to animal studies with the possibility of making the findings obtained in animals more applicable to humans.

Possible uses of in vitro methods:

Detection of damage in defined organs

Testing for specific toxicity (e.g., phototoxicity)

Tests using receptors or ion channels in the context of safety pharmacology studies

Genotoxicity testing

Some of the common in vitro methods and their possible uses are described below.

## Tests with Liver Cell Cultures (Biotransformation and Cytotoxicity)

The liver plays a central role in the metabolism of foreign substances. Its extraordinary capacity to convert and break down substances is largely attributable to the parenchymal cells of the liver or hepatocytes. From a toxicological point of view, the liver is one of the most important target organs for toxicity. It is therefore not surprising that, in the context of in vitro testing, particularly great importance is attached to tests using isolated liver cells. Hepatocytes from the common study animal species (mouse, rat, dog, or monkey) are easy to obtain because most toxicological studies end with necropsy of the animals. Obtaining human tissue is more difficult, and use must be made here of surgical specimens obtained, for example, from resected liver tissue, following tumor surgery. The hepatocytes obtained at necropsy or from surgical specimens via perfusion are used as primary cultures. They can be maintained in culture for a while, but lose their full functionality over time, with a particularly sharp decline in their cytochrome P450 enzyme activity. The use of newer culture methods such as sandwich culture, in which primary hepatocytes are sandwiched between layers of a collagen matrix, or a sandwich technique involving coculture with non-parenchymatous liver cells, significantly prolongs the period for which metabolic activity can be maintained. Under these conditions, it is possible to maintain hepatocytes in culture for up to 14 days without any significant loss of metabolic activity.

Tests on the metabolism and cytotoxicity of substances can be performed with liver cells obtained and maintained in culture in this way. Preliminary statements can thus be made about biotransformation without animal studies or trials in humans having been conducted. Within the context of drug development, these tests are therefore also of particular importance because the results obtained in human hepatocytes can be compared with the results from the hepatocytes of the study animal species in which the toxicological studies have been or should be carried out. On the basis of the comparability of the metabolic pattern (human compared with animal), conclusions can then be drawn as to whether the study animal species used in the toxicological studies can be classified as relevant in terms of their applicability to humans.

Cytotoxicity tests with liver cells involve measuring the levels of certain enzymes in the culture supernatant. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) can be mentioned here as examples. Increased levels of these (cellular) enzymes in the supernatant are an indicator of cell damage, following the same principle as is applied in the diagnosis of liver damage in patients (increased levels of liver-specific enzymes in the blood as an indicator of liver damage). The determination of mitochondrial dehydrogenase activity (MTT assay) represents another test criterion. The use of liver cell cultures in the context of early screening has proven particularly effective when previous information is available from in vivo studies. Under these conditions ("screening via knowledge"), tests with hepatocytes can be used for further chemical optimization with a view to avoiding or eliminating hepatotoxic properties in

a new substance. Inconclusive findings from animal studies or significant species differences resulting in uncertainty in assessing the possible risk to humans provide the basis for another possible use. In these cases, testing with human specimens can improve the basis for the risk assessment. Although tests with liver cell cultures are not a regulatory requirement, they represent an important internal decision-making criterion in the context of substance preselection or supplementing/supporting in vivo data with regard to their applicability to humans.

## Tests with Mouse Fibroblasts (Phototoxicity)

The term "phototoxicity" is used to refer to reactions triggered when an organism exposed to light or the sun shows particular sensitivity to certain (phototoxic) substances, resulting in harmful health effects. These kinds of reactions can range from local symptoms resembling sunburn (redness) to severe burns (extensive skin necrosis) and general health effects. A large number of substances (especially cosmetics, medicinal products) are now known to have phototoxic potential. It is therefore appropriate that the legislation requires manufacturers to provide information on phototoxicity in the presence of relevant grounds for suspicion (photo instability, presence/accumulation of the substance in the skin) or for certain indications (dermally applied substances). Traditionally, these kinds of tests were performed in animals and involved mice, rats, guinea pigs, or rabbits being irradiated with UV light after being treated with the substances to be tested.

The most widely used in vitro assay for phototoxicity is the "in vitro 3T3 Neutral Red Uptake 186 Phototoxicity Test" (3T3 NRU-PT) for which a guideline (OECD 2004) is available. The assay was developed under the leadership of the Center for the Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) of the Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV). It is performed using a permanent mouse fibroblast cell line (Balb/c 3T3) and is based on the testing and comparison of the cytotoxic effects of a substance in the presence or absence of exposure to UV light. This in vitro assay is also part of a sequential phototoxicity testing strategy proposed in a CPMP guidance document (CPMP/SWP/398/01 2002), and it is also mentioned in an ICH draft guideline for the testing of pharmaceutical drugs which is currently under discussion (CHMP/ICH/752211/2012 2012). While it is acknowledged that the 3T3 NRU-PT assay is a very sensitive test and many positive findings are not confirmed in in vivo follow-up studies, the importance of the 3T3 NRU-PT assay within the context of a sequential testing strategy lies in the fact that if a negative result is obtained, i.e., if evidence is obtained of the absence of phototoxicity, no other tests, and specifically no animal studies, need to be performed, something which is to be greatly welcomed from the point of view of limiting the number of animal studies.

However, a positive result in the 3T3 NRU-PT should not be regarded as indicative of a likely clinical phototoxic risk but rather a flag for a follow-up assessment.

## Tests with Isolated Ion Channels (Cardiotoxicity, ECG Changes)

In the context of the risk assessment of medicinal products, possible cardiotoxic properties, particularly in medicinal products used primarily in non-cardiovascular indications, have been the focus of attention for some time now. The properties concerned are characteristic ECG changes (prolongation of the QT interval as evidence of delayed cardiac repolarization) which are considered predictive in respect of the induction of arrhythmias. This kind of potential must be identified at an early, i.e., preclinical stage, and the legislation therefore consistently requires appropriate nonclinical (safety pharmacology) studies. Reference should be made in this connection to an ICH test guideline entitled "The non-clinical evaluation of the potential for delayed ventricular repolarisation (QT interval prolongation) by human pharmaceuticals" (CPMP/ICH/423/02 2005). Among the tests stipulated in this test guideline are tests using human potassium channels which are expressed in a cell line in a stable manner (hERG). The background for the tests is the fact that cardiac repolarization is essentially mediated by potassium flow, and that drug-induced inhibition of the ion channels leads to prolongation of the action potential, which makes itself apparent in the ECG in the form of prolongation of the QT interval. Tests using hERG channels represent an additional and new example of in vitro studies that are established in regulatory terms.

## Tests with Mammalian Cell Cultures (Genotoxicity)

In vitro methods are already long established and stipulated by test guidelines as standard in the field of genotoxicity testing. These kinds of test were in fact already in use even before the development and use of in vivo methods. For the testing of a substance for genotoxic properties, it is assumed that no single test system is capable of predicting a possible risk to humans in a reliable manner. This is why batteries of tests are used to test substances for possible genotoxic effects. A typical battery of tests, stipulated for the testing of medicinal products, for example, consists of two in vitro tests (gene mutation test in bacteria, chromosomal aberration test in mammalian cell cultures) and one in vivo test (micronucleus test in bone marrow). To perform chromosomal aberration tests under in vitro conditions, cells in culture are treated with the substance to be tested in both the presence and absence of external metabolic activation, arrested at metaphase by administration of a spindle inhibitor, fixed, and then evaluated under the microscope. Permanent fibroblast cells originating from various Chinese hamster tissues are most often used for these tests,

including v79, CHO, or CHL cells. These are particularly suitable for chromosome analyses because of their small number of chromosomes and especially their uncomplicated karyotype. Human peripheral lymphocytes are also used.

## Tests with Incubated Chicken Eggs (Various Parameters)

Incubated chicken eggs have long been a well-established test system in biomedical research. Development stages without any sensitivity to pain are also used as a model. Both the embryo and the extraembryonic vascular systems are considered as target structures. The substances to be tested are administered directly or via the intravascular route. Functional and/or morphological parameters are end points. The results of numerous studies show that incubated chicken eggs are used to determine the irritation potential, to screen for cardiovascular effects, phototoxicity and angiogenesis, and in cancer research.

## Human Embryonic Stem (hES) Cells for Use in Toxicity Testing, e.g., Early Development Toxicity Testing

Early developmental toxicity assays for screening of various compounds for the potential risks for abnormal development in the growing embryo have been traditionally based on animal cells. As species differences might affect the accuracy of the assessments, there is an increasing need for alternative cell sources. In vitro differentiation of hES cells bears a resemblance to the early stages of human embryonic development and offers in principle the possibilities for alternative toxicity testing.

Developmental toxicity evaluations, as used in safety assessment assays, are sometimes suffering from a lack of normal, reproducible, and easily available human cell systems. In this context, pluripotent hES cells and their derivatives have the potential to improve the quality of targets, hits, and leads, thus reducing late-stage attrition. The promise of hES cells for in vitro toxicology is the indefinite access to starting material of identical origin in combination with highly human relevant assays for, e.g., developmental toxicity testing.

## Possibilities and Limitations of In Vitro Toxicity Testing

It is no longer possible to imagine modern toxicity testing without in vitro test systems. They enable initial information on toxicological properties to be obtained within the context of early screening and can provide chemical research with important indications with regard to the possibilities for optimization. The reliability of such screening is enhanced considerably if previous information is already available on the substance (“screening via knowledge”). Mechanistic investigations represent a second focus of in vitro methods. The same principle applies here as

with "screening via knowledge," i.e., that the real role of in vitro methods lies in the targeted investigation of specific questions based on previous information obtained from in vivo studies. In vitro methods can add major value, however, e.g., by making it possible for tests to be carried out using human specimens, and thus improve the basis for the risk assessment. Quantitative assessments aimed at extrapolating concentrations from in vitro tests to the in vivo situation (doses) are problematic. It is therefore not surprising that the in vitro test systems established so far for regulatory purposes (e.g., in vitro genotoxicity, phototoxicity, or cardiotoxicity testing) are almost always part of an integrated battery of in vitro/in vivo tests, and the results of in vitro testing are assessed in the sense of a yes/no answer and not in terms of a quantitative assessment of the risk.

## References

CHMP/ICH/752211/2012 (2012) Release for consultation Dec 2012, guidance on photosafety evaluation of pharmaceuticals

CPMP/ICH/423/02 (2005) The non-clinical evaluation of the potential for delayed ventricular repolarisation (QT interval prolongation) by human pharmaceuticals

CPMP/SWP/398/01 (2002) Note for guidance on photosafety

OECD (2004) Test No. 432: in vitro 3T3 NRU phototoxicity test, OECD guidelines for the testing of 485 chemicals, Section 4. OECD Publishing

# New and Future Toxicological Assays and Their Regulation

Horst Spielmann

## Contents

Challenges for Regulatory Testing in the Twenty-First Century ..... 115
Classical Toxicity Testing Is an Expensive "Patchwork Approach" ..... 116
How New Technologies Could Transform Existing Approaches ..... 116
The US Vision "Toxicity Testing in the 21st Century" (Tox21) ..... 117
The Adverse Outcome Pathway (AOP) Concept ..... 118
Human-on-a-Chip (Multiorgan-Chip) Technology Applied to Toxicity Testing ..... 119
Virtual Organs ..... 120
Application of Virtual Models in Predictive Toxicology ..... 120
Virtual Liver Projects ..... 120
The Virtual Embryo ..... 121
References ..... 121

**Abstract**

Twenty-first-century tools are now providing high-dimensional data at the molecular and cellular level that can advance predictive toxicology.

## Challenges for Regulatory Testing in the Twenty-First Century

Regulators and the public face increasingly complex challenges that require harnessing the best available science and technology on behalf of patients and consumers. Therefore we need to develop new tools, standards, and approaches that efficiently and consistently assess the safety, efficacy, quality, and performance of products. However, so far regulatory science has not sufficiently been appreciated and underfunded. Today, we are not sufficiently applying scientific discoveries to ensure

H. Spielmann
Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany
e-mail: horst.spielmann@fu-berlin.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_34, 

the safety of drugs and chemicals to which consumers are exposed. Thus we must bring twenty-first-century approaches to twenty-first-century products and problems.

Most of the toxicological methods used for regulatory assessment rely on high-dose animal studies and default extrapolation procedures and have remained relatively unchanged for decades, despite the scientific revolutions in the biosciences over the past 50 years. We need better predictive models to identify concerns earlier in the product development process to reduce time and costs. We also need to modernize the tools used to potential risks of consumers who are exposed to drugs, food, and other chemical products.

The challenge today is that the toxicological evaluation of chemicals must take advantage of the ongoing revolution in biology and biotechnology. This revolution is making it increasingly possible to study the effects of chemicals using cells, cellular components, and tissues – preferably of human origin – rather than whole animals.

With an advanced field of regulatory science, new tools, including functional genomics, proteomics, metabolomics, high-throughput screening, and systems biology, can replace current toxicology assays with tests that incorporate the mechanistic underpinnings of disease and of underlying toxic side effects. This should allow the development, validation, and qualification of preclinical and clinical models that accelerate the evaluation of toxicities during the development of drugs and other chemicals to which humans are exposed. The goals include developing biomarkers to predict toxicity and screen at-risk human subjects during clinical trials, as well as after new products are on the market. The new methods also should enable rapid screening of chemicals, which could reduce the large number of industrial chemicals that have not yet been evaluated under the current testing system, e.g., according to the EU REACH chemicals regulation.

## Classical Toxicity Testing Is an Expensive "Patchwork Approach"

Today, companies planning to register drugs, industrial or consumer chemicals, have to conduct a series of tests by exposing animals to chemicals to screen for cancer, birth defects, and other adverse health effects. In the past, agencies have typically responded to scientific advances mostly by altering animal-based toxicity tests or adding more animal tests – such as studying offspring of exposed mothers – to existing toxicity-testing regimens. That approach has led to a testing system that is lengthy and costly and that uses many animals. In combination with the various legal authorities, this system has resulted in many toxicants not being tested at all, despite potential human exposure to them – even as other contaminants receive significant research attention and decades of scrutiny.

## How New Technologies Could Transform Existing Approaches

Since more innovative approaches to toxicity testing should be implemented, the US National Research Council asked the US National Academy of Sciences to

develop a long-range vision and a strategy to advance toxicity testing in the twenty-first century.

In 2007 the US National Academy of Sciences published the report "Toxicity Testing in the 21st Century – a Vision and Strategy" (Natl. Acad. Sci., USA 2007). The report takes into account a number of emerging fields and techniques, which are contributing new insights for understanding the biologic responses to chemicals in human tissues. For example, new high-throughput techniques developed by the pharmaceutical industry use efficient automated methods to test specific biologic activities of thousands of chemicals that used to be studied in animals. Emerging fields also include systems biology, a powerful approach that uses computational models and laboratory data to describe and understand biologic systems as a whole and how they operate. Systems biology, bioinformatics, and rapid assay technologies will help to better understand how cellular networks or pathways in the human body carry out normal functions that are essential to maintaining health. When important pathways are significantly altered by chemical exposures, they can cause adverse health effects. But these effects only occur when exposures are of sufficient intensity or duration, or if they occur in susceptible individuals or during sensitive life-stages.

## The US Vision "Toxicity Testing in the 21st Century" (Tox21)

A new concept of the report *Toxicity Testing in the 21st Century* is a toxicity-testing system that relies mainly on understanding "toxicity pathways" – the cellular response pathways that can result in adverse health effects when sufficiently perturbed (Natl. Acad. Sci., USA 2007). Such a system would evaluate biologically significant alterations without relying on studies of whole animals. In addition "targeted testing" has to be conducted to clarify and refine information from toxicity pathway tests for use in chemical risk assessments. For the foreseeable future, some targeted testing in animals will need to continue, since it is not yet possible to sufficiently understand how chemicals are metabolized in the human body, when applying only tests in cells and tissues. Such targeted tests will complement the new rapid assays and ensure the proper evaluation of chemicals.

*Toxicity pathway* refers to a chemically induced chain of events that leads to an adverse effect such as tumor formation. These pathways ordinarily coordinate normal processes such as hormone signalling or gene expression. Estrogen-receptor signalling, for instance, is an ordinary feature of normal cell biology, but if it is inappropriately up- or downregulated, it can cause fertility problems. Molecular biologists are now attempting to identify and map toxicity pathways and the ways chemicals interact with the biochemical processes involved in cell function, communication, and the ability to adapt to environmental changes. For example, a protein that – upon chemical binding – blocks or amplifies estrogen-receptor signalling could alter the pathway's normal function and induce a *pathway perturbation*.

After identifying a perturbation at the cellular level, the effects have to be put into a broader context of toxicity in living animals. In order to extrapolate a toxic blood or

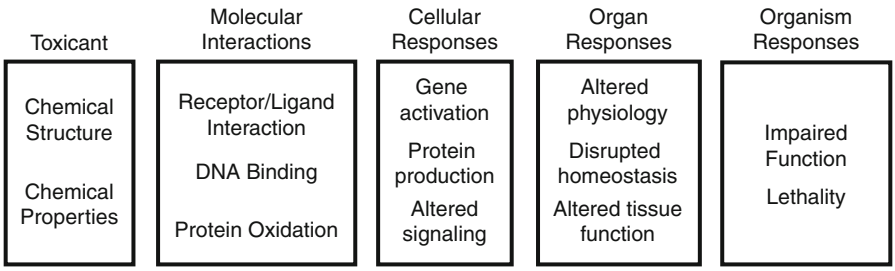



**Fig. 1** An adverse outcome pathway (AOP) represents existing knowledge concerning the linkage between a the molecular initiating event and an adverse outcome at the individual or population levels (Ankley et al. 2010). As such, AOPs by definition, span multiple levels of biological organization. AOPs often start out being depicted as linear processes, however, the amount of detail and linearity characterizing the pathway between a molecular initiating event and an adverse outcome within an AOP can vary substantially, both as a function of existing knowledge and risk assessment needs

tissue dose from a cell-based response, PBPK modelling and computational methods based on human cell circuitry should be applied. Cell-based assays offer some advantages in comparison to animal tests, which are limited by cost and resource constraints to just a few doses, while chemicals can be tested in in vitro assays at a broad range of doses that might provide better information about low-dose human effects. Dose-response and extrapolation modelling will enable the translation of cellular tests to whole human systems. Specifically, the modelling will estimate exposures that would lead to significant perturbations of "toxicity pathways" observed in cellular tests.

An *adverse outcome pathway (AOP)* describes the critical alteration of a "toxicity pathway" by an agent or its metabolites that can impair normal biological function to such an extent that an adverse health effect may occur. It relates to a linear sequence of events from the exposure of an individual to a chemical substance through to an understanding of the adverse (toxic) effect at the individual level for human health. Each AOP is a set of chemical, biochemical, cellular, or physiological responses, which characterize the biological effects cascade resulting from a particular exposure. The key events in an AOP should be definable and make sense from a physiological and biochemical perspective; AOPs span multiple levels of biological organization (Fig. 1).

## The Adverse Outcome Pathway (AOP) Concept

An AOP is a conceptual framework that links a molecular-level initiating event with adverse effects relevant for risk assessment. Each AOP consists of a set of chemical, biochemical, cellular, and physiological responses, which characterize the biological effects cascade resulting from a particular toxic insult. AOPs span multiple levels of biological organization. AOPs often start out being depicted as linear processes; however, the amount of detail and linearity characterizing the

pathway between a molecular initiating event and an adverse outcome within an AOP can vary substantially, both as a function of existing knowledge and assessment needs.

Meanwhile the new concept has been accepted by the international scientific community, and in 2011 the OECD has accepted the AOP concept and introduced it into the OECD Test Guidelines (TGs) program. As a consequence, the OECD requires that the molecular or mechanistic AOP concept should be taken into account, when new toxicity tests are introduced or existing ones are updated (OECD 2011), e.g., the new draft TG on "in vitro skin sensitization."

It should be kept in mind that the terms "adverse outcome pathway" (AOP) and "toxicity pathway" should be used with caution, since the disturbed pathways are in the first place "normal" cellular pathways that are being altered by toxic agents and as a result "toxic" or "adverse" effects are resulting.

Human exposure data are an also important element of the vision. For this purpose biomonitoring data from exposed human populations is required, e.g., concentrations of chemicals measured in human blood, hair or other tissues. As testing is developed and refined, other markers of human exposure, health effects, and susceptibility will be identified that will allow to evaluate chemicals of concern and to develop programs for risk management.

## Human-on-a-Chip (Multiorgan-Chip) Technology Applied to Toxicity Testing

Pressures to change from traditional animal models to novel technologies arise from the limited predictivity of human health effects and animal welfare considerations (Natl. Acad. Sci., USA 2007). This change requires human organ models combined with the use of new technologies in the field of -omics and systems biology, as well as respective evaluation strategies. In vitro organ emulation needs an appropriate model for each organ system, i.e., what makes a heart a heart, a liver a liver, etc.? In this context, it is important to consider combining such organs into systems. Miniaturization of such systems to the smallest possible chip-based scale is envisioned to minimize human tissue demand and to match with the test throughput required in industry (Esch et al. 2011; Huh et al. 2011, 2012). A multiorgan-chip technology has been established based on a self-contained smartphone-size chip format, within a German BMBF 3-year GO-Bio project (Marx et al. 2012). A micro-pump has been successfully implemented into the microcirculation system for long-term operation under dynamic perfusion conditions. Performance of the technology has been proven by 28-day chip-based bioreactor runs of single perfusion circuits combining human 3D liver equivalent and human foreskin tissue cultures. The inclusion of organ equivalents for the intestine, kidney, and bone marrow will extend the multiorgan-chip (MOC) use to ADMET testing in a follow-up program.

Since 2012 the US NIH and FDA are funding a major multicenter program for the development of a technology platform that will mimic human physiological systems in the laboratory, using an array of integrated, interchangeable engineered

human tissue constructs – "a human body on a chip" (NIH 2012). The program will combine the technologies to create a microfluidic platform that can incorporate up to 10 individual engineered human microphysiological organ system modules in an interacting circuit. The modules will be designed to mimic the functions of specific organ systems representing a broad spectrum of human tissues, including the circulatory, endocrine, gastrointestinal, immune, integumentary, musculoskeletal, nervous, reproductive, respiratory, and urinary systems.

The goal of the program is to create a versatile platform capable of accurately predicting drug and vaccine efficacy, toxicity, and pharmacokinetics in preclinical testing. The research team anticipates that the platform will be suitable for use in regulatory review, amenable to rapid translation to the biopharmaceutical research community, and adaptable for integration of future technologies such as advances in stem cell technologies and personalized medicine.

## Virtual Organs

### Application of Virtual Models in Predictive Toxicology

Cell-agent-based models are useful for modelling developmental toxicity by virtue of their ability to accept data on many linked components and implement a morphogenetic series of events. These data may be simulated (e.g., what is the effect of localized cell death on the system?) or data derived from in vitro studies. In the latter case, perturbed parameters are introduced as simple lesions or combinations of lesions identified from the data, where the assay features have been annotated and mapped to a pathway or cellular process implemented in the virtual model. Whereas in the US EPA ToxCast program, predictive models are built with computer-assisted mapping of chemical assay data to chemical end point effects (Judson et al. 2010), the virtual tissue models incorporate biological structure and thus extend the in vitro data to a higher level of biological organization. As such, a developing system can be modelled and perturbed "virtually" with toxicological data, and then the predictions on growth and development can be mapped against real experimental findings.

### Virtual Liver Projects

The goal of the US "Virtual Liver project" (www.epa.gov/ncct/virtual_liver.html) is to develop models for predicting liver injury due to chronic chemical exposure by simulating the dynamics of perturbed molecular pathways, their linkage with adaptive or adverse processes leading to alterations of cell state, and integration of the responses into a physiological tissue model. When completed, the Virtual Liver Web portal and accompanying query tools will provide a framework for incorporation of mechanistic information on hepatic toxicity pathways and for characterizing interactions spatially and across the various cells types that comprise liver tissue.

The German BMBF-funded "Virtual Liver" project (http://www.virtual-liver.de/about/) focuses on the establishment of a three-dimensional model of the liver that correctly recapitulates alterations of the complex micro-architecture, both in response to and during regeneration from chemically induced liver damage. There is currently limited knowledge on how cells behave in a coordinated fashion to establish functional tissue architecture and to respond to chemically induced tissue damage during regeneration. A vision of this project is that the spatial-temporal events during tissue damage and regeneration can be simulated in silico. Since the exact position and metabolic capacity of the individual cells of the model are known, it should also be possible to simulate to what degree a certain pattern of chemically induced liver damage compromises the metabolic capacity at the organ level. Finally, a long-term goal will be to integrate intracellular mechanisms into each cell of the model, as many of the critical intracellular key mechanisms still need to be elucidated.

## The Virtual Embryo

The US EPA program "The Virtual Embryo Project (v-Embryo™): A computational framework for developmental toxicity" (http://epa.gov/ncct/v-Embryo/index.html) is focused on the predictive toxicology of children's health and developmental defects following prenatal exposure to environmental chemicals. The research is motivated by scientific principles in systems biology as a framework for the generation, assessment, and evaluation of data, tools, and approaches in computational toxicology. The long-term objectives are to determine the specificity and sensitivity of biological pathways relevant to human developmental health and disease, predict and understand key events during embryogenesis leading to adverse fetal outcomes, and assess the impacts of prenatal exposure to chemicals at various stages of development and scales of biological organization.

## References

Ankley GT, Bennett RS, Erickson RJ (2010) Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29:730–741

Esch MB, King TL, Shuler ML (2011) The role of body-on-a-chip devices in drug and toxicity studies. Annu Rev Biomed Eng 13:55–72

Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs-on-chips. Trends Cell Biol 21:745–754

Huh D, Torisawa Y, Hamilton GA (2012) Micro-engineered physiological biomimicry: organs-on-chips. The Royal Society of Chemistry, London

Judson RS, Houck KA, Kavlock RJ (2010) Predictive in vitro screening of environmental chemicals – the ToxCast project. Environ Health Perspect 118:485–492

Marx U, Atac B, Brincker S (2012) Multi-organ-chips developing a human chip-based platform for repeated dose toxicity testing. In: Spielmann H, Seilde T (eds) Alternative testing strategies – progress report 2012. FU, Berlin, pp 210–217

National Academy of Sciences, Committee on Toxicity Testing and Assessment of Environmental Agents, National Research Council (2007) Toxicity testing in the 21st century: a vision and strategy. National Academies Press, Washington

National Institutes of Health (NIH), National Center for Advancing Translational Sciences (2012) NIH funds development of tissue chips to help predict drug safety:2012 Tissue Chip Project Awards. http://www.ncats.nih.gov/research/reengineering/tissue-chip/projects/awards-2012.html

OECD (2011) The adverse outcome pathway for skin sensitization. OECD Publication Office, Paris, 33 pp

# Computer-Based Prediction Models in Regulatory Toxicology

Thomas Steger-Hartmann and Scott Boyer

## Contents

Principles of Predictive Systems ..... 124
Assessment of Predictive Systems ..... 125
Definitions ..... 126
Use of Predictive Tools in Regulatory Toxicology ..... 128
Future Perspectives ..... 130
References ..... 130
Resources ..... 131

**Abstract**

The increasing regulatory safety demands for the submission and registration of chemicals, pesticides, and pharmaceuticals as well as several amendments in animal protection legislation have exacerbated the dilemma of regulatory toxicology, where on the one hand the required scientific contributions for the protection of workers, consumers, or patients are constantly augmented while on the other hand the number of experimental animal studies should be reduced for ethical and economic reasons.

One way to resolve this dilemma could be the use of computer-assisted systems to predict toxic effects. These "in silico" tools have experienced improvements in their performance and predictive power over the past three decades. They could therefore contribute to hazard identification and risk

T. Steger-Hartmann (✉)
Head of Investigational Toxicology, Global Early Development, Bayer Healthcare, Berlin, Germany
e-mail: thomas.steger-hartmann@bayer.com

S. Boyer
Global Head of Molecular and Investigational Toxicology, Global Safety Assessment, AstraZeneca R&D, Mölndal, Sweden
e-mail: scott.boyer@astrazeneca.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_36, 

assessment at least for some toxicological endpoints. The number of areas of application of in silico tools and systems will undoubtedly increase in the future. However, knowledge of how these systems work, on the underlying data quality, and their respective limitations are prerequisites for a sensible application.

## Principles of Predictive Systems

Providing a current overview of available predictive systems is not a meaningful exercise for a textbook since the pace of development would outdate such a list at the time of print. Therefore, this chapter will rather focus on the illustration of the basic principles of predictive tools in toxicology, give an overview of the current fields of application, and provide an outlook on expected future developments.

Comparing a new substance of unknown toxicity to compounds with reasonable structural or biological similarities for which useful toxicological information exists is a very significant technical problem. Searching the scientific literature to compare an unknown compound with a structure which has been toxicologically characterized would involve knowing all of the synonyms of the old and new compounds, searching the right scientific journals, and then carefully reading all of the information that is found. For these reasons, simple literature searching plays only a minor role in risk assessment and is most often replaced by database searches, especially if these databases allow for structure or substructure searches. During a database search, the structural comparison is performed in the context of the toxicological endpoint of interest. Once the users are convinced that the chemical structure that has been located in the database search is similar enough to the new compound, they can then ***read-across*** any findings from the existing compound and data in the database to the new compound and thereby reach conclusions regarding potential toxicological issues of the new compound. A detailed compendium of publically available and commercially available toxicology databases has recently been published (Boyer et al. 2012). A list of these is included at the end of this chapter.

With the accumulation of toxicological data around a specific endpoint, it has become possible to generate relationships between chemical structure and toxicological effect. The manual derivation of structure activity relationships (SARs) can be performed by qualified annotators familiar with both the chemical structures and the toxicological data. The resulting SAR can then be coded into a computer program which can scan a new chemical structure and recognize the presence of structures or substructures (parts of the larger chemical structure) and present the user with an estimate of the toxicity of the new structure. These systems, sometimes referred to as "expert" systems, have the advantage that they contain SAR conclusions based on data that have been assembled and quality-controlled.

*Expert systems*, the most commonly used of which is DEREK from Lhasa, Ltd., analyze the compound under investigation for substructures with toxicological relevance. Usually an expert system provides the information (rule) why

a specific (sub)structure causes a warning ("alert") such as "aromatic amines may cause mutagenicity." This offers the opportunity to the user to assess the plausibility of the alert. In most cases, however, such an alert relates only to the graphical comparison of the structure. Other properties, such as the physicochemical nature of the compound, which can significantly influence the toxicological properties of the chemical under investigation, are not necessarily accounted for. This problem may be illustrated for the prediction of the endpoint skin sensitization. For a chemical to cause skin sensitization, a particular reactivity with proteins is required to elicit the immune response. The reactivity can be derived from the chemical structure. However, the reaction with skin proteins can only occur if the compound shows a significant penetration into the skin. Skin absorption is a parameter which cannot always be derived from the two-dimensional structure of the chemical, but rather needs additional descriptors to determine the lipophilicity or the octanol-water partition coefficient of the compound. Advanced predictive systems calculate the parameters for reactivity and absorption individually and then combine these values for a prediction using "if. . .then" rules.

A significant development of traditional SAR is the ***quantitative structure activity relationship*** (QSAR). QSAR systems (represented by commercial systems like Multicase, TOPCAT, ECOSAR) combine physicochemical properties with other "descriptors" such as molecular fragments, indices of electron density or polarity with either the categorical outcome of an experiment (positive or negative) or a quantitative outcome, e.g., number of revertants in an Ames mutagenicity assay, inhibition constants of a enzyme, receptor, or ion channel (Tropsha 2004). This combination is most frequently achieved by the use of multivariate statistical correlations. Thus, QSARs use statistical correlation to perform the analysis of structure and toxicological activity that are performed by annotators as described above for SAR Expert systems. The statistical methods to establish these correlations are numerous and beyond the scope of this chapter; however, it is quite clear that the choice of the statistical method to correlate toxicological outcome to the "description" of the chemical structure is quite important and also highly dependent on the nature of the dataset, toxicological endpoint, and the desired performance of the resulting "model." The current trend in the modeling of toxicological endpoints is to use relatively flexible and powerful statistical machine learning algorithms which sometimes do suffer from a lack of interpretability, although recent research has established that these methods can be interpreted under some conditions (Hasselgren et al. 2013). To enhance the interpretation of QSAR models, the dataset that has been used to "train" the model is usually made available in various forms. This is critical for the interpretation of the outcome of the model as discussed in the following sections.

## Assessment of Predictive Systems

The prediction quality of in silico tools can be assessed with statistical parameters also used for medical diagnostic procedures. The predominant criteria are

**Table 1** Confusion matrix for a categorical, two-class model

| | | Experimental outcome | | |
|---|---|---|---|---|
| | | Test positive | Test negative | |
| Prediction outcome | Predicted positive | **True positive (TP)** | **False positive (FP)** | Positive predictive value |
| | | | | TP/(TP + FP) |
| | Predicted negative | **False negative (FN)** | **True negative (TN)** | Negative predictive value |
| | | | | TN/(FN + TN) |
| | | Sensitivity | Specificity | |
| | | TP/(TP + FN) | TN/(FP + TN) | |

***sensitivity*** and ***specificity***. A definition for these parameters and further assessment criteria are provided in Table 1.

## Definitions

*Sensitivity*: The ability to predict positives when they actually exist
*Specificity*: The ability to predict negatives when they actually exist
*Positive Predictive Value*: The ability of the predictive system to distinguish between true positives and false positives
*Negative Predictive Value*: The ability of the predictive system to distinguish between true negatives and false negatives
*Overall Accuracy*: The total number of correct predictions divided by all examples
In order to determine sensitivity and specificity, a certain amount of compounds with known toxicological properties will normally be predicted in the given model or system. The predicted effects are then compared to the effects observed in the actual experiment. The simple example of a two-class categorical model is shown in Table 1 but as noted above, some toxicological data can be recorded as continuous variables. In these cases, the confusion matrix cannot be used, but rather the user evaluates the model's predictive performance using either a correlation coefficient established between predicted values and observed values for compounds which are not included in the training set or measures of the root mean squared error (RMSE) of prediction. In most cases, the RMSE evaluation is preferred as it relates the prediction error, as an expression of RMSE, with the experimental error which is frequently expressed as RMSE as well.

Regardless of the data type or evaluation method, the results of such comparisons are not absolute, but rather dependent on the selection of the test set compounds, i.e., the selection of the structures for building the expert or QSAR system determines the quality of the predictions. Most systems are built on data from industrial chemicals or pesticides as abundant data sets are available for these compounds. As a consequence, these systems are predictive primarily for compounds possessing such inherent properties as being reactive, herbicidal,

insecticidal, etc. Predictions for other areas of chemical use, e.g., pharmaceuticals tend to perform worse in these systems for above-mentioned parameters. Comparisons of industrial chemicals, pesticides, pharmaceuticals, and food-related chemicals show that these various classes are chemically distinct and thus probably should be modeled separately. As a consequence, it is now common practice to provide information on the prediction limits of the model or system, otherwise known as the "domain of applicability". The domain of applicability represents the chemical and biological domain for which robust prediction can be made. A common approach to assess the domain of applicability lies in the analysis of chemical similarity of the compound under investigation with the list of compounds used to build the system. Alternatively, a system could be split up on several "local" models, each of which represent a distinct chemical class for which it can be applied (e.g., prediction of ecotoxicity of different classes of environmental pollutants in the EPA in silico tool ECOSAR).

Contrary to what one might think, a complete concordance between prediction and the experimental result is usually not the ultimate goal of a computational system. Several factors can confound achieving a perfectly predictive SAR or QSAR system. Paramount among these factors is the toxicological endpoint itself. Most endpoints are observations of a complex series of chemical and biological events that culminate in an observable toxicity. In addition, nearly every toxicological observation can be produced by more than one mechanism and thus expecting a single chemical structure-toxicity relationship to reflect these various mechanisms is expecting too much from both the method and the data. The second layer of complexity in toxicological data has to do with data reproducibility. Interlaboratory variability means that even well-known and mechanistically simple (relatively speaking) endpoints like the Ames mutagenicity assay have an imperfect concordance and thus a "positive" in one laboratory may be "negative" in another. While perfectly understandable, data variability means that SAR or QSAR models built with these data will never perfectly reflect the experimental outcome because the experiment cannot always replicate itself. Finally, another layer of complexity is that of translatability of toxicological outcomes. If one considers a comparison of effects observed in animal studies with those observed in humans, values in the range of 60 % concordance are reported for some endpoints (Olson et al. 2000). Thus, predictive systems built on data from animal studies will not be able to achieve a better prediction of human effects than the animal studies themselves.

*Data quality assessment* is one of the cornerstones of good SAR and QSAR modeling practice. Whereas expert systems often reference the scientific literature and thus allow the user to assess the quality, such a quality assessment (was the study performed according to international guidelines, according to GLP regulations, was the study published in a peer reviewed journal, etc.?) is often difficult in QSAR systems because the supporting quality information behind the data upon which the model is built is frequently not available. However, the development of data quality assessment schemes has made progress and may provide a more systematic way to combine data of similar quality (Klimisch et al. 1997).

In addition to questions regarding data quality, often there are issues with data completeness as well. The construction of toxicological QSARs mainly relies on data from experiments for which a toxic effect was recorded. The unfortunate consequence of this is that negative effects, i.e., the absence of any effect, are only rarely published in the literature. Thus QSAR training data sets sometimes show an overabundance of compounds which cause toxicity even though in the experimental system very few positive compounds are actually observed. For example, in the Registry of Toxic Effects of Chemical Substances (RTECS) which was initially curated by the US National Institute for Occupational Safety and Health (NIOSH) and is now commercially available the endpoint mutagenicity contains only compounds which showed a positive effect in the different assays for genotoxicity. Negative results, i.e., non-genotoxic outcomes are not reported. Systems built on such databases risk overpredicting the effects under investigations, i.e., many compounds are forecast to be mutagenic, even though they might contain certain properties (e.g., groups causing steric hindrance) which prohibit the activation to a mutagenic intermediate because compounds containing these mitigating features have been tested and found to be negative. Such inhibiting substructures or other conditions mitigating toxic effects cannot be calculated in QSAR systems if the data are not available. This situation is improving however, as the scientific community begins to see the utility of publishing and sharing data showing that a certain compound has been characterized for a toxicological effect and been found to be negative.

In order to resolve some of these issues, initiatives or consortia which gather unpublished data for the construction of new predictive tools are being formed. The advantage of such initiatives is the possibility of sharing unpublished, proprietary data and structures in a controlled way. This approach is particularly important for the pharmaceutical industry, where only very few results are published in comparison to the number of compounds that are actually evaluated. Examples of such consortia are the RepDose database of the Fraunhofer institute (ITEM, Hannover) or the eTOX-Project under the Innovative Medicines Initiative.

## Use of Predictive Tools in Regulatory Toxicology

In 2004, the OECD guidance document on principles for the validation of (Q)SAR was adopted (OECD 2004) and led to subsequent in silico activities which resulted in the release of the OECD QSAR Toolbox in 2008. The OECD Toolbox was intended to be used in the context of European ***industrial chemical assessment*** and registration: REACH (Regulation, Evaluation, Authorization, and Restriction of Chemical Substances) (OECD 2013a). This predictive system allows the user to find related compounds based on structural comparisons which are already assessed or registered and for which toxicological data are available. Based on these comparisons, it provides expert hypotheses on potential effects of the unknown compound. While the focus of the OECD Toolbox approach is to "read-across" to

existing data, the EU-funded project OpenTox works on establishing common standards and open access technologies for the exchange and distribution of SAR and QSAR models which should also contribute to improve transparency and validation of the tools for chemical assessment under REACH.

A completely different approach is followed by the US-EPA initiative ToxCast. In this project, numerous environmental chemicals have been investigated in a multitude of in vitro assays for different endpoints (gene expression, receptor binding, etc.) with relatively high throughput. The obtained data sets are analyzed with multivariate statistics while comparing them with the existing in vivo data. The objective of this approach is to identify robust predictors in the vitro assays which correlated with the in vivo results and to then build a predictive system based on combinations of these predictive in vitro assays.

For ***cosmetics and toiletries***, the EU directive 76/768/EEC ("Cosmetics Directive") aims to phase out animal studies for the risk assessment of cosmetics by the year 2013. While there are in vitro replacement methods for the endpoints such as skin or eye irritation that have found international regulatory acceptance, this is not the case for the more complex endpoints such as skin sensitization, organ toxicity, or in vivo toxicity. The OECD (OECD 2013b) proposed a concept to approach these complex endpoints with the help of "adverse outcome pathways" (AOPs), which "delineates the documented, plausible, and testable processes by which a chemical induces molecular perturbations and the associated biological responses which describe how the molecular perturbations cause effects at the subcellular, cellular, tissue, organ, whole animal and population level." The objective is to identify the crucial mechanistic steps which lead to the effect observed in vivo. Based on this analyses replacement, methods or predictive tools can be built which represent these mechanisms. The European project SEURAT ("Safety Evaluation Ultimately Replacing Animal Testing") embraced this approach and has begun to develop not only in vitro replacement methods but also in silico tools for the prediction of in vivo toxicity. However, despite these efforts, a complete replacement of animal studies for cosmetics assessment is not possible within the foreseeable future.

Computer-assisted prediction tools have found their entry into the regulatory registration of ***pharmaceuticals*** in the context of genotoxicity assessment of impurities (EMA 2009; FDA 2009). Impurities may occur in the drug product from degradation of the active pharmaceutical ingredient or as remaining traces of synthetic intermediates. In many cases, these impurities occur in extremely low concentration and/or are unstable or reactive making it impossible to be isolated and tested separately in in vitro or in vivo assays. The genotoxic potential of such impurities may be assessed with predictive tools solely based on the identified chemical structure (Glowienke and Hasselgren 2010). In case such an assessment does not identify a structural alert for mutagenicity, and the prediction of a lack of mutagenic potential can be supported by an argument of validity with respect to the domain of applicability (usually involving a read-across step), a prediction of low/no genotoxic risk is usually accepted by the regulatory authority.

## Future Perspectives

Some success has been seen in the prediction of complex toxicological endpoints using the methods outlined above. However, the number of failures still far outweighs the number of true successes and very large leaps in the underlying science of toxicology prediction are required before these tools and approaches can be relied upon for a wide variety of applications. While the statistical tools for associating experimental data have been very well proven in other areas, their ultimate success in toxicology prediction will depend on three basic factors:

1. Improvements in our understanding of data quality and what data can be used together to build predictive models
2. Additions of complimentary datasets like those generated in the ToxCast project to identify high throughput in vitro "surrogates" for in vivo studies or the OECD AOP approach which aims at understanding and categorizing key biological steps to enhance our understanding of the biological pathways affected by a particular compound
3. More sophisticated methods for evidence combination which take into account the sources of uncertainty (including applicability domain) and express the resulting prediction as a probability while still maintaining transparency regarding the sources of uncertainty in the conclusion

In the end, all three of these areas will play a very large role in the improvement of both our understanding of the mechanisms of toxicological outcomes and in our ability to predict them, and perhaps more importantly, our understanding of when we cannot predict outcomes using computational tools.

## References

Boyer S, Muthas D, Greene N (2012) Information, informatics and modeling predictive toxicology. In: Wilson AGE (ed) New horizons in predictive toxicology: current status and application. Royal Society of Chemistry, London, pp 70–100

EMA (2009) Assessment of genotoxic impurities: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf

FDA (2009) Assessment of genotoxic impurities: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079235.pdf, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002907.pdf

Glowienke S, Hasselgren C (2010) Use of structure activity relationship (SAR) evaluations as a critical tool in the evaluation of the genotoxic potential of impurities. In: Teasdale A (ed) Genotoxic impurities: strategies for identification and control. Wiley, New York, pp 97–120

Hasselgren C, Muthas D, Ahlberg E, Andersson S, Carlsson L, Noeske T, Stålring J, Boyer S (2013) Chemoinformatics and beyond – moving from simple models to complex relationships in pharmaceutical computational toxicology. In: Bajorath J (ed) Chemoinformatics: case studies and pharmaceutical applications. Wiley, New York, pp 49–62

Klimisch HJ, Andreae M, Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5

OECD (2004) OECD principles for the validation, for regulatory purposes, of (quantitative) structure-activity relationship models: http://www.oecd.org/dataoecd/33/37/37849783.pdf,

http://www.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282007%292&doclanguage=en
OECD (2013a) The OECD QSAR toolbox. http://www.oecd.org/env/ehs/risk-assessment/theoecdqsartoolbox.htm
OECD (2013b) Guidance document on developing and assessing adverse outcome pathways. Series on testing and assessment No. 184: http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono%282011%298&doclanguage=en
Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A (2000) Concordance of the toxicity of pharmaceuticals in humans and animals. Regul Toxicol Pharmacol 32:56–67
Tropsha A (2004) Application of predictive QSAR models to database mining. In: Oprea T (ed) Chemoinformatics in drug discovery. Wiley-VCH, Weinheim, pp 437–456

## Resources

List of toxicological internet data sources:
- ToxNet http://toxnet.nlm.nih.gov/index.html
- DSSTox http://www.epa.gov/ncct/dsstox/
- ACToR http://actor.epa.gov/actor/faces/Download.jsp
- RTECS http://www.cdc.gov/niosh/rtecs/default.html
- CTD http://ctd.mdibl.org
- GENETOX http://www.nlm.nih.gov/pubs/factsheets/genetxfs.html
- DART http://www.nlm.nih.gov/pubs/factsheets/dartfs.html
- KEGG http://www.kegg.jp/kegg/drug/
- DITOP http://bioinf.xmu.edu.cn/databases/ADR/search.php
- T3DB http://www.t3db.org

Selection of freely available systems and projects:
- CAESAR http://www.caesar-project.eu/index.php?page=index
- ECOSAR: http://www.epa.gov/oppt/newchems/tools/21ecosar.htm
- eTOX http://www.etoxproject.eu/
- OASIS http://toolbox.oasis-lmc.org/?section=download
- OECD toolbox http://www.qsartoolbox.org/
- OpenTox http://www.opentox.org/toxicity-prediction
- RepDose http://www.fraunhofer-repdose.de/
- SEURAT http://www.seurat-1.eu/, http://www.seurat-1.eu//pages/the-cluster-projects/cosmos.php
- ToxCast http://www.epa.gov/ncct/toxcast/, http://www.epa.gov/ncct/download_files/factsheets/ToxCast%20Models%20Fact%20Sheet-Nov%2010%202011.pdf
- Tox21 http://www.epa.gov/ncct/Tox21/

Selection of commercial systems:
- DEREK https://www.lhasalimited.org/derek_nexus/
- HazardExpert http://www.compudrug.com/?q=node/35
- Multicase http://www.multicase.com
- QSAR Workbench http://accelrys.com/services/qsar-workbench.html
- TOPKAT http://accelrys.com/products/discovery-studio/predictive-toxicology.html

# Metabolism Tests in Regulatory Toxicology

Gert Ulrich Kuerzel and Christine Mauriac

## Contents

Metabolite Profiles and Structure Elucidation ..... 134
In Vitro Test Systems ..... 134
In Vivo Test Systems ..... 135
Structure Elucidation of Metabolites ..... 135
Investigation of Drug-Drug Interactions ..... 136
Elucidation of Enzymes Involved in the Metabolism of a Drug ..... 136
Enzyme Inhibition ..... 137
Enzyme Induction ..... 138
Quality Assurance ..... 138
Recommended Reading ..... 138
Resources ..... 139

**Abstract**

Investigations on the metabolism of drugs are meaningful for two reasons. Firstly, drug metabolites can be pharmacologically active or cause adverse reactions via on-target or off-target interactions. Secondly, interactions with other co-medications are possible via drug metabolizing enzymes which can be investigated in suitable in vitro test systems. Both provide predictive information to assess the potential risk to patients before approval of a drug and even before the first clinical trials and, thus, contribute to the minimization of risk for healthy subjects and patients.

G.U. Kuerzel (✉)
Sanofi-Aventis Deutschland GmbH, Industriepark Hoechst, Frankfurt am Main, Germany
e-mail: gertulrich.kuerzel@sanofi.com

C. Mauriac
Sanofi R&D, Chilly-Mazarin, France
e-mail: christine.mauriac@sanofi.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_37, 

## Metabolite Profiles and Structure Elucidation

To properly assess the impact of metabolites on efficacy and safety, it is necessary to have some knowledge of their structure and concentrations in the body. Since 2008, Health Authorities and consortia published several guidance documents on Safety Testing of Drug Metabolites. These provide recommendation on when and how to identify and characterize drug metabolites whose nonclinical toxicity needs to be evaluated. A threshold for relative metabolite abundance was considered in plasma at steady state to define those metabolites for which adequate exposure must be demonstrated in toxicological test species. According to these guidances, safety assessment of metabolites more highly exposed in humans than in toxicologically relevant species should be completed prior to the conduct of large-scale clinical trials.

As a rule of thumb, 70 % of drug-related material present either as parent compound or metabolites and excreted via urine and feces or circulating in blood should be structurally identified in animals and human. These investigations typically employ radiolabeled drugs in order to quantify drug and metabolite concentrations in various biological matrices regardless of the knowledge of the chemical structure. Radionuclides applied are typically carbon-14 and tritium due to ease of use, the synthetic accessibility, and the analytical traceability without change of the biological properties of the investigational compound.

## In Vitro Test Systems

In vitro investigations on metabolism give rise to basic information on the biotransformation of a compound before animal studies or clinical investigations. Drug metabolism is due to biotransformation during transfer into the systemic circulation, e.g., for orally administered compounds, a potential degradation in the gastrointestinal tract and in the gut mucosa or for inhaled compounds in lung tissue. After oral absorption, the liver is the main site for drug metabolism and can be involved in the first-pass elimination of a drug before it attains the systemic circulation. Special compound classes, e.g., peptides, predominantly undergo metabolism in the kidneys, other compounds such as prodrugs are prone to metabolism by plasma esterases.

For the selection of the suitable in vitro test system, e.g., to investigate the metabolism in the liver, prediction of in vitro-in vivo extrapolation decreases from whole liver perfusion studies to recombinant enzymes, as shown in Fig. 1. In other words, decreasing complexity of a test system decreases the relevance to clinical state but allows rapid implementation and higher throughput. Selection of a particular test system depends on the required information at key decision points along the value chain of research and development.

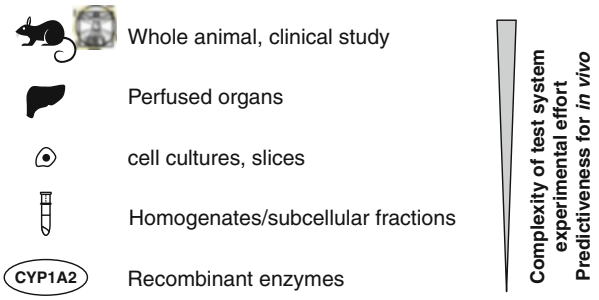



**Fig. 1** In vitro test systems used for metabolism studies in comparison to in vivo models

## In Vivo Test Systems

Metabolism investigations in the whole body are key to obtain a complete picture of qualitative and quantitative biotransformation of a drug. Radioactive studies are the method of choice in order to exhaustively detect and quantify all metabolites, including structurally unknown ones or those for which no reference compounds are available (e.g., to allow quantification using standard bioanalytical assay methods). Moreover, use of radiolabeled compounds facilitates validation of sample processing for matrices like feces, organs, and tissue homogenates by determination of extraction yields or recoveries.

The selection of animal species for these investigations is determined by the species used in chronic toxicology studies, both a rodent and non-rodent species (in general rat and dog), as well as those additional species used in carcinogenicity (mouse) and embryo-fetal studies (rabbit). The human ADME study, investigating *a*bsorption, *d*istribution, *m*etabolism, and *e*xcretion in humans, is of particular importance to detect and quantify all human metabolites and to deduce their margin of exposure in humans at the NOAEL (**N**o **O**bserved **A**dverse **E**ffect **L**evel) dose defined in chronic toxicological studies. Important matrices for analysis are blood, plasma, urine, fecal homogenates, and, in addition for animals, bile, milk, organs, and tissues (see Fig. 2).

Of particular importance is the difference in the metabolism due to sex, dose, race, age as well as possible persistent accumulation of the drug and/or its metabolites in organs and tissues, as indicated in tissue distribution studies.

## Structure Elucidation of Metabolites

Samples from in vitro studies, radioactive ADME studies in animals and humans, and high dose non-radioactive in vivo studies are collected in order to elucidate metabolite structures by suitable techniques such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, often coupled with liquid

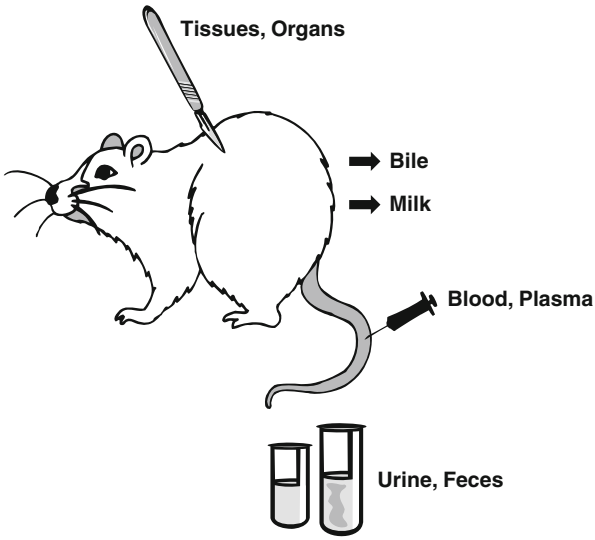



**Fig. 2** Important matrices for in vivo metabolism studies

chromatographic methods ("LC-MS," "LC-NMR"), if needed after complex and time-consuming concentration and purification steps. A definitive identification of the structure of relevant metabolites (abundant or pharmacologically active) is the prerequisite for their chemical synthesis. This allows its bioanalytical quantification in all toxicological and clinical pharmacokinetic studies.

## Investigation of Drug-Drug Interactions

A drug is intended not only to be pharmacologically active with as little toxicity as possible but also as safe as possible in relation to both individual genetic differences of patients and concomitant administration of other drug(s) (see Fig. 3).

In the latter cases, only human in vitro metabolism systems are able to predict safety in human due to the lack of relevance of animals (significant enzymatic differences between animals and human). These in vitro experiments are the prerequisite to time-consuming, costly, and perhaps risky clinical studies and provide the first assessment of the requirement to conduct a clinical interaction study.

## Elucidation of Enzymes Involved in the Metabolism of a Drug

The majority of drugs are eliminated from the body after biotransformation into more hydrophilic metabolites which facilitates their excretion. The safety or the efficacy of a drug in patients can be affected by the interindividual differences in enzyme activities or by co-medication, possibly leading to inhibition or induction of these enzymes.

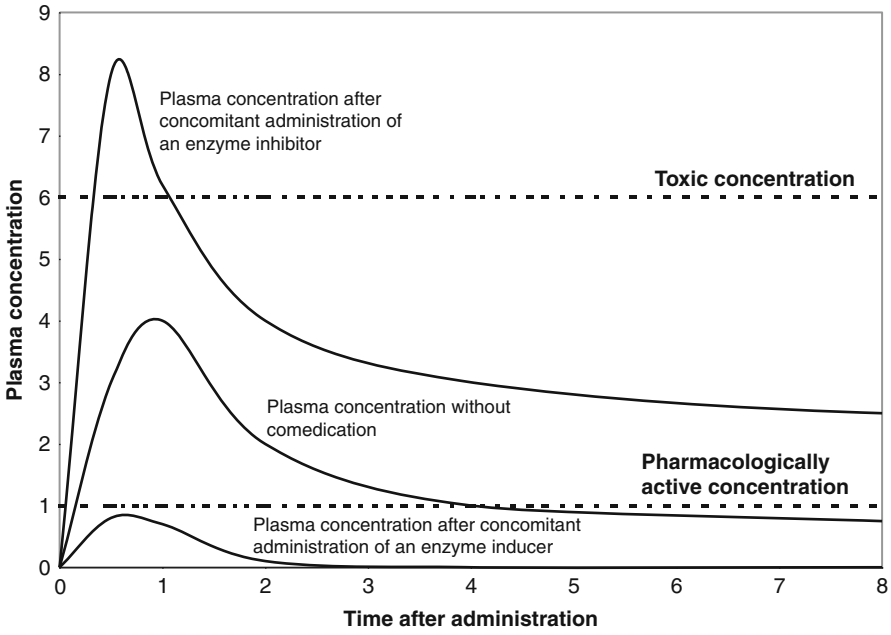



**Fig. 3** Potential effect of co-medications on plasma levels of a drug by inhibition or induction of drug-metabolizing enzymes

Thus, enzymes involved in the biotransformation of a drug have to be elucidated as early as possible. Of importance are enzymes revealing a genetic polymorphism. The major enzymes implicated in the biotransformation of the majority of marketed drugs are the cytochrome P450s, a superfamily of enzymes. Inhibition experiments in human liver microsomes can be performed using selective inhibitors or antibodies. In addition, human recombinant P450 enzymes are available to identify enzymes capable for metabolizing a drug. Correlation analysis of turnover observed in individual donors with different enzyme activities can be done. All these investigations contribute to verify or rule out the involvement of a particular enzyme. Similar studies can be applied to investigate non-P450 enzymes, although availability of recombinant enzymes or selective inhibitors might be limited.

The quantitative effect on the pharmacokinetics of the drug and a possible interaction with other co-medications has to be determined in clinical studies based on these in vitro findings.

## Enzyme Inhibition

A drug can act as an inhibitor of drug-metabolizing enzymes through a reversible mechanism, e.g., competitive inhibition, or irreversible, e.g., mechanism-based inhibition. This can lead to a prolonged elimination of the victim drug and, thus,

to higher plasma concentrations, which are potentially toxic. The determination of the inhibition potential of a new drug is therefore important. This can be performed in human hepatocytes or liver microsomes applying enzyme-specific marker substrates or in recombinant human enzymes. Reversible inhibitors are characterized by determination of the IC50 or inhibition constant (Ki). Risk assessment based on these data is acknowledged by Health authorities to conduct or not a clinical interaction study.

## Enzyme Induction

After repeated administration, drug-metabolizing enzymes can be induced. The resulting increased enzyme activity can lead to a more extensive metabolism of a co-medication metabolized by this particular enzyme. Decreased plasma levels can lead to the loss of efficacy. Enzymatic induction can be evaluated at mRNA, protein, or enzyme activity levels and, thus, can be studied by real-time PCR, western blot, or enzyme activity determination. Suitable in vitro test systems for the determination of enzyme induction are human hepatocyte cultures using a 3-day incubation of the test drug. High-throughput screening in discovery is possible using reporter gene assays in cell lines, such as PXR.

## Quality Assurance

Health authorities do not require metabolism studies to be conducted under GLP (**G**ood **L**aboratory **P**ractice) or GCP (**G**ood **C**linical **P**ractice). However, due to the importance of these investigations for drug safety, it is recommended to conduct these studies in the spirit of GLP to ensure quality and validity of the data. This requires study plans, standard operating procedures (SOP), proper documentation of analytical methods, and results, but no need for Quality Audits.

## Recommended Reading

Dudda A, Kuerzel GU (2006) Metabolism studies in vitro and in vivo. In: Vogel HG (ed) Drug discovery and evaluation, safety and pharmacokinetic assays. Springer, Heidelberg, pp 493–520, and references cited there-in

Krone V, Kuerzel GU, Shackleton G, Zimmer M (2011) The human ADME study. In: Vogel HG (ed) Drug discovery and evaluation: methods in clinical pharmacology. Springer, Heidelberg, pp 73–104

Weaver RJ, Jochemsen R (2009) Non clinical pharmacokinetics and toxicokinetics. In: Cartwright AC, Matthews BR (eds) International pharmaceutical product registration. Informa Healthcare, New York, pp 336–376

## Resources

International Conference on Harmonization. http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html
US-Dept of Health and Human Services Food and Drug Administration. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
European Medicines Agency. http://www.ema.europa.eu/ema/index.jsp?curl=pages/document_library/landing/document_library_search.jsp&mid=
Ministry of Health Labour and Welfare, Japan:.http://www.mhlw.go.jp/english/

# Toxicokinetic Tests

Jürgen Pauluhn

## Contents

Objectives of Toxicokinetic Investigations ..... 142
Extrapolation Across Species and Systems ..... 144
Extrapolation Across Routes ..... 146
Toxicokinetic Data and Risk Assessment ..... 149
Toxicokinetic Readouts ..... 149
  Time-Course Changes ..... 149
  Selection of Compartment ..... 150
Absorption, Distribution, and Elimination ..... 151
  Absorption ..... 151
  Absorption from the Skin ..... 151
  Gastrointestinal Absorption ..... 152
  Pulmonary Deposition, Retention, Clearance, and Absorption ..... 152
  Compartmental Pulmonary Biokinetics of Poorly Soluble Particles ..... 153
  Distribution ..... 155
  Excretion ..... 157
Toxicokinetic Parameter ..... 157
  Time-Course Analyses ..... 157
  Volume of Distribution ..... 157
  Area Under the Curve ..... 158
Significance of Toxicokinetics in Regulatory Toxicology ..... 158
Future Directions ..... 160
References ..... 160
  Recommended Reading ..... 160
  Resources ..... 160

J. Pauluhn
Experimental Toxicology, Bayer HealthCare, Wuppertal, Germany
e-mail: juergen.pauluhn@bayer.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_38, 

**Abstract**

Toxicokinetics is an integral component of toxicological studies in order to interrelate the administered dose with the associated effect. Pharmacokinetic data are essential for judging absorption, bioavailability, and bioefficacy across routes, regimens, and species. In case substances are administered by inhalation, it provides a means to identify substance accumulation and clearance in the lung. Also in other disciplines of toxicology, toxicokinetics is important for the assessment of persistence and bioaccumulation. Toxicokinetics includes analyses of temporal change of concentration profiles of the parent substance, its metabolites, or degradation products. Accordingly, toxicokinetic data provide indispensable estimates for systemic and organ-specific substance burdens and contribute significantly to the interpretation of toxicological data and their significance for humans.

## Objectives of Toxicokinetic Investigations

Toxicokinetics deals with the study and with the mathematical description of the time dependence from the disposition (absorption, distribution, biotransformation, and excretion) of xenobiotics in the whole organism. Absorption is the translocation of the administered substance to the blood stream. Once in the blood, the substance is distributed through the body and delivered to tissues, where it may leave the blood and enter the cells of the tissue or it may remain in the blood, particularly when bound to plasma proteins, and simply pass through the tissue. In certain tissues, such as the liver, the substance may be effectively removed from the body by metabolism. Other tissues, such as the kidney and lung, serve to eliminate xenobiotics from the body by excretion. The factors influencing the disposition are conceptualized in Fig. 1. Substances may show complex pattern distribution within an organism depending on the partition to and affinity of the particular tissue for the parent compound or its metabolite. When its absorption and distribution is complete, the concentration in blood depends on the amount absorbed and the extent of tissue distribution.

Knowledge of the specific time profiles of concentrations within tissues or specific compartments is important for assessing the total organ dose (AUC, area under the curve) as well the associated peak concentration ($C_{max}$). The AUC is represented best by the dose or concentration administered per unit of time $\times$ dosing duration ($C \times t$). Depending on whether any specific toxic outcome is AUC- or $C_{max}$-dependent, the same AUC can produce markedly stronger effects when $C_{max}$-dependent mechanisms play a role (Fig. 2). In case the rate of dosing exceeds that of elimination or clearance, a substance may accumulate at the portal of entry or the organ showing the highest partitioning/affinity to the substance administered. Typically, such accumulation occurs in the lung following long-term exposure to insoluble dust particles deposited and retained in the lower respiratory tract. Cumulative doses lead to a compartmental (alveolar macrophages) lung overload that may over-proportionally increase their residence time in the lung as detailed in the later sections.

For the inhalation route, as long the dosing variables are kept constant, the well-known reciprocal relationship of concentration and time, that is,

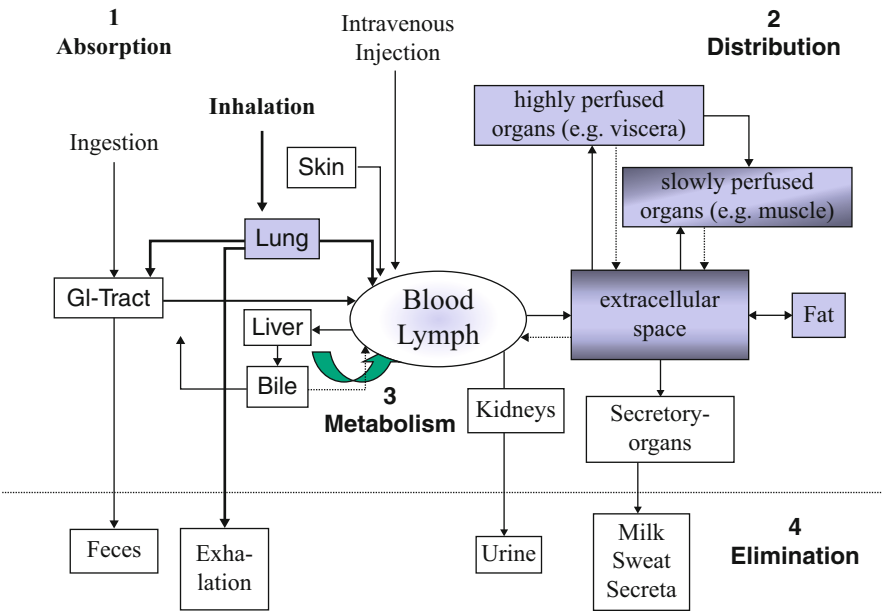



**Fig. 1** The four steps of toxicokinetics: absorption, distribution, metabolism, and excretion (ADME)

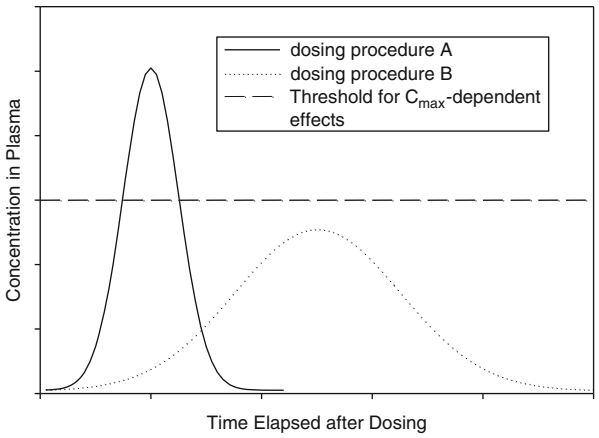



**Fig. 2** Theoretical curves representing the plasma concentration of a chemical at different times of dosing. *Curve A* represents the systemic dose from a substance administered by gavage, which is rapidly absorbed. *Curve B* represents the systemic dose from a dermally applied substance that is slowly absorbed. Assuming the area under the two curves is the same and the elimination capacity becomes saturated at a plasma concentration shown by the *broken line*, the toxic effect in A will be greater than in B and extrapolation between the two routes will either underestimate (B to A) or overestimate (A to B) the hazard. $C_{max}$-dependent toxicity, such as neuroexcitation, can reliably be estimated by route A only

$C^n \times t = constant\ effect$, is a valid approach (Haber's rule is fulfilled with $n = 1$). This rule is commonly understood in inhalation toxicology as a constant product of the two factors "exposure concentration in the inspired air" (C) and the "duration" (t) during which this concentration is inhaled to yield an identical intensity of biological response. A third factor has been considered, namely, the actual volume inhaled by the animal during the exposure period (t). The negligence of this last variable implies that the inhaled volume of a specified concentration of a hazardous substance in air is constant across exposure groups and need not be considered any further. This simplification is subject to challenge, especially when analyzing $C^n \times t$ relationships of irritant gases in small laboratory rodents which are known to instantly change their ventilation and respiratory patterns concentration dependently. Historically, Haber's rule has been used for time × concentration extrapolations assuming that each unit of damage is irreversible, that no repair takes place during the exposure period, and, therefore, that each unit of exposure is 100 % cumulative.

Physiologically based pharmacokinetic (PBPK) models are being used increasingly by regulatory agencies to estimate the internal dose of toxic/drug agents or their metabolites to target tissues. Using this technique, risk assessment for toxic substances can be based on estimates of the amount of the agent that reaches the target tissue, rather than the applied dose. In PBPK modeling, the pharmacokinetic behavior of a compound in the various compartments of the body is represented by equations that attempt to quantitatively describe actual physiological processes. The parameters of these equations are key anatomical and physiological descriptors of the organism. Thus, compartmental pharmacokinetic modeling is a powerful in silico tool for interrelating the accumulated target organ dose with the associated toxicological effect. Such models are useful for designing organ-specific drugs, "equivalent dose" testing protocols across species at differing exposure regimens, and to verify route-to-route extrapolations. Toxicokinetic studies pursue multiple objectives; these are summarized in Table 1.

## Extrapolation Across Species and Systems

Testing guidelines recommend standardized approaches on selected animal species for the evaluation of specific toxicological endpoints. The use of common laboratory animals for inhalation toxicity studies continually supplements the database and furthers the understanding of toxicity data in experimental animals and their relevance for man. Kinetic cornerstones become ultimately important when examining novel substances with a species-specific mode of action. When using alternative species or testing approaches kinetic data from target organs are indispensable to distentangle effects in regard to their dynamic (susceptibility) or kinetic (disposition) cause. The choice of animal species for regulatory testing is usually based on guideline requirements and practical considerations rather than validity for use in human beings. An animal species must be small enough to allow handling and exposure in sufficient numbers in relatively small inhalation chambers. An animal species, however, must be large enough to allow measurement of

**Table 1** Requirements for kinetic analyses

| Data requirement | Test system | Finding |
|---|---|---|
| Disposition "kinetic base set" | Single-dose studies to evaluate route-specific kinetic cornerstones | $C_{max}$, $t_{max}$, AUC, $t_{1/2}$ bioavailability, absorption rate (flux), parameterization for PBPK modeling, invasion and evasion kinetics |
| Disposition, "accumulation," organ-specific toxicity | Repeated dose study (e.g., oral, dermal, inhalation, intranasal, intravenous) | Parameterization for PBPK modeling, distribution within the organism at steady state, organ burden vs. associated organ-specific toxicity, saturation and/or adaptation, accumulation |
| Dosimetry and biomonitoring | Single to repeated administration/ exposure (e.g., oral, dermal, inhalation) | Proportionality of external or administered dose with "internal" biomarkers of exposure, including fate. Each exposure pathway displays its own relationship |
| Across-species comparisons/ extrapolations | Single to repeated administration/ exposure (e.g., oral, dermal, inhalation, intranasal, intravenous) | Species-specific differences in disposition, protein binding, organ burden, and associated toxic effects |
| Route-to-route extrapolation, exposure regimens | Single to repeated administration/ exposure (e.g., oral, dermal, inhalation) | $C_{max}$ and AUC of the parent chemical or metabolite in the blood or selected tissues |
| Modulation of absorption overload | Single to repeated administration/ exposure (e.g., oral, dermal, inhalation) | Vehicle, particle size, including solubility, surface area, and surface functionalization; amorphous or crystalline; excipients to modify absorption/clearance for drug delivery applications; competitive effects |
| PBPK-based study design | Single to repeated administration/ exposure (e.g., inhalation) | Optimization of particle size for pulmonary deposition, dose selection based on TK properties (accumulation, dissolution, translocation) of particles to attain lung burdens at non-overload to overload |

all endpoints relevant to identifying the inherent toxicity of the substance under investigation. Exposure paradigms may vary from small to larger animals, from animal bioassays to humans, as well as within the human population.

In summary, no animal species mimics man in all respects. Therefore, animal models are, at best, a necessary compromise and must be used because they offer the advantages of experimental control and reproducibility. Accordingly, animal model selection may often be contingent upon toxicodynamic or pathological identification of early changes consistent with the pathomechanism of the test substance. In general, the uncertainties of extrapolation of toxicological results across different species are minimized if a maximum of mechanistic understanding

is gained from a study. This is achieved by measurement of a sufficient number of procedures, endpoints, and incorporation of kinetic endpoints accounting for any species-specific differences in dosimetry and fate.

Recent progress in in vitro toxicology makes it necessary to consider pharmacokinetics also on cellular level to make comparisons possible of in vivo and in vitro models. New methods in the cultivation and exposure allow the direct exposure of lung cells thus providing means to analyze biological responses of cells during and following direct exposure to airborne materials at the air/liquid interface. Such systems are amenable of using cultured cells as an integrating biological dosimeter. Nonetheless, one has to recall that in vitro systems are typically designed for homogenous systems with water-soluble substances rather than insoluble and lipophilic particles which may have limited access to cells in aqueous culture media. To make cellular in vitro systems more practicable to human risk assessment, improved concepts for cellular dosimetry and kinetics are urgently needed.

## Extrapolation Across Routes

Risk assessors frequently have to use data obtained using a single route of administration. Most studies are carried out using the oral route (by gavage or in diet or drinking water). This is because such studies tend to be the most straightforward to perform and interpret, and dosimetry is easiest to quantify, particularly when a chemical is given by gavage. However, the differences that may occur due to exposure via high "bolus" systemic dosing resulting from administration by gavage, intratracheal or intranasal instillation, compared with prolonged systemic dosing resulting from administration via food or drinking water, the skin, or inhalation, need to be recognized. lf the metabolism or action at the target site of a chemical is critically influenced by concentration at any one time, rather than the total integrated systemic dose, the toxicity of that chemical may differ with route of administration or dosage regime if these affect the systemic concentration $\times$ time relationship (AUC) as shown schematically in Fig. 2.

The toxicity of a chemical may also be altered by previous exposure, for example, as a result of induction of metabolizing enzymes. Although these effects of the dosing regimen are important factors in the assessment of hazard, they are not specific to route-to-route extrapolation. Inhalation toxicity studies are technically much more complex: It is necessary to monitor the levels to which the animals are exposed, and in the case of aerosols, their size matters as it will influence the amounts that penetrate the upper respiratory tract (inhalable) and the alveoli (respirable). Other factors that govern deposition include water solubility and reactivity. Calculation of dose is much more complex compared with using the oral route, being dependent on the lung morphology, ventilation, etc., as well as the applied $C \times t$. There is therefore commonly a need to extrapolate toxicity data obtained using the oral route of administration in order to assess human health risks resulting from inhalation exposure.

Toxicity via oral and inhalation routes can differ remarkably when a dose rate-specific activating first-pass metabolism occurs. Aniline is given as an example.

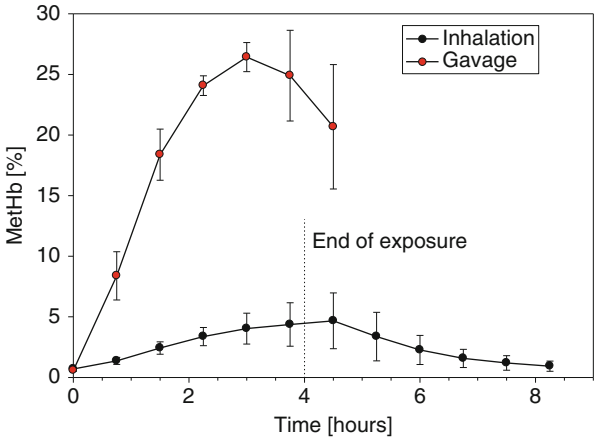



**Fig. 3** Time course of methemoglobin (MetHb) formation in beagle dogs. The dogs were either head-only exposed to aniline vapor for 4 h to 174 mg/m$^3$ or received 15 mg aniline/kg body weight by gavage. Blood was collected before exposure and thereafter in 45 min intervals (Data reproduced from Pauluhn (2002))

This substance is known to be bioactivated in the gastrointestinal mucosa and especially in the liver to become a MetHb-forming agent. It exerts toxicity to the red blood cells (RBC) through an active/reactive metabolite mechanism, mainly N-hydroxylated metabolites, which take part in cyclic redox processes. This *vicious cycle* depletes the RBC of factors to regenerate oxidized hemoglobin. Dogs were exposed by head-only inhalation to exclude dermal uptake for 4 h to 0.174 mg aniline/L air. When applying the typical respiratory minute volume of dogs, the total exposure dose is equal to 15 mg/kg body weight (0.36 L/kg-min $\times$ 240 min $\times$ 0.174 mg/L $=$ 15 mg/kg). The same dose was administered by gavage (Fig. 3). A fivefold lower potency of MetHb formation was observed following inhalation as compared to the bolus gavage. This appears to be related to the more efficacious hepatic first-pass bioactivation when administered via the gastrointestinal tract. Thus, for agents known to be bioactivated by a hepatic first-pass metabolism, the conversion of findings obtained from oral dosing to inhalation exposure concentrations is subject to errors. Likewise, in contrast to studies where the uptake is by the gastrointestinal route, the passage time-dependent modification of the test agent may be decisive for the toxic outcome.

Likewise, complex molecules can spontaneously decompose pH dependently in the gastrointestinal (GI) tract as shown for the zinc-propylenbisdithiocarbamate fungicide propineb (Fig. 4). The degree of decomposition can be estimated by the nonenzymatically formed reaction product TTCA (2-thiazolidinethione-4-carboxylic acid) in urine, a metabolite and biomarker of exposure to $CS_2$, through direct reaction with cysteine or glutathione. The dithiocarbamate formation is reversible under physiological conditions and provides a reservoir of $CS_2$ within biological systems. As can be deduced from Fig. 4, the intermediate concentrations of $CS_2$ and TTCA, including the new toxic entity PTU (propylenthiourea), and ionized zinc are substantially different following gastrointestinal and pulmonary exposure. Portal-of-entry-specific pulmonary changes may occur at lower doses due to the higher local concentrations of zinc and a diminished capacity of metabolizing $CS_2$. This makes different patterns of distribution likely to occur. In contrast to

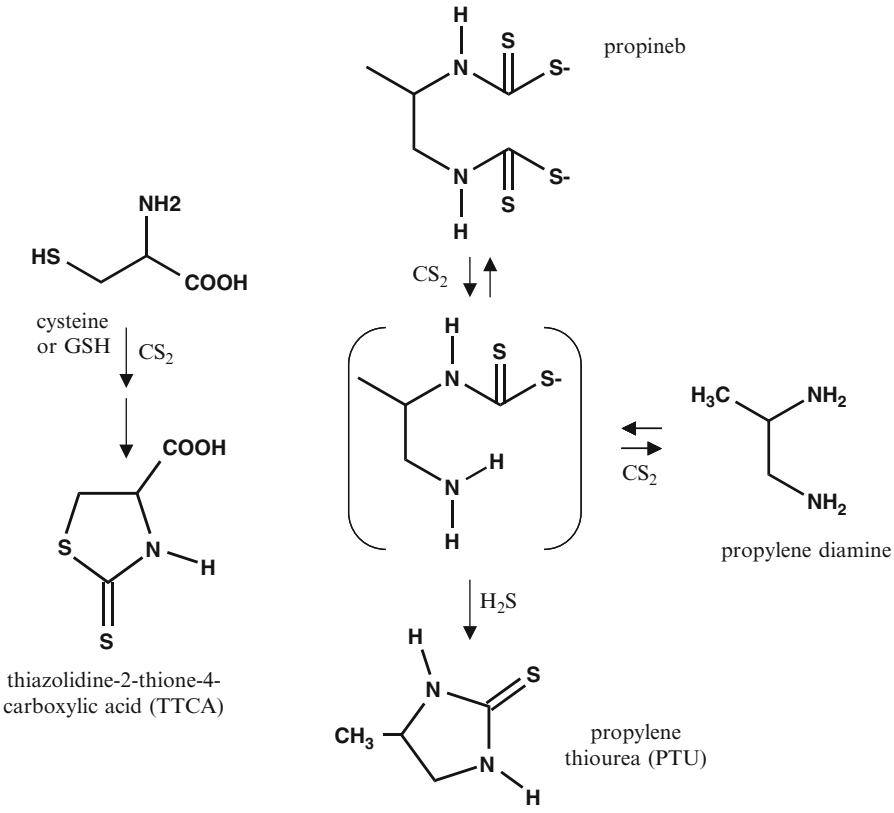



**Fig. 4** Conceptual scheme of decomposition of the zinc-propylenbisdithiocarbamate fungicide propineb under physiological conditions showing the generation of carbon disulfide ($CS_2$) and the subsequent reaction with free cysteine (or the cysteinyl moiety of glutathione) to form a metabolite that cyclizes to TTCA. In concept, this decomposition may also lead to the formation of propylene thiourea (PTU), a thyreotropic substance, (Adapted from Pauluhn and Rosenbruch (2003))

dietary routes, thyroidal (goitrogenic) effects have not been found following inhalation exposure, presumably because PTU is unlikely to be formed within the pulmonary environment. Thus, the formation of new toxicophoretic entities and portal-of-entry specific types of toxicities, including site-specific, often rate-dependent compensatory mechanisms, need to be accounted for before attempting such extrapolations.

The above considerations suggest that extrapolations from the oral to inhalation routes are by far not straightforward in the absence of adequate toxicity and biokinetic data. For route-to-route extrapolation to be appropriate, the toxicological concern often relates to systemic toxicity, that is, toxicity expressed in tissues/organs distant from the site of administration. However, even at this level of

simplification, expert judgment and PBPK data are needed to arrive at scientifically sound conclusions.

The key factors that need to be considered are briefly summarized below:

- Is the toxicity of concern a local or systemic effect?
- Is there any dependence on the extent of absorption and on the balance between any activation (where appropriate) and detoxification mechanisms? In case this can be materialized, the systemic toxicity may be the result of complex interactions, some of which could be route specific as well as dose-rate specific.
- Absorption is a determinant as to how efficiently a substance can be transported across biological barriers of the target organ dose, and implications of first-pass-like metabolism.
- Decomposition of a substance within the gastrointestinal tract with the formation of new toxicological entities (toxicophoresis) needs to be appreciated. This means the toxicodynamics and spectrum of organ toxicity change from one route to another.

## Toxicokinetic Data and Risk Assessment

In the application of pharmacokinetic models in risk assessment, the differences in compound disposition between humans and experimental animals are of particular interest. Therefore, there is a need to establish appropriate physiological values for humans and for the more commonly used laboratory species. To address this need, representative values and biologically plausible ranges of these values are provided for a number of anatomical and physiological parameters in multiple species. Representative values are available from the literature for most of the species commonly used in toxicology and risk assessment. As a result, these values can serve as reasonable, empirically based defaults that can be used in PBPK modeling when case-specific data are unavailable. Clearly, it is preferable to determine physiological and anatomical values directly on the individuals under study or, at least, on individuals known to be drawn from the same population and subjected to similar conditions.

Toxicokinetic studies provide historically an integral part of toxicological studies with drugs in order to allow direct comparisons of internal doses from preclinical animal studies to humans dosed with optimized drug delivery systems under clinical conditions. They also allow a specific search for the most relevant human-like animal species in regard to metabolism and pharmacodynamics.

## Toxicokinetic Readouts

### Time-Course Changes

Toxicokinetics commonly captures concentration measurements in plasma or blood of the parent substance or its metabolites. Also concentrations in organs,

such as the lung in inhalation studies, give not only invaluable information on the efficacy of the inhalation exposure; these data provide a robust basis whether measurements from blood can be used at all for the process of risk assessment. The elimination kinetics of insoluble particles from the lung depends on the degree of lung overload (see below). Thus, kinetic hallmarks characterizing the threshold dose of lung overload are a basic requirement for any meaningful extrapolation from animals to humans. Commonly, toxicokinetics focuses on the following parameters, AUC, which is a measure of the total systemic exposure to the chemical. AUC is an integral of the rate of change of concentration in plasma as a function of time. $C_{max}$ is the maximum plasma concentration (see Fig. 2), and $t_{max}$ is the time required to reach this maximum concentration after administration. They are determined at various time points after a dosing. Bioavailability, which is the fraction of chemical that is absorbed, is determined for extravascular administration with reference to an intravenous dose. An example for the compartmental kinetics of poorly soluble inhaled particles from the lung is given below (see Fig. 5).

## Selection of Compartment

The pharmacological efficacy of a drug substance depends on its time-dependent concentration at the site of targeted action (target organ). Especially in humans, this target site is often not directly accessible for specimen collection. As a surrogate, concentration of drug is commonly determined in the blood assuming a state of equilibrium among organs. Drugs targeted to the lung are preferentially administered by inhalation to increase the $C_{max}$ and AUC within this organ at equally lower systemic $C_{max}$ and AUC which then substantially reduce the systemic dose with less side effects. As a result of this strategy, the concentrations in the blood and in the target tissue may differ considerably. PBPK models can be used to calculate the most appropriate dosing interval to prevent accumulation and local toxicity to occur.

Aminoglycosides have several potential antibiotic mechanisms and are frequently administered by inhalation to attenuate/cure pulmonary infections. This class of drugs is known to have strong, irreversible binding to the ribosome and remains intracellular long after plasma levels drop. This allows a prolonged dosage interval. Depending on their local concentration, they act as bacteriostatic or bactericidal agents. These properties make these drugs amenable to interact inhaled dose proportionally with the phospholipids lining the lung resulting in dysfunctional surfactant due to aminoglycoside-phospholipid complexes which are engulfed by alveolar macrophages. Dose-proportional elevations suggestive of drug accumulation in the lung provide evidence of reversible drug sequestration in the lung with clearance proportional to the degradation of drug-phospholipoprotein complexes. By virtue of PBPK modeling, the complex relationship of drug sequestration and elimination can be estimated by PBPK modeling in spite of its potential to accumulate.

## Absorption, Distribution, and Elimination

### Absorption

For drugs, the absorption is usually as large as technically feasible to maximize systemic bioavailability or alternatively small when limited to the site of application (e.g., the lung). Absorption can potentially take place from all exposure routes (oral, dermal, inhalation, intranasal). The absorption rates and yields are highly route and excipient dependent or formulation dependent. Therefore, PBPK modeling requires exposure information to account for these differences. Biomonitoring using biomarkers of short-term or long-term exposure integrates the dose from all exposure routes. Caution is therefore advised to attribute data to one single route without considering the potential impact of other routes.

For rapidly absorbed chemicals, equilibrium may be established between blood and the site of absorption. The rate of entry into the blood is limited by the blood flow rather than any diffusion across the membrane barrier. In this case, any increase in blood flow will also increase the rate of absorption and absorption is said to be perfusion- or blood-flow-limited. For poorly absorbed substances, absorption is not dependent on the blood flow which means it is diffusion-rate-limited. Absorption is taking place by passive (mainly via pores or simple diffusion processes), active (transport against a concentration or electrochemical gradient) utilizing transcellular (through the cell) or paracellular (between cells) pathways. Solubility is an important factor driving the rate of absorption. For instance, particles deposited in the lower respiratory tract, in the absence of any specific protein binding or chelation, even poorly soluble substances (solubility in the range $\leq$1 mg/L water at physiological pH) are still rapidly cleared from the lung by dissolution.

### Absorption from the Skin

For the penetration and absorption of substances through the skin, the flux needs to be calculated using Fick's first law of diffusion to relate the flux rate (J in mg/cm$^2$/h) to the permeability, concentration, area of exposed surface, and length of exposure. An additional important parameter that needs to be utilized is the chemical's permeability coefficient (Kp). The permeability coefficient (in cm$^2$/h) should be consistent regardless of exposure concentration (provided that the concentration is infinite) and surface area for any given exposure site and chemical, but it can vary between exposure sites. Unless a mathematical model is used, the calculation of flux or permeability coefficient must be assessed at steady state. Mathematical models have been developed to attempt to describe percutaneous absorption kinetics. In almost every case, absorption through rodent skin is more than threefold higher than through human skin, and the increased absorption through rodent skin does not show a consistent pattern between compounds. The higher absorption in rodent skin may be due to differences in skin appendages (e.g., hair follicles) and different morphology of the individual skin layers.

An additional difference between human and rodent dermal absorption is the difference in the lag phase before the appearance of chemical in the blood; human absorption is delayed, whereas chemical absorption through the skin of rodents occurs with no apparent delay.

## Gastrointestinal Absorption

The rate of absorption from the gastrointestinal tract depends on the $pK_a$, solubility, lipophilicity, degree of ionization, as well as its residence time which is influenced by the tract's filling state, pH of microenvironment, and the active surface area of the respective segment. The milieu of the GI tract and the respective bacterial microflora can promote spontaneous intraluminal decomposition or chemical modification of substances (e.g., formation of nitrosamines in the stomach). Metabolic activation or deactivation of the absorbed substance from the intestine can take place in the subsequent passage through the liver (first-pass metabolism). The rate and extent of absorption of weak organic acids and bases varies with the location in the GI tract; weak acids are nonionized and are absorbed in the stomach, whereas weak bases are nonionized and are absorbed in the intestine. Removal from the site of absorption by blood flow maintains a concentration gradient, thus enhancing absorption of chemicals. The protonation of lipophilic chemicals within any specific sub-compartment may lead to intracellular substance accumulation by a "pH trap."

## Pulmonary Deposition, Retention, Clearance, and Absorption

The various species used in inhalation toxicology studies do not receive identical doses in comparable respiratory tract regions when exposed to the same external particle or gas concentration. The total body burden per unit body weight may also differ from one species to another because of differences in respiratory patterns and the respiratory minute volume. The dose metrics is also dependent of the local and/or systemic pathomechanism and may range from "total body burden" to "critical dose per alveolar macrophage" or "critical dose per cell volume or surface area of the most susceptible lung region." The biologic endpoint or health effect of concern may be more directly related to the quantitative pattern of mass deposited within the respiratory tract than to the external exposure concentration. Retention is the actual amount of inhaled agent found in the lungs at any time and is determined by the relative rates of deposition and clearance. Retention and the toxicologic properties of the inhaled agent are related to the magnitude of the pharmacologic, physiologic, or pathologic response. For particles, deposition mechanisms include inertial impaction, sedimentation (gravitational), diffusion, interception, and electrostatic precipitation. Generalizations regarding the site of deposition of particles of a given size are problematic due to the many factors involved. However, in the average adult human, most particles larger than 10 μm in aerodynamic diameter are

deposited in the nose or oral pharynx and are unlikely to penetrate to tissues distal to the larynx. Very fine particles (0.01 μm and smaller) are also trapped relatively efficiently in the upper airways by diffusion. Particles that penetrate beyond the upper airways are available to be deposited in the bronchial region and the deeper-lying airways. Sedimentation brings about deposition in the smaller bronchi, the bronchioles, and the alveolar spaces, where the airways are small and the velocity of airflow is low. As a particle moves downward through air, buoyancy and the resistance of air act on the particle in an upward direction, while gravitational force acts on the particle in a downward direction. Eventually, the gravitational force equilibrates with the sum of the buoyancy and the air resistance, and the particle continues to settle with a constant velocity known as the terminal settling velocity. Diffusion is an important factor in the deposition of submicrometer particles or gases. The clearance of deposited particles is an important aspect of lung defense. Rapid removal lessens the time available to cause direct tissue damage. Particles are cleared by the mucociliary escalator from the airways or may be phagocytized by alveolar macrophages and are ultimately transported to the mucociliary escalator. Even moderately soluble particles dissolve relatively rapidly in the lining fluids of the lung.

The mechanisms important for gases include convection, diffusion, chemical reaction (including metabolism), dissolution, and perfusion. Especially in obligate nose-breathing animals, absorption or "scrubbing" of a relatively water-soluble and/or reactive gas may occur from the inspired airstream as it travels from the extrathoracic to the pulmonary region. That is, the dose to the peripheral regions is affected by the dose to the region immediately proximal. For lung irritants, commonly an anterior-posterior gradient of intensity of damage of airways is observed, whereas the severity of toxicity also progresses distally with increased exposure concentrations. Although the deposition, clearance mechanisms, and physicochemical properties of the agent are often described as distinct properties, assessment of the overall toxicity requires integration of the various factors. Regional deposition pattern determines not only the initial lung tissue doses but also the specific pathways and rates by which the inhaled agents are cleared and redistributed or translocated.

## Compartmental Pulmonary Biokinetics of Poorly Soluble Particles

Elimination is usually a logarithmic process – that is, a constant proportion of the substance is eliminated per unit time which is described by a first-order relationship: $C_t = C_0 e^{-kt}$ where $C_t$ is the concentration after the time t, $C_0$ is the initial concentration at t = 0, and k is the elimination constant ($k_e$). The relationship between the elimination rate constant (ke) and halftime is given by the following Eq. 1:

$$C = C_0 e^{-k_e t} \quad \text{with} \quad ke = \frac{\ln 2}{t_{1/2}} \tag{1}$$

Halftime is dependent on the clearance (CL) and the volume of distribution ($V_d$) which are combined using the following relationships:

$$t_{1/2} = \frac{\ln 2 \times V_d}{CL} \tag{2}$$

with

$$CL = V_d \times ke \tag{3}$$

There is a common strong relationship between $V_d$ and body weight across species. This aspect has been observed when adjusting this endpoint across species. Any increase in the volume of distribution also increases the mean residence time of retained particulate matter (PM).

Following exposure to insoluble particles, there is an adaptive influx of alveolar macrophages. Thus, this concept considers possible changes in the dynamic increase of the phagocyte pool with increasing lung particle burdens. The clearance of deposited particles via phagocytosis is an important aspect of lung defense. Rapid removal lessens the time available to cause direct tissue interaction and damage. Retention is the actual amount of inhaled particles found in the lungs at any postexposure time and is determined by the relative rates of deposition and clearance. Those deposited in the alveoli are primarily phagocytized by alveolar macrophages which are ultimately transported to the mucociliary escalator and cleared mechanically via the airways. A small fraction of particles is also cleared via the lymphatic system draining the lung. The translocation of particles to the draining hilar lymph nodes (LALNs) commonly increases significantly at lung burdens high enough to cause pulmonary inflammation and barrier disruption. In regard to their alveolar and interstitial retention as well as lymphatic drainage, species differences between rats and humans exist. In rats particles are retained predominately in the airspaces, whereas in humans chronically inhaled PM are retained in the interstitium.

As a corollary of their function, alveolar macrophages engulf and retain inhaled particles. With increasing lung burdens, this may lead to a macrophage-load-dependent increase in cellular volume and/or an increased recruitment of phagocytic cells. Both relationships are presented in Fig. 5. Data are from rats exposed for 4 weeks to aerosolized dust (iron oxide, $Fe_3O_4$). This schematic representation of lung burdens demonstrates that no-adverse-effect levels can be predicted by PBPK modeling and that under conditions of lung overload, lung burdens (and toxic effects) increase overproportionally as a result of kinetic lung overload. It also shows that the retention kinetics increases with increasing lung burdens.

Unlike other laboratory animals and humans, rats appear to be more susceptible to overload-related effects due to impaired macrophage-mediated alveolar clearance. It was proposed that the threshold of causing particle-induced chronic effects is the pulmonary dose that results in a first reduction in macrophage-mediated clearance. Prevailing evidence suggests that a rats' macrophage-mediated clearance

is impaired at an estimated volumetric loading of 6 % or 1 μl particle-volume/g-lung. Significant impairment has been postulated to occur at 10 μl particle-volume/g-lung volumetric loading. The halftime of insoluble particles of alveolar clearance in rats under non-overloading conditions has been reported to be in the range of 60–90 days. Under conditions of lung overload, this halftime can increase to an extent exceeding the lifetime of rats.

The comparison of PBPK-modeled data with empirical data (Fig. 5) demonstrates the in silico attributes are an invaluable adjunct to inhalation toxicity studies in which particokinetics is indispensably linked to toxicity. PBPK-assisted study design of bioassays may reduce substantially the number of animals used in such bioassays and minimize testing of unduly high cumulative doses.

## Distribution

Once in the bloodstream, the substance is available for distribution and elimination throughout the body as detailed in Fig. 1. Metabolism and excretion, which are components of elimination, are discussed in other chapters.

Factors that influence the rate and extent of distribution of a chemical to a particular tissue include blood flow to the tissue (rate of delivery), the mass of the tissue, the ability of the chemical to cross membranes, and the affinity of the chemical for the tissue relative to blood. The rate of distribution of a chemical from blood to tissues can be perfusion- or diffusion-rate-limited. For lipophilic chemicals that rapidly cross membranes, the rate of delivery to tissues is limited by blood flow (perfusion-rate-limited). For polar and ionized chemicals that do not readily cross the plasma membrane, the rate of delivery to tissues is limited by diffusion (diffusion-rate-limited). Plasma protein binding increases the rate of distribution to tissues for toxicants that are not diffusion-rate-limited. The free toxicant may readily cross the capillary wall, effectively decreasing its free concentration in blood. Bound toxicant then dissociates from plasma proteins to maintain the equilibrium between the bound and free forms, yet the new free molecules rapidly leave the blood, which further increases dissociation of bound toxicant, and so on. In contrast, distribution of more polar compounds that are diffusion-rate-limited is dependent on the extent of protein binding.

Initial distribution is influenced primarily by blood flow to tissues, whereas final distribution is influenced primarily by the relative affinity of the chemical for various tissues relative to blood (i.e., the tissue partition coefficient). In the early phase of distribution, tissues that receive a high blood flow (e.g., liver, kidney, and brain) may achieve high concentrations of the chemical even though the tissue partition coefficient for that chemical is low. Likewise, tissues that are slowly perfused (e.g., adipose) may achieve a low concentration of the chemical in the early phase of distribution even though the tissue partition coefficient for that chemical is very high. Later in the distribution phase, however, the chemical redistributes to tissues based on tissue partition coefficients, and the chemical is more concentrated in tissues with relatively high partition coefficients.

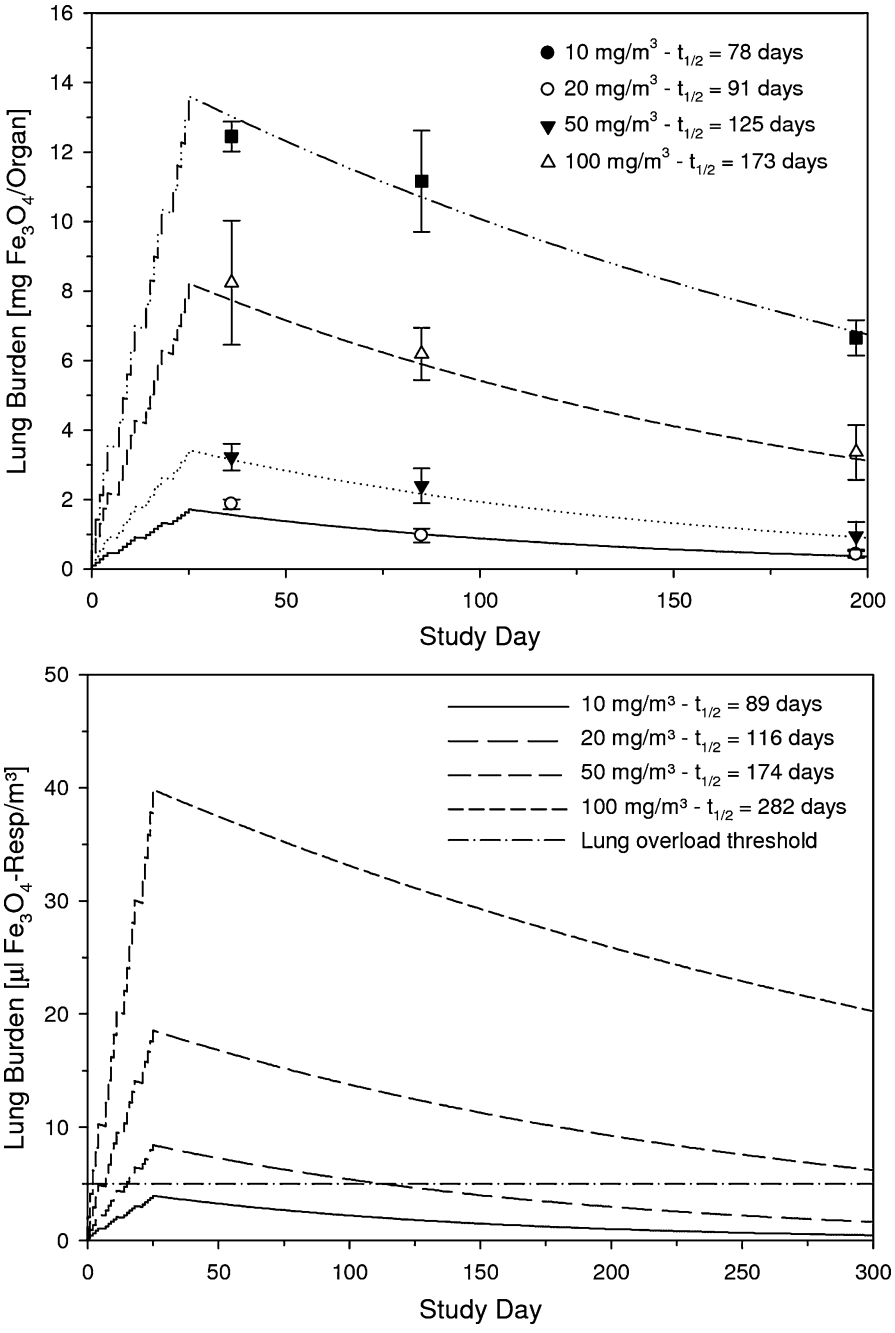



**Fig. 5** (continued)

### Excretion

In mammalian organism, several routes of elimination are available. The main excretory organ is the kidney. The kidneys receive 25 % of the cardiac output; about 20 % of this volume is glomerular filtration. Substances with a molecular weight less than 60 kDa are filtered by the glomeruli. As a result of this, protein-bound chemicals cannot readily be eliminated via this pathway. For effective renal elimination, lipophilic substances require biotransformation to more water-soluble metabolites, as lipophilic substances are subject to renal reabsorption, whilst hydrophilic metabolites may preferentially utilize active transport systems for organic anions and cations. Other excretory pathways are the gastrointestinal tract (excretion via the bile), secretory glands (e.g., milk, sweat, tears), and, for volatile substances, the lung (Fig. 1).

## Toxicokinetic Parameter

### Time-Course Analyses

Toxicokinetic processes are commonly described by either zero-order or first-order kinetic profiles. When applying a zero-order kinetics, the same amount of the substance, regardless of its initial concentration, is eliminated. Alcohol is a good example that is eliminated by zero-order kinetics; that means it is eliminated at a rate of about 25 mg/dL/h, a steady amount rather than a steady percentage in case of a first-order kinetics. Most substances are eliminated in something close to first-order kinetics. The biological half-life or elimination half-life of a substance is the time it takes for a substance or its metabolite to lose half of its biological activity. In a toxicological context, half-life describes the time it takes for the organ or blood plasma concentration of a substance to halve ("organ or plasma half-life") its steady state. The relationship between the biological and plasma half-lives of a substance can be complex depending on the substance in question, due to factors including accumulation in tissues, active metabolites, and receptor interactions. A typical example of a first-order retention and elimination kinetics has already been shown above (Fig. 5).

### Volume of Distribution

The apparent volume of distribution ($V_d$) is a proportionality constant that relates the amount of substance in the body to its concentration in plasma. It is the

**Fig. 5** Comparison of modeled and empirical lung burden of iron oxide in the lungs of rats exposed for 4 weeks (6 h/day, 5 days/week) (*top*). PBPK modeling of time and particle volume dependence of lung burdens relative to the kinetic lung overload threshold is defined as 5 μl/$m^3$ particle volume (alveolar fraction). The 4-week study was designed that this threshold was not attained at 10 mg/$m^3$ and minimally exceeded at 20 mg/$m^3$ at the end of the exposure period. At 50 and 100 mg/$m^3$, pulmonary toxicity was expected to occur without complete reversibility at the end of the 6-month postexposure period

theoretical volume of fluid the substance would occupy to achieve the observed concentration in plasma. For instance, a substance that is sequestered in a particular tissue will have a low concentration in plasma and a corresponding high volume of distribution, which may in fact be greater than the total body water. Thus, the volume of distribution has nothing to do with the actual volume of the body or its fluid compartments but rather involves the distribution of the drug within the body. Volume of distribution provides a reference for the plasma concentration expected for a given dose but provides little information about the specific pattern of distribution. Each drug is uniquely distributed in the body. Some drugs distribute mostly into fat, others remain in extracellular fluid, and others are bound extensively to specific tissues. Only unbound drug is available for passive diffusion to extravascular or tissue sites where the pharmacologic effects of the drug occur. Therefore, the unbound drug concentration in systemic circulation typically determines drug concentration at the active site and thus efficacy. The initial volume of distribution describes blood concentrations prior to attaining the apparent volume of distribution and uses the same formula.

### Area Under the Curve

The degree of systemic exposure is defined as the integral area under the concentration-time curve, area under the curve (AUC). It represents the change in concentration over time:

$$AUC = \int_0^\infty C dt$$

Bioavailability (F) is the absorbed fraction of a substance according to extravascular (e.g., by inhalation, oral, or dermal), relative to the intravenous dose. The appropriate surface integrals under the plasma concentration-time curve represents the bioavailability and is dependent on many factors such as route of administration, vehicle, or species.

$$F = \frac{D_{iv}\ AUC_{ex}}{D_{ex}\ AUC_{iv}}$$

## Significance of Toxicokinetics in Regulatory Toxicology

Toxicokinetic data are indispensable cornerstones to describe the fate, ecotoxicity, and mammalian toxicity of xenobiotics in the environment, to understand differences of their behavior in in vivo and in vitro bioassay systems, and are an essential prerequisite for risk assessment. The uncertainty involved in the extrapolation of

animal-based dosimetry to humans is significantly reduced in case in-depth pharmacokinetic is available. Kinetic information is used for hazard classification and PBT (persistent, bioaccumulative, toxic) assessment as well as wildlife and human food chain exposure modeling for the chemical safety assessment. It is also a factor in deciding whether long-term toxicology testing might be necessary. This is because chemical accumulation may result in internal concentrations of a substance in an organism that cause toxic effects over long-term exposures even when external concentrations are very small. Highly bioaccumulative chemicals may also transfer through the food web, which in some cases may lead to biomagnification.

The expression of toxicity arising from exposure to a substance is a consequence of a chain of events that results in the affected tissues of an organism receiving the ultimate toxicant in amounts that cause an adverse effect. The factors that confer susceptibility to certain species and lead to major differences between animals and humans, in their response to such chemical insults, are based either on the nature and quantity of the ultimate toxicant that is presented to the sensitive tissue (toxicokinetics, TK) or in the sensitivity of those tissues to the ultimate toxicant, that is, the toxicodynamic response. While their toxicokinetic data are mandatory for pharmaceuticals, there is no specific requirement to generate toxicokinetic information in the notification or authorization process of chemicals. Nonetheless, in the European REACH regulation, Annex I states that "the human health hazard assessment shall consider the toxicokinetic profile (i.e., absorption, metabolism, distribution and elimination) of the substance." Likewise, REACH announces in Annex VIII that one should perform "assessment of the toxicokinetic behavior of the substance to the extent that can be derived from the relevant available information."

Although TK is not a toxicological endpoint and is not specifically required by chemical regulations, the generation of TK information is definitely encouraged as a means to better interprete and amalgamate data from different sources as well as to assist testing strategy and study design, as well as category development, thus helping to optimize test designs: Prior to any animal study, it is crucial to identify the benefits that will be gained from conducting such a study. The TK behavior derived from available data might make further testing unnecessary in terms of predictability of other properties. The most critical factor influencing toxicity is the concentration of the ultimate toxicant at the actual target site (tissue dose). In this context, bioavailability is a relevant parameter for the assessment of the toxicity profile of a test substance. It links dose and concentration of a substance with the mode of action, which covers the key events within a complete sequence of events leading to toxicity.

The definition of actual TK studies on a case-by-case basis might further improve the knowledge about substance properties in terms of expanding knowledge on properties sufficiently to enable risk assessment. Overall the formation of data that are unlikely to be used and that constitute an unnecessary effort of animals, time, and resources shall be avoided using any supporting data to do so. Moreover, it can provide important information for the design of (subsequent) toxicity studies, for the application of read-across and building of categories. Taken together, along with other approaches, TK can contribute to reduction of animal use in toxicology and reduces uncertainty in risk assessment.

## Future Directions

With the advent of nanotechnology, nanostructures are increasingly investigated in in vitro cell culture systems. Particokinetics need to be improved and refined for insoluble structures for comparative in vivo/in vitro cellular dosimetry. Toxicokinetics may be playing a more significant role in predictive in silico toxicology with PBPK-modeled study design to illustrate the complex relationship between toxicity and physicochemical characteristics which make generalizations possible with less numbers of experimental animals. Theranostics (a portmanteau of *therapeutics* and *diagnostics*) is a proposed process of a targeted diagnostic therapy for individual patients for new principles of medication and to tailor treatment by a combination of pharmacokinetics and pharmacodynamics.

## References

Pauluhn J (2002) Aniline-induced methemoglobinemia in dogs: pitfalls of route-to-route extrapolations. Inhal Toxicol 14:959–973

Pauluhn J, Rosenbruch M (2003) Inhalation toxicity of propineb. Part I: results of subacute inhalation exposure studies in rats. Inhal Toxicol 5:411–434

Pauluhn J (2010) Multi-walled Carbon Nanotubes (Baytubes®): Approach for Derivation of Occupational Exposure Limit. Regulatory Pharmacology and Toxicology 57:78–89

Pauluhn J (2011) Poorly Soluble Particulates: Searching for a Unifying Denominator of Nanoparticles and Fine Particles for DNEL Estimation. Toxicology 279:176–188.

Pauluhn J, Wiemann, M (2011) Siderite ($FeCO_3$) and Magnetite ($Fe_3O_4$) Overload-Dependent Pulmonary Toxicity is determined by the Poorly Soluble Particle, not the Iron-Content. Inhalation Toxicology 23:763–783

## Recommended Reading

Environmental Protection Agency (EPA) (1994) Method for derivation of inhalation reference concentrations and application of inhalation dosimetry. United States Environmental Protection Agency, Washington, DC, EPA/600/8-90/066F

Milton MN, Christopher J (2009) Horvath the EMEA guideline on first-in-human clinical trials and its impact on pharmaceutical development. Toxicol Pathol 37:363–371

Zhang D, Comezoglu SN (2008) Chapter 18. ADME studies in animals and humans: experimental design, metabolite profiling and identification, and data presentation. In: Zhang D, Zhu M, Griffith Humphreys W (eds) Drug metabolism in drug design and development: basic concepts and practice. Wiley, Hoboken, pp 503–604. Food and Drug Administration. http://www.fda.gov/cder/guidance/index.htm

## Resources

REACH-Guidance on information requirements and chemical safety assessment, Chapter R.7c: endpoint specific guidance Chapter R.11: PBT assessment. http://echa.europa.eu/documents/

# Toxicodynamic Tests

Dieter Schrenk

## Contents

Isolated Target Molecules ..... 162
Nucleic Acids ..... 162
Proteins/Enzymes ..... 162
Lipids ..... 163
Subcellular Fractions/Organelles ..... 163
Membranes: Cytoplasmic Fraction ..... 163
Receptors ..... 164
Transfer Through Biological Membranes (Ion Channels, Transporters, and Pumps) ..... 166
Mitochondria ..... 166
Nuclei ..... 166
Cells ..... 166
Primary Cells ..... 166
Permanent Cell Lines ..... 167
Tissues ..... 168
Isolated Organs ..... 168
Tissue Slices ..... 168
Experimental Animals ..... 169
Acute Toxicity/Organ Toxicity ..... 169
Chronic Toxicity/Organ Toxicity ..... 169
Other Modes of Action ..... 169
Genotoxic and Carcinogenic Effects ..... 170
Teratogenicity and Developmental Toxicity ..... 171
Reproductive Toxicity ..... 171
Investigations in Humans ..... 171
Recommended Reading ..... 172

D. Schrenk
Food Chemistry and Toxicology, Technical University of Kaiserslautern, Kaiserslautern, Germany
e-mail: schrenk@rhrk.uni-kl.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_39, 

**Abstract**

Toxicodynamic testing is aimed at the elucidation of adverse effects of chemicals including understanding of their mode of action. In many cases, the standard program of toxicological testing on acute, subchronic, or chronic toxicity, genotoxicity, carcinogenicity, teratogenicity, developmental and reproductive toxicity already provides important information on the mode(s) of action of a compound. Targeted mechanistic investigations often follow which use specifically designed models such as genetically modified cells or animals, studies using specific cell types, subcellular fractions, enzymes, etc. The understanding of the mechanisms underlying a certain mode of action is of crucial importance in human risk assessment/regulatory toxicology of chemicals since it allows decisions on the options for extrapolation of experimental data to the human situation.

This text follows the different levels of experimental models in toxicodynamic testing, as shown in Fig. 1.

## Isolated Target Molecules

### Nucleic Acids

Isolated nucleic acids of various degrees of purification can be obtained from different sources (DNA from calf thymus, herring sperm, tissue cultures, etc.) incubated with a chemical and/or its metabolites. The latter can be generated by adding activating enzymes ("S9 mix") to the incubation. Subsequently, the nucleic acids are extracted and analyzed, e.g., for covalent binding of the chemical and/or its metabolites. The chemicals can be labeled, e.g., by radioactive isotopes in order to facilitate screening for adducts. Alternatively, the nucleic acids or nucleosides (after hydrolysis) including modified nucleosides can be post-labeled for further separation and identification.

### Proteins/Enzymes

The chemical or material of interest can be incubated with tissue or cell homogenates or with purified enzymes or other proteins. Assays are aimed at testing covalent or noncovalent binding, but also functional effects on proteins. Well-known examples are the inhibition of acetylcholine esterase by organic phosphates, binding of inhibitors of mitosis to tubulin in the spindle apparatus, or enzyme inhibition by certain heavy metals such as mercury ions. In the course of such tests, information on the type of inhibition can be derived from concentration-effect analysis using a variety of inhibitor concentrations.

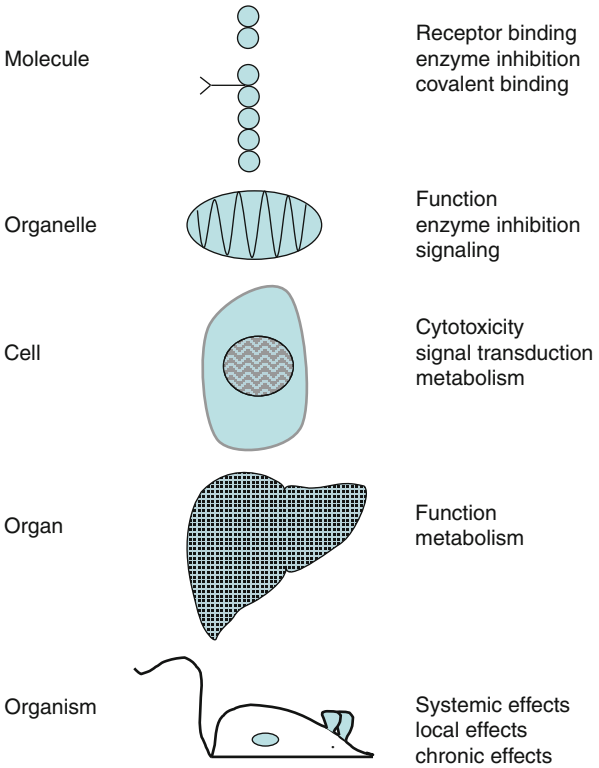



**Fig. 1** Levels of experimental models in toxicodynamics testing

## Lipids

Incubating purified lipids with test compounds or their metabolites can also be used to identify possible covalent or noncovalent binding. Again, addition of an enzyme preparation can be used to modify, e.g., activate, the test compound.

## Subcellular Fractions/Organelles

### Membranes: Cytoplasmic Fraction

The common method to isolate membrane fractions is sequential centrifugation. Likewise, a total membrane fraction can be isolated from a liver homogenate by ultracentrifugation at 100,000 × g, after nuclei, mitochondria, etc. have been sorted out at lower g numbers. The supernatant of the membrane fraction represents the soluble cytosolic fractions, sometimes called "cytosol." The sediment ('membrane fraction') can be re-suspended and subjected to additional (gradient) centrifugation in order to enrich certain types of membranes. Following this approach, fractions

enriched in endoplasmic reticulum– or outer cellular membrane–derived membranes can be prepared. The degree of enrichment can be verified by measuring the presence or activity of marker proteins.

Such fractions can be used for the investigation of membrane-bound (CYPs, UGTs, etc.) or cytoplasmic (GSTs, STs, etc.) enzyme activities, induction, inhibition, etc. Furthermore, the metabolism of chemicals including genotoxic carcinogens, leading eventually to mutagenicity, DNA-binding etc., can be analyzed.

## Receptors

In a strict sense, receptors acts as triggers of signaling chains responding to agonistic molecules by binding and change in receptor conformation. A typical consequence of receptor activation is the formation of an intracellular signal molecule called "second messenger." Likewise, the binding of noradrenalin to $ß_1$-adrenoceptors can result in enhanced intracellular formation of the second messenger cAMP. Xenobiotic chemicals can act on both membrane-bound receptors on the outer cellular membrane and on intracellular receptors, being located, e.g., in the cytoplasm or the nucleus. Also, trafficking of activated receptors, i.e., translocation from the site of ligand binding to the site of effect, is common. Xenobiotic ligands can mimic endogenous ligands, thus activating receptors thought to be responsive to hormones, transmitters, etc. In some cases, endogenous ligands are unknown ("orphan receptors") or there is no scientific agreement on the identity of "the endogenous ligand" although a variety of endogenous compounds can bind to the receptor.

Effects of xenobiotic chemicals on receptors have been widely described and are considered as a central field in toxicodynamics research. In many instances, such effects are wanted, representing a fundamental mode of action of many therapeutic drugs. In toxicology, receptor activation can be crucial for many adverse effects: The binding of dioxins to the aryl hydrocarbon receptor (AhR) is the most prominent example. A major field of research on xenobiotic-responsive receptors is the adaptive response of drug-metabolizing enzymes called "induction." This phenomenon, which can have adverse consequences for the organism, is used as a marker for certain types of induction being monitored as a battery regulated of genes/enzymes. Some important examples for such concerted responses are given in Table 1 listing, e.g., the AhR, CAR (constitutive androstane receptor), PXR (pregnane-X receptor; Fig. 2), or the PPARs (peroxisome proliferator-activated receptors).

Ligand binding to the receptor can be agonistic, partially agonistic, or antagonistic. This classification can depend on receptor subtype, cell type, species, etc. Furthermore, a compound can bind to an alternative ("allosteric") binding site on the receptor, thus modulating the affinity and/or effect transmission capacity of the "real" ligand which binds to the ligand binding site. These phenomena can be studied including bindings assays in receptor-enriched tissue fractions or transfected cell lines which (over)express express the receptor of interest, e.g., combined with a specific reporter gene construct.

**Table 1** "Xenobiotic" receptors regulating expression of drug-metabolizing enzymes

| Receptor | Chemical/compound | Inducible enzyme(s) |
|---|---|---|
| Arylhydrocarbon receptor (AhR) | DL-PCBs, PAHs, TCDD | CYP1A1, 1A2, 1B1 |
| Constitutive active (androstane) receptor (CAR) | DDT, NDL-PCBs, phenobarbital, | CYP2B1, 2B2, 2B6, UGT2 |
| Peroxisome proliferator-activated receptors (PPARs) | Fibrates, phthalates (diethylhexyl phthalate) | CYP4A |
| Pregnane-X-receptor (PXR) | Clotrimazole, dexamethasone (rodents), HBCD, pregnenolone-16α-carbonitrile, rifampicin | CYP3A, 7A1, OATP2, MRP2, MDR1/Pgp |
| Nrf2 (via antioxidant-responsive element; ARE) | BHA, BHT, t-butylated hydroquinone, | GSTYa, M, P1, NQO1 |

Abbreviations: *BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene, *CYP* cytochrome P450, *DL-PCB* dioxin-like polychlorinated biphenyls, *GST* glutathione-S-transferase, *HBCD* hexabromocyclododecane, *MRP* multidrug resistance-associated protein, *MDR*/*Pgp* multidrug resistance protein/P-glycoprotein, *NQO* NAD(P)H-quinone oxidoreductase, *NDL-PCB* non-dioxin-like polychlorinated biphenyls, *OATP* organic anion transporter, *PAH* polycyclic aromatic hydrocarbons, *TCDD* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, *UGT, UDP*-glucuronosyl transferase

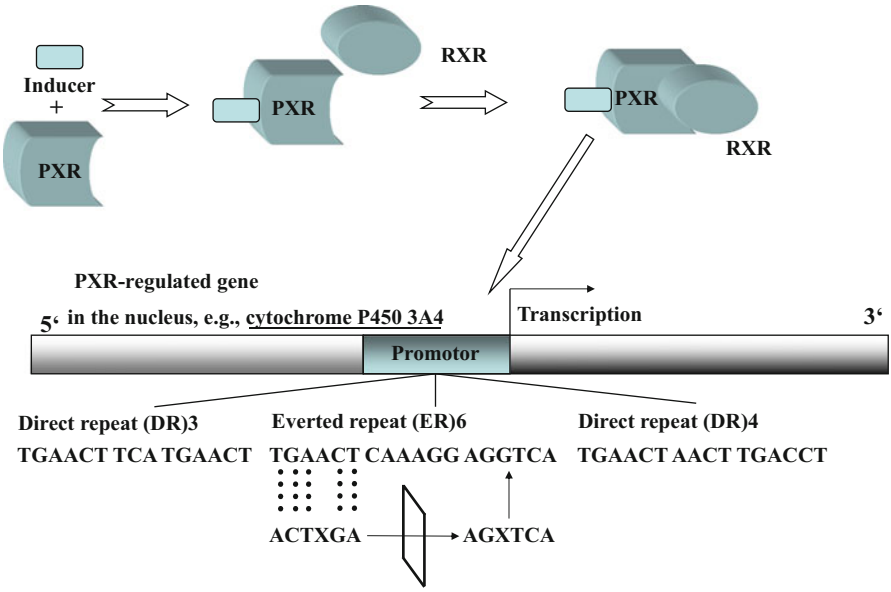



**Fig. 2** Induction of gene expression via the pregnane-x-receptor (*PXR*). Upon ligand binding, the receptor dimerizes with the retinoid-X-receptor (*RXR*). The dimer binds to consensus sequences (direct repeats, everted repeats) in the 5′-flanking region of responsive genes, thus modulating their transcription

### Transfer Through Biological Membranes (Ion Channels, Transporters, and Pumps)

In most cases, the function of ion channels, transmembrane transporters, and pumps is investigated using membrane fractions since most of these proteins are embedded in membranes. From the latter, vesicles can be prepared which can be used for transport studies, e.g., with radioactively labeled transport substrates. Such models are suitable for the analysis of: the binding affinity of standard substrates, modulation of transport function, the properties of a test compound as transport substrate, conformational changes in protein structure upon substrate binding, etc. Furthermore, cell cultures can be applied in order to investigate the consequences of a targeted overexpression of a certain transmembrane protein, its genetic elimination ("knockout"), or selective inhibition by antagonists.

Finally, transmembrane transfer proteins can be regulated at the level of gene expression and localization within the cell ("trafficking") or tissue, in tissue culture or whole organisms.

### Mitochondria

Mechanistic studies in isolated mitochondria comprise the investigation of mitochondrial damage (loss of physiological function) and mitochondrial signaling. Mitochondrial enzymes involved in oxidative phosphorylation/ATP production and oxygen consumption ("respiratory chain") are typical targets of chemicals (blocking of respiration, uncoupling of oxygen consumption and ATP formation, etc.). Signaling compounds released by damaged mitochondria comprise cytochrome C, calcium ions, and many others. Gross change in mitochondrial function can be measured as changes in membrane potential, proton concentrations, oxygen consumption, calcium flow, ATP/ADP ratio, etc.

### Nuclei

Isolated nuclei are used for mechanistic studies investigating effects of chemicals on gene transcription (nuclear run-on assays), covalent and/or noncovalent ("intercalation") binding to DNA, modifications of chromatin, effects on nucleosomes or on DNA/chromatin processing enzymes (topoisomerases, nucleic acid polymerases, etc.).

## Cells

### Primary Cells

Cells isolated from certain organs or tissues of humans or experimental animals such as liver, lung, kidney, or immune cells usually comprise a mixture of several cell

types. The cell preparations are obtained, e.g., by perfusion of the organs with media which disintegrate the tissue or by lavage of the organ surface (e.g., pulmonary epithelia). Individual cell types, e.g., hepatocytes (liver), alveolar cells type I (lung), or macrophages (blood, tissues), can be prepared from mixtures of different cell types by sequential centrifugation/density gradient centrifugation. Many primary cell types can be seeded and adhere on uncovered or specifically covered Petri dishes or tissue culture flasks. The culture conditions usually aim at keeping the cells as long as possible in their differentiated state, i.e., to maintain their tissue-specific ("in situ") properties and functions. In most instances, this aim cannot be achieved completely and/or differentiation is partially lost during culture. Usually, permanent cells undergo senescence or lose their specific phenotype after a certain time in culture.

Parameters which allow conclusions on the mode of action of a chemical in cell cultures include cytotoxicity and cell death, effects on cell culture density, proliferation, apoptosis as well as changes in protein synthesis or growth behavior (e.g., loss of contact inhibition, growth in soft agar). Likewise, the mechanisms leading to necrosis or apoptosis in cell culture are investigated in detail. Hallmarks of molecular pathways are activation of receptors (Fas receptor; TGF-ß1 receptor, etc.), mitochondrial signaling, changes in apoptosis-regulating factors (TNF alpha, bcl-2, bax, p53, etc.), or activation of caspases. In such investigations, various cell types equipped with different receptors as well as various derivatives of the test compound can be used. Furthermore, "omics" analyses detecting changes in gene expression (gene arrays, etc.), protein patterns (proteomics), and endogenous metabolites (metabonomics) play a more and more important role in identifying the cellular mode of action of a chemical. In more specific studies, secretion of certain growth factors or tissue hormones, matrix-cell interactions, release of transmitters, etc. are analyzed. The effects of such changes can be measured directly in co-cultures with respective responder cells (e.g., immune cells). In addition, certain biochemical effects such as enzyme inhibition, binding to nucleophilic targets, generation of reactive oxygen species, etc. can also be analyzed in primary cell cultures.

Of particular interest in toxicology is the investigation of genotoxic events in primary cells. These analyses comprise the determination of modified DNA bases, DNA fragmentation, mutations, micronuclei formation, chromosomal changes, DNA repair, etc.

## Permanent Cell Lines

In contrast to many primary cells in culture, permanent cell lines always proliferate in culture being harvested from the culture plate and seeded onto empty Petri dishes. This "passaging" can virtually be used as an infinite source of cells. However, permanent cells frequently change their properties after several rounds of passaging. Thus, the passage number should be provided as an additional source of information in experiments with permanent cells.

Permanent cell lines are of limited use in the study of the mode of action of a chemical because they usually differ more or less from the corresponding primary

cell type. In many instances, permanent cell lines are derived from tumors exhibiting profound changes in genotype and phenotype when compared to normal cells. For the successful use of permanent cells lines, their properties should be investigated as far as possible. A focused analysis of effects on defined signaling pathways, which are known to be regulated in a similar way in primary cells, is a typical example for such use.

## Tissues

### Isolated Organs

Isolated perfused organs such as liver, lung, heart, intestine, or kidney from rat, rabbit, or guinea pig represent widely used models for the study of the mode of action of a chemical in toxicological research. They allow, e.g., the study of necrotic cell damage and its modulation by inhibitors of metabolic activation or by the addition of protective substances (e.g., of acetylcysteine in paracetamol-mediated liver damage). Furthermore, the issue of localization of the damage or of the underlying biochemical pathway can be addressed. Likewise, perfusion with an acute nephrotoxicant allows the determination of the exact site of tubular damage or the role of glutathione depletion in such a scenario. The perfusion rate (flow) and pressure characteristics itself can be of interest in analyzing the pathogenesis of a damage, e.g., in particular in lung or kidney. In addition, "functional" effects in an isolated organ such as changes in heart rate, uterus contraction, etc. can be detected. The duration of experiments with isolated organs is limited by the lifespan of the organ being between one and a few hours. In many cases, this time is sufficient, however, to obtain relevant amounts of metabolites from a chemical or sufficient organ damage, depending of course on the start concentration of substrate. A novel development in tissue research is the use of organs isolated from domestic animals such as pigs or cows from slaughterhouses. This method allows the reduction in numbers of experimental animals, and benefits from the relatively close relationship between porcine and human physiology when compared to rodents.

### Tissue Slices

Studies in tissue slices allow one to address many questions which can also be dealt with in isolated perfused organs or in cell culture. Thus, this model is positioned between cells and intact organs. Tissue slices are easy to prepare and use (no difficult preparation, no perfusion equipment, etc.) but lack the physiological perfusion via the blood vessels. Nevertheless, tissue slices in many instances allow relevant conclusions about the type of tissue damage, xenobiotic metabolism, and its modulation or complex changes in gene expression.

## Experimental Animals

### Acute Toxicity/Organ Toxicity

Experimental animals represent the most relevant model for the comprehensive prediction of adverse effects of chemicals in humans. Also studies on the mode(s) of action of a chemical can be performed in animals covering many various aspects. For example, the effects of a chemical on certain enzyme activities, levels of hormones, growth factors, etc. in blood or target tissues can be investigated. Furthermore, a broad spectrum of parameters of organ function and morphology (histopathological analysis) can be carried out. Form the complex picture thus obtained, conclusions can be drawn on the possible mode of action. These can be substantiated by the target application of modulators such as enzyme inhibitors. Furthermore, studies on effects on gene expression ("genomics"), protein levels ("proteomics"), endogenous metabolites ("metabonomics"), or the metabolism of the xenobiotic chemical of interest ("metabolomics") are essential parts of the current broad approach in toxicological research.

Using modern methods of genetic engineering and breeding, genetically modified strains can be obtained which allows further conclusions on molecular targets. Examples are rodent strains with deleted or silenced genes ("knockout" animals) or strains which overexpress a certain homologous or heterologous ("humanized") gene. Likewise, the study of Ah receptor-knockout mice has provided crucial insight into the biology of this receptor and its role in dioxin toxicity.

### Chronic Toxicity/Organ Toxicity

The investigations (and prediction) of chronic adverse effects of a chemical represent the most challenging task in toxicological research (see chapter "► Examination of Acute and Chronic Toxicity"). The relevant changes are mostly unknown when the experiment starts. Furthermore, exposure in a certain time window may be the most relevant. In any case, animal experiments still are the only reliable tool in predicting chronic toxicity in humans. Accompanying in vitro studies can be applied to obtain more information on the molecular mechanisms or mode of action underlying adverse effects observed in chronic animal studies.

### Other Modes of Action

Targeted analyses in animal testing are aimed at understanding mode(s) of action. They make use of the broad pattern of biochemical and pharmacological testing approaches such as changes in intestinal passage, blood flow, arterial blood pressure, bile flow, renal blood flow, inulin clearance, to mention a few.

However, a minor temporal change in bile flow or blood pressure does not necessarily represent an adverse effect since it also occurs under physiological conditions representing reversible, adaptive responses (see chapter "► Adverse Effects versus Non-adverse Effects in Toxicology"). Such observations can be very helpful, however, in the understanding of a mode of action and may even be useful in the development of new therapeutic drugs. Additional experiments frequently follow in order to clarify the molecular mechanisms leading to the observed mode of action, e.g., an induction of a biliary export pump in increased bile flow. The induction of drug-metabolizing enzymes is another example of a frequently observed, adaptive consequence of xenobiotic exposure in laboratory animals.

## Genotoxic and Carcinogenic Effects

Mechanisms of genotoxic effects can be found in most of the aforementioned experimental models. Following the paradigm that mutagenic effects and carcinogenic primary ("initiating") lesions are permanent changes in nuclear DNA, the investigation of genotoxic events is focused on DNA. They include bacterial (Ames test) or yeast cells, mammalian cell lines (sister chromatid exchange, micronucleus test, HPRT assay, comet assay, etc.), or intact animals (mouse micronucleus assay) identifying DNA strand breaks, mutations, and aneugenic or clastogenic effects.

The enormous complexity of the carcinogenic process does not allow a comprehensive short-term testing for carcinogenicity. The multistage concept of carcinogenesis suggests the existence of a primary lesion, which predisposes the "initiated" cell for a development into a malignant cell passing various stages. These stages, also termed as promotion and progression, require the presence of additional factors which allows the cell to proceed on this way. It is unclear if these additional steps involve or even require specific genetic changes. Furthermore, predisposing genetic changes in "normal" cells may make those cells vulnerable to additional factors and may even be inherited by the organism. Examples for such predispositions are the familial polyposis coli with respect to colon cancer or the hereditary disposition for breast cancer. A widely used tool to investigate the multistage development of cancer is hepatocarcinogenesis in rodents. In this model, certain mutations in critical genes (hot spots), e.g., in the H-Ras proto-oncogene, are linked to the initiation step. The subsequent phase of promotion can be facilitated by chemical factors (tumor promoters) which may inhibit apoptosis of initiated cells, e.g., by suppression of pro-apoptotic pathways or by inhibition of intercellular signaling, etc. Likewise, certain receptors, such as CAR, PPAR alpha, ER, and growth hormone receptor, can mediate the promotion effect. Detailed studies, e.g., with humanized mice have led to the suggestion that receptor-mediated liver tumor promotion, e.g., with phenobarbital, can markedly differ

between rodents and human, depending on receptor-mediated signaling. These studies illustrate the difficulties in the use of rodent-derived tumor-promotion data in regulatory toxicology.

### Teratogenicity and Developmental Toxicity

These investigations make use of almost all aforementioned experimental models using subcellular, cellular, organ, tissue, or whole animal systems. In addition to animal experiments in rodents, birds, and amphibians, mechanistic studies are aimed at the role of receptors (Retinoid receptors, PPARs). Exposure of dams during pregnancy/lactation does ideally not lead to maternal toxicity. While malformations are frequently seen after birth, developmental effects can occur at later life stages or even only become visible at more advanced stages (learning behavior, etc.) or when the fertility of the offspring is investigated ("multi-generation study").

### Reproductive Toxicity

Detailed studies on reproductive toxicity of a chemical in experimental animals comprise macroscopic and microscopic investigation of changes in the reproductive organs, reproductive behavior, perturbations of steroid hormone homeostasis and metabolism, receptor-linked effects etc. including an analysis of fertility and reproductive success.

## Investigations in Humans

Toxicodynamic studies in humans include those during development of new drugs. Here, pharmacological studies can provide information on possible unwanted/adverse effects. Furthermore, interferences of chemicals with the signaling or metabolism of other compounds or substrates including endogenous compounds are of interest. In the field of receptors and drug-metabolizing enzymes, genetic polymorphisms have been identified in humans such as polymorphisms in the CYP2D6, CYP2C19, NAT2, GST μ, genes, etc. These can result in toxicokinetic effects on the fate of chemicals which may have strong implications for the toxicodynamics. The methods used to identify those polymorphisms comprise DNA investigations looking for point mutations or single nucleotide polymorphisms, as well as gene expression analysis such as RT-PCR, western blotting, or enzyme assays. Metabolism tests in healthy human volunteers are widely used to investigate the consequences of genetic polymorphisms of this type on the kinetics of standard substrates such as caffeine.

## Recommended Reading

Anderson MW, Reynolds SH, You M, Maromnpot RM (1992) Role of proto-oncogene activation in carcinogenesis. Environ Health Perspect 98:13–24

Gregus Z, Klaassen CD (2001) Mechanisms of toxicity. In: Klaassen CD (ed) Casarett and Doull's toxicology. McGraw-Hill, New York

Hayes AW (2001) Principles and methods of toxiocology, 4th edn. Taylor and Francis, New York

Pumford NR, Halmes NC (1997) Protein targets of xenobiotic reactive intermediates. Annu Rev Pharmacol Toxicol 37:91–117

Wyllie AH (1997) Apoptosis: an overview. Br Med Bull 53:451–465

# Omics in Toxicology

Heidrun Ellinger-Ziegelbauer and Hans-Juergen Ahr

## Contents

Omics Technologies ..... 173
Toxicogenomics ..... 174
Proteomics ..... 176
Metabolomics ..... 176
Toxicogenomics in Investigational Toxicology ..... 176
Areas of Applications of Toxicogenomics in Toxicology ..... 178
References ..... 179

**Abstract**

The tremendous progress in the development of new technologies in the areas of molecular biology and bioinformatics enables interrogation of cellular responses to toxicant treatment at a global molecular level, allowing evaluation of toxic effects in the context of molecular pathways.

The major techniques currently employed, especially transcriptomics, but also proteomics and metabolomics, are being used and further evaluated in investigational toxicology. Since they already have been shown to provide increased insight into molecular mechanisms of toxicological effects, regulatory authorities are evaluating such data to prepare themselves for use in regulatory toxicology.

## Omics Technologies

Advancements in molecular biology research, especially the development of the microarrays in the 1990s, allowed the development of new technologies to

H. Ellinger-Ziegelbauer (✉) • H.-J. Ahr
Investigational Toxicology, Bayer Pharma AG, Wuppertal, Germany
e-mail: heidrun.ellinger-ziegelbauer@bayer.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_40, 

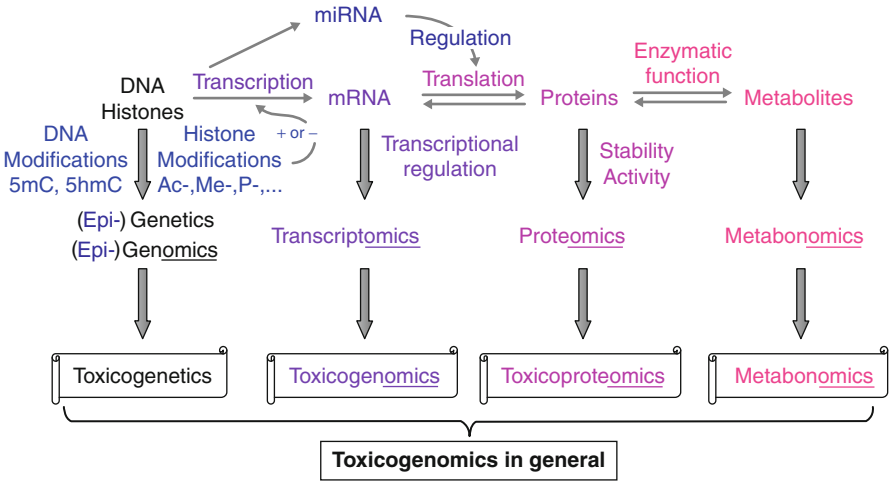



**Fig. 1** Omics technologies applied in toxicology for global analyses of the major molecules present in biological samples represent the major levels of gene expression and cellular pathways. Toxicogenomics may also be used as a general term for omics applied to toxicological studies

perform detailed analyses of fundamental processes in living organisms. These so-called omics technologies enable the simultaneous measurement of all definable entities of an "-ome," corresponding to a certain class of molecules in biology. Due to rapid development of DNA sequencing techniques, many genomes have now been completely sequenced, including human, mouse, and rat (genomics). Parallel analysis of all expressed genes in an organ or cell at the mRNA (transcriptomics) and protein (proteomics) level provides much increased insight into biological processes at the molecular level. Recently similar analyses of noncoding RNAs, including microRNAs, have been added as omics tool. Finally, measurement of all metabolites in cells and tissues or in body fluids indicates functional changes of cellular metabolism (metabolomics).

In general, the different omics technologies deliver complementary data, and thus one technology is likely not sufficient to reveal all molecular processes interacting at the cellular or organ level (Fig. 1). Yet even if just one technology is used, it does increase our knowledge of toxicological processes. Currently, the technical requirements and maturity of the individual technologies are different.

## Toxicogenomics

Measurement and analysis of gene expression profiles (transcriptomics) under the influence of chemical stressors or toxic compounds (toxicogenomics) or in disease states is technically the most advanced among omics technologies. This is due to the underlying low complexity of the molecules to be measured, which are

represented by a combination of only four bases obeying to clear principles of complementary base pairing, and to the relatively simple chemistry behind these molecules being experimentally easily accessible. Efficient techniques for quantitative measurement of the expression levels of many genes in a single sample could therefore be developed.

The first methods for global expression profiling were relatively elaborate open-profiling methods like *differential display*, which are based on conversion of all mRNAs in a sample into cDNA and extensive further processing to display differential expression levels. These are hardly used anymore. The emergence of whole genome *microarrays* represented a breakthrough then enabling routine whole genome profiling analysis. Microarrays allow interrogation of the expression level of essentially all known genes and/or sequenced transcripts of a species of interest. They are available in different designs, yet a few major ones have penetrated the market. Those based on oligonucleotides, either printed on a solid support, attached to beads, or synthesized in situ onto a wafer chip, seem to be preferred over printed cDNA arrays, probably due to ease of production or reproducibility. The general method encompasses adding a label to the isolated mRNAs via enzymatic steps, hybridizing the labeled molecules to the array with immobilized DNA molecules, and then quantifying the label, which may be fluorescent on its own, or via binding of fluorescently labeled molecules allowing signal amplification. The strength of the signal at a certain localization representing a specific gene then is proportional to the amount of the corresponding mRNA in the original sample.

A comparison between major microarray types, performed by the MicroArray Quality Control (MAQC) consortium, with participation by microarray platform providers, the US FDA National Center of Toxicological Research, and others, revealed high repeatability between and within platforms and high correlation between microarray results and other quantitative gene expression measurements (Guo et al. 2006).

Another major technique is quantitative polymerase chain reaction (Q-PCR), which allows sensitive quantification of one to several hundreds of known genes; Q-PCR may be used to validate microarray results or measure certain genes in many samples.

The newest addition to the genomics toolbox is next-generation sequencing (NGS) technology, which allows much faster and cheaper sequencing of whole genomes than the sequencing technologies available before (Woollard et al. 2011). It is applied in pharmacogenomics research to search, e.g., for mutations in cancer genomes. Since NGS can also count how many times the same sequence is available in a DNA pool, it can also be used for expression profiling especially of such RNAs which have not yet been identified, including microRNAs and other noncoding RNAs.

Issues such as reproducibility of gene expression profiling techniques, standard practice for assays and analysis, relevance of the results to conventional end points, and robustness of statistical models on diverse data sets have been and are addressed by public consortia in addition to MAQC. Due to recommendations for

technical aspects developed in such efforts, highly standardized microarrays with reproducible performance and reasonable sensitivity are now available for toxicogenomics investigations.

### Proteomics

Proteomics, which encompasses identification and quantification of all proteins within a given proteome, is quite challenging and therefore does not allow high throughput use in most cases.

Reasons are (1) the much bigger complexity of the proteome compared to the genome due to the many possible protein modifications, (2) the wide dynamic range, (3) detection limits of the available technologies, (4) challenges with respect to characterization of membrane proteins, and (5) requirement for pre-fractionation (Ly and Wasinger 2011). Pre-fractionation can be performed with diverse methodologies, including various electrophoretic and chromatographic techniques. Then the separated proteins need to be extracted and identified with mass-spectrometric methods. Due to this rather elaborate workflow, proteomics analysis on a global scale is mostly used for specific mechanistic investigations.

### Metabolomics

Metabolomics has the goal to comprehensively and quantitatively analyze all metabolites in a given sample, which could be cell or organ extract, or any biofluid. In toxicological studies, metabolomics may reveal affected metabolic pathways via altered metabolite patterns which could either be causally involved in the toxic phenotype or represent the downstream effect of a toxic insult.

The large diversity in chemical and physical properties and the wide range of metabolite concentrations (9 orders of magnitude) pose great challenges to metabolomics methods. The two major technological platforms used are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based approaches, the latter usually with prior chromatographic separation like liquid (LC) or gas (GC) chromatography. Although NMR is the more quantitative method, it is less sensitive than MS. Furthermore, since annotation of NMR peaks is rather time-consuming, NMR may be used to define peak patterns for different classes of toxicants yet is of less use for clarification of toxicity mechanism. Since MS-based methods allow construction of databases containing spectra for known metabolites, they can be employed in mechanistic toxicology.

## Toxicogenomics in Investigational Toxicology

Fundamental assumptions of toxicogenomics are that all toxicological relevant effects are accompanied by gene expression changes and that similar toxicological

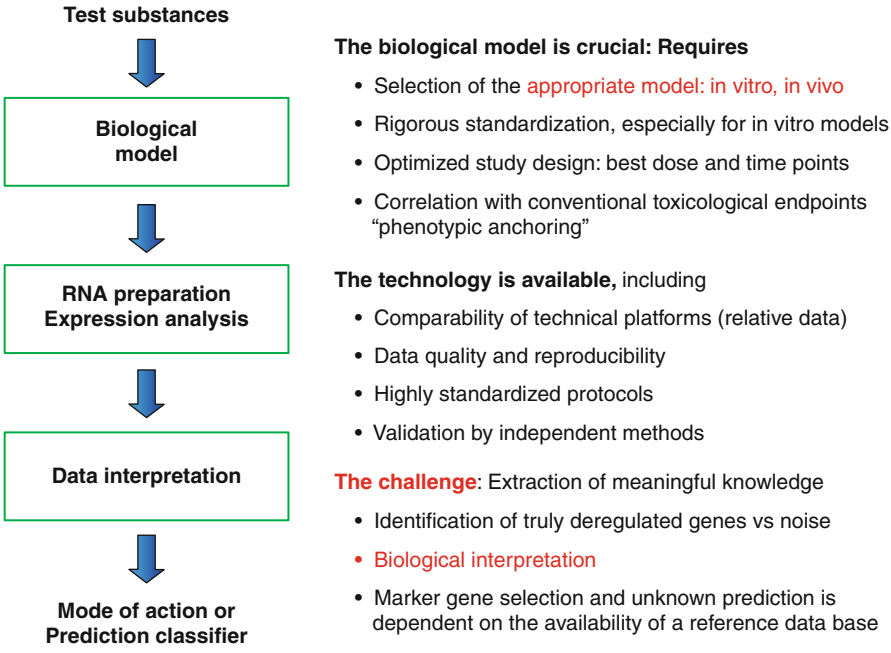



**Fig. 2** Proper preparation and performance of the three major components of a toxicogenomic study is important to obtain good quality results

mechanisms cause comparable expression changes, with potential exceptions being acute necrotic effects.

The idea to obtain insight into toxicological mechanisms from measurement of compound-induced gene expression has been around already before the appearance of microarrays, yet establishment of the latter permitted an analysis of these changes in their entirety, enabling to derive hypotheses about causative mechanisms.

A toxicogenomics study in general has three major components (Fig. 2), the biological model, the technological platform, and data analysis and interpretation, leading from application of a substance to a mechanistic hypothesis, to biomarker candidates, or to prediction of a potential toxicity. Both in vivo and in vitro models may serve as model, yet the studies need to be appropriately designed with respect to, e.g., time course and doses, and preferably should allow correlation of the expression profiles with conventional toxicological end points.

As outlined above, powerful technological platforms are nowadays available which enable examination of the influence of compound effects on essentially all genes in the corresponding target organs of the principle tox species. The major challenge then lies in analysis of the huge amounts of data being generated. Adequate methods must be applied for identification of a useful number of truly

deregulated genes to derive a biological interpretation for the observed toxicological effects of a compound or compound class.

Analysis of toxicogenomics data (Afshari et al. 2011) can follow two major paths: (1) mechanistic analysis and (2) classification or prediction analysis. Biomarker genes or signatures of certain toxic effects may be derived from both approaches.

Mechanistic toxicogenomics encompasses the assignment of functional categories to significantly deregulate genes in a biological model in the context of dose and time dependence and their relation to possible mechanisms of toxic action. From this analysis, mechanistic hypotheses may be derived or mechanistic similarities between different toxic agents may be uncovered. This approach can nowadays be seen as "State of the Art" in toxicogenomics analysis, as revealed, e.g., by the many publications to characterize toxic compound effects with gene expression profiling.

Predictive toxicogenomics relies on a database of expression profiles from samples representing organs or cells following treatment with compounds of predefined toxic classes. Then marker genes are selected and classifiers are calculated by statistical or other algorithms to allow classification of unknown samples with respect to potential induction of these toxicity classes. It would be a major step forward if long-term effects could be predicted with short-term expression profiling experiments. Yet due to challenges including compilation of a sufficient number of expression profiles derived from studies with well-defined compounds and appropriate bioinformatics methods, toxicogenomics is currently not yet widely used for prediction. Collaborative projects are still evaluating such approaches in different biological models.

## Areas of Applications of Toxicogenomics in Toxicology

As alluded to above, toxicogenomics is used in investigational toxicology to complement conventional data collected in toxicological studies. It does increase our understanding of mechanistic networks underlying toxicological effects. This allows identification of new safety biomarkers for organ toxicity. It also allows comparison of compound effects on such networks in different species, helping to characterize phenotypic differences between species upon compound treatment, which may then enable evaluation of human relevance. Therefore, toxicogenomics does and will have an influence on toxicological risk assessment. Furthermore, application of toxicogenomics for classification of later-occurring compound effects may be used to predict toxicological effects in a faster and more sensitive manner as currently achievable with the available tests systems.

To advance the use of toxicogenomics in the regulatory toxicology setting, various challenges must still be overcome, including standardization of all elements of a toxicogenomics study and of data sharing, population of public expression profile databases associated with standardized metadata, and qualification of

signatures and biomarkers derived from such a study. Several mostly collaborative efforts are ongoing to address these challenges, e.g., by working groups of the Predictive Safety Testing Consortium (PSTC) initiated by the FDA Critical Path Initiative, by groups belonging to the Health and Environmental Science Institute (HESI), and by certain EU projects, e.g., the IMI MARCAR project (biomarkers and molecular tumor classification for non-genotoxic carcinogenesis, http://www.imi-marcar.eu/). In most of these projects, industry, regulators, and academia collaborate. Furthermore, regulatory authorities, especially FDA and EMA, are evaluating (Goodsaid et al. 2010) and asking for submission of omics data and are developing guidance documents for biomarker qualification to enable use of omics data in decision making during drug development. Similarly the US EPA is eager to analyze such data for risk assessment of agrochemicals. Therefore, it has to be expected that these technologies will have significant impact on regulatory decisions in the future.

## References

Afshari CA, Hamadeh HK, Bushel PR (2011) The evolution of bioinformatics in toxicology: advancing toxicogenomics. Toxicol Sci 120(Suppl 1):S225–S237

Goodsaid FM, Amur S, Aubrecht J, Burczynski ME, Carl K, Catalano J, Charlab R, Close S, Cornu-Artis C, Essioux L, Fornace AJ Jr, Hinman L, Hong H, Hunt I, Jacobson-Kram D, Jawaid A, Laurie D, Lesko L, Li HH, Lindpaintner K, Mayne J, Morrow P, Papaluca-Amati M, Robison TW, Roth J, Schuppe-Koistinen I, Shi L, Spleiss O, Tong W, Truter SL, Vonderscher J, Westelinck A, Zhang L, Zineh I (2010) Voluntary exploratory data submissions to the US FDA and the EMA: experience and impact. Nat Rev Drug Discov 9:435–445

Guo L, Lobenhofer EK, Wang C, Shippy R, Harris SC, Zhang L, Mei N, Chen T, Herman D, Goodsaid FM, Hurban P, Phillips KL, Xu J, Deng X, Sun YA, Tong W, Dragan YP, Shi L (2006) Rat toxicogenomic study reveals analytical consistency across microarray platforms. Nat Biotechnol 24:1162–1169

Ly L, Wasinger VC (2011) Protein and peptide fractionation, enrichment and depletion: tools for the complex proteome. Proteomics 11:513–534

Woollard PM, Mehta NA, Vamathevan JJ, Van Horn S, Bonde BK, Dow DJ (2011) The application of next-generation sequencing technologies to drug discovery and development. Drug Discov Today 16:512–519

# International Regulation of Toxicological Test Systems

Horst Spielmann

## Contents

International Harmonization of Guidelines for Toxicity Testing ..... 182
Validation and Acceptance of New OECD Test Methods ..... 184
Example of the Successful Validation and Regulator Acceptance of a New Test Method ..... 185
New Developments During the Last Decade ..... 186
References ..... 188

**Abstract**

Toxicity testing for regulatory purposes began after the thalidomide (Contergan®) tragedy in Germany around 1960, when unexpectedly the hypnotic drug caused severe limb malformations in newborns whose mothers had taken the drug during pregnancy. This accident initiated in the first place regulatory testing for drugs, while safety testing of other chemicals, e.g., pesticides, industrial chemicals, cosmetics, and food ingredients, has become mandatory at the international level about 20 years later. Meanwhile regulatory safety testing of chemicals is mandatory in all OECD (*Organization for Economic Cooperation and Development*, http://www.oecd.org/) member states, which are the major industrial countries except Brazil, China, and India. Initially test requirements as well as individual tests were developed at the national level, and therefore, they differed quite significantly due to experience at the national level of both in industry and regulatory agencies. As a consequence, international industry had to conduct different sets of safety tests to meet the legal requirements in each of these countries. In essence, differences in test requirements created a considerable financial burden both for the industry and

H. Spielmann
Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany
e-mail: horst.spielmann@fu-berlin.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_41, © Springer-Verlag Berlin Heidelberg 2014

also for patients and consumers, who in the end have to pay for testing. Thus, the differences in testing requirements, which were not based on scientific grounds, resulted in barriers to international trade.

Finally, the major industrial nations agreed to correct the situation by harmonizing the safety testing requirements. At the OECD, industry and regulatory agencies took the lead in this activity, since regulatory testing has a higher priority for them than for research-driven academic institutions. It was the goal of the harmonization activity to achieve *mutual acceptance of data* (MAD) by OECD member countries when the data were produced according to standardized testing requirements. Today harmonization of test guidelines has been achieved for regulatory testing in all areas of toxicology. In addition, the criteria for development and validation of new test methods have been harmonized, since test guidelines have to be updated continuously according to scientific progress and also to meet the needs of testing for newly emerging endpoints.

In this chapter the concept of the *mutual acceptance of data* (MAD) produced in standardized and harmonized toxicity tests is described and also the criteria for developing, validating, and achieving international acceptance of new tests methods.

## International Harmonization of Guidelines for Toxicity Testing

For historical and economic reasons, standardization and harmonization of test guidelines were developed independently for the major areas of toxicology, e.g., safety testing of drugs, cosmetics, industrial chemicals, and pesticides/biocides. The major reason for this are differences in regulatory requirements, which take into account differences in use and exposure of humans and the environment. The most important step was in 1982 the adoption of the *OECD Guidelines for the Testing of Chemicals* (OECD TGs) http://www1.oecd.org/ehs/testguid which are mandatory today for testing of all chemicals except human and veterinary drugs. It is important to note that OECD TGs have been accepted not only for toxicity testing but also for physicochemical properties and for environmental safety including accumulation and degradation of biotic and abiotic systems.

The most important consequence of the adoption of harmonized OECD TGs by OECD member states is the concept of *mutual acceptance of data* (MAD) that are generated by testing according to OECD TGs by regulatory agencies of all OECD member countries. Another important requirement for MAD is that testing must be conducted according to GLP (*good laboratory practice*), which is an accepted measure to enforce quality assurance. If a toxicity test has been conducted according to an OECD TG and to GLP, the test must only be performed once and not any more for each national regulatory agency. The harmonization of TGs by the OECD has not only financial advantages for industry and consumers, but it also

improves ethical standards from the animal welfare perspective. The latter aspect is a major driving force in OECD member countries. Finally, from the scientific perspective, harmonization of TGs is most welcomed since data for specific endpoints of toxicity are now produced according to the same TG, which provides for a better comparison of the results of studies conducted for the same endpoint.

It has been criticized that OECD TGs are quite rigid, and it is time consuming and laborious to update them once they have been accepted, since at the OECD decisions are not made by majority vote, but all OECD member states have to agree. Although progress may be delayed by unanimous agreement, this has not really happened during the past 20 years, since the procedure for submitting new TGs or for updating existing TGs has continuously been improved.

In Germany and all EU member states, testing of chemicals has in the first place to be conducted according EU TGs, which are almost identical to OECD TGs. When the EU legislation was changed in 2007 from the EU Directive 76/769/EEC on the regulation of dangerous substances and preparations to the EU Directive 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (*REACH*, http://echa.europa.eu/web/guest/regulations/reach/), only testing according to OECD TGs is accepted in Europe.

Since the concept of using standardized OECD TGs for toxicity testing has proven successful and also the *mutual acceptance of data* (MAD), OECD TGs are now used for safety testing of all chemical substances and products except drugs, e.g., biocides, pesticides, cosmetics, as well as food and feed additives.

Taking into account the successful implementation of the OECD TGs into regulatory practice, in 1990 the national and international agencies that are responsible for the regulation of drug safety agreed on an international harmonization of the test guidelines for human and veterinary drugs. They were harmonized by the ICH (International Conference on Harmonisation, http://www.ich.org/), which is formed by regulatory agencies and the drug industry of the major economic regions Europe, Japan, and the USA. ICH test guidelines (TGs) are used not only for toxicity testing but also for all other areas of preclinical drug testing, e.g., efficacy testing and pharmaceutical quality control. As described for OECD TGs, results of tests that were conducted according to ICH guidelines will only be accepted internationally according to MAD, if testing has been conducted according to "GLP," and produced according to "good manufacturing practice (GMP)." Again, the harmonization of TGs has led to significant reduction of testing in animals, since regulatory agencies around the world are now accepting the results of a test that was conducted according to ICH guidelines.

Table 1 summarizes the most important areas, which require safety testing in animals and in which the test guidelines have been harmonized at the international level. Table 1 shows that in addition to drugs, industrial chemicals, and pesticides, international TGs have also been harmonized for hormones and biologicals by the pharmacopoeias and for vaccines by the WHO. So far, the harmonization of international TGs for toxicity and safety testing has been the most successful approach to reduce animal testing for regulatory purposes.

**Table 1** International harmonization of methods for safety testing

| |
|---|
| 1. Industrial chemicals, cosmetics, pesticides, biocides, and food and feed additives |
| OECD Test Guidelines for the Testing of Chemicals (OECD 1982–2013) |
| EU REACH Regulation, Annexes VII, VIII, IX, and X (EU Commission 2006) |
| 2. Drugs and medicinal products |
| ICH Test Guidelines of the "International Conference on Harmonisation" (http://www.ich.org/) |
| 3. Safety testing of hormones and biological |
| Pharmacopoeias (US Pharmacopoeia, EU Pharmacopoeia) |
| 4. Vaccines and immunologicals |
| WHO recommendations, EU and US Pharmacopoeias |

## Validation and Acceptance of New OECD Test Methods

Regulators will only accept new nonanimal tests, also termed "alternative tests" (e.g., in vitro or in silico tests), if the new tests allow to classify and label chemicals in the same way as the current animal tests. The OECD has, therefore, decided that in vitro toxicity tests can be accepted for regulatory purposes only after a successful experimental validation study has been conducted. To approach this problem scientifically, European and American scientists agreed in 1990 in Amden, Switzerland, on a definition of experimental validation and the essential steps in this process. At this workshop, validation was defined as the process by which reproducibility and relevance of a toxicity testing procedure are established for a particular purpose (Balls et al. 1990), regardless of whether the method is an in vitro or in vivo test. The essential steps of the experimental validation process were defined in the following manner:

1. Test development in one or several laboratories
2. Experimental validation under blind conditions in several laboratories in a ring trial
3. Independent assessment of the results of the validation trial
4. Regulatory acceptance

Steps 2 and 3 are the essential part of a formal validation study conducted for regulatory purposes. The report of this workshop (Balls et al. 1990) encouraged scientists to initiate several international validation studies. Since the Draize eye test has been the most widely criticized toxicity test, a worldwide validation study on nine nonanimal alternatives to the Draize eye test was coordinated by the EU Commission's Centre for the Validation of Alternative Methods (ECVAM, http://ecvam.jrc.it/) and the British Home Office. However, this and other extensive international validation attempts failed (Balls et al. 1995b).

Therefore, the leading scientists involved met for a second validation workshop in Amden in 1994 to improve the concept of the validation procedure. The second Amden validation workshop recommended the inclusion of new elements into the validation process (Balls et al. 1995a), which had not sufficiently been identified in the first Amden validation workshop. The following three essential elements were added:

1. The definition of a biostatistically based *prediction model*
2. The inclusion of a *prevalidation stage* between test development and formal validation under blind conditions
3. A well-defined *management structure*

As to in vitro tests, a *prediction model* should allow the prediction of in vivo endpoints in animals or humans from the endpoints determined. The prediction model must be defined mathematically in the *standard operation procedure* (SOP) of the test that will undergo experimental validation under blind conditions with coded chemicals (Balls et al. 1995a). In order to assess the limitations of a new test before it will be evaluated in a validation study, the test should be standardized in a *prevalidation study* with a few test chemicals in a few laboratories (Curren et al. 1995). This will ensure that the in vitro test method, including the prediction model, is robust and that the formal validation study with coded chemicals is likely to be successful. Finally, the goal of a validation study has to be defined clearly, and the *management structure* has to ensure that within the study the scientists who are responsible for essential tasks can conduct their duties independently from the sponsors and the managers of the study, e.g., biostatistical analysis, and the selection, coding, and shipment of the test chemicals.

The improved concept of experimental validation for regulatory purposes defined in the second Amden workshop was accepted by ECVAM, in 1995, and in 1996 by US regulatory agencies and also by the OECD (OECD 1996). After this agreement at the international level, scientists have tried to follow the ECVAM/US/OECD principles for validation in new validation trials. The improved validation concept was immediately introduced into ongoing validation studies, e.g., the ECVAM/COLIPA validation study on in vitro phototoxicity tests and the ECVAM validation study of in vitro skin corrosivity test. Taking into account the experience from successful validation studies, in 2005 the OECD has published a "guidance document on the validation and international acceptance of new or updated test methods for hazard assessment" (OECD 2005).

## Example of the Successful Validation and Regulator Acceptance of a New Test Method

Since no standard guideline for the testing of photoirritation potential, either in vivo or in vitro, for regulatory purposes existed at the international level in 1991, the OECD, the European Commission (EC), and the European Cosmetics, Toiletry, and Perfumery Association (COLIPA) established a joint program to develop and validate in vitro photoirritation tests. In the first phase of the study, which was funded by GD Research and Technology of the EU Commission and coordinated by ZEBET (Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch, the national German validation center), in vitro phototoxicity tests established in laboratories of the cosmetics industry were evaluated and also a new assay, the 3T3 NRU PT test, which is a photocytotoxicity test using the mouse fibroblast cell line 3T3 and neutral red uptake (NRU) as the endpoint for cytotoxicity.

In the prevalidation study conducted with 20 test chemicals, the 3T3 NRU PT in vitro phototoxicity test was the only in vitro test in which all of the 20 test chemicals were correctly identified as phototoxic or non-phototoxic. Quite independently, a laboratory in Japan subsequently obtained the same correct results in the 3T3 NRU PT, when testing the same set of 20 test chemicals. In the second phase of the study, which was funded by ECVAM and coordinated by ZEBET, the 3T3 NRU PT test was validated with 30 carefully selected test chemicals in 11 laboratories in a blind trial. A representative set of test chemicals covering all major classes of phototoxins was selected according to results from standardized photopatch testing in humans. The results obtained in this in vitro test under blind conditions were reproducible, and the correlation between in vitro and in vivo data was almost perfect (Spielmann et al. 1998a).

Therefore, the ECVAM Scientific Advisory Committee (ESAC) concluded in 1998 that the 3T3 NRU PT is a scientifically validated test which is ready to be considered for regulatory acceptance (ESAC 1998). However, the EU expert committee on the safety of cosmetics, the Scientific Committee on Cosmetology and Non-Food Products (SCCNFP), criticized that an insufficient number of UV filter chemicals (widely used as sun blockers) were tested in the formal validation study. In a subsequent blind trial on UV filter chemicals, which was again funded by ECVAM and coordinated by ZEBET, the phototoxic potential of all test chemicals was predicted correctly in the 3T3 NRU PT in vitro phototoxicity test (Spielmann et al. 1998b). Therefore, in 1998, the EU, having accepted the 3T3 NRU PT test as the first experimentally validated in vitro toxicity test for regulatory purposes, officially applied to the OECD for worldwide acceptance of this in vitro toxicity test. Early in 2000, the European Commission has officially accepted and published the 3T3 NRU PT phototoxicity test in *Annex V* of *Directive 67/548 EEC on the Classification, Packaging and Labelling of Dangerous Substances* (EU Commission 1983). Thus, this in vitro test is the first formally validated in vitro toxicity test that has been accepted into Annex V, and it is the only phototoxicity test that is accepted for regulatory purposes in Europe. In 2002 the OECD has accepted the 3T3 NRU PT phototoxicity test at the worldwide level as the *first* in vitro *toxicity test* into the OECD guidelines for the testing of chemicals (OECD 2004).

## New Developments During the Last Decade

Recent EU legislations, e.g., the seventh Amendment of the EU Cosmetics Directive (EU Commission 2003) and the EU chemical regulation REACH (EU Commission 2006), are enforcing the use of nonanimal methods to replace the use of toxicity testing in animals. In fact, in 2013 the EU Cosmetics Directive stipulates a marketing ban of cosmetic ingredients that were tested in animals. Due to public and private research funding activities in Europe, this deadline may be met as far as testing for local side effects on eye and skin is concerned. As a consequence, during the past decade, several nonanimal test methods for skin and eye irritation have successfully been developed and validated, and some of them have been accepted

**Table 2** In vitro toxicity test for local eye and skin toxicity accepted by the OECD for regulatory purposes (classification and labelling)

| |
|---|
| 1. Skin absorption: OECD TG 428 "Skin absorption: in vitro method" |
| 2. Skin corrosion: OECD TG 430 "In vitro skin corrosion: transcutaneous electrical resistance test method (TER)" |
| 3. Skin corrosion: OECD TG 431 "In vitro skin corrosion: reconstructed human *epidermis* (RhE) test method" |
| 4. Phototoxicity: OECD TG 432 "In vitro 3T3 NRU phototoxicity test" |
| 5. Severe eye irritation and corrosion: OECD TG 437 "Bovine corneal opacity and permeability (BCOP) test for ocular corrosives and severe irritants" |
| 6. Severe eye irritation and corrosion: OECD TG 438 "Isolated chicken eye (ICE) test method for ocular corrosives and severe irritants" |
| 7. Skin irritation: OECD TG 439 "In vitro skin irritation: reconstructed human epidermis test method" |
| 8. Severe eye irritation and corrosion: OECD TG 460 "Fluorescein leakage test method for identifying ocular corrosives and severe irritants" |

for regulatory purposes at the international level by the OECD. Testing of new chemicals for local toxicity can now be conducted according to the following OECD in vitro toxicity test methods (http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788) shown in Table 2:

In addition for eye irritation, several "human cornea construct" in vitro models are currently undergoing prevalidation and also a tiered in vitro testing scheme and prediction model for the detection of skin sensitizers based on in vitro assays, addressing three different steps in the development of skin sensitization, an in silico method, a protein reactivity assay, and a dendritic cell activation assay. A prevalidation study with 54 chemicals showed a high predictivity for sensitization potential in humans, which was better than that of the in vivo local lymph node assay (LLNA) (Bauch et al. 2012).

In 2012 the OECD has also accepted two in vitro hormone receptor assays for assessing the estrogenic and antiestrogenic potential of chemicals (http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788):

1. OECD TG 455 the "Performance-based test guideline for stably transfected transactivation in vitro assays to detect estrogen receptor agonists"
2. OECD TG 457 the "BG1Luc estrogen receptor transactivation test method for identifying estrogen receptor agonists and antagonists"

The past two decades have seen unprecedented scientific and technological advances, including the birth of functional genomics, the fast-paced growth of computing power and computational biology/bioinformatics, the establishment of robotic platforms for high-throughput screening of chemicals, and the sequencing of the human genome. Together, these advances have triggered a revolution in molecular biology and have made available a wide range of new tools for studying the effects of chemicals on cells, tissues, and organisms in a rapid and cost-efficient manner.

This convergence of factors, coupled with increased recognition of the limitations of conventional in vivo tests and the need to evaluate the safety of an increasingly large number of chemical substances and mixtures, has led authorities such as the US National Research Council and other to call for a shift in toxicity testing towards the elucidation of *toxicity pathways* at the cellular level – an approach commonly referred to as "Toxicity testing in the 21st century" (US National Research Council 2007). According to this concept an *adverse outcome pathway* (*AOP*) delineates the documented, plausible, and testable processes by which a chemical induces molecular perturbations and the associated biological responses which describe how the molecular perturbations cause effects at the subcellular, cellular, tissue, organ, whole animal, and population levels of observation. The AOP can then be used to form categories by integrating knowledge of how chemicals interact with biological systems (i.e., the molecular initiating events) with knowledge of the biological responses. Taking into account the new concept, an OECD expert group is working on developing an in vitro sensitization test based on the *adverse outcome pathway* (*AOP*) approach.

Since the new concept of *toxicity pathways* has been welcomed by scientists in academia, industry, and regulatory agencies, the OECD is proposing that all new test methods should take into account the AOP concept and it should also be considered when existing tests are updated.

## References

Balls M, Blaauboer B, Brusik D (1990) Report and recommendations of the CAAT/ERGATT workshop on the validation of toxicity test procedures. ATLA 18:313–337

Balls M, Blaauboer BJ, Fentem J (1995a) Practical aspects of the validation of toxicity test procedures. The report and recommendations of ECVAM Workshop 5. ATLA 23:129–147

Balls M, Botham PA, Bruner LH (1995b) The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro 9:871–929

Bauch C, Kolle SN, Ramirez T (2012) Putting the parts together: combining in vitro methods to test for skin sensitizing potentials. Regul Toxicol Pharmacol 63:489–504

Curren RD, Southee JA, Spielmann H (1995) The role of prevalidation in the development, validation and acceptance of alternative methods. ATLA 23:211–217

ESAC (ECVAM Scientific Advisory Committee) (1998) Statement on the scientific validity of the 3T3 NRU PT test (an in vitro test for phototoxic potential). ATLA 26:7–8

European Commission (1983) Directive 83/467EEC adapting to technical progress for the fifth time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. EU DG Environment, Brussels, 122 pp

European Commission (2003) Directive 2003/15/EC amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. OJ Eur Union Law 66:26–35

European Commission (2006) Directive 1907/2006 concerning the Registration, Evaluation, and Authorization of Chemicals, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ Eur Union Law 396:1–845

OECD (1982–2013) Guidelines for the Testing of Chemicals, Section 4, Health Effects, http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788.

OECD (1996) Final report of the OECD workshop on harmonization of validation and acceptance criteria for alternative toxicological tests methods. OECD Publication Office, Paris, 53 pp

OECD (2004) OECD guidelines for the testing of chemicals: test guideline 432 "in vitro 3T3 NRU phototoxicity test". OECD Publication Office, Paris, p 15

OECD (2005) Guidance document on the validation and international acceptance of new or updated test methods for hazard assessment. OECD Publication Office, Paris, 96 pp

Spielmann H, Balls M, Dupuis J (1998a) The international EU/COLIPA in vitro phototoxicity validation study: results of Phase II (blind trial); part 1: the 3T3 NRU phototoxicity test. Toxicol In Vitro 12:305–327

Spielmann H, Balls M, Dupuis J (1998b) A study on UV filter chemicals from Annex VII of European Union Directive 76/768/EEC, in the in vitro 3T3 NRU phototoxicity test. ATLA 26:679–708

US National Research Council (2007) Toxicity testing in the 21st century: a vision and strategy. National Academic Press, Washington, DC, 216 pp

# Epidemiological Methods in Regulatory Toxicology

Ulrich Ranft and Gregory A. Wellenius

## Contents

Basics ..... 192
Definition ..... 192
Population ..... 192
Exposure and Effect ..... 193
Association Versus Causation ..... 194
Measures of Disease Occurrence and Association ..... 194
Common Epidemiologic Study Designs ..... 196
Descriptive and Analytical Epidemiology ..... 196
Cohort Study ..... 197
Case–Control Study ..... 197
Cross-Sectional Study ..... 198
Panel Study ..... 198
Ecological Study ..... 199
Study Quality ..... 199
Validity ..... 199
Confounding ..... 200
Selection Bias ..... 200
Information Bias {Bias, Information} ..... 201
Effect Measure Modification and Interactions ..... 201
Random Error and Precision ..... 202
Study Planning ..... 203

U. Ranft (✉)
IUF - Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
e-mail: ranft@uni-duesseldorf.de

G.A. Wellenius
Department of Epidemiology, Brown University, Providence, RI, USA
e-mail: Gregory_Wellenius@brown.edu

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_42, 

Statistical Analysis ..... 203
Estimation, Confidence Intervals, and Testing ..... 203
Regression Models ..... 204
Recommended Reading ..... 205
Resources ..... 205

**Abstract**

A major challenge in the field of toxicology is extrapolating the findings from in vitro and in vivo animal experiments to infer a causal effect of exposure on disease in people. Specifically, differences in species, exposure dose, route of administration, duration of follow-up, and co-exposures may lead to substantially different effects of exposures in animals or cells than in humans. Randomized trials or intervention studies in people provide an opportunity for assessing the health effects of exposures, but high costs and safety concerns often limit the types of exposures that can be evaluated with this approach. In the context of regulatory toxicology of potentially hazardous toxicants with little potential for benefit, safety and ethical concerns are paramount, leaving very few toxicants which can be ethically studied with randomized trials or intervention studies. Observational epidemiology provides a means to study the links between potentially harmful exposures and disease in people.

## Basics

### Definition

Epidemiology is often defined as the study of the distribution and determinants of diseases in people. By studying how specific exposures influence the distribution of diseases or physiologic variables, one can find evidence in support of or against the presence of an association between a given exposure and disease. Therefore, epidemiology offers an important alternative to human or animal experimentation in studying the etiology of disease and identifying the health effects of potentially harmful toxicants.

The distinguishing feature of epidemiologic studies compared to experimental studies is that in epidemiologic studies, exposure is determined by each individual (or their circumstances, environment, etc.) rather than by the investigator. Because each *individual's exposure is not assigned at random*, the major challenge of epidemiology is being able to interpret the results of epidemiologic studies as evidence in support of the presence or absence of causal effects.

### Population

Toxicologic experiments typically compare the average or expected level of a given outcome in exposed versus unexposed cells or animals. Implicit in this approach is that the results are based on average effects across the population under study and may not apply to any single cell or animal. Similarly, the target of inference in

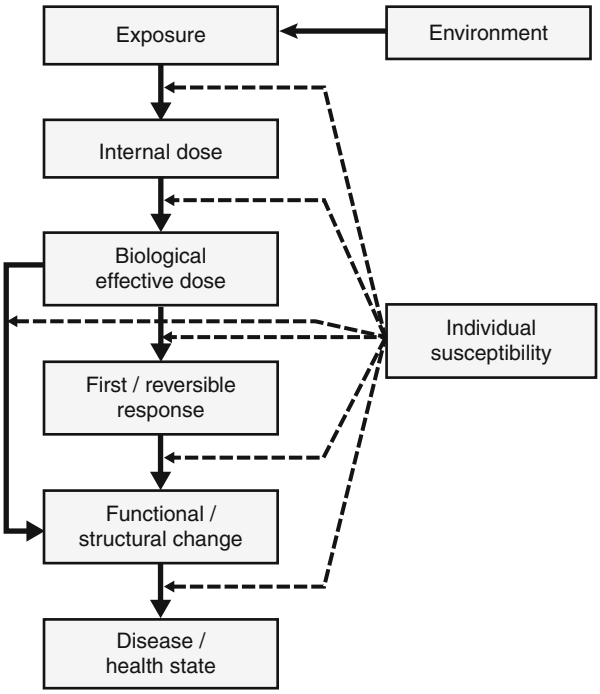



**Fig. 1** Causal chain from exposure to disease

epidemiologic studies is the population under study rather than the individual. For example, while we might find that exposure increases the average risk of a specific type of cancer, we generally cannot determine whether an individual that developed this cancer developed it as a result of the exposure under study. However, under certain assumptions, population effects can be used as *statements of probability* about the health risks of individuals. For instance, extending the prior example, we might be able to say that the risk of developing this cancer is higher in an exposed individual as compared to an unexposed individual.

## Exposure and Effect

Epidemiologic studies are designed to quantify the association between a given exposure and a given outcome or health effect. However, an important aspect of epidemiologic study design is clearly defining what is meant by "exposure" and "effect." Figure 1 illustrates the *causal chain from exposure to outcome* as frequently conceived. For example, in a study of the health effects of water disinfection by-products, we must clearly specify whether the exposure of interest is the concentrations of these by-products in the water supply (potentially combined with some estimates of individual water consumption rates), the concentration of some biomarker of internal dose, or some estimate of the biologically effective dose of the by-products. Similarly, we need to be very specific about the definition of the outcome, which can range from subtle shifts in

**Table 1** Hill criteria for the evidence of a causal relationship

| |
|---|
| Strength of association |
| Consistency |
| Temporality |
| Biological plausibility |
| Dose-effect relationship |
| Coherence among epidemiologic studies |
| Specificity |
| Coherence between epidemiologic and laboratory findings |
| Analogy |

physiological variables to incidence of overt disease. Due to its importance, estimating exposure has its own chapter. To reliably differentiate the health effects of several simultaneous exposures presents a particular challenge for statistical analysis and interpretation.

## Association Versus Causation

As alluded to above, epidemiologic studies quantify the statistical association between a given exposure and disease. Two variables might be statistically associated because of the following: (1) uncontrolled confounding (as occurs when the two variables have a common cause), (2) selection bias (as occurs when participants are chosen in a way that is related to both exposure and outcome), (3) chance, and/or (4) one variable actually causes the other. A fundamental challenge of epidemiologic studies is to minimize the potential for confounding, selection bias, and chance so that the results of the study provide evidence in support of (or against) the presence of a causal effect of exposure on disease.

While much can be done during the study design and data analysis to minimize the potential for observing noncausal associations, in the end the interpretation of a statistical association as reflecting causality is based on judgment. An early formulation of guidelines for *inferring causation from observation* studies was provided by Sir Austin Bradford Hill (Table 1). Of the criteria shown, only temporality (exposure occurs before the outcome) is necessary for a causal relationship to exist. While the remaining Hill criteria are neither necessary nor sufficient to infer causation, they provide a useful framework for judging the strength of the evidence.

## Measures of Disease Occurrence and Association

Traditional epidemiologic studies aim to quantify the association between exposure and a dichotomous outcome, generally the presence or absence of a given disease. Disease occurrence is generally quantified using estimates of *prevalence*, *risk* (also called incidence proportion or cumulative incidence), and *incidence rate* (see Table 2, Fig. 2).

**Table 2** Common measures of disease occurrence and association

| |
|---|
| **Measures of disease occurrence** |
| *Prevalence*: *the* ratio of the number of existing cases of disease observed at a given point (*point prevalence*) or during a certain time period (*period prevalence*) to the size of the population under observation. A proportion ranging from 0 to 1 |
| *Risk*, *incidence proportion*, *cumulative incidence*: the ratio of the number of new (incident) cases of disease within a given time period of observation to the number of people at risk of the disease at the start of observation. A proportion ranging from 0 to 1 |
| *Incidence rate*: the number of new (incident) cases of disease within a given time period of observation divided by person-time at risk of the disease. Person-time is defined as the sum of the time spent under observation and at risk of disease by each member of the population. Incidence rates are not proportions and range from 0 to positive infinity |
| **Measures of associations** |
| *Risk ratio*: risk of disease among the exposed divided by the risk of disease among the unexposed |
| *Incidence rate ratio* (*rate ratio*): incidence rate of disease among the exposed divided by the incidence rate of disease among the unexposed |
| *Odds ratio*: odds of disease among the exposed divided by the odds of disease among the unexposed where the odds of disease are defined as the probability of disease divided by (1−the probability of disease) |
| *Relative risk*: a nonspecific term that can refer any ratio measure of association (i.e., risk ratio, rate ratio, or odds ratio) |
| *Risk difference*: risk of disease among the exposed minus the risk of disease among the unexposed |
| *Incidence rate difference*: incidence rate of disease among the exposed minus the incidence rate of disease among the unexposed |

Common measures of association between exposure and a dichotomous outcome include the *risk ratio* (also called the cumulative incidence ratio), the *incidence rate ratio*, and the *odds ratio* (Table 2). When there is no association between exposure and outcome, the risk, rate, and odds of the disease will be the same in the exposed and unexposed groups, and the risk ratio, rate ratio, and odds ratio will all be equal to 1.

The nonspecific term *relative risk* {risk, relative} is often used to refer to any of these ratio measures. However, it is important to note that estimates of the risk ratio, rate ratio, and odds ratios have different mathematical properties and interpretations, and generally these terms cannot be used interchangeably. In particular, when exposure truly increases the risk of disease, estimates of the odds ratio will always be further from the null hypothesis of no association than the risk ratio, making the results appear more extreme.

The association between exposure and a dichotomous outcome can also be quantified using difference measures, including the *risk difference* and *incidence rate difference* (Table 2). When there is no association between exposure and outcome, the risk (or incidence rate) in the exposed and unexposed groups will be the same and the risk (or incidence rate) difference will equal zero. Note that measures of relative risk assume that exposure acts to multiply the baseline risk (or rate or odds), while difference measures of association assume that exposures add to the baseline risk (or rate).

**Fig. 2** Definition of epidemiologic measures

Contingency table

| | | Exposure: yes | Exposure: no | |
|---|---|---|---|---|
| Disease | yes | a | b | a+b |
| | no | c | d | c+d |
| | Total | a+c | b+d | n = a+b+c+d |

| Measure | Formula |
|---|---|
| Risk of exposed | a / (a+c) |
| Risk of non-exposed | b / (b+d) |
| Relative risk | a(b+d) / b(a+c) |
| Odds ratio | ad / bc |
| Risk difference | (ad – bc) / (a+c)(b+d) |

Note:

When a, b, c and d are observed frequencies, then the formulae are estimates of the measures.

When a, b, c and d are probabilities (n=1), then the formulae are the true measures.

The above measures of disease occurrence and association are applicable to situations where the outcome is dichotomous. For simplicity, in the above discussion, we have implicitly also considered exposure as a dichotomous variable (i.e., comparing outcomes in those exposed vs. unexposed), but this is not necessary. The above measures of association can be generalized to situations where exposure is measured as a continuous variable.

A somewhat different set of metrics are used when studying the association between exposure and a continuous outcome such as blood pressure, heart rate, or levels of a disease biomarker. In these settings, the expected values of the outcome (generally the arithmetic mean) among the exposed and unexposed subjects are compared.

## Common Epidemiologic Study Designs

### Descriptive and Analytical Epidemiology

*Descriptive epidemiology* is limited to the description of disease distribution using suitable measures, such as incidence rate, to enable comparisons between

populations across space, time, or other contrasts. Descriptive studies are often carried out using routinely collected administrative or survey data. The results of descriptive epidemiologic studies are frequently used for public health planning or to generate new hypotheses about disease etiology. In contrast, analytical epidemiologic studies are used to test hypotheses about exposure-effect relationships. Study design and statistical evaluation focus on the initial assumptions and aim to make quantitative statements about associations which can be used to interpret the cause. The most important types of study in *analytical epidemiology* are briefly described below.

## Cohort Study

A cohort study is an epidemiologic study design where participants that are initially free of a disease are followed for a specified period of time and monitored for new cases of (i.e., incident) disease. Exposures of interest and potential confounders are measured at study entry and, optimally, at multiple times throughout the follow-up period. In a *closed cohort study*, participants enter the study at baseline and remain under observation until they develop the disease of interest, die, or are otherwise lost to follow-up. Prominent examples of closed cohort studies include the Women's Health Initiative, the Cardiovascular Health Study, and the Nurses' Health Study. In an *open cohort study*, participants may enter and leave the study multiple times during the follow-up. Examples of open cohort studies include studies of all current members of a health insurance plan or all current residents of a state.

Cohort studies can be either *prospective* or *retrospective*. In a prospective study, data on exposure and confounders are obtained before the development of disease. In a retrospective study, historical data on exposure and confounders are assembled from existing data sources, often after disease has already occurred. Prospective cohort studies can require long follow-up of a large number of participants and are therefore frequently very expensive. Retrospective studies that make use of existing data can be very cost-effective but may be subject to additional potential biases.

## Case–Control Study

A cohort study can be very inefficient if the disease of interest is rare. For example, suppose that we are interested in studying a disease that in a given population has an incident rate of 20 cases per 100,000 person-years. In this example, we could follow 100,000 people for 5 years and still only expect about 100 new cases of melanoma. Put another way, we would need to assess and follow for 5 years about 90,000 people that will not develop melanoma in that time frame.

Because the statistical power of a study is driven largely by the number of incident cases, in the above example, we could gain almost as much information

if we were to assess only the 100 new cases of melanoma and a random sample of those participants that have not yet developed melanoma. Thus, the fundamental principle of a *case–control study* is that one can assess exposures and confounders in a *group of cases* (those with disease) and a (potentially small fraction) random sample of participants that have not yet developed the disease (*group of controls*). The strategy for selecting controls from the source population is very important for the interpretation and validity of the resulting estimate. For example, depending on the control sampling strategy, the odds ratio estimated from a case–control study may approximate either the incidence rate ratio or the risk ratio. The details of control selection strategies are beyond the scope of this chapter.

Sometimes a case–control study is nested within a larger cohort study such that the cases are those identified by the cohort and controls are sampled from among those participants in the cohort that have not yet developed the disease of interest. This is referred to as a *nested case–control study* and has the advantage that the source population giving rise to the cases is easily identified (i.e., the cohort participants). In other case–control studies, identification of the source population may be more challenging. For example, in a hospital-based case–control study, complicated referral patterns and changing catchment areas often make it difficult to clearly identify – and hence choose a random sample of – the source population that gave rise to the cases. Like cohort studies, case–control studies can be either prospective or retrospective in nature.

## Cross-Sectional Study

In a *cross-sectional study*, participants are selected at random from a population of interest and then examined for risk factors and disease at the same time. Thus, the key feature of a cross-sectional study is that exposures and prevalent (existing) cases are assessed simultaneously. Because cross-sectional studies study *prevalent disease*, they generally provide estimates of prevalence ratios or prevalence odds ratios rather than risk ratios or incidence rate ratios. Moreover, because cross-sectional studies assess exposure and outcomes simultaneously, it is often not possible to clearly determine the temporal direction of the association. However, the duration of these studies is generally comparably short, making cross-sectional studies a cost-effective type of study appropriate for exploring certain hypotheses.

## Panel Study

A *panel study* is a special type of cohort study where participants are followed longitudinally and both exposures and outcomes are assessed repeatedly over time within each participant. Panel studies are most appropriate for outcomes that vary

over a relatively short time period such as blood pressure, weight, or levels of most serum biomarkers, but are not appropriate for most disease end points.

### Ecological Study

All of the study designs presented above make use of data collected from individuals. In contrast, *ecological studies* quantify associations between a given exposure and outcome, but both the exposure and outcome are measured in *aggregate* rather than in individuals. Prominent examples of ecological studies include studies showing that average per capita meat consumption is associated with incidence rates of certain types of cancer compared across several countries. The key feature of an ecological study is that the unit of observation is the country (or county, neighborhood, school, etc.) rather than the individual. Because data are aggregated, there is considerable potential for uncontrolled confounding in ecological studies and incorrect conclusion (i.e., *ecological fallacy*). For instance, continuing the above example, smoking is also an important determinant of many cancers and may also be associated with higher meat intake in individuals. However, in an ecological study, controlling for average population smoking prevalence would not necessarily control for confounding by smoking at the individual level. Thus, ecological studies are most useful for offering initial evidence in favor of novel hypotheses.

## Study Quality

### Validity

The overall objective of an epidemiologic study is to obtain a valid and precise *estimate* of disease occurrence or of the association between exposure and disease. It is useful here to differentiate between internal and external validity. *Internal validity* {validity, internal} refers to whether the results of the epidemiologic study can be used to make inferences about the source population for that particular study. On the other hand, *external validity* {validity, external} refers to whether the results of the current study can be generalized to other populations.

Errors in epidemiologic estimates can be classified as either *systematic errors* {error, systematic} (*bias*) or *random errors* {error, random} (*chance*). Of note, while the potential for random error decreases with increasing sample size, the potential for systematic errors is independent of sample size. Moreover, the precision or amount of random error present in an estimate can be easily quantified with routine statistical methods. In contrast, predicting the direction or magnitude of the bias induced by systematic errors is quite challenging and seldom done in practice.

Systematic errors can be further classified as due to confounding, selection bias, or information bias. Each of these sources of systematic bias is discussed in more detail below.

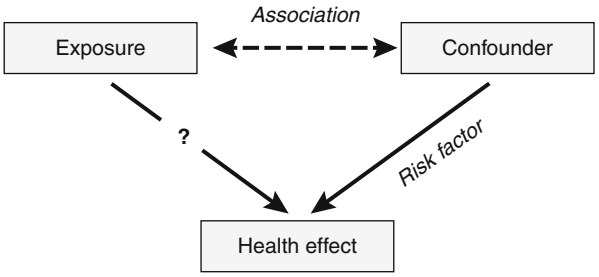



**Fig. 3** Confounding: relationship between exposure, health effect, and confounder

## Confounding

In contrast to experimental research, confounding is a major threat to validity in epidemiologic studies. *Confounding* {bias, confounding} can occur when one or more factors exist in the study population which are associated with both the outcome and the exposure, but are not caused by either the exposure or the outcome (Fig. 3). A known risk factor is a potential confounder and, if associated with the exposure in the study population, will become a *confounder*. Importantly, confounding can bias the health effect estimates either towards or away from the null hypothesis of no association.

The potential for confounding can be minimized through appropriate study design. For example, if sex is an important confounder (because it is associated with both exposure and disease but not caused by either), a study restricted to men only or women only would not be susceptible to confounding by sex. More commonly, analytic methods are used to reduce the potential for confounding. For instance, continuing the above example, if sex is an important confounder, we can stratify the analyses on sex, that is, consider the association between exposure and disease conditional on sex. More generally, one can use regression models to quantify the association between exposure and disease conditional on (or adjusting for) a number of potential confounders.

## Selection Bias

*Selection bias* {bias, selection} generally arises from the manner in which participants were selected for the study. A typical example of selection bias is the so-called *nonresponder bias* {bias, non-responder} in which people that agree versus those who do not agree to participate in a study differ in terms of both exposure and their risk of the outcome. If the factors which potentially influence the selection are known and therefore measured, the selection bias can be controlled in the statistical analysis (see confounding).

Selection bias may also occur in the context of case–control studies (see below) where the controls are meant to represent the distribution of exposure among the source population from which the cases arose. *Selection bias in a case–control*

*study* occurs when the controls are sampled in such a way that the exposure distribution among the controls does not estimate the exposure distribution in the source population.

Another common source of selection bias arises in the setting of missing data or when participants are *lost to follow-up*. If the risk of a subject missing data is related to both exposure and outcome, the missingness is said to be informative. Ignoring informative missing data can lead to selection bias. Similarly, loss to follow-up in a cohort study (see below) leads to missing data in some study participants. If the risk of being lost to follow-up is associated with both the exposure and outcome, selection bias may result.

## Information Bias {Bias, Information}

Exposures and outcomes in epidemiologic studies are always measured with error. Measurement error of dichotomous or categorical variables is often referred to as *misclassification*. Misclassification which depends on another variable is termed differential misclassification. Misclassification that does not depend on other variables is termed *non-differential misclassification*.

For example, differential misclassification of the exposure would occur if an exposure were measured with more error among those with the outcome of interest as compared to those not experiencing the outcome of interest. *Recall bias* {bias, recall} in the context of case–control studies is a well-known example of differential exposure misclassification; the health effect estimates are biased because those diagnosed with a specific disease (the cases) may recall their past exposures better than the controls without the disease. Differential misclassification can bias the estimated associations between exposure and disease either towards or away from the null hypothesis of no effect.

The impact of non-differential misclassification is sometimes predictable. Specifically, non-differential misclassification of a dichotomous exposure is expected to bias health effect estimates towards the null. This observation is often cited as a reason why one should not be overly concerned with measurement error of the exposure. However, non-differential misclassification of a categorical exposure with more than two categories, or a continuous exposure, can lead to bias either towards or away from the null, because in these cases the impact on study validity will depend on the pattern of classification and the measure of association.

## Effect Measure Modification and Interactions

*Effect measure modification* {modification, effect measure} (frequently simply referred to as *effect modification* or *interaction*) is present when the association between exposure and disease differs across levels of a third variable (the modifier). For example, the relative risk between exposure to asbestos and lung cancer is

known to be greater among smokers than among nonsmokers. In this example, it would be tempting to conclude that smokers are more susceptible to the effects of this exposure. However, for any given exposure-disease relationship, the presence of effect measure modification will depend on the measure of association being used, hence the term effect *measure* modification. For example, for a truly harmful exposure and in the absence of any other biases, the absence of effect modification when considering the risk ratio guarantees that effect modification will be present when one instead considers the risk difference. Effect modification is equivalent to the concept of *statistical interaction* {interaction, statistical} and represents a departure from a multiplicative or additive model, depending on the measure of association being modeled. Thus, it is entirely possible that effect measure modification will exist only in multiplicative models, only in additive models, or in both. Thus, the presence of statistical interaction or effect measure modification must be distinguished from the concept of biological interaction.

Students of epidemiology often have trouble distinguishing the concepts of confounding and effect modification. To clarify, effect modification is present when the strength of the association differs (aside from random variation) across strata of the potential modifier. On the other hand, confounding represents a mixing of the effect of the exposure with effects of other factors (confounders) on the outcome. Furthermore, confounders, by definition, are associated with exposure and outcome, and need to be controlled for in analyses. In contrast, an effect modifier need not be associated with either exposure or disease and need not to be adjusted for in analyses. A given factor potentially can be a confounder, an effect modifier, both, or neither.

## Random Error and Precision

The precision of a health effect estimate (i.e., magnitude of the random error) in an epidemiologic study can be quantified and will depend on the *sample size*. This means the *precision* of study results can generally be improved by increasing the sample size of the study. In *study planning*, this is used to determine the sample size needed to answer a specific research question. Furthermore, it leads to the concept of *statistical power*, i.e., the ability of a study to demonstrate an association of a given magnitude between exposure and outcome, if such an association actually exists.

One can often increase the precision of estimates from an epidemiologic study by improving study efficiency. For example, for the same number of study participants, some epidemiologic study designs may be much more efficient than others. For instance, a case–control study with 1,000 cases and 1,000 control subjects is expected to be more informative than a case–control study with 500 cases and 1,500 controls or a cohort study that follows 2,000 subjects, 100 of which develop the outcome of interest. In the above example, study efficiency can be assessed as the amount of information per subject with some study designs yielded more or less information per subject. However, depending on the resource constraints, one may

**Table 3** Principles and elements of "Good Epidemiological Practice"

| |
|---|
| Accordance with ethical principles |
| Formulation of explicit and operationalizable research questions |
| Detailed and binding study protocol |
| Well-documented biological sample banks |
| Quality assurance |
| Data management and documentation |
| Analysis |
| Observance of applicable data protection regulations |
| Legally binding agreements between all stakeholders (researchers, sponsors, collaborators) |
| Publication and interpretation of results |

wish to optimize the amount of information per research dollar spent rather than per subject. For example, if recruiting cases for a case–control study is much harder (i.e., more expensive) than recruiting control subjects, a study with fewer cases and relatively more control subjects may be the most cost-effective.

## Study Planning

A high *quality standard* in an epidemiologic study, usually conducted as a multidisciplinary cooperation, is only achieved when a *study plan* is developed at the start of each study which follows the generally recognized *Good Epidemiological Practice* (*GEP*) guidelines. In the international technical literature, guidelines have been developed which have found broad recognition and which contribute significantly to quality assurance if they are carefully followed. Some of the important principles and elements of the GEP are listed in Table 3.

## Statistical Analysis

### Estimation, Confidence Intervals, and Testing

Statistical procedures and statistical inference are material parts of epidemiologic methodology. Since statistics is accorded its own chapter (see chapter "▶ Statistical Evaluation Methods in Toxicology"), this section will merely address three important viewpoints, and in the following section, an introductory explanation of the regression models will be given.

The estimation of measures of association, such as a risk difference or odds ratio, is subject to random error. Virtually always, quantitative study results on the exposure-outcome association are given as a combination of an estimate of the strength of the association (*point estimate*) and an estimate of the uncertainty or precision associated with the point estimate. Depending on the target audience and the statistic being used, the uncertainty may be expressed using the *standard error*

of the point estimate or the 95 % *confidence interval* around the point estimate. Since the confidence interval provides information on both the magnitude and the probability of a potential error, it is often the preferred method for quantifying estimate uncertainty in epidemiologic studies.

An important question in epidemiology relates to the significance of *testing hypotheses* {testing, hypotheses}. The purpose of testing a hypothesis is to come to a decision as whether to accept or to reject a hypothesis on the basis of the results of a trial which was carried out for this purpose. This methodological starting point of the statistical test theory does not, strictly speaking, apply to the problem of an epidemiologic study, which is to quantify the exposure-outcome relationship rather than to make a decision about whether or not the relationship exists. However, it is quite sensible to take the *p-value* from the hypothesis testing, which is a continuous evidence measure of the compatibility of a hypothesis with the data observed, but the p-value should not merely be used with a predetermined threshold value such as a 5 % threshold to classify the results of the study as "significant" or "not significant."

Another point which is often hotly debated is *multiple testing* {testing, multiple} or more specifically the simultaneous investigation of several relationships in a study. Whether or not using procedures for simultaneous testing is recommended depends on whether the problem actually requires the simultaneous testing of a whole list of relationships (exposure to several end points of effect) or whether the various exposure-effect combinations are based on independent problems and, for example, are only being investigated together in one study because of practicability. In this latter case, no multiple testing is carried out. If multiple testing with a few simultaneous hypotheses is necessary, simple methods such as the *Bonferroni* method are available. For the analysis of more extensive sets of related hypotheses, modern but more complex procedures have to be used.

## Regression Models

As discussed above, epidemiologic studies are susceptible to confounding, and potential confounders need to be considered either in the design of the study (through restriction) or in the analysis of the resulting data (through stratification or statistical adjustment). In very simple cases involving only a small number of key confounders, tabular analyses with simple hand calculations can be used to obtain valid point estimates and confidence intervals. However, as the number of potential confounders increases, tabular analyses become impractical and statistically inefficient and regression models are preferred. Choosing the most appropriate *statistical model* {model, statistical} is complex and requires advanced understanding of the statistical issues, as well as a clear understanding of the hypotheses to be tested and the structure of all the relevant cause-effect relationships. Thus, *interdisciplinary cooperation* between epidemiologists, statisticians, and experts of all the affected specialties to create the model and evaluate and interpret the results is highly recommended.

## Recommended Reading

Rothman KJ, Greenland S, Lash TL (2008) Modern epidemiology, 3rd edn. Lippincott, Williams & Wilkins, Philadelphia

Woodward M (2005) Epidemiology, study design and data analysis, 2nd edn. Chappman & Hall/CRC press, Boca Raton

## Resources

Good Epidemiological Practice. http://dgepi.de/fileadmin/pdf/leitlinien/GEP_LL_english_f.pdf. Accessed 3 June 2013

# Studies in Volunteers and Its Regulation

Klaus Mörike

## Contents

Definitions ..... 207
Medicinal Product ..... 207
Drug ..... 208
Ethics Committee ..... 208
Important Documents ..... 209
Types of Clinical Trials ..... 210
Testing of Other Substances ..... 211
References ..... 211
Recommended Reading ..... 212

**Abstract**

Studies in volunteers are most important in the research and development of a medicinal product or, as defined by the FDA (see below), of a drug. Clinical trials in humans can be performed only if preceding toxicological studies and practical considerations rule out a risk for the subjects/patients. The tests provide evidence of safety, efficacy (drugs), and thresholds. Many regulations, such as insurance, privacy, and ethics committee, must be observed.

## Definitions

### Medicinal Product

The German Federal Institute for Drugs and Medical Devices (BfArM 2005) provides a definition according to the German Medicinal Products Act (Drug Law):

K. Mörike
Division of Clinical Pharmacology, Eberhard Karls University Tübingen, Tübingen, Germany
e-mail: klaus.moerike@med.uni-tuebingen.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_43, 

*Medicinal products are substances or preparations made from substances which:*

1. *Are intended for use on or in the human or animal body and are intended for use as remedies with properties for the curing, alleviating, or preventing of human or animal diseases or disease symptoms or*
2. *Can be used in or on the human or animal body or can be administered to a human being or an animal, either:*
   (a) *To restore, correct, or influence the physiological functions through a pharmacological, immunological, or metabolic effect, or*
   (b) *To make a medical diagnosis*

## Drug

The United States Food and Drug Administration, according to the Federal Food, Drug, and Cosmetic Act, defines drugs, in part, by their intended use, as "articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" *and* "articles (other than food) intended to affect the structure or any function of the body of man or other animals" (FDA 2002).

Studies in humans are subject to legal regulations (national and supranational, e.g., the European Union) which include Good Clinical Practice directives and professional regulations (Declaration of Helsinki, ethical committee). In addition, institutional regulations (e.g., standard operating procedures) usually apply also.

*Good Clinical Practice* is a standard for the design, conduct, performance, monitoring, auditing, recording, analyzes, and reporting of clinical trials that provides assurance that the data and reported results are credible and accurate and that the rights, integrity, and confidentiality of trial subjects are protected. It includes ethical and scientific quality standards for designing, conducting, recording, and reporting trials that involve participation of human subjects to ensure that the rights, safety, and well-being of the trial subjects are protected. It is also to ensure the credibility of clinical trial data (ICH-GCP 2012, and European Union 2005).

The *investigator* is a person responsible for the conduct of the clinical trial at a trial site. If a trial is conducted by a team of individuals at a trial site, the investigator is the responsible leader of the team and may be called the principal investigator. The *sponsor* is an individual, company, institution, or organization which takes responsibility for the initiation, management, and/or financing of a clinical trial. One of the sponsor's responsibilities is to get a *EudraCT* (European Union Drug Regulating Authorities Clinical Trials) number. EudraCT is the European Clinical Trials Database of all clinical trials commencing in the European Union from 1 May 2004 onwards (EudraCT 2012).

## Ethics Committee

An *Independent Ethics Committee* (IEC) is an independent body (a review board or a committee, institutional, regional, national, or supranational), constituted of

medical professionals and nonmedical members, whose responsibility is to ensure the protection of the rights, safety, and well-being of human subjects involved in a trial and to provide public assurance of that protection, by, among other things, reviewing and approving/providing favorable opinion on the trial protocol, the suitability of the investigator(s), facilities, and the methods and material to be used in obtaining and documenting informed *consent* of the trial subjects. The legal status, composition, function, operations, and regulatory requirements pertaining to IECs may differ among countries but should allow the IEC to act in agreement with GCP as described in the ICH-GCP Guideline (ICH-GCP 2012). It is important to note that, by incorporation into national law, the ICH-GCP Guideline is more than just a guideline (see also chapter "▶ Ethical Issues in Science. Focus on Regulatory Toxicology").

## Important Documents

The most important documents to be submitted to and reviewed by the IEC include:

- The *study protocol*. The protocol is a document that describes the objective(s), design, methodology, statistical considerations, and organization of a trial. The protocol usually also gives the background and rationale for the trial, but these could be provided in other protocol referenced documents. Throughout the ICH-GCP Guideline, the term protocol refers to protocol and protocol amendments (ICH-GCP 2012). The protocol also described the investigator's and sponsor's responsibilities. This is particularly important with the documentation, reporting, and assessment of adverse events.
- The *informed consent* form. The informed consent is a process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form (ICH-GCP 2012).
- The *Investigator's Brochure* (IB): The IB is a compilation of the clinical and nonclinical data on the investigational product(s) that is relevant to the study of the product(s) in human subjects. Its purpose is to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, many key features of the protocol, such as the dose, dose frequency/interval, methods of administration, and safety monitoring procedures. The IB also provides insight to support the clinical management of the study subjects during the course of the clinical trial (ICH-GCP 2012). Obviously, the IB needs to include all the current knowledge about the investigational product(s).

Clinical trials should be conducted in accordance with the ethical principles that have their origin in the *Declaration of Helsinki* and that are consistent with GCP and the applicable regulatory requirement(s) (ICH-GCP 2012). The World Medical

Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

## Types of Clinical Trials

Clinical trials are conducted in a series of steps, called *phases* (U.S. National Library of Medicine 2008; Pasqualetti et al. 2010; Rang et al. 2012):

Phase I: Researchers test a new drug or treatment in a small group of people (normally 20–80) for the first time to evaluate its safety, determine a safe dosage range, identify side effects, evaluate pharmacokinetics, and obtain preliminary data on pharmacodynamics. Phase I begins with the first administration of a new compound in humans. The first dose of this phase I is a fraction of the dose that causes harm in animal testing. Usually, phase I studies are performed in healthy volunteers. However, studies evaluating high-risk drugs should be performed in ill patients when the potential therapeutic effects of the test drug are expected to overcome its well-known toxicity (e.g., cytotoxic agents) or when the expected risks are not acceptable for healthy volunteers. Recently, the new "phase 0" (Marchetti and Schellens 2007) or "early phase I" studies were introduced, according to which the so-called high-risk drugs can be administered to a small number of healthy subjects in subtherapeutic micro-dosing studies, with a consequent reduction of toxicity risk.

A tragedy occurred at a London hospital in March 2006, when TGN1412, a new monoclonal antibody directed against a human lymphocytic antigen, was studied in a first-in-man clinical trial and had been shown to be well tolerated by nonhuman primates. A severe inflammatory response, causing catastrophic systemic organ failure in healthy subjects, led to the hospitalization of all six volunteers in intensive care units, despite being administered at a supposed subclinical dose (Kenter and Cohen 2006). After this tragedy, in an attempt to mitigate the risk associated with phase I clinical trials, the European Medicines Agency issued new guidelines to aid sponsors in the transition from nonclinical to early clinical drug development (European Medicines Agency 2007a, b). In general, a careful study protocol, a high level of attention, and a close clinical observation of the volunteers by the doctors are cornerstones for a safe conduction. This is especially true in the case of novel drugs and biologics.

Phase II: The drug or treatment is given to a larger group of people (normally 100–300, normally patients) to see if it is effective and to further evaluate its safety. If efficacy is confirmed, phase II studies are designed to establish the dose to be used in the definitive phase III study.

Phase III: The drug or treatment is given to large groups of people (thousands of patients) to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the drug or treatment to be used safely. At the end of phase III, the drug will be submitted to the relevant regulatory authority for licensing. The probability that a molecule will successfully

emerge from clinical trials was estimated to be about 21.5 % or even 11.5 % only (Munos 2009).

Phase IV: Studies are done after the drug or treatment has been marketed to gather information on the drug's effect in various populations and any side effects associated with long-term use. Serious adverse effects, if rare, may emerge with large-scale use only, and continuous safety reassessment by regulatory authorities may, in some specific cases, necessitate the restriction of the use or the withdrawal of the drug.

## Testing of Other Substances

To assess the tolerability of working materials and chemicals, controlled studies are occasionally performed in volunteers. Examples are studies of ozone effects on lung function or the study of the neuropsychological effects of low doses of solvents. Special rooms and equipment allow the inhalation of defined substance concentrations. Such studies are important in toxicology as for the revaluation of limit values for humans. The formalities required to perform such studies are usually similar to those of phase 1 clinical trials.

## References

BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte) (2005) Federal institute for drugs and medical devices, created 15 Apr 2005, updated 16 Jan 2007. http://www.bfarm.de/EN/drugs/drugs-node-en.html. Accessed 1 Nov 2012

EudraCT (European Union Drug Regulating Authorities Clinical Trials) (2012) last updated 9 Oct 2012. https://eudract.ema.europa.eu. Accessed 1 Nov 2012

European Medicines Agency (EMEA) (2007a) Guideline on requirements for first-in-man clinical trials for potential high-risk medicinal products, CHMP/SWP/28367/2007. http://www.ema.europa.eu/pdfs/human/swp/2836707en.pdf. Accessed 3 Nov 2012

European Medicines Agency (EMEA) (2007b) Strategies to identify and mitigate risks for first-inhuman clinical trials with investigational medicinal products, CHMP/SWP/28367/07. http://www.ema.europa.eu/pdfs/human/swp/2836707enfin.pdf. Accessed 11 Jan 2010

European Union (2005) Commission Directive 2005/28/EC of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:091:0013:0019:EN:PDF. Accessed 1 Nov 2012

FDA U.S. Food and Drug Administration (2012) Is it a cosmetic, a drug, or both? (or is it soap?), created 8 July 2002, updated 30 Apr 2012. http://www.fda.gov/cosmetics/guidancecomplianceregulatoryinformation/ucm074201.htm. Accessed 28 Oct 2012

ICH-GCP (2012) International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use, Good clinical practice. http://ichgcp.net. Accessed 1 Nov 2012

Kenter MJ, Cohen AF (2006) Establishing risk of human experimentation with drugs: lessons from TGN1412. Lancet 368:1387–1391

Marchetti S, Schellens JHM (2007) The impact of FDA and EMEA guidelines on drug development in relation to phase 0 trials. Br J Cancer 97:577–581

Munos B (2009) Lessons from 60 years of pharmaceutical innovation. Nat Rev Drug Discov 8:959–968
Pasqualetti G, Gori G, Blandizzi C, Del Tacca M (2010) Healthy volunteers and early phases of clinical experimentation. Eur J Clin Pharmacol 66:647–653
Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G (eds) (2012) Rang and Dale's pharmacology, 7th edn. Elsevier Churchill Livingstone, Edinburgh, Chapter 60: Drug discovery and development
U.S. National Library of Medicine (NLM) (2008) Clinical trial phases (last reviewed and updated 18 Apr 2008). http://www.nlm.nih.gov/services/ctphases.html. Accessed 1 Nov 2012

## Recommended Reading

Dowsing T, Kendall MJ (2007) The Northwick Park tragedy–protecting healthy volunteers in future first-in-man trials. J Clin Pharm Ther 32:203–207
World Medical Association (2008) WMA Declaration of Helsinki – ethical principles for medical research involving human subjects. http://www.wma.net/en/30publications/10policies/b3/index.html. Accessed 3 Nov 2012

# Statistical Evaluation Methods in Toxicology

Ludwig A. Hothorn

## Contents

The Falsification Principle ..... 214
Decision Scheme ..... 214
Proof-of-Hazard Approach ..... 214
Proof-of-Safety Approach ..... 218
Designs Including a Positive Control ..... 221
Impact of Sample Size ..... 221
Further Aspects ..... 222
References ..... 222

**Abstract**

What is specific to the statistics in toxicology, and why not just use textbook statistics? The reason is the aim of regulatory toxicology: "be confident in negative results." By toxicological studies, one would like to prove the harmlessness of new drugs. By means of the so-called proof-of-safety approach, the false-negative error rate (consumer's risk) is directly controlled. Unfortunately, in most of the statistical textbooks and publications, the alternative proof of the efficacy of new drugs with the direct control of the false-positive error rate is used, denoted in toxicology as proof of hazard. Therefore, in this chapter, the basics of the falsification principle are presented simplistic. The commonly used proof-of-hazard approach is discussed hereinafter, focusing on testing a dose-related trend. Finally, the proof-of-safety methods for selected study types are explained by means of examples.

L.A. Hothorn
Leibniz University Hannover, Hannover, Germany
e-mail: hothorn@biostat.uni-hannover.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_44, 

## The Falsification Principle

Most of the tests used in biostatistics base on Popper's falsification principle, briefly "An effect can never be proved directly, only in that the probability of its opposite is very low." This very small probability is the p-value of a test. It is a probability between 0 % and 100 %, where only very small levels, e.g., 0.01 %, argue against the null hypothesis. As an arbitrary limit, 5 % has been established, alternatively, for common tumors, a level of 1 % was proposed as relevance criteria. Commonly, the decision for either the null hypothesis (harmless substance) or the alternative hypothesis (substance of concern) is usually performed by a statistical test, e.g., the Wilcoxon test (proof of hazard). On harmlessness is conclude, if the p-value is greater than 5 %, i.e., the null hypothesis of equal expected values is not rejected.

## Decision Scheme

The type I error rate ($\alpha$) is the false-positive rate, i.e., the probability of false rejection of $H_0$, while in "truth" no difference between treatment and control exists. The type II error rate ($\beta$) is the false-negative rate, i.e., the probability of erroneous retention (i.e., non-rejection) of $H_0$, although in the "truth" a difference between treatment and control exists; see the following decision scheme (Table 1):

This results in the two fundamental problems of the confirmatory test statistic: (i) Only one of two errors – $\alpha$ or $\beta$ – is directly controlled, and (ii) the second error can only be controlled indirectly by a priori sample size determination (statistically) or definition (regulatory). It follows that the hypotheses is formulated in such a way that the content's more meaningful error was chosen as type I error. Thus, there are two test options: (i) tests of efficacy, in the case in screening research with the direct control of false-positive rate (proof of hazard), and (ii) tests of equivalence (two-sided hypotheses) respective test of non-inferiority (one-sided hypotheses) with the direct control of false-negative rate (proof of safety). For toxicological studies, therefore two concepts exist (see Tables 2 and 3).

Here $\delta > 0$ is minimal tolerable toxic effect, whereas we assume increasing values are toxic.

## Proof-of-Hazard Approach

The common design in regulatory toxicology includes a negative control, several (commonly 2–4) dose groups, and sometimes a positive control. For normal distributed endpoints, such as organ weights, the US National Toxicology Program recommends the use of either Dunnett (1955) or Williams (1972) procedure for pairwise comparisons between the dose group and the zero-dose control group. Both procedures control a familywise type I error (false positive) rate. The first

**Table 1** Error rates

| | | Computer output | Absolute, unknown truth | |
|---|---|---|---|---|
| | | | $H_0$ is true, i.e., no effect | $H_0$ is not true, i.e., effect |
| Test decision | $H_0$ not rejected | – (empty) | True | Type II error (β) false-negative rate |
| | $H_0$ rejected | * | Type I error (α) false-positive rate | True |

**Table 2** Proof of hazard

| | |
|---|---|
| Null hypothesis | $H_0^{Hazard}$: $\mu_{Treatment} - \mu_{Control} \leq 0$ (Substance harmless) |
| Alternative hypothesis | $H_A^{Hazard}$: $\mu_{BTreatment} - \mu_{Control} > 0$ (Substance harmful) |

**Table 3** Proof of safety

| | |
|---|---|
| Null hypothesis | $H_0^{Safety}$ $\mu_{Treatment} - \mu_{Control} \geq \delta$ (Substance harmful) |
| Alternative hypothesis | $H_1^{Safety}$ $\mu_{Treatment} - \mu_{Control} < \delta$ (Substance harmless) |

procedure tests chances against control, whereas the second tests a monotonic trend including to control. As long as monotonicity can be assumed, the Williams test is the recommended test in the proof of hazard. When downturn effects at high doses are possible, the Dunnett test or a related Williams test modification robust against such specific non-monotonicity at high doses should be used. Through the multiplicity adjustment (control of familywise type I error rate), these tests however intensify the control of the actually less relevant false-positive rate substantially. For example, the false-negative rate increases from 17.6 % to 34.4 % when comparing three groups with a control by the Dunnett procedure compared with independent t-tests (endpoint body weight, to be detected difference $\Delta = 10$ g, $\sigma = 10$ g sample sizes 14, $\alpha = 5$ %, Bonferroni adjustment). Therefore, pairwise two-sample tests "control vs. dose," each at level $\alpha$, may represent a compromise.

The evidence of a global trend represents a relevance criterion; on the other hand, the maximum safe dose (no observed effect level, NOEL) would be determined in toxicology also. The determination of a global trend appears as an easy task; nonlinear models or linear regression after data transformation is used frequently. The dilemma is that these approaches much depend on the shape of the dose–response. But the shape is not an assumption; it is just an outcome of the experiment. To post hoc data view and selecting from this impression out a specific model, is statistically incorrect. Therefore, it requires methods which are sensitive to all the possible shapes of the dose–response dependency. Tests with restriction order (trend test), based on the restricted alternative hypothesis,

$$H_0: \quad \mu_C = \mu_{D_1} = \mu_{D_2} = .... = \mu_{D_k}$$
$$H_A: \quad \mu_C \leq \mu_{D_1} \leq \mu_{D_2} \leq .... \leq \mu_{D_k}$$

can be used where at least one (any one) inequality in the alternative must hold true.

For this purpose, there are two principles: the likelihood ratio test and multiple contrast tests. Since the second approach is easier, numerically feasible confidence intervals are available and power can be directly estimated, this should be shown here shortly. The Williams procedure is a special order-restricted test including the zero-dose control – an important argument for its use in toxicology. The idea will be illustrated with reference to the experimental design $[C, D_1, D_2]$.

Here, precisely, there are two possible dose–response profiles with respect to the control:

$$H_A^1: \quad \mu_C = \mu_{D_1} < \mu_{D_2}$$
$$H_A^2: \quad \mu_C < \mu_{D_1} = \mu_{D_2}$$

For each profile, a contrast test can be used:

$$T_j = \frac{\sum_{i=C}^{k} c_i \bar{X}_i}{\sqrt{S^2 \sum_{i=C}^{k} \frac{c_i^2}{n_i}}}$$

The two sets of contrast coefficients $c_i$ are (simplified for a design with equal sample sizes)

| | | | |
|---|---|---|---|
| $c_i^1$ | $-1$ | 0 | 1 |
| $c_i^2$ | $-1$ | 0.5 | 0.5 |

The maximum test

$$T_{max} = \max\left(T_{1,} \ldots, T_q\right)$$

(in our example q = 2) is multivariate (q) distributed with a correlation matrix defined by the contrast coefficients and the sample size (using R library multcomp) or can be calculated by resampling approach (SAS procedure multtest).

This approach is demonstrated by Ames assay data (Table 4).

The following contrasts and their multiplicity adjusted p-values result using the R code:

```
library(multcomp)
mymod<−lm(y∼Dose, data=salmonellaTA98)
summary(glht(mymod, linfct=mcp(Dose="Williams"), alternative="greater"))
```

See Table 5.

**Table 4** Ames assay data

| I | Dose | Revertants | Mean |
|---|---|---|---|
| 0 | 0 | 23, 22, 14 | 19.7 |
| 1 | 100 | 27, 23, 21 | 23.7 |
| 2 | 333 | 28, 37, 35 | 33.3 |
| 3 | 1,000 | 41, 37, 43 | 40.3 |
| 4 | 3,333 | 28, 21, 30 | 26.3 |
| 5 | 10,000 | 16, 19, 13 | 16.0 |

**Table 5** Contrast coefficients and adjusted p-values

| Contrast | Coefficients ci | | | | | | p-value |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 3 | 4 | 5 | 6 | 0.26 |
| | −1 | 0 | 0 | 0 | 0 | 1.0 | |
| 2 | 1 | 2 | 3 | 4 | 5 | 6 | 0.095 |
| | −1 | 0 | 0 | 0 | 0.5 | 0.5 | |
| 3 | 1 | 2 | 3 | 4 | 5 | 6 | 0.037 |
| | −1 | 0 | 0 | 0.33 | 0.33 | 0.33 | |
| 4 | 1 | 2 | 3 | 4 | 5 | 6 | 0.069 |
| | −1 | 0 | 0.25 | 0.25 | 0.25 | 0.25 | |
| 5 | 1 | 2 | 3 | 4 | 5 | 6 | 0.142 |
| | −1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | |

For contrast 3, a slightly significant p-value results, indicating a global significant trend, whereas a plateau including the doses 1,000, 333, and 100 μg (contrast 3 with a p-value of 0.037) is most likely (Fig. 1).

However, the boxplots indicate an increase up to doses 100 μg; hereafter a downturn effect occurs. Therefore, the modified Williams procedure protected against downturn effect at higher doses is used (Bretz and Hothorn 2003). The idea is to test the global Williams trend together with a trend up to dose 333 μg, 100 μg, and up to 10 μg, i.e., for all possible peak points of the dose–response simultaneously (Table 6).

Contrast 7 reveals the smallest p-value, i.e., the peak dose is 333 μg (not the 100 μg guessed from the boxplot), and a plateau including doses 333 and 100 μg is most likely.

A different objective is the estimation of the NOEL. Commonly it is estimated by step-down significant trend tests, whereas NOEL is the next lower dose after the last significant trend test. But this classic proof of hazard has the disadvantage that with small sample sizes, lower doses would be characterized as safe. An alternative concept of maximal safe dose (MAXSD) is described in the following section for the proof of safety.

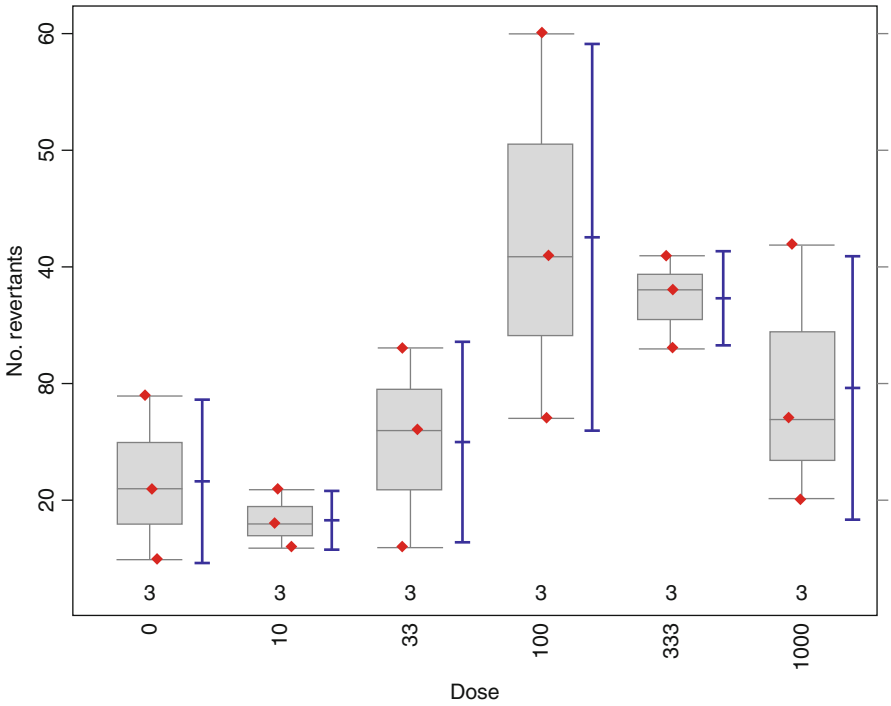



**Fig. 1** Box-plots for Ames assay data

## Proof-of-Safety Approach

The toxic response of most endpoints is either decreasing or increasing, such as declining numbers of offsprings in the Daphnia aquatic assay or rising number of micronuclei in the micronucleus assay. That is, the other direction is irrelevant for a toxicological perspective. Therefore, one-sided tests are appropriate for these assays. The harmlessness can be concluded by rejection of the null hypotheses that the difference between the treatment and dose effect is larger than an irrelevance threshold $\delta$. This decision can be achieved by so-called non-inferiority tests. However, the a priori definition of irrelevance threshold $\delta$ is needed. Because this threshold is endpoint specific and scale dependent, a consensus is hard to find for different toxicological assays. For aquatic toxicity assays recently instead of difference to control, ratio-to-control tests were proposed (Denton et al. 2011) which allows a percentage, i.e., scale-independent definition of the threshold $\eta$. For chronic assays $\eta = 75$ % and acute assays $\eta = 80$ % was proposed. The approach provides several advantages: (i) the proof-of safety concept controls the more important false-negative

**Table 6** Contrast coefficients and adjusted p-values

| Contrast | Peak dose | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 1,000 | 1 | 2 | 3 | 4 | 5 | 6 | 0.42 |
| | | −1 | 0 | 0 | 0 | 0 | 1 | |
| 2 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.17 |
| | | −1 | 0 | 0 | 0 | 0.5 | 0.5 | |
| 3 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.071 |
| | | −1 | 0 | 0 | 0.33 | 0.33 | 0.33 | |
| 4 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.13 |
| | | −1 | 0 | 0.25 | 0.25 | 0.25 | 0.25 | |
| 5 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.25 |
| | | −1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | |
| 6 | 333 | 1 | 2 | 3 | 4 | 5 | 6 | 0.12 |
| | | −1 | 0 | 0 | 0 | 1 | **0** | |
| 7 | | 1 | 2 | 3 | 4 | 5 | 6 | **0.038** |
| | | −1 | 0 | 0 | 0.5 | 0.5 | **0** | |
| 8 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.104 |
| | | −1 | 0 | 0.33 | 0.33 | 0.33 | **0** | |
| 9 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.25 |
| | | −1 | 0.25 | 0.25 | 0.25 | 0.25 | **0** | |
| 10 | 100 | 1 | 2 | 3 | 4 | 5 | 6 | 0.04 |
| | | −1 | 0 | 0 | 1 | **0** | **0** | |
| 11 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.16 |
| | | −1 | 0 | 0.5 | 0.5 | **0** | **0** | |
| 12 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.39 |
| | | −1 | 0.3333 | 0.3333 | 0.3333 | 0 | 0 | |
| 13 | 33 | 1 | 2 | 3 | 4 | 5 | 6 | 0.68 |
| | | −1 | 0 | 1 | **0** | **0** | **0** | |
| 14 | | 1 | 2 | 3 | 4 | 5 | 6 | 0.88 |
| | | −1 | 0.5 | 0.5 | **0** | **0** | **0** | |
| 15 | 10 | 1 | 2 | 3 | 4 | 5 | 6 | 0.93 |
| | | −1 | 1 | **0** | **0** | **0** | **0** | |

decision rate directly, (ii) it focuses on the toxicological relevant direction of decreasing effects, and (iii) it avoids the false claim of harmlessness when designs with insufficient small sample sizes are used (Hauschke et al. 1999).

The two-sample test for ratio-to-control comparison is a modification of the t-test (Fig. 2, Table 7):

$$T^{\text{Ratio}} = \frac{\bar{X}_T - \eta \bar{X}_C}{\sqrt{S^2\left(\frac{1}{n_T} + \frac{\eta^2}{n_C}\right)}}$$

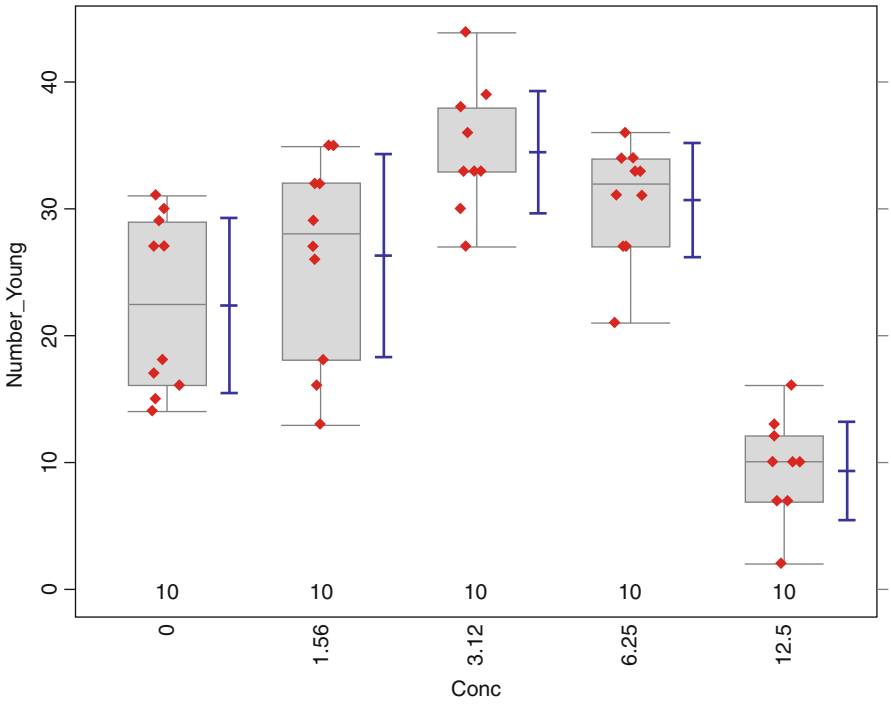



**Fig. 2** Box-plots for Daphnia data

**Table 7** Reproduction data for the Daphnid Ceriodaphnia dubia exposed to an effluent for 7 days

| Concentration/% | No. Young per adult |
|---|---|
| Control | 27, 30, 29, 31, 16, 15, 18, 17, 14, 27 |
| 1.56 | 32, 35, 32, 26, 18, 29, 27, 16, 35, 13 |
| 3.12 | 39, 30, 33, 33, 36, 33, 33, 27, 38, 44 |
| 6.25 | 27, 34, 36, 34, 31, 27, 33, 21, 33, 31 |
| 12.5 | 10, 13, 7, 7, 7, 10, 10, 16, 12, 2 |
| 25.0 | 0, 0, 0, 0, 0, 0, 0, 0, 0, 0 |

Whereas the p-values for the two-sample tests for the concentrations 1.56, 3.12, and 6.25 are very small, i.e., these concentrations are harmless, the p-value for the concentration 6.25 is p = 0.99, i.e., this concentration is not harmless; see the R code:

```
library(mratios)
t.test.ratio(Number_Young~Conc, data=daph, rho=0.8, base=1,
alternative="greater", var.equal=TRUE)
```

**Table 8** Decision tree Daphnia example

| Step | Comparison | p-value | Decision |
|---|---|---|---|
| 1 | $\mu_{1.56}/\mu_{Control}$ | 6.9e−04 | Significant, i.e., harmless |
| | | | Go to step 2 |
| 2 | $\mu_{3.12}/\mu_{Control}$ | 2.5e−10 | Significant, i.e., harmless |
| | | | Go to step 3 |
| 3 | $\mu_{6.25}/\mu_{Control}$ | 4.9e−07 | Significant, i.e., harmless |
| | | | Go to step 4 |
| 4 | $\mu_{12.5}/\mu_{Control}$ | 0.99 | Not significant, i.e., not harmless, i.e., dose 6.25 is MAXSD |
| – | $\mu_{25.0}/\mu_{Control}$ | Not tested | – |

The estimation of the maximally safe dose (MAXSD) can be performed by step-up non-inferiority tests (Table 8); the related R code is:

```
library(mratios)
simtest.ratio(Number_Young~Conc, data=daphnia, type="Dunnett", alternative="greater", Margin.vec=c(0.8,0.8,0.8,0.8))
```

## Designs Including a Positive Control

In some assays, a positive control (C+) is used, i.e., a substance of particularly known toxicity in appropriate dose. Especially in assays using a single endpoint, such as the number of micronuclei, this positive control is used to check the sensitivity of the current assays and/or to evaluate the relevance of the observed effects. Since "confidence in negative results" is the goal, the insensitivity of current assays would be a serious problem. By a significant pretest (C vs. Dj), the sensitivity is ensured. This test may also like the test (C vs. Dj) be performed to the level $\alpha$ while is aborted in the case of resistance. The relevance of the dose effects can be demonstrated by means of the relative ratio test:

$$T^{\text{Relevance}} = \frac{\bar{X}_D - \bar{X}_{C-}}{\bar{X}_{C+} - \bar{X}_{C-}}$$

## Impact of Sample Size

The sample size has a central position in decisions of tests, because the secondary error rate is determined only by an a priori estimation of the sample sizes. This will be illustrated for the proof of hazard and proof of safety on the basis of parametric tests (see Table 9).

For example, for the terminal body weight, a standard deviation estimator from historical studies of $\sigma = 10$ g and normal distribution can be derived.

**Table 9** Estimated sample sizes

| Proof of hazard | | Proof of safety | | |
|---|---|---|---|---|
| Detectable difference $\Delta$ | Sample size | $CV_{Control}$ | k-fold threshold $\eta$ | Sample size |
| 2.5 g | 199 | 10 % | 0.8 | 8 |
| 5 g | 51 | | 0.667 | 3 |
| 7.5 g | 23 | | 0.5 | (1) |
| 10 g | 14 | 25 % | 0.8 | 28 |
| 12.5 g | 9 | | 0.667 | 10 |
| 15 g | 7 | | 0.5 | 5 |
| 17.5 g | 5 | 50 % | 0.8 | 109 |
| 20 g | 4 | | 0.667 | 35 |
| 25 g | (1) | | 0.5 | 14 |

For the proof-of-hazard approach, the necessary sample size per group can be estimated for several detectable differences $\Delta$ (one-sided *t*-test, design with equal sample sizes, type II error rate = 20 %, type I error 5 %); see Table 9.

For the proof of safety using the ratio-to-control test, the sample sizes depend on the coefficient of variation of the control ($CV_{Control}$) and the irrelevance threshold $\eta$. Sample size depends seriously on the accuracy requirements. Therefore, sample size should be chosen "per chance" but by either a statistical calculation or regulatory recommendation.

## Further Aspects

Other relevant statistical problems in toxicology are (i) the evaluation of tumor incidences (in competition to mortality) (see Fairweather et al. (1998)), (ii) the maternal to fetal relationship in reproduction studies (Chow and Liu 1998, Chap. 10), and (iii) the interlaboratory comparison between new in vitro assays (Hothorn 2003).

## References

Bretz F, Hothorn LA (2003) Statistical analysis of monotone or non-monotone dose-response data from in vitro toxicological assays. ATLA (Alternat. Lab. Animals) 31(1):81–96

Chow S-C, Liu J-P (eds) (1998) Design and analysis of animal studies in pharmaceutical development. Marcel Dekker, New York

Denton DL, Diamond J, Zheng L (2011) Test of significant toxicity: a statistical application for assessing whether an effluent or site water is truly toxic. Environ Toxicol Chem 30(5):1117–1126

Dunnett CW (1955) A multiple comparison procedure for comparing several treatments with a control. Journal of the American Statistical Association 50(272):1096–1121

Fairweather WR, Bhattacharya A et al (1998) Biostatistical methodology in carcinogenicity studies. Drug Inf J 32:401–421

Hauschke D, Kieser M, Hothorn LA (1999) Proof of safety in toxicology based on the ratio of two means for normally distributed data. Biom J 41:295–304

Hothorn LA (2003) Statistics of interlaboratory in vitro toxicological studies. ATLA-Altern Lab Anim 31(Suppl 1):43–63

Hothorn LA, Hauschke D (2000) Identifying the maximum safe dose: a multiple testing approach. J Biopharm Stat 10:15–30

US-EPA (2013) Section 13, Test Method, Daphnid, Ceriodaphnia Dubia, survival and reproduction test. http://water.epa.gov/scitech/methods/cwa/wet/upload/2007_07_10_methods_wet_disk3_ctf13.pdf. Accessed 3 March 2013

Williams DA (1972) The comparison of several dose levels with a zero dose control. Biometrics 28(2):519–531

# Dose-Response Analysis, Identification of Threshold Levels for Chemicals

Hans-Karl Heim and Peter Mayer

## Contents

Dose-Effect Relationships for Toxic Effects on the Individual Level ..... 226
Interaction of Toxicants with Target Molecules in Living Organisms ..... 226
Reversible and Irreversible Damage, Accumulation of Toxic Effects ..... 226
Receptor-Mediated Toxic Effects, Law of Mass Action, and $K_D$ Value ..... 227
Sigmoid Shape of Toxicant-Receptor Binding Curves ..... 227
Toxicant-Receptor Binding and Agonistic Respectively Antagonistic Effects, Intrinsic Activity ..... 228
Characterization of Toxicological Efficacy and Potency ..... 230
Identification of NOEL and LOEL as Toxicological Threshold Levels ..... 230
Problems in Determination of NOEL- and LOEL-Levels ..... 231
Reporting of NOAEL- and LOAEL-Values ..... 232
Dose-Effect Relationships for Toxic Effects in Collectives ..... 232
Differences in the Individual Sensitivity Versus Toxicants ..... 232
$LD_{50}$ and $TD_{50}$ ..... 232
Distribution of Individual Sensitivity, Therapeutic Range ..... 234
Specific Dose-Effect Relationships ..... 234
Extrapolation of Threshold Levels to Application in Humans ..... 234
Use of More Than one Animal Species ..... 234
Quantification of Exposure ..... 235
Toxic Threshold Levels and Safety Margin in Humans ..... 235
Recommended Reading ..... 236
Resources ..... 236

**Abstract**

Following the initial observation of a toxic effect of a substance in humans or animals ["hazard identification", step 1 of the National Academy of Sciences (NAS)-scheme, see chapter "► Toxicological Risk Assessment"], the

H.-K. Heim (✉) • P. Mayer
Federal Institute for Drugs and Medical Devices, Bonn, Germany
e-mail: H.K.Heim@bfarm.de; p.mayer@bfarm.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_45, 

determination of the dose-effect relationship for the observed toxic effect represents the second step in the NAS-scheme. The toxicological threshold levels identified in this process (e.g., NOAEL) are then used for a quantitative toxicological risk assessment (NAS step 4), taking into account the available exposure data (NAS step 3).

## Dose-Effect Relationships for Toxic Effects on the Individual Level

### Interaction of Toxicants with Target Molecules in Living Organisms

Pharmaco-toxicological effects of chemical compounds, whether desired (e.g., in the case of therapeutic effects of medicinal products) or unwanted (e.g., in the case of detrimental effects of environmental toxicants), result in most cases from an interaction with specific target molecules in living organisms.

An important exemption from this rule is that chemically reactive compounds or compounds from which reactive metabolites (e.g., free radicals) are formed, which modify biomolecules more or less unspecifically. In this case, the resulting toxicological effects are dependent on numerous factors, for example, the regenerative or repair capacity of the affected cell or organism, so that no simple model exists to describe the pharmaco-toxicological effects of such compounds.

However, usually defined macromolecules, for example, proteins or nucleic acids, are the specific targets of toxicants. The interaction of a toxicant/drug with its target(s) is in most cases mediated by binding to the target molecule, which is characterized by a specific *affinity*. In the case of toxic compounds, the normal physiological function of the target molecule is usually affected by this process, whereby toxic (pathophysiological) consequences can be induced.

### Reversible and Irreversible Damage, Accumulation of Toxic Effects

A toxicant may bind to its target either in a reversible (e.g., by ionic or van der Waals binding) or irreversible (e.g., by covalent binding) way. However, whether a toxic effect is in the end reversible or irreversible does not only depend on the kind of interaction of the toxicant with its target molecule but also on the capacity of the respective target tissue for regeneration.

For example, the covalent, irreversible inactivation of the enzyme cholinesterase by a sublethal dose of the insecticide parathion (E605) may not necessarily lead to an irreversible damage, since new cholinesterase is continuously synthesized by the organism, whereby the toxic effect of parathion may be reversed. Also in the case of toxic effects on the liver, an organ with a high capacity for regeneration, tissue damage induced by toxicants is often reversible. On the other hand,

a toxicant-induced damage of the peripheral or central nervous system is often irreversible, since differentiated nervous cells have no or only limited capacity for regeneration (e.g., irreversible damage of the sensory cells of the inner ear by aminoglycoside antibiotics with permanent deafness/hardness of hearing as consequence).

In the event of irreversible effects, not only the administered single dose is important but also the cumulative dose which is taken up during lifetime (summation toxicants). For example, for the anthracycline derivative doxorubicin (used as a cytostatic), the cumulative total lifetime dose should not exceed 450 mg/m$^2$ body surface, since for cumulative doses exceeding this empirically defined value the risk of manifest heart damage is strongly increasing. In practice, threshold levels can be identified for many irreversible toxic effects.

## Receptor-Mediated Toxic Effects, Law of Mass Action, and $K_D$ Value

Hormone and neurotransmitter receptors represent important targets for many toxicants. In case of receptor-mediated toxic effects, the intensity of these effects depends on the number of receptors which are affected by the toxicant. The receptor affinity of the toxicant (*ligand*) determines its potency. The higher the affinity, the higher the number of occupied receptors at a given concentration of the ligand. The affinity is quantitatively described by the so-called *dissociation constant* $K_D$. This constant describes the disintegration of the receptor-ligand complex (RL) in case of reversible interactions:

$$RL \rightarrow R + L \tag{1}$$

This process is the reversion of the binding reaction of the ligand to its receptor (which is often easier to determine experimentally than the binding reaction itself). Since the reaction obeys the *law of mass action*, the dissociation constant is given as

$$K_D = \frac{[R]\ [L]}{[RL]} \tag{2}$$

where [L], [R], and [RL] are the concentration of the free ligand, the receptor, and the ligand-receptor complex, respectively. The $K_D$ value indicates at which ligand concentration half of the receptors are occupied. The *smaller* the $K_D$, the *higher* the affinity of the ligand to its receptor.

## Sigmoid Shape of Toxicant-Receptor Binding Curves

The magnitude of the pharmaco-toxicological effect of a ligand depends on the amount of ligand-receptor complex formed, that is, [RL], because usually only

a receptor that carries a bound ligand is biologically active. [RL] depends on the ligand concentration [L] in a way that can be derived by transformation from Eq. 2 above:

$$[\mathrm{RL}] = \frac{[\mathrm{R}]_t[\mathrm{L}]}{\mathrm{K_D} + [\mathrm{L}]} \tag{3}$$

In this equation, $[R]_t$ stands for the total receptor concentration, that is, for the sum of free receptor and receptor carrying ligand, [R] + [RL]. When [RL] is plotted against [L] according to the function provided in Eq. 3, then a hyperbolic curve results (Fig. 1a).

With a logarithmic scale of the x-axis, a sigmoid shape of the curve results (Fig. 1b). This logarithmic presentation more clearly indicates that a significant amount of ligand-receptor complex is only formed when the ligand concentration exceeds a certain limit (in the example of Fig. 1b a ligand concentration above around $10^{-7}$ mol/L). For smaller ligand concentrations, formation of ligand-receptor complexes and thereby biological activity is virtually negligible. The $K_D$ value can be estimated by determining the concentration which elicits the half-maximal response. This concentration is called $EC_{50}$ and is equal to $K_D$ as long as the assumptions made above are valid, that is, that the effect size is only determined by [RL] and that [RL] only depends on the affinity of the ligand to its receptor. Derivations from this ideal case can occur, for example, if the receptor has additional binding sites for other ligands, leading to allosteric effects, or if there are more receptors than signaling molecules (so-called *spare receptors*) so that not every occupied receptor can contribute to the effect.

## Toxicant-Receptor Binding and Agonistic Respectively Antagonistic Effects, Intrinsic Activity

A ligand, which is bound to its receptor, may activate (receptor agonist) or block (receptor antagonist) this receptor. Both processes may result in either desired or unwanted effects for the organism. A classical example for a toxic agonistic effect is observed for the mushroom poison muscarine, which can result in an overstimulation of the parasympathetic nervous system by an activation of cholinergic receptors. Contrarily, atropine, an alkaloid contained in belladonna, can elicit severe toxic effects by an antagonistic interaction with cholinergic receptors. However, atropine, by virtue of its cholinergic receptor-blocking properties, can be used as an antidote in case of intoxication with muscarine.

While muscarine is designated as an *agonist* at certain cholinergic receptors, atropine is designated as an *antagonist*. More specifically, atropine is called a *direct antagonist* of muscarine, because it binds to the same receptors. On the other hand, atropine may indirectly abrogate, as a *functional antagonist*, the decrease in heart rate induced by ß-receptor antagonists, by blocking inhibitory effects of the parasympathic nervous system on heart rate (For completion of definitions: a *chemical antagonist*

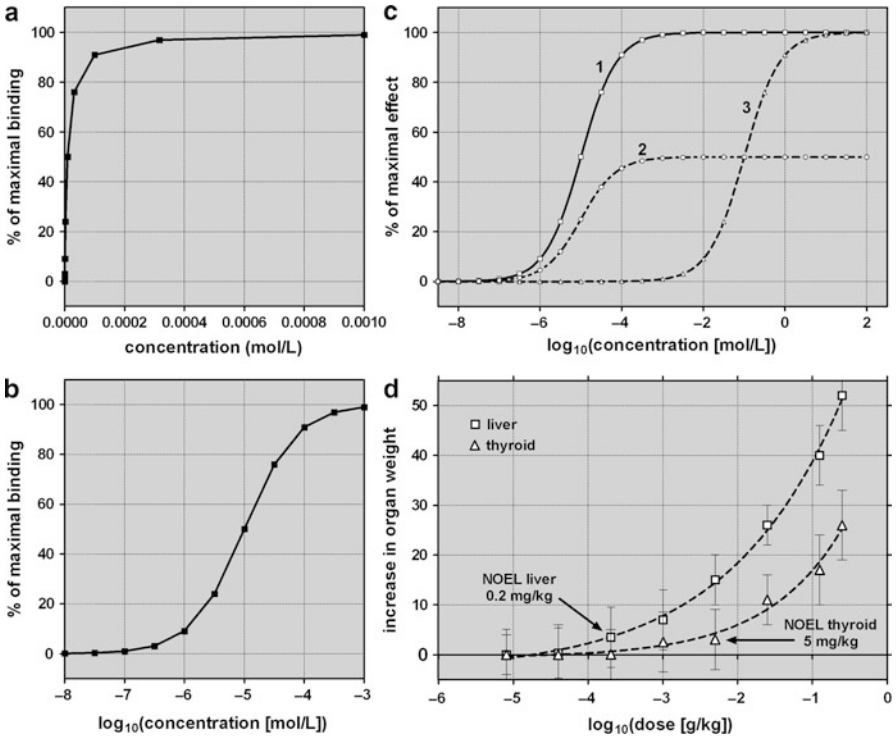



**Fig. 1 Ligand binding, dose-response relationship and toxicological threshold levels**. (**a**) shows the relationship between the ligand concentration and the resulting extent of binding of the ligand (e.g., a toxin) to the receptor. The relationship follows the law of mass action. The graphical view on a linear x-axis scale yields a hyperbolic curve. (**b**) shows the same as (**a**) but now with a logarithmic display of the ligand concentration. A sigmoid curve results. (**c**) shows dose-response relationships for ligands with different potency (substance 1 = substance 2 > substance 3) and different efficacy (substance 1 = substance 3 > substance 2). In (**d**), the deduction of the NOEL level for the effects of a substance on the organ weights of liver and thyroid is visualized. At the NOEL, the effect size is significantly different from zero. See text for further explanations

can abrogate the effects of another substance by direct chemical inactivation, for example, dimercaptopropane sulfonic acid in the case of toxic effects of lead).

A receptor ligand may act as an agonist or as an antagonist. This behaviour is described by the so-called *intrinsic activity* which quantifies the potential to activate, via binding to its receptor, downstream signal transduction mechanisms in the target cell (e.g., the cAMP system). It is possible to differentiate between "full agonists," which result in a maximal activation (intrinsic activity = 1) and *partial agonists*, which only result in a submaximal activation of signal transduction mechanisms (intrinsic activity >0 but <1). Pure antagonists have an intrinsic activity of 0, so-called *inverse agonists* have an intrinsic activity <0.

## Characterization of Toxicological Efficacy and Potency

The considerations outlined above assumed that the complete amount of administered ligand is directly available for receptor interaction, as it is the case in vitro. In vivo there is also usually a positive correlation between the total dose administered to the organism, the plasma concentration, and the concentration in the compartment where the receptor is located (often in a tissue outside the vasculature, either on the cell surface or intracellularly). Ideally, there is a linear relationship between the administered dose and the resulting ligand concentration [L] in the vicinity of the receptor. Therefore, the relationship between ligand concentration and response given in Eq. 3 can in principle also be applied to characterize the relation between administered dose and response.

The term *efficacy* describes the maximal effect ($E_{max}$) a substance can elicit if administered in sufficient dose. This situation is usually reached if all available receptor molecules have bound a ligand molecule, that is, in the case of maximal binding ($B_{max}$). A further increase of dose (and thereby of [L]) is not capable to increase the effect size further. In the graphical presentation of the dose-response relationship, this is reflected by the leveling off of the sigmoid curve at high ligand doses. Depending on the intrinsic activity of the ligand at its receptor, its efficacy may differ. By definition, full intrinsic activity is achieved with an agonist which activates the receptor to the highest extent possible. In the example of Fig. 1c, substance 2 shows a lower efficacy (intrinsic activity = 0.5, designated as partial agonism), whereas substances 1 and 3 display full agonistic activity (intrinsic activity = 1).

The *potency* of a ligand expresses how much (respectively how little) of this substance is needed to elicit a certain magnitude of response (usually the half-maximal response). The lower the required dose, the higher the potency. A higher potency manifests itself graphically as a shift to the left of the dose-response curve. In the example of Fig. 1c, the substances 1 and 2 possess a higher potency than substance 3 because the half-maximal effect ($ED_{50}$) is already reached at a dose of $10^{-5}$ mol/L, whereas for substance 3 the $ED_{50}$ is $10^{-1}$ mol/L.

## Identification of NOEL and LOEL as Toxicological Threshold Levels

Toxicants can have pharmaco-toxicological effects on different organs, for each of which a separate dose-response relationship can be established. This is illustrated in Fig. 1d, which shows the increase in organ weight of liver and thyroid in relation to the administered dose of a test substance. An increase in liver weight, often accompanied by histopathological signs of hyperplasia, is a relatively frequent toxicological finding. This is because foreign substances often induce the expression of metabolizing enzymes in the liver, an effect which in turn may lead to liver cell hypertrophy and hyperplasia in the long term. Thyroid hypertrophy on the other hand may be caused either by a direct thyrostatic effect of a test substance or may

occur secondary to enzyme induction in the liver, because the induced enzymes may degrade thyroid hormones more intensively and the thyroid gland has to produce a higher amount of hormones to keep the thyroid hormone plasma levels constant. Nevertheless, even under these circumstances liver and thyroid hypertrophy can display different dose-response relationships. This is because a considerable extent of enzyme induction in the liver is often necessary until first signs of secondary thyroid hypertrophy may become evident and there may be toxicant/drug doses at which an increased liver weight is found but no effects on thyroid weight can yet be observed.

The lowest dose at which an effect is observed is called the *LOEL* (*Lowest-Observed-Effect Level*). In the example shown in Fig. 1d, a statistically significant effect (organ weight increase) was observed at a dose of 1 mg/kg or above in the liver but not until 25 mg/kg in the thyroid.

The *NOEL* (*No-Observed-Effect Level*) is the tested dose level just below the LOEL. In the example provided in Fig. 1d, the NOEL was 0.2 mg/kg for liver and 5 mg/kg for thyroid. It should be emphasized that the LOEL (and thereby the NOEL) is usually defined by a statistically significant effect at this dose level although small, not statistically significant effects may be obvious already at lower dose levels. Furthermore, although in Fig. 1d a curve is fitted on the data points for clarity, fitted curves are usually disregarded for LOEL/NOEL determination and only the actual data points considered relevant. It should be pointed out that for statistical calculations, the number of replicates (animals per group in this case) and the statistical model used are important parameters. In the example shown in Fig. 1d, each dose level is regarded separately and statistical significance assumed if the 95 % confidence interval (95 % CI) no longer contains zero. Other approaches would also be conceivable, for example, a trend analysis. In any case, the selection of the statistical model used has to be justified.

## Problems in Determination of NOEL- and LOEL-Levels

On basis of the aforementioned considerations, it is obvious that the calculated NOEL- and LOEL-values, respectively, will reflect the true "no effect level" and "lowest effect level" all the better if the number of dose levels evaluated and the number of measured values per tested dose level (i.e., in animal studies, the number of evaluated animals) is high, because then statistical significance can already be reached for small deviations from the control value. In case that only few measured values are available, statistical uncertainty may be so high, that a NOEL can only be assigned far in the ascending part of the curve. To avoid this, toxicity studies should be performed with a sufficient number of animals. For example, for performance of nonclinical chronic toxicity and carcinogenicity studies in rodents in context with marketing authorization of medicinal products, use of at least 20, respectively, 50 animals per gender and dose level is recommended (OECD Guidelines 451 and 452).

### Reporting of NOAEL- and LOAEL-Values

Particularly during assessment of nonclinical study data for medicinal products it may, in certain circumstances, be a matter of discretion: which effects should be considered as unwanted (and therefore be avoided) and which effects can be tolerated with regard to the therapeutic benefit of the medicinal product. In the example shown in Fig. 1d, it would be conceivable to tolerate liver hyperplasia, if this is not related to irreversible liver damage, however, to consider impairment of thyroid function as being not tolerable.

For effects which are regarded as disadvantageous for the individual, instead of the NOEL-value (which includes desirable effects) the *NOAEL* (*No-observed-adverse-effect-level*) is given. Accordingly, the lowest dose level at which a significant adverse effect is observed is designated as *LOAEL* (*Lowest-observed-adverse-effect level*). In the example presented in Fig. 1d, the NOAEL would be 5 mg/kg, if liver hyperplasia is considered as "not adverse," and 0.2 mg/kg, if it is considered as "adverse."

## Dose-Effect Relationships for Toxic Effects in Collectives

### Differences in the Individual Sensitivity Versus Toxicants

In the preceding paragraphs, the dose-dependency of toxicant/drug effects was described, which should in principle be valid for each individual of the evaluated species. However, it should be considered that not each animal or human being will react in an exactly identical way to a given toxicant/drug. In practice, some individuals will react more sensitive and others will react less sensitive than the average. For the more sensitive individuals, a given toxic effect will therefore occur already at a lower toxicant dose when compared with the less sensitive individuals.

### $LD_{50}$ and $TD_{50}$

The dose-response relationship in collectives shall be exemplified for a long-known toxicological parameter, the $LD_{50}$ (LD stands for *Lethal Dose*), which indicates the dose of a test substance at which half of the treated animals die. The $LD_{50}$ is a first, orientating but rather crude measure for the toxicity of a substance. Ideally, differences in the individual sensitivity of the animals toward the substance result in a bell-shaped Gaussian curve for lethal dose (see Fig. 2a). Few animals die already at a rather low dose. With increasing doses the mortality reaches a maximum (at the $LD_{50}$), and again few animals survive until a rather high dose. Integration of the Gaussian distribution results in a function *of cumulative mortality* versus dose which expresses the number of animals that die *until* (instead of *at*) a given toxicant/drug dose. This integrated Gauss curve again has

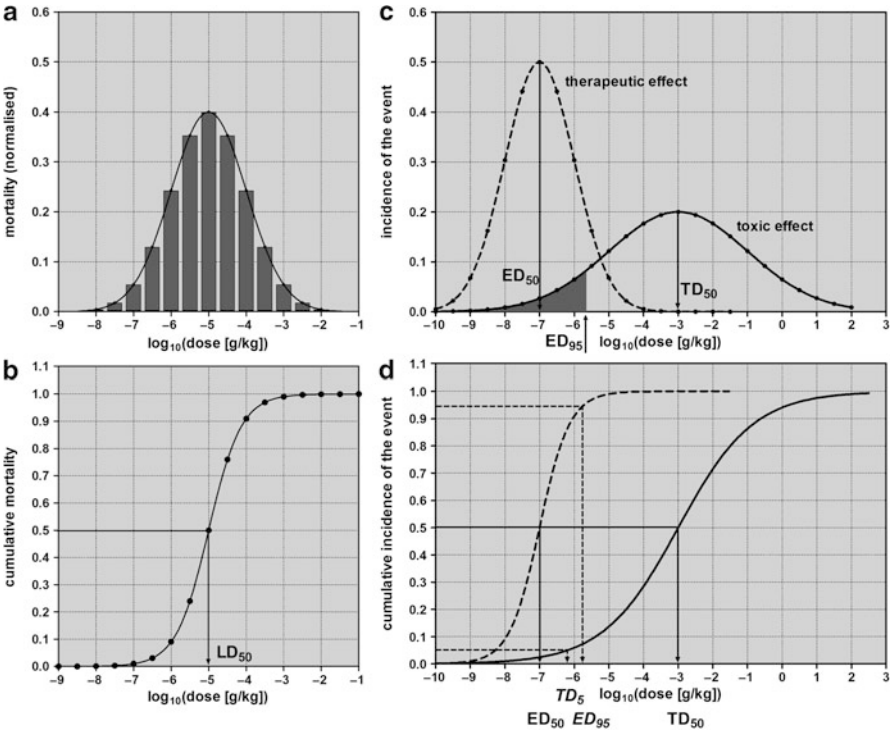



**Fig. 2 Dose-response relationship in collectives**. (a) displays the statistical fluctuation of the individual lethal dose of a toxin within a collective. Ideally, as shown, a typical Gaussian distribution results. The maximum of the Gauss curve lies at the mean lethal dose ($LD_{50}$); in (**b**) the curve of cumulative mortality is shown which was obtained by integrating the curve displayed in part (**a**). From this cumulative presentation, the $LD_{50}$ value can be derived. (**c**) shows the frequency distribution, in relation to the dose, for a desired therapeutic effect and for an unwanted toxic effect. From the large interindividual variability an overlap results, so that in some individuals (*shaded area*) toxic effects already occur at doses in the therapeutic range (i.e., up to $ED_{95}$). (**d**) provides the data shown in (**c**) in a cumulative, that is, integrated, manner with deduction of the parameters $TD_5$ and $ED_{95}$ together with the standard parameters $TD_{50}$ and $ED_{50}$

a sigmoid shape, and, in analogy to the determination of $ED_{50}$ from the dose-response curve, $LD_{50}$ can be determined (graphically or with the help of an adequate computer program) from this function (See Fig. 2b). In toxicological tests, usually different groups of animals are treated with different, ascending doses of the test substance and the event rate (in case of $LD_{50}$ the event "death") is counted in each group. This approach immediately yields the integrated Gauss function. Similar calculations can be performed for other parameters of interest, for example, organ toxicity, behavioral changes, etc. In this case, the resulting quantitative parameter is not called $LD_{50}$ but $TD_{50}$ (TD stands for *Toxic Dose*).

### Distribution of Individual Sensitivity, Therapeutic Range

The $TD_{50}$ is an orientating parameter which gives no information about the interindividual variability of the effect. In case of a large variability, unwanted effects can occur in a relatively large part of the population already at doses that are far below the $TD_{50}$. This is visualized in Fig. 2c, there the distribution of the desired (therapeutic) effect and the unwanted (toxic) effect of a drug is plotted. For the toxic effect, a $TD_{50}$ of $10^{-3}$ g/kg is identified, whereas the $ED_{50}$ lies at $10^{-7}$ g/kg, which implies a large safety margin, with a *therapeutic ratio* (defined as quotient $TD_{50}/ED_{50}$) of $10^4$. However, a more detailed consideration of the dose-response relationships reveals that there is considerable overlap of the therapeutic and toxic effect curves and that a considerable fraction of the total population experiences toxic effects already at doses needed to elicit the full desired effect. Quantitatively spoken, the dose that elicits the desired effect in 95 % of the population ($ED_{95}$) also leads to toxic effects in more than 5 % of the collective (shaded area in Fig. 2c), that is, is higher than the $TD_5$ value (Fig. 2d). Thus, a more safety-related definition of the therapeutic ratio uses the $TD_5/ED_{95}$ quotient instead of the $TD_{50}/ED_{50}$ quotient. In the example shown in Fig. 2c, d the $TD_5/ED_{95}$ ratio is about 0.25 and thus markedly below 1.

### Specific Dose-Effect Relationships

Dose-response curves with a U-shape (*hormetic curves*) can occur, for example, in the case of essential nutrients or trace elements. For example, vitamin A, when given in high doses, has a marked teratogenic effect. However, since vitamin A in a low dosage is essential for the correct embryo-fetal development, vitamin A-deficiency may also result in the occurrence of malformations.

## Extrapolation of Threshold Levels to Application in Humans

NOAEL and other threshold levels determined in animal studies are valid at first only for animals of the investigated species. Extrapolation of these threshold levels to the human situation in context of a marketing authorization procedure for a medicinal product is shortly exemplified below.

### Use of More Than one Animal Species

According to ICH and European guidelines for evaluation of repeated dose toxicity of medicinal products, toxicological tests should usually be performed in more than one animal species. Hereby it is ensured that potential species-specific effects, that is, toxic effects which only occur in a specific animal species, are revealed. Often, such effects are not representative for human toxicology.

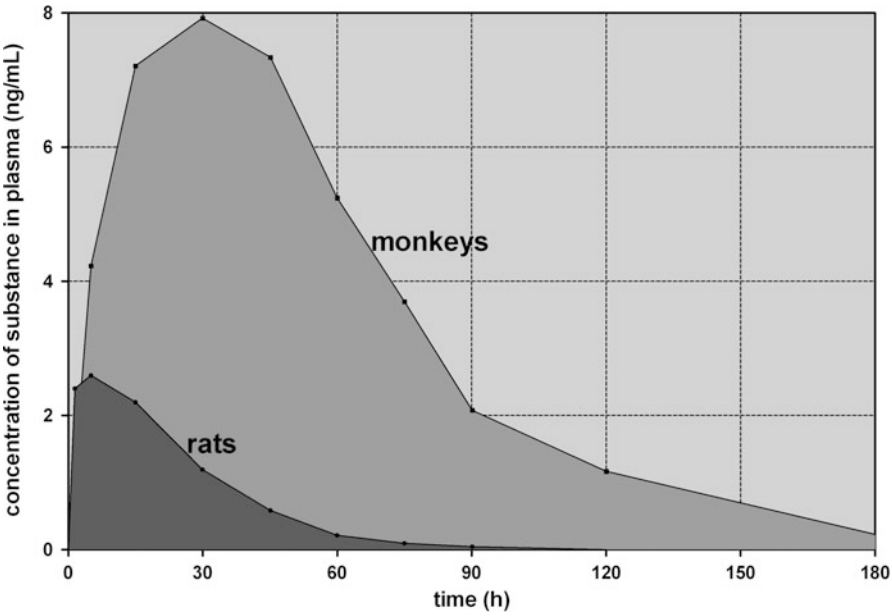



**Fig. 3 Measurement of plasma levels for determination of drug exposure**. The plasma levels (means) observed at different time points after single subcutaneous administration of a substance at a dose of 10 mg/kg at $t = 0$ is shown for rats and monkeys. Note that the maximal plasma levels ($C_{max}$) and the area under curve (AUC), reflecting integration of plasma levels over time and hence total systemic exposure, are much larger in monkeys (AUC 26 ng·h/mL) than in rats (AUC 1.4 ng·h/mL) in this example

## Quantification of Exposure

Usually, dosage in toxicological studies makes reference to the bodyweight (mg/kg bodyweight) of the used laboratory animals. However, a direct extrapolation of these values to the human situation may be problematic, since in smaller animals, at the same weight-based dosage, often a considerably lower systemic exposure than in larger animals or in humans is achieved (see Fig. 3). In such cases, standardization of the dosage to *body surface* ($mg/m^2$) often provides a better correlation. However, instead of relying on data extrapolation to estimate exposure, it may be more favorable to measure the actual systemic exposure of experimental animals and of humans directly, for example, by determination of plasma levels following drug administration (see below).

## Toxic Threshold Levels and Safety Margin in Humans

For marketing authorization of medicinal products, it is recommended to collect pharmacokinetic data in humans and in laboratory animals for comparison.

Under the assumption that at a systemic exposure at which toxic effects are observed in the animal study (LOAEL), toxic effects can also be expected to occur in humans, a *safety margin* can be estimated for drug application to humans. For this purpose, the ratio of the systemic exposure (*Area Under Curve, AUC*) at the LOAEL (or alternatively the NOAEL) in the animal study and the systemic exposure at the (maximal) therapeutic dose level in humans is calculated. This quotient is called *exposure multiple* and indicates how far the dose range, to which humans are exposed during therapeutic dosing of a medicinal product and the dose range, at and above which toxic effects have to be feared, are separated. In practice, often additional factors have to be taken into consideration, for example, the fact that pharmacokinetics may show dose-dependency, may change after repeated administration (e.g., by induction of enzymes involved in drug degradation/metabolism), or may be influenced by genetic polymorphisms.

By taking into account the aforementioned parameters, finally a *risk-benefit analysis* is performed for the medicinal product in which the main toxicological findings, the calculated safety margin, the expected therapeutic benefit, and specific factors related to the exposed patient population, are taken into consideration. A more detailed description of the benefit-risk evaluation is provided in other chapters of this book.

## Recommended Reading

Blumenthal DK, Garrison JC (2011) Chapter 3. Pharmacodynamics: molecular mechanisms of drug action. In: Brunton LL, Chabner BA, Knollmann BC (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 12th edn. McGraw-Hill, New York

OECD Guidelines for the testing of chemicals: 451 – carcinogenicity studies

OECD Guidelines for the testing of chemicals: 452 – chronic toxicity studies

## Resources

toxnet.nlm.nih.gov/
www.oecd.org

# Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds

Lutz Edler

## Contents

Study Types and Evaluation Principles ..... 238
A Road Map for Extrapolation ..... 238
Choice of Risk Parameters ..... 239
Choice of Risk Measures ..... 240
Dose Extrapolation ..... 242
Risk Assessment Under the Threshold Dose Assumption ..... 243
Risk Assessment Without Threshold Dose Assumption ..... 244
The Limit Risk ..... 246
Benchmark Dose ..... 247
Other PoDs ..... 248
Margin of Exposure (MoE) ..... 249
References ..... 250

**Abstract**

One fundamental goal of regulatory toxicology is to establish safe levels of human exposure to toxic compounds. This is usually performed within the framework of risk assessment (see chapter "▶ Toxicological Risk Assessment") and risk management: Using both data describing human exposure (*exposure assessment*) and results from the characterization of the toxicity (*hazard characterization*), the risk of the compound can then be characterized (*risk characterization)* in a framed approach through health-based guidance values (HBGVs), or related measures are used for that purpose. In the absence of information to establish dose–response relationships at exposure levels such low as they are generally experienced by humans, high-dose to low-dose extrapolation is generally used. Whereas epidemiological findings of the agent's

L. Edler
Department of Biostatistics –C060, German Cancer Research Center (DKFZ), Heidelberg, Germany
e-mail: edler@dkfz.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_46, © Springer-Verlag Berlin Heidelberg 2014

toxicity are directly applicable to humans, toxicological results detected in experimental animals need in addition the extrapolation from the specific animal species to human. To estimate the magnitude of expected effects at lower doses reflecting human exposure or corresponding to acceptable risks for humans set by regulatory practices, appropriate extrapolation is required. This chapter focuses on the low-dose extrapolation of animal data but presents at the same time general methodology also applicable to human data to establish HBGVs for humans.

## Study Types and Evaluation Principles

Although human studies, epidemiological studies (see chapter "► Epidemiological Methods in Regulatory Toxicology") in particular, would be the gold standard for the risk assessment of compounds to which humans are exposed, those studies are almost always of observational nature with retrospective elements and confounded by other risk factors (e.g., personal, behavioral, and environmental characteristics, co-exposure to other agents) and background exposure. Therefore, specialized statistical and epidemiological methods are required to analyze these data. It should be noted that the most valuable human data are often obtained from highly exposed populations (e.g., occupational cohorts) and do neither cover dose ranges relevant for regulatory practice and such they need also the extrapolation to effects at low doses. In contrast to human data of high variability and heterogeneity are data from studies in usually inbred strains of experimental animals which exhibit very low heterogeneity and moderate variability. Furthermore, confounding can be efficiently controlled by prospective and randomized designs. Therefore, animal studies have been considered as gold standard for human risk assessment as well, even when two steps of extrapolation – from high to low doses and from animals to humans – are required.

Although the nature of statistical methods is general enough to be applied to both carcinogenic and noncarcinogenic data, the statistical methods for risk extrapolation must account for a risk management principle, e.g., the biologically based paradigm that genotoxic and/or directly DNA reactive carcinogens would not allow, assuming the existence of a threshold exposure level below which no biological effect is possible. Even when the existence of a threshold could be assumed for noncarcinogenic compounds or carcinogens which do not directly react with DNA, estimating that threshold dose would require the use of statistical methods and in most cases extrapolation methods as well since that dose may also range in a low-dose region.

## A Road Map for Extrapolation

Risk extrapolation of both carcinogenic and noncarcinogenic compounds is preferably performed in a carefully planned investigation which should account for a number of critical check points listed in Table 1 as sort of road

**Table 1** Road map and checklist for extrapolation

| |
|---|
| 1. **Data examination**: Screening and assessment of the available data on the compound, their quality, their relevance, and their suitability to construct dose–response relationships |
| a. When not only one but a whole class of compounds is assessed (e.g., dioxins), clarify how to proceed (lead compound, group risk index, toxic equivalence) |
| b. When more than one study and more than one endpoint are to be considered, check for pivotal studies and critical endpoints, respectively |
| 2. **Risk parameters and measures**: Definition of risk parameter (the "risk") by appropriate choice(s) of critical effects and of the type of risk measure used for characterizing the risk and for which an extrapolation to low(er) doses is planned |
| 3. **Dose–response relationship**: Construction and critical evaluation of the presence and of the type (linear versus nonlinear, monotone versus non-monotone, steepness at low and/or saturation at high doses) of the dose–response relationship. Check for availability of data from step 1 and discuss (risk-based) effect sizes regarding the choices made in step 2 |
| 4. **Assumption of a threshold dose**: Decision about the biological nature of the critical effect on the basis of all available biological data (structure-activity relationship, in vitro and in vivo tests, short- and long-term animal studies, epidemiological studies). Assess available evidence for the existence of a threshold dose only when the substance is not a genotoxic carcinogen |
| 5. **Extrapolation**: Determination of the data suitable data for a fitting dose–response models and choice of a set of models or model classes which may fit the regulatory purpose |
| a. Derive in a first step a *point of departure (*PoD*) or reference value (*RP*)* from the available dose–response data and assess its statistical quality (e.g., standard error, confidence interval) and the degree of extrapolation used thereby |
| b. Establish in a second step a health-based guidance value (HBGV), e.g., an acceptable/tolerable daily intake value (ADI/TDI), or characterize the gap between the PoD/RP and the estimate of current human exposure, e.g., through a margin of safety or margin of exposure (MoE) |
| 6. **Outcome assessment**: Critical evaluation of the uncertainty of the regulatory value established in step 5 (e.g., by means of probabilistic methods), determination of data gaps, and formulation of recommendations of further research and additional data if appropriate |

map. Working through these points cannot be without considering the resources available for the assessment (e.g., available scientist and their profile of expertise, access to data, computational facilities including software) and the time frame for delivering the low dose extrapolation results. It should also be noted that this checklist may be applied iteratively for refining the assessment process.

## Choice of Risk Parameters

The critical effects which define the risk parameter for extrapolation should have been identified in an earlier step of risk assessment (*"hazard identification"*) as adverse effects which are potentially relevant for risk characterization and which can be assessed quantitatively for extrapolation from high to low doses. Methodological statistical considerations differentiate between three major

classes of data types which express increasing statistical (not necessarily biological) content of information:

- Quantal (e.g., the occurrence of a defined illness)
- Categorical-ordinal (e.g., severity of allergies)
- Quantitative-metric (e.g., concentration of a liver enzyme).

Carcinogenic effects seen in animal studies usually fall into the class of *quantal data*, since the occurrence of cancer (*cancer incidence*) and death from cancer (*cancer mortality*) are the relevant endpoints for human cancer risk assessment. Both are still considered as the most relevant indices for cancer risk assessment and to control cancer disease in a population. For time-to-tumor data, both the biological database and the statistical tools available are still not well developed. In contrast to the quantal data describing carcinogenic effects, the assessment of noncarcinogenic effects is much more diverse and needs special considerations for selecting the relevant adverse events and identifying the parameters which describe these effects best. On the other hand, the database for noncarcinogenic endpoints is often richer, and there are often *quantitative data* available which allow powerful dose–response analysis with smaller numbers of subjects. Data of the type *categorical-ordinal* are rarely analyzed for extrapolation purposes and require in general more specialized methods.

## Choice of Risk Measures

Based on the critical effect which could be a disease incidence or the change of a quantitative marker of a health effect (e.g., beta-2-microglobulin, a biomarker of renal tubular effects), a quantitative risk measure $R$ must be defined, which describes the risk as a mathematical function $R(d)$ of the exposure dose, denoted $d$. In animal experiments the dose is usually expressed in units of mg/kg body weight administered per day. Alternatively one may formulate the risk measure also in terms of the concentration of the substance, e.g., in a target organ (e.g., blood, liver, kidney).

In the case of quantal data, $R(d)$ expresses the probability of the occurrence of the critical effect in the subject of investigation exposed to dose $d$:

$$R(d) = P\ (Effect \mid Dose = d).$$

The symbol P stands for *probability* (unfortunately, sometimes also denoted as risk). For many compounds one must assume that there exists background exposure, either from exogenous or endogenous origin that adds to the total exposure (total exposure = background exposure + exposure through administered dose = d). Denoting the risk due to background by $R_0 = R(0)$, one may distinguish between additional and extra risk:

- Additional/added risk (above background): $R^*_{Add} = R(d) - R_0$.
- Extra risk (of the substance): $R^*_{EXCESS} = \frac{R(d)-R_0}{1-R_0}$.

**Table 2** Estimates for unit risks (UR) and unit doses (LAI 1992)

| Pollutant | UR[a] per µg/m$^3$ | UD (1 µg/1 m$^3$) |
|---|---|---|
| Arsenic[b] | $4 \times 10^{-3}$ | 2.5 ng/m$^3$ |
| Asbestos[c] | $2 \times 10^{-5}$ | 50 F/m$^3$ |
| Benzene | $9 \times 10^{-6}$ | 1.1 µg/m$^3$ |
| Cadmium[d] | $1.2 \times 10^{-2}$ | 0.83 ng/m$^3$ |
| Diesel particles | $7 \times 10^{-5}$ | 0.14 µg/m$^3$ |
| PAH (benzo(a)pyren) | $7 \times 10^{-2}$ | 0.14 ng/m$^3$ |
| 2,3,7,8-TCDD | 1.4 | 7.1 pg/m$^3$ |

[a]Estimated cancer risk for a person who had constant inhalation exposure to a concentration of 1 µg pollutant per cubic meter of air over 70 years
[b]Arsenic and its inorganic compounds
[c]Based on 100 F/m$^3$ (F fibers)
[d]Cadmium and its compounds

Risk measures for quantitative-metric data where *R(d)* simply represents the effect size associated with the toxic compound can be defined accordingly as:

- Additional effect: $R^*_{Add} = R(d) - R_0$.
- Relative effect (size): $R^*_{Rel} = \frac{R(d)-R_0}{R_0}$.

In quantitative risk assessments of environmental contaminants, in particular, when chronic inhalation exposure is assessed in epidemiological studies on cancer incidence or mortality, the *unit risk (UR)* has been used as an international agreed risk measure, defined as the extra risk when a constant concentration of the toxic compound of 1 µg/m$^3$exists in the inhaled air. Formally, this can be written:

$$Unit\ risk = P\left(C|constant\ exposure\ 1\,\mu g/m^3\right) - P(C|no\ exposure)$$

where C represents the occurrence of the observed disease, e.g., cancer. Similar as for the additional risk, the first term on the right describes the probability of disease due to the exposure (1 µg/m$^3$) and, respectively, the second due to background, i.e., when the substance is absent. *UR* is then the excess lifetime cancer risk from continuous lifetime exposure to an agent at a concentration of 1 µg/m$^3$ in air. The interpretation of an $UR = 3 \times 10^{-6}$ per µg/L means that three excess cancer cases are expected to develop per 1,000,000 people if exposed to the *unit dose (UD)*, i.e., the daily exposure for a lifetime to 1 µg of the substance in 1 m$^3$ in air, analogously, when exposed to drinking water in units of 1 µg/L water or through food in units of 1 µg/kg food, see, e.g., http://www.epa.gov/risk_assessment/glossary.htm. In a specific situation, the UR is simply multiplied by the exposure dose, say µg/m$^3$, to calculate a risk estimate (see, e.g., Becher and Steindorf 1993). UR is the preferred measure for comparing the carcinogenic potentials of different toxic compounds (see, e.g., Table 2 where some important airborne environmental carcinogens are compared with polycyclic hydrocarbons which show a 1,000 higher carcinogenic potency compared to diesel soot

particles). It should be noted that without additional specification, all these risk measures assume lifelong constant exposure to the substance, in the past often assuming life length of 70 years.

## Dose Extrapolation

Extrapolating from an established dose–response relationship available for the dose range

$$D_{\text{Experimental}} : d_{\min} < d < d_{\max}$$

to a lower dose range

$$D_{\text{Extrapolation}} : d_L < d < d_U, \text{ where } d_U < d_{\min}$$

should distinguish between low-dose extrapolation with or without assuming a threshold dose. This distinction has guided risk assessment (WHO 1999), although the question of the existence of biological thresholds has hardly been unequivocally resolved for any compound. Interindividual differences of responses both of carcinogenic as well as noncarcinogenic substances are just one observation which questions the existence of universally applicable thresholds (*"heterogeneity in the population"* argument) (see also Rhomberg et al. (2011)). Nevertheless, the threshold concept has been introduced in regulatory toxicology as pragmatic mean and has been applied even though lower doses may show a biological effect but considered as irrelevant or may be indistinguishable from background in the presence of statistical variation including measurement error. An overview on possible extrapolation scenarios for human or animal data depending on the assumption on the existence of threshold doses is given in Table 3.

**Table 3** Four possible scenarios for extrapolation

| | Assumptions concerning the biological nature of action | |
|---|---|---|
| Data source | No threshold | Threshold |
| Epidemiologic | **EKS** | **ES** |
| | Dose–response model | Approximation of the threshold |
| | Extrapolation in the model | Determination of a PoD/RP and using safety factor $SF_{intraspec}$ |
| Animal experiments | **TKS** | **TS** |
| | Dose–response model | Same as ES but in addition using safety factor $SF_{interspec}$ |
| | Extrapolation in the model | |
| | Extrapolation to humans | |

## Risk Assessment Under the Threshold Dose Assumption

When the existence of a threshold is assumed, below which no biologically relevant effect of the compound can be expected, the aim of a regulatory approach may be to estimate that biological threshold, say D*, as close and precise as possible. Accounting for the uncertainty of that estimate, a sufficiently large safety margin represented by a safety factor (SF) would establish an *intervention dose* (ID) below which no biologically significant effects would be expected:

$$\mathrm{ID} = \mathrm{D}^{*}/\mathrm{SF},$$

also referred as reference dose (RfD) (see WHO (1999)) defined as the maximum dose without significant or appreciable adverse effect on human health.

In a first step toward estimating D*, traditionally the smallest experimental dose at which no adverse effect is observed has been determined using the dose–response data available. Practically, this is pursued through statistical hypothesis testing of each dose group against the control group, stepwise, starting with the lowest dose until one finds the highest dose at which there is still no statistically significant difference of the effects compared to control (significance usually defined by a p value $< 0.05$). Consequently, the next higher dose such tested must show a statistically significant effect. The highest dose with no statistically significant effect is then denoted NOAEL (no observed adverse effect level) and serves as estimate of the biological threshold D* and is used PoD/RP, Table 1 step 5a. When no NOAEL can be identified in a dose–response data set (e.g., when all doses tested were statistically significant different from the controls), the smallest dose that caused a statistically significant effect denoted LOAEL (lowest observed adverse effect level) has been suggested to serve as PoD/RP. Since the LOAEL would in general overestimate D*, a higher safety factor (usually by a factor of ten higher) is used. It should be noted that the estimation of the NOAEL may be significantly above or below D* and that the use of the NOAEL has been criticized therefore (EFSA 2009), predominantly for three reasons:

- *Strongly depending on the number of cases tested per dose group*. The larger the number of the examined subjects per dose, the higher is the statistical sensitivity (power) of the approach and thus the chance that a statistically significant effect is found at a dose. In converse, the smaller the sample sizes have been chosen per dose group, the higher will be the NOAEL, eventually higher than the highest dose tested.
- *Depending on the sensitivity of the biological assay*. The higher the sensitivity of the experimental determination of the biological effect, the smaller will be the NOAEL.
- *Strongly depending on the choice of doses and dose range*. The selection of the doses in $\mathrm{D}_{\mathrm{Experimental}}$ is crucial for the identification and localization of the NOAEL. If doses are widely spread in relation to true range where the dose–response curve increases, the NOAEL can be determined only very vaguely and can be far above or below D*.

Safety factors (SFs) are applied in the second step of the establishment of the PoD/RP, e.g., by dividing the NOAEL by SFs representing different types of

uncertainty. Traditionally, two types of SFs have been used (cf. Edler et al. 2002) when extrapolating from animals to humans:

- $SF_{interspec} = 10$ to take into account the interspecies variability between animals and humans. It allows for the possibility that the average exposed person is up to ten-fold more sensitive than the average exposed animal for which the NOAEL was determined (see the case TS in Table 3).
- $SF_{intraspec} = 10$ to take into account the interindividual variability. This is to ensure that a ten-fold more sensitive individual than that for which the PoD/RP value was derived will still be protected by the PoD/RP (see the case ES in Table 3).

For a refinement of these SFs accounting for both toxicokinetic and toxicodynamic data, if available, see, e.g., Dorne and Renwick (2005). It should be noted that even then these SFs are default factors not accounting for specific toxicokinetic and toxicodynamic knowledge of the toxic compound. A biologically based extrapolation would transform the dose–response relationship from animals to humans using toxicokinetic information by applying two physiologically based toxicokinetic (PBTK) models, one for the animal strain and another for humans permitting the calculation of concentrations in target organs. A precondition, however, is that sufficient biological information is available to construct both PBTK models.

If based on an animal experiment, dose has been converted from animal experiments to humans using interspecies extrapolation (USEPA 2005; ECHA 2012). For that extrapolation from animals to humans oral exposures, an allometric scaling is used where the administered doses are adjusted with body weights to the power of ¾ based on allometric scaling.

## Risk Assessment Without Threshold Dose Assumption

For compounds for which no threshold dose is assumed, there are two approaches (see Fig. 1a). At first, one can try to expand the dose–response curve *F(d)* to the entire dose range with the inclusion of the "zero dose," i.e., where only background exposure may exert an effect. The dose interval D: $0 \leq d \leq d_{max}$ serves then as base of the dose–response assessmen and estimates of the risk, could be made at any exposure level. However, this implies that four to six orders of magnitude both in terms of response or in terms of dose must be bridged by extrapolation. Although mathematical dose–response models are fit for this purpose, the biological database is not and a dose–response relationship *F(d)* in the experimental range can only provide limited information on the relationship in the extrapolation range $D_{Extrapolation}$. It was found that different mathematical models equally good fitting the data in $D_{Experimental}$ provided largely deviant risk estimates when extrapolated to the low-dose range of interest, differing by several orders of magnitude. When, e.g., the one-hit model, the multistage model, and the two empirical models derived from the Weibull distribution and the log-normal distribution would all fit the

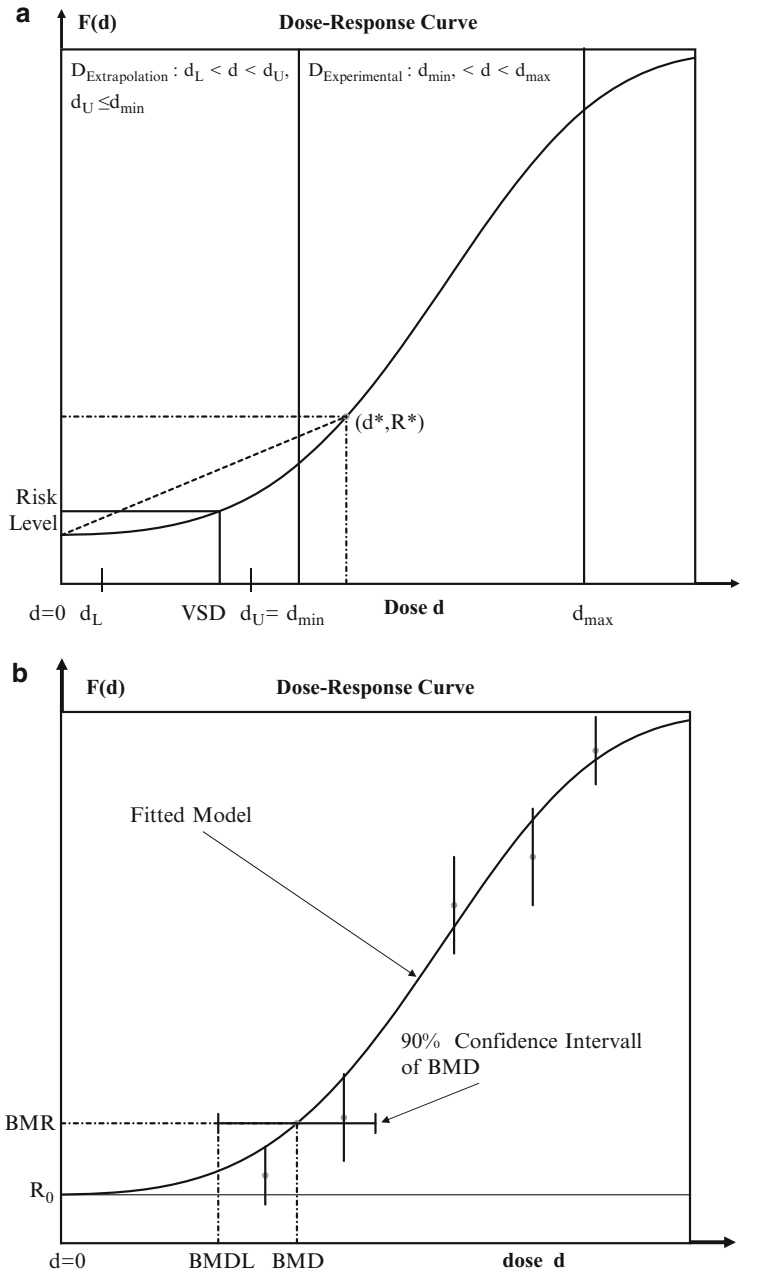



**Fig. 1** (**a**) Dose–response curve $F(d)$ in the observed range $d_{min} < d < d_{max}$ and in the extrapolation range $0 < d < d_{min.}$ (**b**) Benchmark dose (BMD) approach restricted to a left truncated dose range combining that $D_{Experimental}$ : $d_{min}$, $< d < d_{max}$ and a limited extrapolation range $D_{Extrapolation}$ of the dose–response curve (the author thanks Annette Kopp-Schneider for providing the figure)

data, one would obtain increasingly differing risk results when going to lower doses, always in the same order of

$$One\ Hit < Multistage < Weibull < Lognormal,$$

when excess risk is considered (see Krewski and van Ryzin 1981). This strong dependence of the risk estimates on the models selected and lack of biological justification for using a particular model has significantly compromised the use of these models for regulatory purposes.

An alternative approach focuses on modeling dose–response for doses from $D_{Experimental} : d_{min}, < d < d_{max}$ allowing only a limited extrapolation to $D_{Extrapolation} : d_L < d < d_U$, where $d_U < d_{min}$, and $0 < d_L$ using the data available. Modeling determines the dose associated with a predetermined but identifiable risk. The best investigated approach therefore is the benchmark dose (BMD) approach (EFSA 2009) described below.

## The Limit Risk

A limit risk $R_{limit}$ is interpreted as lifetime risk or lifetime cancer risk (LCR), the probability that the exposure will cause cancer (incidence type of risk) or death from cancer (mortality type of risk) within average lifetime.

A first version of the limit risk approach stems from the second half of the last century as "virtually safe dose" (VSD) concept in response to difficulties in complying with US Food, Drug, and Cosmetic Act, when in the context of the Delaney Clause, food additives found to induce cancer at any dose level were banned and the VSD was defined as dose associated with one additional tumor per one million (1,000,000) subjects through lifetime exposure in the belief that such a low risk would be acceptable for a population of several millions, corresponding to a lifetime cancer risk (LCR) of $10^{-6}$.

It should be noted that in a population of 100 million, people of the order of 500 000 persons will be diagnosed with cancer every year (IARC 2008). An LCR of $10^{-5}$ would then result in 13 additional persons with cancer per year in case the whole population is exposed during its whole lifetime assuming an average lifetime of 75 years, whereas an LCR of $10^{-6}$ would represent 1.3 additional cancer case per year in a population of 100 million (see SCCS 2012).

In the context of a risk management decision, it should be noted that the WHO and the US EPA as well as the US OSHA recommended an LCR of $10^{-5}$ for carcinogenic compounds. ECHA (2012) states that "based on experiences, cancer risk levels of $10^{-5}$ and $10^{-6}$ could be seen as indicative tolerable risk levels when setting DMELs (derived minimal effect levels) for workers and the general population, respectively." Higher risks up to 1/1,000 have been accepted in the regulation in the working environment. The measurable risk in a test group of animals is generally not below 1/20, at best 1/50.

The most extensively used model for calculating an LCR has been the so-called linearized multistage (LMS) model (USEPA 1986). Based on the multistage mutation model of Armitage and Doll, this model is in essence a linear approximation of the dose–response curve. In praxis it has provided robust risk assessments and limit values, and it has become the basis of the slope factor (SF) approach used by the USEPA as convenient descriptor of cancer potency (see http://www.epa.gov/iris/carcino.htm). The LMS model is also a member of the set of models recommended for the BMD.

## Benchmark Dose

The benchmark dose (BMD) approach is a general method of fitting dose–response models applicable for any dose–response data based on four gross steps:

Specification of type of dose–response data
Specification of the BMR
Selection of candidate dose–response model(s)
Identification of acceptable models

The BMD approach aims at determining a PoD/RP on an empirically and objectively verifiable basis and is applicable for all four scenarios described in Table 3. The BMD was introduced into regulatory practice by the US EPA (USEPA 1999) as the lower confidence limit of the dose at which no such response above background occurs that would exceed a previously defined level, the benchmark response (BMR) (Fig. 1b). The benchmark dose (BMD) is the dose level derived from the dose–response data associated with a specific change in the response defined through the benchmark response (BMR) level which has the following properties:

- The BMD approach uses all available dose–response data from a study and fits a set of mathematical models. It accounts for the statistical variability of the dose–response data by calculating the confidence interval of the BMD ranging from the lower bound (the BMDL) to the upper bound (the BMDU). The lower one-sided confidence bound BMDL ($BMDL_{10}$ when setting BMR = 10 %) accounts for the statistical uncertainty in the data (with the statistical certainty level of 95 %) and is used as PoD/RP. The BMD approach has been increasingly used and recommended (EFSA 2009).
- The BMR should be set equal to a low but measurable response level, reflecting an effect that is negligible or non-adverse. Choosing the BMR too low would normally result in an extrapolation outside the range of the observed data and could induce severe model dependence of the BMDL. Such a low BMR could let different models return drastically different BMD and BMDL values, reducing confidence in the modeling, characterized as a situation where "the risk assessment would be driven by the models fitted to the data and not by the data." A BMR = 10 % of extra risk over background has been set as a default level (EFSA 2009) when analyzing quantal data such as tumor incidence in animal experiments. For continuous data a BMR = 5 % of change related to background was proposed as default.

- When different models are fitted to the data and when some models fit equally well but result in different BMDs and BMDLs, selecting the BMDL of the best-fitting model is likely to underestimate the uncertainty in the BMD approach, while selecting the model with the lowest BMDL generally results in an overestimate of the risk. A stepwise and decision tree-based procedure has been proposed by Davis et al. (2010) and iterated by USEPA (2012) which differs from the EFSA approach in that it uses an adaptive approach to find the best-fitting model in contrast to the EFSA approach which is based on finding all models which are compatible with the dose–response data, i.e., those with an acceptable fit, once the data have been selected.
- Recommended models are for quantal data usually:
  - Probit
  - Log-probit
  - Logistic
  - Log-logistic
  - Weibull
  - Multistage including the LMS
  - Quantal-linear
  - Gamma multihit

  and for continuous data, the
  - Exponential family
  - Hill family

  where each family contains a set of hierarchically nested models allowing for the determination of a best-fitting model.
- The BMD approach should always be accompanied by appropriate reporting not only of the results finally obtained but also of all relevant information that allows other risk assessors to judge and eventually repeat the analysis.

It should be noted that the outcome of a BMD analysis depends on the criteria used to decide on the acceptability and on the significance level of a goodness-of-fit test chosen. The BMDL depends on the study design, in particular, on the sample size, but much less than the NOAEL.

## Other PoDs

Depending on the dose–response data available, two other methods concur with the BMD approach in practice:

**T25:** Defined as the chronic dose which will give tumors at a specific tissue site in 25 % of the animals after correction for spontaneous incidence and within the standard lifetime of the species (Dybing et al. 1997), the T25 has values that are likely to be within the range of the experimental data. An adjusted T25 is obtained as

$$HT25 = T25/(bw_{human}/bw_{animal})^{0.25}$$

and an LCR can be calculated as

$$LCR = exposure\ dose/(HT25/0.25)$$

The T25 can be – and has been – applied even when besides the control group, only one dose group was available.

**TD50:** The TD50 value was introduced primarily for ranking of carcinogens in the Carcinogenic Potency Database (CPDB) (see http://potency.berkeley.edu/). It characterizes the dose which, if administered chronically for the standard lifespan of the species, will halve the probability of the remaining tumor free throughout that period; for details see Sawyer et al. (1984). The determination of the TD50 value is complicated by intercurrent deaths due to causes other than tumorigenesis and the non-observability of the time of onset. The TD50 has been used as PoD when the toxic substance was administered chronically for the standard lifespan of the species, but is not recommended for low-dose extrapolation.

## Margin of Exposure (MoE)

Risk assessment of compounds that are both genotoxic and carcinogenic presents particular difficulties, since the effects of such compounds are normally regarded as being without a threshold and no safe level can therefore be defined. Therefore, low-dose extrapolation has been found inappropriate for genotoxic carcinogenic compounds, and pragmatic risk management approaches such as the application of the ALARA (As Low as Reasonably Achievable) and the TTC (Threshold of Toxicological Concern), which establishes exposure thresholds for chemicals present in food, dependent on chemical structure, have been applied. However, such approaches cannot inform risk managers on urgency and extent of the risk reduction measures needed.

More recently the margin of exposure (MoE) approach has been applied by both the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) *not to bridge* the gap between the PoD/RP and human exposure but to *describe* the extension of that gap (Barlow et al. 2006). The MoE is numerically defined as the ratio of the point of departure (PoD/RP) of the critical effect to the theoretical, predicted, or estimated exposure level (WHO 2009). Therefore, the BMD approach provides a practical tool when it defines a PoD/RP.

The magnitude of the MoE gives an indication of the level of concern without extrapolation to the substantially lower exposure levels usually encountered in human situations: the larger the MoE, the smaller the potential risk posed by exposure to the compound under consideration. The MoE should, however, not be used for a numerical quantification of risk but must be considered as practical approach for the formulation of advice to risk management; as a consequence, extrapolation using the MoE has not been recommended to derive a risk estimate or

a level of actual risk in the exposed population (Barlow et al. 2006). The EFSA Scientific Committee considered that a MoE of 10,000 or more, based on animal cancer bioassay data, would be of low concern (EFSA 2005). A MoE higher than 10,000 based on $BMDL_{10}$ can, in cases of lifelong exposure, be associated with an LCR lower than $3.5 \times 10^{-5}$ if based on a male rat experiment and lower than $7 \times 10^{-5}$ if based on a male mice experiment and using linear extrapolation (ECHA 2012; USEPA 2005).

## References


Barlow S, Renwick AG, Kleiner J, Bridges JW, Busk L, Dybing E, Edler L, Eisenbrand G, Fink-Gremmels J, Knaap A, Kroes R, Liem D, Müller DJG, Page S, Schlatter J, Tritscher A, Rolland V, Tueting W, Würtzen G (2006) Risk assessment of substances that are both genotoxic and carcinogenic. Report of an International Conference organised by EFSA and WHO with support of ILSI Europe. Food Chem Toxicol 44:1636–1650

Becher H, Steindorf K (1993) Epidemiologische Methoden und Wege der Risikoabschätzung. Informatik, Biometrie und Epidemiologie in Medizin und Biologie 24:14–27

Davis JA, Gift JS, Zhao QJ (2010) Introduction to benchmark dose methods and U.S. EPA's benchmark dose software (BMDS) version 2.1.1. Toxicol Appl Pharmacol 254:181–191

Dorne JL, Renwick AG (2005) The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol Sci 86(1):20–26

Dybing E, Sanner T, Roelfzema H, Kroese D, Tennant RW (1997) T25: a simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity. Pharmacol Toxicol 80:272–279

ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.8: ECHA- 2010-G-19-EN Version 2.1, November 2012, European Chemicals Agency, Helsinki, Finland http://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf

Edler L, Poirier K, Dourson M, Kleiner J, Mileson B, Nordmann H, Renwick A, Slob W, Walton K, Würtzen G (2002) Mathematical modelling and quantitative methods. Food Chem Toxicol 40:283–326

EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic. The EFSA Journal; 282:1-31. (http://www.efsa.europa.eu/en/efsajournal/doc/282.pdf)

EFSA (2009) Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1–72

EFSA (2011) Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal (2009) 1150, 1–72

IARC (2008) Atlas of cancer mortality in the European Union and the European economic area 1993-1997. IARC Scientific Publication No. 159. Edited by P. Boyle and M. Smans, ISBN 978 92 832 2159 3, Lyon http://www.iarc.fr/en/publications/pdfs-online/epi/sp159/index.php

Krewski D, van Ryzin J (1981) Dose response models for quantal response toxicity data. In: Csörgö M et al (eds) Statistics and related topics. North-Holland, Amsterdam, pp 201–231

LAI (Bund/Länder-Arbeitsgemeinschaft für Immissionsschutz) -Regional Committee on Pollution Control (1992) Krebsrisiko durch Luftverunreinigungen. Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfalen, Düsseldorf. http://www.lai-immissionsschutz.de/servlet/is/8048/

Rhomberg LR, Goodman JE, Haber LT, Dourson M, Andersen ME, Klaunig JE, Meek B, Price PS, McClellan RO, Cohen SM (2011) Linear low-dose extrapolation for noncancer health effects is the exception, not the rule. Crit Rev Toxicol 41(1):1–19. doi:10.3109/10408444.2010.536524

Sawyer C, Peto R, Bernstein L, Pike MC (1984) Calculation of carcinogenic potency from long-term animal carcinogenesis experiments. Biometrics 40:27–40

SCCS (2012) Opinion on NDELA in cosmetic products and nitrosamines in Scientific Committe on Consumer Safety (SCCS), European Union, SCCS/1486/12, Revision of 18 September 2012 http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_100.pdf

USEPA (1986) US Environmental Protection Agency guidelines for carcinogen risk assessment. Fed Regist 51:33992–34003

USEPA (1999) Guidelines for carcinogen risk assessment. NCEA-F-0644, July 1999. Review Draft. Risk Assessment Forum, US Environmental Protection Agency, Washington, DC. http://www.epa.gov

USEPA (2005) Guidelines for carcinogen risk assessment. Risk Assessment Forum, U.S. Environmental Protection Agency Washington, DC. EPA/630/P-03/001FMarch 2005. http://www.epa.gov/osa/mmoaframework/pdfs/CANCER-GUIDELINES-FINAL-3-25-05[1].pdf

USEPA (2012) US Environmental Protection Agency benchmark dose technical guidance. EPA/100/R-12/001

WHO (1999) Principles for the assessment of risks to human health from exposure to chemicals. International Programme on Chemical Safety. Environmental Health Criteria 210. World Health Organization, Geneva

WHO (2009) Principles for modeling dose-response for the risk assessment of chemicals. Environmental Health Criteria 239. WHO, Geneva. ISBN 978 92 4 157239 2, ISSN 0250-863X, http://www.inchem.org/documents/ehc/ehc/ehc239.pdf

# Probabilistic Methods in Toxicology

Odile Mekel and Rainer Fehr

## Contents

Background ..... 253
Methods for Quantifying Variability and Uncertainty in Risk Assessment ..... 255
Point Estimates ..... 255
Probabilistic Estimates ..... 256
Sensitivity Analysis ..... 256
Application Potential in Dose–Response Assessment ..... 258
Software for Probabilistic Exposure and Risk Assessment ..... 258
References ..... 259

**Abstract**

The concepts of "variability" and "uncertainty" play a central role in exposure and risk assessment. Traditionally applied worst-case scenarios do not adequately reflect the requirements of modern practice. Methods of probabilistic analysis, such as Monte Carlo simulations, are promising developments for sound consideration of these aspects.

## Background

Regardless of the topic in question, variability and uncertainty are aspects of modeling and assessing health risks which need to be taken into account

O. Mekel (✉)
Department of Prevention and Innovation, Innovation in Health, NRW Centre for Health, Bielefeld, Germany
e-mail: odile.mekel@lzg.gc.nrw.de

R. Fehr
Department of Public Health, University of Bielefeld, Bielefeld, Germany
e-mail: rainer.fehr@uni-bielefeld.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_47, 

(Mekel and Fehr 2000; US EPA 2011). "Variability" refers to the (statistical) distribution of the studied phenomena, while "uncertainty" refers to those parameters, factors, and models which are lacking or incomplete. This chapter will expand upon the concept of uncertainty and variability, describe methods of probabilistic estimation and sensitivity analysis, and provide an overview of suitable software.

**Variability** refers to real heterogeneity with respect to space, time, or persons and represents a feature of the system studied. Subdividing sources of variability according to space, time, and population provides a useful means for their understanding. Examples of temporal variability include, for instance, seasonal food consumption patterns or patterns of activities varying on a weekly basis. Both small and wide-area variations are observed in environmental pollution. Examples of intra-individual variability concern behavioral and personal features (Table 1).

In practice, variability can be taken into account through subdividing the studied system into a number of subgroups which are then analyzed separately. In research design and classical statistics, this is called "stratification." The phenomenon of variability cannot be resolved by additional studies, these can serve solely to characterize the degree of variability more precisely. This results in a need for political-administrative decision-making on the desired level of safety in environmental policy.

**Uncertainty**, in contrast, is a researcher's feature. It results from incomplete or lacking knowledge on aspects of the studied system. Uncertainty, just as variability, contributes to variation of analytical results. Types of uncertainty include: scenario, parameter, and model uncertainties. The former concerns, e.g., an exposure pathway which was overlooked. Parameter uncertainty can result from samples lacking representativeness. The third type of uncertainty regards the modeling quality, as, e.g., inclusion or exclusion of a relevant model parameter. In principle, uncertainty can be reduced by doing additional research (Table 2).

Both phenomena, variability and uncertainty, are relevant to each step of risk assessment. Distinguishing between sources of variability and uncertainty is important regarding two aspects: Firstly, with respect to interpreting the results; when assessing toxicity, for instance, it is important to know which variability exists within the population in question. Additionally, the reliability of this toxicity assessment matters: How sure are we that the toxicity and its variability was estimated correctly?

Secondly, the distinction between variability and uncertainty is important for the following reason: While variability impacts on the assessment's precision and its generalizability, uncertainty can lead to incorrect statements.

Variability and uncertainty of variables often occur together. If certain aspects of variability are unknown and stratification therefore is not possible, this lack of knowledge contributes to the uncertainty of the analysis. The quantification of soil ingestion from mouthing behavior of small children can serve as an example: It is well known that there are large differences between children concerning the daily soil ingestion. The study design and methods of most recent studies still leave many open questions. For instance, it is questionable to which extent the soil ingestion was determined correctly; what is the variance between children; which type of statistical distribution can best describe the variability, and how do seasonal factors influence these values.

**Table 1** Sources for variability (based on US-EPA 2011)

| Category | Variability source concerning... | Examples |
|---|---|---|
| Time | Long-term resp. short-term variation | Concentration level |
| | | Weather |
| | | Dietary intake |
| | | Seasonal variation |
| | | Long-term trends |
| | | Weekly interval of activity patterns |
| Space | Regional; small scale | Spatial variable concentration |
| | | Regional dietary habits |
| Population | Interindividual variability | Personal characteristics: e.g., bodyweight, age |
| | | Behavior: e.g. time budget, activity pattern |

**Table 2** Sources for uncertainty (Based on US-EPA 2011)

| Category | Uncertainty source concerning... | Examples |
|---|---|---|
| Scenario uncertainty | Descriptive errors | Incorrect or incomplete information |
| | Aggregation errors | Spatial and temporal approximations |
| | Judgments errors | Selection of a wrong model |
| | Incomplete analysis | Overlooking important exposure pathways |
| Parameter uncertainty | Measurement error | Imprecise or biased measurements |
| | Sample uncertainty | Small or nonrepresentative sample size |
| | Variability | In time, space, or activity |
| | Surrogate data | Chemicals with similar structure |
| Model uncertainty | Relation error | Incorrect conclusions from correlations |
| | Modeling error | Non-consideration of relevant parameter |

## Methods for Quantifying Variability and Uncertainty in Risk Assessment

### Point Estimates

In traditional risk assessment, single values or point estimates are commonly being used for representing the input model variables. In order to describe the typical conditions, for model variables having an empirically describable variability, measures of central tendency, i.e., mean or median, are being used. Such an estimate is referred to as "typical case." For the purpose of considering variability and uncertainty adequately, especially with respect to sufficient health protection, assumptions are mostly conservative or "unfavorable." So far, upper percentiles like 90th or 95th percentiles of variables or – if such measures

were not available – the worst conceivable assumptions were used for exposure assessment. This results in the so-called worst-case approach. Worst-case assumptions are usually a combination of variability and uncertainty concerning model variables. It is problematic that worst-case estimates often do not describe realistic exposure situations.

## Probabilistic Estimates

Probabilistic assessments make use of the entire distribution of all or several model variables (Cullen and Frey 1999). Simulated values are randomly chosen from these distributions according to their statistical parameters and then linked to other randomly chosen values according to the model's algorithms. An example of this principle using the "nutrition" pathway in probabilistic exposure assessment is illustrated in Fig. 1. From the distribution of each of the three input variables, randomly chosen simulation values are being selected, e.g., 1.14 kg/day for food consumption, 4.9 ng/kg for pollutant concentration, and 9.7 kg for body weight. According to the model equation, the resulting exposure is 0.58 μg/kg body weight-day. This procedure is repeated through Monte Carlo simulation many times. The results of these simulations, in turn, can be displayed in a distribution, too. This distribution then represents the exposure assessment's results and can be described by its statistical parameters such as mean, standard deviation, and percentiles.

By using entire distributions for estimation, each possible feature of a variable, including the "tails" of the distribution, is combined with other model variables according to its respective probability. This results in better insights about the populations' exposure and more meaningful information regarding the spread and confidence interval of the calculated exposure or risk. Additionally, probabilistic methods provide the possibility to include all available information into the assessment, as opposed to an arbitrary selection of percentiles.

## Sensitivity Analysis

By conducting sensitivity analysis, model variables that contribute most to the spread of the results can be isolated: If, e.g., the distribution of input variables that are identified as being influential to the final results relies on sound data, the estimation can be considered sound. Body weight, for instance, could have strong influence on the final results. If the probability distribution of body weight applied is based on a representative population sample, the calculated variation can be considered reliable. If, in contrast, the input variables that are identified as influential to the final results rely on a relatively weak data basis, the results, correspondingly, are unreliable. Such findings can also point at further need for research regarding that variable. From this background, variables, which rely on a weak data basis but are identified as not significantly impacting the final results, will not necessarily require an effort to improve the data basis.

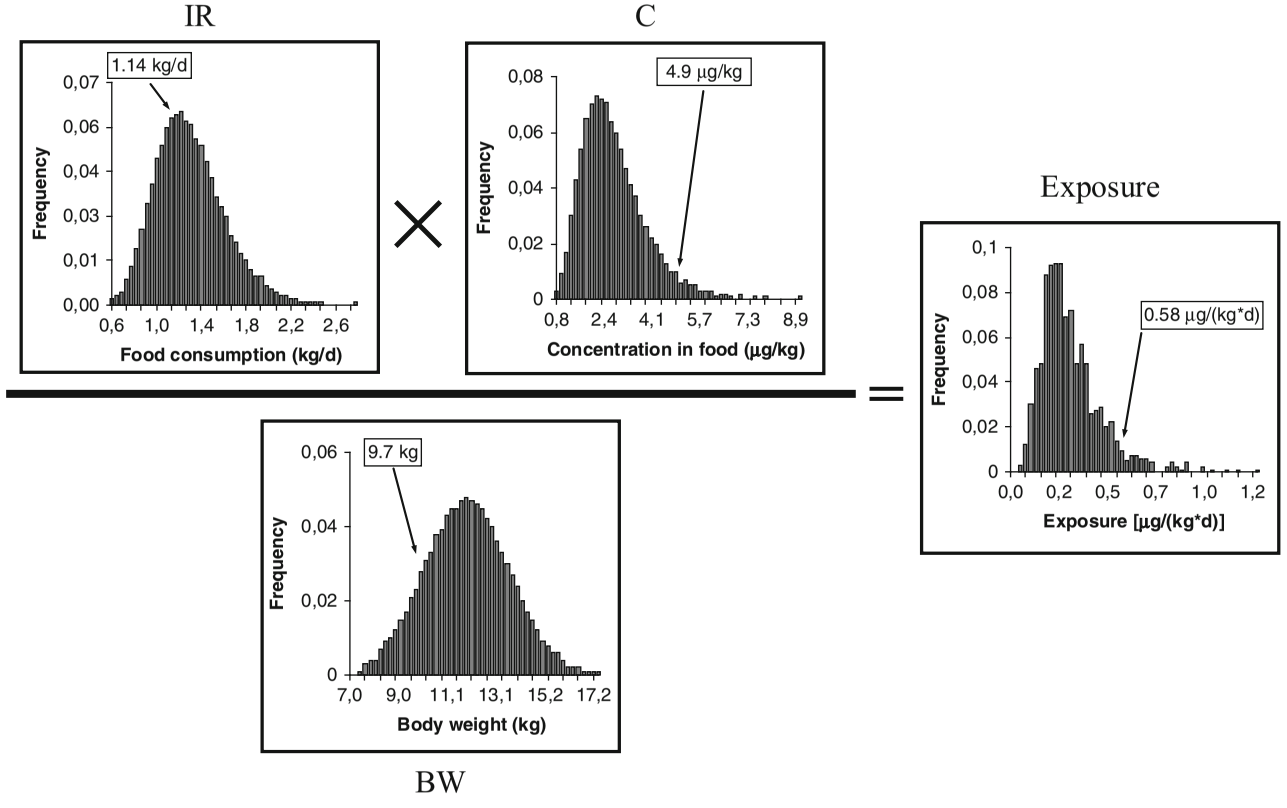



**Fig. 1** Exemplification of a Monte Carlo simulation for the example of dietary exposure of children (*IR* = food consumption [kg/day], *C* = concentration in food [µg/kg], *BW* = body weight [kg])

The simplest type of sensitivity analyses are *What-if*-analyses: The size of each input variable is modified (e.g., in steps of 10 %), respectively, while the other variables are kept constant, studying the respective influence on the final result. Itemizing for input values, the most sensitive variables can be identified.

Meaningful sensitivity analysis requires data on variation that usually cannot be obtained from point estimates, but are easily available from probability distributions. Sensitivity analysis is not meaningful when using worst-case point estimates, because the maximum value is used for several input variables (e.g., 100 % resorption). The combination of probabilistic estimates and sensitivity analysis provides information about the reliability of the estimates and its possible consequences regarding risk management.

## Application Potential in Dose–Response Assessment

Research and development in the area of probabilistic modeling so far have focused on exposure assessment (Mekel et al. 2007). In recent years only, efforts were made to investigate their application potential in dose–response assessment as an alternative or addition to the application of so-called uncertainty factors that traditionally have been used when transferring data from animal studies to humans. In the Netherlands, these methods are applied in parallel to traditional, deterministic risk assessment of new and existing chemicals and pesticides (Vermeire et al. 2001). Similar developments can be observed in other countries, but often have not become part of regulatory practice yet.

## Software for Probabilistic Exposure and Risk Assessment

Faster computers have enabled the application of computationally intensive probabilistic modeling in recent years. Specific commercial software tools for conducting probabilistic simulations are available. These software tools are not specifically designed for use in areas like toxicology or environmental health, but are used in a variety of disciplines where risk and decision analysis is an issue, in particular, in areas like economy and finance.

For performing a probabilistic exposure and risk assessment, the two most popular commercial systems are @Risk (www.palisade.com) and Crystal Ball (www.oracle.com). Both systems work directly as add-ins for spreadsheet software like Excel. @Risk is now available in seven different languages.

Both systems work in similar ways: Both require (i) a user-defined model to be implemented in a spreadsheet, and (ii) the specification of the probability distributions for the model input variables. Differences exist in performance, e.g., in terms of clarity, provision of (partly) automatic functions, graphs, etc. Both systems offer a large amount of different options for performing probabilistic

analysis, necessitating, however, considerable intensity of training. Standard statistical packages like SAS or SPSS can be used for probabilistic assessment, too, but all simulation steps need to be programmed. Again, this requires extensive knowledge of the statistical packages.

**Acknowledgment** We thank Eva Barrenberg for her English translation assistance.

## References

Cullen C, Frey HC (1999) Probabilistic techniques in exposure assessment: a handbook for dealing with variability and uncertainty in models and inputs. Plenum Press, New York

Mekel OCL, Fehr R (2000) Berücksichtigung von Variabilität und Unsicherheit in quantitativen Risikoabschatzungen (QRA). Umweltwissenschaften und Schadstoff-Forschung 12:43–50

Mekel O, Mosbach-Schulz O, Schümann M, Okken P-K, Peters C, Herrmann J, Hehl O, Bubenheim M, Fehr R, Timm J (2007) Evaluation von Standards und Modellen zur probabilistischen Expositionsabschätzung. WaBoLu-Hefte, 02/07 – 05/07, Umweltbundesamt (UBA), Berlin

US-EPA (U.S. Environmental Protection Agency) (2011) Exposure factors handbook 2011 edition (Final). EPA/600/R-09/052F. U.S. Environmental Protection Agency, Washington, DC

Vermeire T, Pieters M, Rennen M, Bos P (2001) Probabilistic assessment factors for human health risk assessment. RIVM report 601516005, TNO report V3489, National Institute of Public Health and the Environment, Bilthoven, the Netherlands

# Toxicodynamic Models

Lutz Edler and Angelika Tritscher

## Contents

General ..... 262
Requirements for Toxicodynamic Modeling ..... 262
Description and Application of TDMs ..... 263
Toxicodynamic Potency ..... 263
Explanatory TDMs ..... 266
Application of TD Models for Risk Assessment/Risk Extrapolation ..... 268
References ..... 268

**Abstract**

A toxicodynamic model (TDM) is used to describe a concentration-response relationship primarily for predicting effects at certain concentrations but also as explanatory tool for investigating mechanisms of action (MOA) of toxic substances or explaining sensitivity differences between exposed subjects. All such information can specifically contribute to the risk assessment of such substances. Suitable mathematical models and statistical analysis methods have to be applied for the toxicodynamic modeling.

L. Edler (✉)
Department of Biostatistics –C060, German Cancer Research Center (DKFZ), Heidelberg, Germany
e-mail: edler@dkfz.de

A. Tritscher
Department of Food Safety and Zoonoses, World Health Organization, Geneva 27, Switzerland
e-mail: tritschera@who.int

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_48, © Springer-Verlag Berlin Heidelberg 2014

## General

Toxicodynamics (TD) describes the relationship between the concentration of a toxic substance or its metabolites in the body (or in a target organ) and the toxic effects attributed or related to exposure from that substance. Therefore, toxicodynamic modeling is often based on statistical models of cell dynamics. Table 1 informs on the major areas of the application of TDMs and related methodological aspects of the modeling.

## Requirements for Toxicodynamic Modeling

In order to provide information on the MOA of the toxic substance and/or to establish concentration-response relationships of toxicity, the toxicodynamic modeling requires careful planning and performing of TD studies. In general, TD studies may proceed as follows:

- Formulation of the TD issue or question (problem formulation)
- Collection of available preliminary information on MOA and toxicological parameters
- Choice of one or a class of toxicodynamic models (TDMs) which appear to fit the purpose
- Selection of the available and appropriate experimental (or observational) data required for modeling
- Identification of model parameters (e.g., known from previous experience) and, if applicable, also of "initial" model parameter values when the model fit requires iterative computational procedures and needs such values to start the calculation
- Fit of the TDMs to the concentration-response data and determination (using statistical estimation procedures) of previously unknown model parameters
- Assessing the quality of the model fit
- Interpretation of the estimated parameter values and application of the modeling outcome to the TD question
- Assessing the robustness and the uncertainty of the modeling

An important part of the TD problem formulation is to determine the TD endpoint (target) and to characterize its statistical quality in terms of scale of its measurement. Three main classes of effect data can be distinguished:

- Quantal measures (e.g., presence/absence of liver toxicity/liver tumors)
- Categorical (ordinal) measures (e.g., degree of impairment of an organ function)
- Quantitative (metric) measures (e.g., number of tumors in animal experiments, values of an enzyme measured in the blood serum)

Besides the statistical information content of a TD endpoint, one should also address at that step the biological information content and toxicological meaning of this target value. This is, in particular, relevant for the ultimate interpretation of the outcome of the TDM. Therefore, it is important to distinguish

**Table 1** Overview on functions, application areas, and procedures of toxicodynamic models

| Function | Application areas | Methods |
|---|---|---|
| **Describe** | Concentration-response curve | Parametric curve fitting |
| | Toxicodynamic potency | Model fit |
| | Hypothesis generation | Statistical estimation of concentration (dose) descriptors |
| **Explain** | Complex biological and toxicological mechanisms | Biologically based models |
| | Check or reject hypotheses | Model selection |
| **Predict** | Extrapolation to low concentrations | (Non-) parametric curve fitting |
| | Interspecies extrapolation | Model fit |

between a direct and an indirect target. A direct target would accurately exhibit the effect to be tested in the TD study. Indirect targets (e.g., biomarkers) are applicable when only a surrogate of the target can be measured.

## Description and Application of TDMs

### Toxicodynamic Potency

Measures of toxicodynamic potency are obtained mainly from descriptive parametric TDMs and their adaptation to concentration-effect curves. Below, three common TDMs for quantitative measures and two TDMs for quantal measures are presented. All five models allow the determination of a quantity which can be interpreted as TD potency of a substance which expresses its activity in the form of a concentration/dose value (dose descriptor) which causes a certain degree of a toxic effect.

**The Linear Concentration-Effect Model**. In cases where a direct proportional relationship between the concentration of the toxic substance $C$ and the effect $E$ can be assumed, the linear model

$$E = E_0 + mC, \tag{1}$$

is the most simplest model to be applied. Here $E_0$ denotes the base effect (background), and m the proportionality of the effect relative to the concentration (slope) factor. The two model parameters ($E_0$, $m$) are statistically determined from concentration-effect data usually available in the form of n data pairs

$$\{(C_i,\ E_i),\ i = 1, \ldots,\ n\}$$

where $E_i$ denotes the effect observed for concentration $C_i$ in a total of n observational units (samples) investigated in the TD study. The statistical analysis

method is linear regression (see, e.g., Draper and Smith 1998). In practice, this model plays only a minor role since a direct linear relationship rarely applies for TD data. However, the linear model can be used for an interpolation of the concentration-response curve over a few concentrations when only a part of the concentration-effect course is considered, and linearity can be assumed for that part. Moreover, linear regression is ideal for teaching purposes and for the presentation of the basic concepts of statistical regression and model fitting as sort of statistical reference standard.

**The Log-Linear Concentration-Response Model**. When the range of concentrations of the TD study is large, covering, e.g., several orders of magnitude, one often uses a logarithmic transformation of the concentration in order to visualize the concentration-effect relationship in a graphic when plotting the $E_i$ values versus the $ln/C_i$ values (ln denotes natural logarithm to the base e). If this results in a graph where $E$ is proportional to $lnC$, then the log-linear model

$$E = E_0 + m\ lnC \tag{2}$$

with the base effect value $E_0$ and the slope parameter m can be fitted using the same statistical methods as for Eq. 1.

Although the log-linear model is analyzed as the linear model, it should be noted that fitting a log-linear model to data where effects at concentration 0 are included is mathematically more complex, and the fit can only be inspected on a restricted concentration range when an effect at concentration value 0 is included since the transformed concentration ln(0) is for mathematical reasons no more a real number but located outside the range of the plot, in mathematical terms at "minus infinity." For this reason, the data and the fit are usually retransformed to a linear plot with a logarithmic scale of the abscissa ("x-axis"). It should also be noted that a logarithmic transformation of the concentration may not remove all nonlinearity from the concentration-effect relationship such that other models (see below) may be investigated. Model fit is obtained by standard linear regression where the concentration ln(0) is replaced by a large negative value to mimic "minus infinity."

**The Emax Model**. Concentration-effect curves, which show a saturation of the effect at high concentrations, exhibit a similarity with curves known from enzyme kinetics and receptor-binding relationships for a long time. They are often evaluated with a model related to the Michaelis-Menten equation. In its simplest form, this so-called $E_{max}$ model takes the form

$$E = \frac{E_{max} \cdot C}{E_{50} + C} \tag{3}$$

with the two model parameters ($E_{max}$, $E_{50}$). Herein, $E_{\max}$ is the maximum effect, achieved at the maximum concentration, theoretically at $C = \infty$. $E_{50}$ is the "half-maximum" concentration at which 50 % of the maximum effect $E_{max}$ is reached.

By definition, the effect at background (i.e., at concentration equal to 0) is $E_0$. In the model Eq. 3, $E_0 = 0$ and such comparable with a linear regression through the origin (0,0) where one is interested in the slope m only. If the TD study must account for a positive effect at background, there are two options to include that in this model: At first, one can add as a background effect a term $E_0$ such that the model Eq. 3 changes to $E = E_0 + (E_{max}\ C)/(E_{50} + C)$ (response-additive background). Secondly, one can postulate a virtual background concentration $C_0$ and replace on the right side of Eq. 3 the concentration $C$ by $C + C_0$ (concentration-additive background). Practically more important is, however, an extension of the $E_{max}$ model by replacing the concentration $C$ on the right side by an exponentiated expression $C^n$ (the n-th power of $C$) which leads to a sigmoidal shape of the concentration-effect curve and an $E_{max}$ model of *Hill type*. Methods of nonlinear curve fitting (regression) are available in standard statistical software packages for the calculation of the model parameters (see, e.g., Gabrielsson and Weiner (1998)).

Note that the models of the type of Eq. 3 are by one more parameter more complex than the linear and log-linear models since they have two parameters for the shape of the curve compared to only one slope parameter. It is obvious that the same class of models can be applied when the toxic effect is also presented on a logarithmic scale. However, the effects will be interpreted on a multiplicative scale in that case.

**The Probit and Logit Model**. Concentration-effect data available as quantal data in a form, where the number of responders $r_i$ and the number of non-responders $s_i$ at the different concentrations ($C_i$, $i = 1, \ldots, n$) have been analyzed traditionally using parametric models. Therefore, it is assumed that the data have the form

$$\{(C_i,\ p_i),\ i = 1, \ldots,\ n\}$$

where $p_i = r_i/(r_i + s_i)$ describes the effect as proportion (or effect rate) at concentration $C_i$. The *probit model* describes the proportion P as a function of the concentration $C$ by

$$P(C) = \Phi(E_0 + m\ lnC) \tag{4}$$

with the base effect parameter $E_0$ and the proportionality (slope) factor m and where $\Phi$ denotes the Gaussian ("Normal") distribution function. A somehow more flexible partner of the *probit model* is the *logit model* which models the likelihood of an effect at concentration $C$ as

$$P(C) = \frac{\exp(E_0 + mC)}{1 + \exp(E_0 + mC)} \tag{5}$$

and thus, after a linearizing logit transformation, it allows for the linear relationship

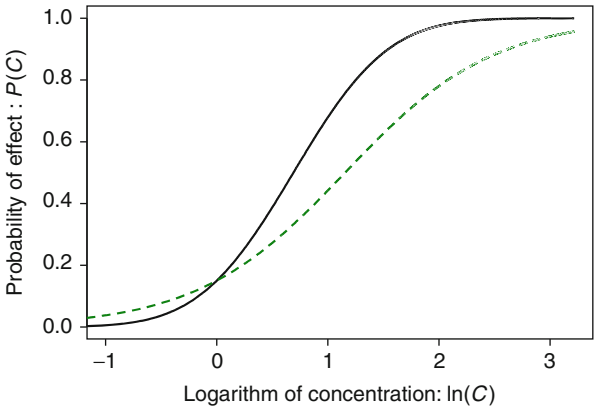



**Fig. 1** The probit (*solid line*) and the logit model (*dashed line*) as descriptive TD models

$$logit\ P(C) = \ln \frac{P(C)}{1 - P(C)} = E_0 + m\ C$$

or when the concentration is analyzed on logarithmic scale,

$$logit\ P(C) = \ln \frac{P(C)}{1 - P(C)} = E_0 + m \ln\ C. \tag{6}$$

with $E_0$ as basal effect parameter m as proportionality factor (see Fig. 1).

## Explanatory TDMs

TDMs that integrate biological knowledge of the MOA of a substance in the modeling process have found special consideration in risk assessment. In contrast to the models presented above where the model parameters primarily express statistical properties of the concentration-effect relationship and are therefore often denoted as empirical models biologically based TDMs contain (at least some) model parameters with a physiological or toxicological interpretation. As a consequence, such models require a high degree of reliable foreknowledge. As another consequence, a more complex computational modeling is needed including the use of special software since the underlying biological or toxicological features do often exhibit a higher order of nonlinearity than what is used in empirical models. On the other hand, fitting a biologically based TD model should always be accompanied with a sensitivity and uncertainty analysis. In the field of carcinogenesis, the two-stage model of clonal expansion of tumors (initiation-promotion model) and the multistage mutation model (Armitage-Doll model) are known examples of biologically based TD models (see Fig. 2 and Kopp-Schneider (1997)).

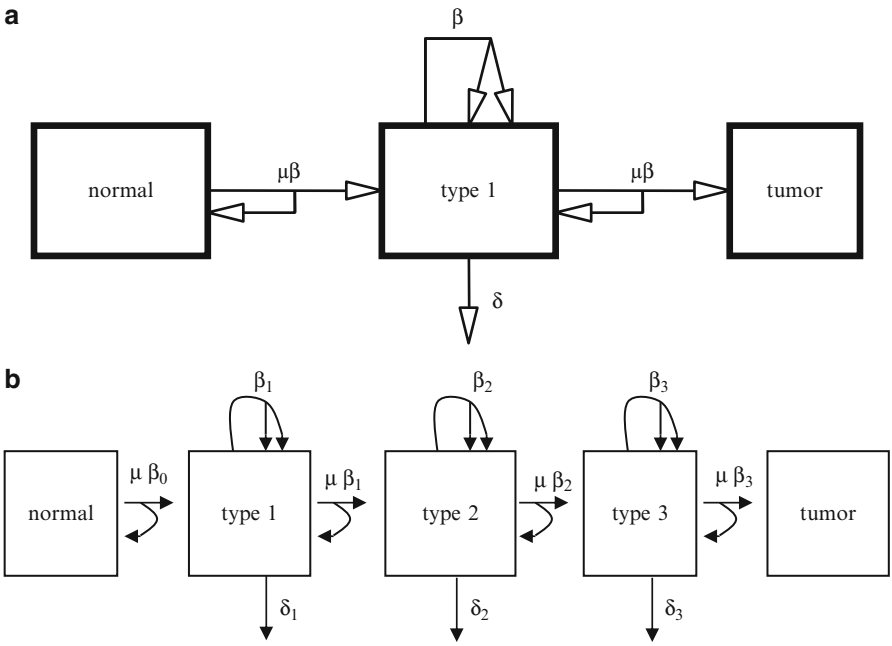



**Fig. 2** The two-stage model of clonal evolution of tumors (**a**) and the multistage mutation model of carcinogenesis (**b**, shown here with three intermediates) represent explanatory toxicodynamic models. In the models, $\mu$ describes the mutation rates, $\beta$ the birth rates, and $\delta$ the death rates of the cells

## Toxicokinetic-Toxicodynamic Models

Toxicokinetic-toxicodynamic (TK-TD) models combine dose-time relationships of toxicokinetics (TK) with concentration-activity relationships of TD and thus allow a holistic view of dose-dependent toxic effects over time. TK-TD models are therefore particularly well suited to represent causal relationships between exposure and the toxic effects and thus contribute to a better understanding of the chronologic sequence of toxic effects. Simple TK models can be replaced by physiologically based TK models (PB-TK models) to calculate concentrations in organs and target tissues.

A further extension of TK-TD models are the TK-TD population models which characterize dose/time-response relationships in populations and can combine individual relationships in a comprehensive modeling approach. They are applicable even with sparse and irregularly sampled individual exposure data, if the sample size is large enough. The statistical analysis of these models is challenging and uses nonlinear mixed effect models or Bayesian hierarchical models. Obviously there exists from a methodological statistical point of view a direct connection to Pharmacokinetic-Pharmacodynamic (PK-PD) models used

for drug research (Derendorf and Hochhaus 1995). For more details on statistical methods and software, see, e.g., Lunn et al. (2002).

## Application of TD Models for Risk Assessment/Risk Extrapolation

An important predictive application of TD models is the extrapolation of an effect to lower concentrations and subsequently the derivation of a value that causes a defined effect in humans. Traditionally, a distinction has been made between approaches, where a threshold is assumed for the toxic effect of a substance and approaches where no threshold is included. The selection of an appropriate TDM is a key step, especially if one uses the benchmark dose approach for risk assessment, when a concentration limit corresponding to a critical effect (size) and the establishment of a health-based guidance value is in the focus of the investigation.

## References

Derendorf H, Hochhaus G (1995) Handbook of pharmacokinetic/pharmacodynamics correlation. CRC Press, Boca Raton

Draper NR, Smith H (1998) Applied regression analysis, 3rd edn. Wiley, New York

Gabrielsson J, Weiner D (1998) Pharmacokinetic/pharmacodynamics data analysis: concepts and applications, 2nd edn. Swedish Pharmaceutical Press, Stockholm

Kopp-Schneider A (1997) Carcinogenesis models for risk assessment. Stat Methods Med Res 6:317–340

Lunn DJ, Best N, Thomas A, Wakefield J, Spiegelhalter D (2002) Bayesian analysis of population PK/PD models: general concepts and software. J Pharmacokinet Pharmacodyn 29(3):271–307

# Toxicokinetic Models

Johannes Georg Filser

## Contents

The Relevance of Toxicokinetics ..... 269
Toxicokinetic Modeling ..... 270
Recommended Reading ..... 276

**Abstract**

Toxicokinetics describes by means of mathematical functions the time- and dose-dependent processes of absorption, distribution, metabolism, and excretion of a substance in animals and humans. For this purpose, toxicokinetic models are used, mostly straightforward compartment models by which toxicokinetic in vivo data are fitted or physiological toxicokinetic models that enable to predict species-specifically the substance burdens in various tissues and organs. Toxicokinetic information is required for the quantitative assessment of the substance-specific health risk carried out by national and international agencies responsible for regulating health and safety.

## The Relevance of Toxicokinetics

Toxicity studies of xenobiotics are generally conducted in a relatively small number of animals. High doses are selected in order to obtain statistically significant increases in toxic effects. Often, toxic effects are not caused by the xenobiotics themselves but by their metabolites. Because elimination of xenobiotics and formation as well as elimination of metabolites are generally

J.G. Filser
Institute for Toxicology and Environmental Hygiene, Technical University of Munich, Munich, Germany
e-mail: johannes.filser@helmholtz-muenchen.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_49, 

characterized by saturation kinetics, a meaningful extrapolation of the dose-effect relationship to low doses, relevant for human exposure, requires robust information on the relationship between the external dose (administered amount and exposure concentration, respectively, of the xenobiotic) and the resulting internal dose (body and tissue burdens) of the toxic chemical species in laboratory animals and in humans. Such information can be obtained by means of toxicokinetic studies in laboratory animals, in tissues of animals and humans, and, if ethically acceptable, in low-dose exposed humans. This is why the use of toxicokinetic data is of utmost relevance for the quantitative assessment of the dose-dependent health risk of a toxicant when it is based on the results of animal studies. Quantitative risk assessment (e.g., the probability of developing cancer for a given scenario of exposure) is generally carried out by national and international agencies responsible for regulating health and safety.

## Toxicokinetic Modeling

Toxicokinetics deals with the fate of a toxicant in animals and humans. It describes by means of mathematical functions the time- and dose-dependent processes of absorption, distribution, metabolic elimination, and elimination by excretion (ADME) of a substance. The required toxicokinetic data are generated in vivo by monitoring at diverse doses the resulting concentration-time courses of a substance in body fluids and, if a gaseous substance is dealt with, even in the inhaled and exhaled air. In vitro, dose-dependent concentration-time courses are monitored in the incubation medium usually containing organ- and species-specific microsomal or cytosolic suspensions. The ADME-characteristic parameters are then obtained from fits of a toxicokinetic model to in vivo data or from predictions made by a toxicokinetic model that uses in vitro data. The most frequently used toxicokinetic models are "compartment models." The distribution of the substance in a compartment that is defined by its volume is considered to be uniform. The compartments are "open" because the investigated substance enters (input) and leaves (output) them. For each compartment, a differential equation can be formulated which describes the change in the amount of substance in the compartment in dependence of time.

"Classical" models, mostly used to describe the fate of a drug in the organism (pharmacokinetics), generally consist of not more than three compartments. Monitored concentration-time courses in plasma or blood define the number of the compartments to be chosen. For instance, if the distribution of a substance in the organism occurs too fast for experimental measurement (instant distribution), a one-compartment model will be chosen (Fig. 1). If the distribution processes can be observed experimentally, a two- or even a three-compartment model might be required to fit the data adequately. In most cases, it is sufficient to use a two-compartment model (Fig. 2). The first compartment, called central compartment, summarizes those organs and tissues that are rapidly perfused (e.g., blood, spleen,

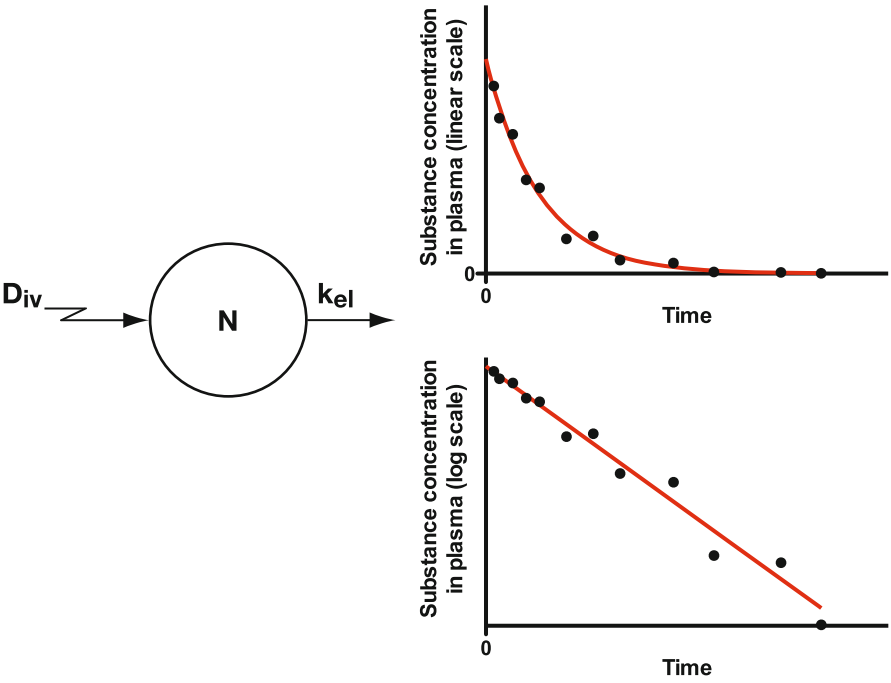



**Fig. 1** One-compartment model for a single intravenous injection of a low dose of a substance that distributes too fast to be experimentally observed and curves fitted to the measured concentration-time course of the substance in plasma (*plotted linearly and semilogarithmically*). Symbols and abbreviations: *filled circles*, measured data; *lines*, fitted first-order decay curves; $D_{iv}$, intravenously administered amount of a substance (dose) at time point zero; $k_{el}$, first-order elimination rate constant; $N$, amount of substance at any time point in the compartment. The fitted concentration-time curves are given by $y = y_{(0)} \cdot e^{-k_{el} \cdot t}$. The substance concentrations at any time point $t$ are given as y and at $t = 0$ as $y_{(0)}$. The linear slope of the curve in the semilogarithmic plot represents $k_{el}$

heart, brain, kidneys, liver). The substance concentrations in them are considered to be always in equilibrium with the substance concentration in the circulating blood. The second, "peripheral", and the third, "deep", compartments combine the slowly perfused tissues (e.g., muscles, adipose tissue, skin) that require distinctly longer periods of time until equilibrium. The volume of distribution generally represents not a physiological space but a fictitious (apparent) one because it is defined as the ratio of the actual amount of the substance in the whole organism (except that in the bladder and in the gastrointestinal tract) to the actual concentration in blood and plasma, respectively. In the one-compartment model, the volume of distribution is constant. In the two- or three-compartment models, however, it changes with time. It increases until the end of the distribution phase and remains constant thereafter.

At low substance concentrations, the rates of distribution and elimination of a substance follow first-order kinetics, and the concentration-time course of the

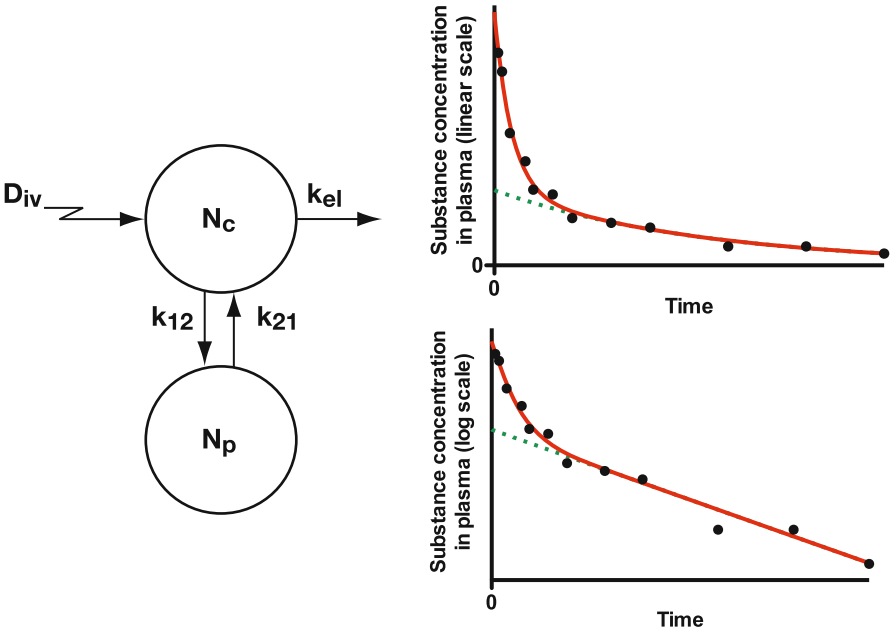



**Fig. 2** Two-compartment model for a single intravenous injection of a low dose of a substance that distributes from the central into the peripheral compartment and curves fitted to the measured concentration-time course of the substance in plasma (*plotted linearly and semilogarithmically*). Symbols and abbreviations: *filled circles*, measured data; *lines*, fitted curves; $D_{iv}$, intravenously administered dose at time point zero; $k_{el}$, first-order elimination rate constant; $k_{12}$ and $k_{21}$, first-order rate constants of substance transport from the central to the peripheral compartment and vice versa, respectively; $N_c$ and $N_p$, amounts of substance at any time point in the central and the peripheral compartment, respectively. The fitted concentration-time curves are given by a function that is composed of two exponential terms: $y = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}$. The substance concentration in plasma (the central compartment) at any time point $t$ is given as $y$. The *green dotted lines* showing the second exponential term of the fitted function have the y-axis intercept $B$. The constant $A$ is the difference between the initial concentration in the central compartment $y_{(0)}$ and $B$. The constants $\alpha$ and $\beta$ consist of the rate constants $k_{12}$, $k_{21}$, and $k_{el}$

substance in the central compartment is given by an explicit function. With increasing substance concentration, saturation kinetics (e.g., according to Michaelis and Menten, see, for instance, Bisswanger 2008) will become evident. Under such conditions, no explicit solution exists for the differential functions describing the concentration-time courses in the compartments. Numerical methods are used for this purpose.

The disadvantages of the classical models are evident. No information can be obtained on the target tissue burden by the toxicant. Also, an extrapolation between different mammalian species including human (species scaling) is highly problematic because the kinetic information gained by such models has no biological or physiological meaning. These shortcomings are drastically reduced when using physiological toxicokinetic models.

In physiological toxicokinetic models, the compartments correspond to organs, tissues, or lumped groups of tissues with actual, well-defined species- and body weight-specific anatomical volumes. The processes of input and output are driven by the physiological organ- or tissue-specific blood flows and depend on physicochemical parameters (e.g., tissue-to-blood partition coefficients) and additionally on biochemical parameters in metabolizing organs (e.g., a chemical- and organ-specific maximum metabolic rate $V_{max}$ together with the corresponding apparent Michaelis constant $K_{map}$ that is related to the whole organ). Figure 3 shows a flow diagram of a physiological toxicokinetic model for exposure of a mammal to a lipophilic gaseous substance that is biotransformed in the liver to an amphiphilic, nonvolatile metabolite. The latter is eliminated by metabolism in the liver and by first-order excretion in the kidneys. The organism is subdivided into several compartments representing those organs and tissues that are relevant to describe the fate of the chemical and its metabolite. The lung, adipose tissue, liver, and kidneys are

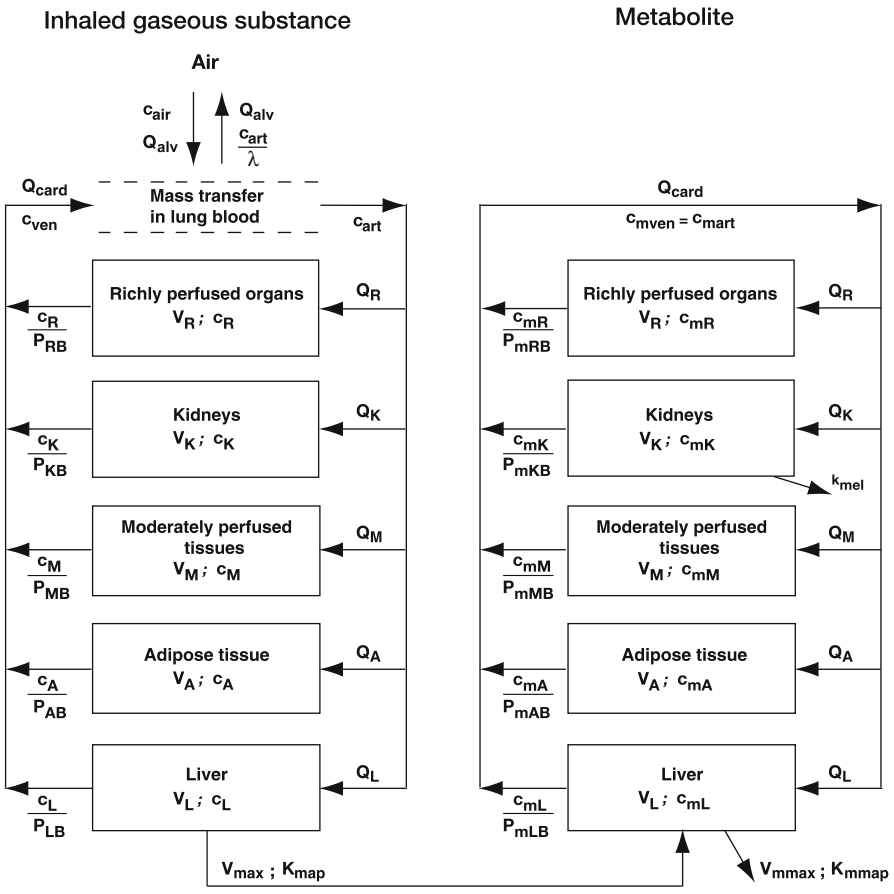



**Fig. 3** (continued)

## Inhaled gaseous substance

$$\text{(a)}\ c_{ven} = \frac{Q_R \cdot \frac{c_R}{P_{RB}} + Q_K \cdot \frac{c_K}{P_{KB}} + Q_M \cdot \frac{c_M}{P_{MB}} + Q_A \cdot \frac{c_A}{P_{AB}} + Q_L \cdot \frac{c_L}{P_{LB}}}{Q_{card}}$$

$$\text{(b)}\ c_{art} = \frac{c_{ven} \cdot Q_{card} + c_{air} \cdot Q_{alv}}{Q_{card} + \frac{Q_{alv}}{\lambda}}$$

$$\text{(c)}\ \frac{dc_R}{dt} \cdot V_R = Q_R \cdot \left(c_{art} - \frac{c_R}{P_{RB}}\right)$$

$$\text{(d)}\ \frac{dc_K}{dt} \cdot V_K = Q_K \cdot \left(c_{art} - \frac{c_K}{P_{KB}}\right)$$

$$\text{(e)}\ \frac{dc_M}{dt} \cdot V_M = Q_M \cdot \left(c_{art} - \frac{c_M}{P_{MB}}\right)$$

$$\text{(f)}\ \frac{dc_A}{dt} \cdot V_A = Q_A \cdot \left(c_{art} - \frac{c_A}{P_{AB}}\right)$$

$$\text{(g)}\ \frac{dc_L}{dt} \cdot V_L = Q_L \cdot \left(c_{art} - \frac{c_L}{P_{LB}}\right) - \frac{V_{max} \cdot c_L}{K_{map} + c_L}$$

## Metabolite

$$\text{(a)}\ c_{mven} = \frac{Q_R \cdot \frac{c_{mR}}{P_{mRB}} + Q_K \cdot \frac{c_{mK}}{P_{mKB}} + Q_M \cdot \frac{c_{mM}}{P_{mMB}} + Q_A \cdot \frac{c_{mA}}{P_{mAB}} + Q_L \cdot \frac{c_{mL}}{P_{mLB}}}{Q_{card}}$$

$$\text{(b)}\ c_{mart} = c_{mven}$$

$$\text{(c)}\ \frac{dc_{mR}}{dt} \cdot V_R = Q_R \cdot \left(c_{mart} - \frac{c_{mR}}{P_{mRB}}\right)$$

$$\text{(d)}\ \frac{dc_{mK}}{dt} \cdot V_K = Q_K \cdot \left(c_{mart} - \frac{c_{mK}}{P_{mKB}}\right) - k_{mel} \cdot V_K \cdot c_{mK}$$

$$\text{(e)}\ \frac{dc_{mM}}{dt} \cdot V_M = Q_M \cdot \left(c_{mart} - \frac{c_{mM}}{P_{mMB}}\right)$$

$$\text{(f)}\ \frac{dc_{mA}}{dt} \cdot V_A = Q_A \cdot \left(c_{mart} - \frac{c_{mA}}{P_{mAB}}\right)$$

$$\text{(g)}\ \frac{dc_{mL}}{dt} \cdot V_L = Q_L \cdot \left(c_{mart} - \frac{c_{mL}}{P_{mLB}}\right) - \frac{V_{mmax} \cdot c_{mL}}{K_{mmap} + c_{mL}} + \frac{V_{max} \cdot c_L}{K_{map} + c_L}$$

**Fig. 3** (continued)

represented by their own compartments. The compartment "richly perfused organs" primarily summarizes the brain, spleen, heart, and intestines; the compartment "moderately perfused tissues" mainly represents the muscle and skin. The scarcely perfused bones and cartilage are not taken into account. The model is flow limited or perfusion limited. This means that the substance in each tissue is described to be always uniformly distributed and in equilibrium with the blood leaving the tissue. Computer software is available for the numerical solution of the differential equations that describe the mass changes of the inhaled substance and its metabolite in each compartment. Figure 4 demonstrates model-predicted tissue-characteristic concentration-time curves of an inhaled lipophilic chemical and its metabolite, which result from a short-term exposure during which steady state (the situation in which input and elimination rates are equal) is not reached.

Physiological toxicokinetic models have the advantage over the classical models that they permit knowledge of the fate of a substance not only in blood or plasma but also in the target and other tissues. They are useful for species scaling of the kinetic information on a substance because sufficient species-specific anatomical and physiological information is usually available. Additionally, the chemical-specific physicochemical and biochemical parameters can easily be obtained from measurements in vitro using animal and human tissues. This is why the use of such models for risk assessment purposes is continuously growing. However, it has to be stressed that species scaling often results in erroneous predictions on the blood and tissue burdens of metabolites. Model predictions should always be treated with caution as long as they are not "validated" by a comparison of predicted data with species-specific experimental in vivo data.

**Fig. 3** Physiological toxicokinetic model for a lipophilic gaseous substance that is biotransformed in the liver to an amphiphilic, nonvolatile metabolite (marked by the suffix "m") which in turn is excreted via the kidneys and metabolically eliminated in the liver. Also shown are the equations that describe uptake and elimination processes in each of the physiological compartments. Equations: (**a**) concentration of the substance or of the metabolite in oxygen-poor "venous" blood and (**b**) in oxygen-rich "arterial" blood. Differential equations giving the mass change in the following: (**c**) the richly perfused organs, (**d**) the kidneys, (**e**) the moderately perfused tissues, (**f**) the adipose tissue, and (**g**) the liver. Symbols: $c_{air}$, concentration of the substance in the air at time t; $c_{art}$ ($c_{mart}$), $c_{ven}$ ($c_{mven}$), concentrations of the substance (the metabolite) in the arterial blood leaving the lung and in the venous blood entering the lung at time t; $c_R$ ($c_{mR}$), $c_K$ ($c_{mK}$), $c_M$ ($c_{mM}$), $c_A$ ($c_{mA}$), $c_L$ ($c_{mL}$), concentrations of the substance (the metabolite) in the richly perfused organs, kidneys, moderately perfused tissues, adipose tissue, and liver at time *t*; $K_{mapp}$ ($K_{mmapp}$), apparent Michaelis constants for the concentration of the substance (the metabolite) in the liver; $V_{max}$ ($V_{mmax}$), maximum rate of metabolic elimination of the substance (the metabolite) in the liver; $\lambda$, substance-specific partition coefficient blood:air; $k_{mel}$, first-order rate constant of urinary metabolite excretion from kidneys; $P_{RB}$ ($P_{mRB}$), $P_{KB}$ ($P_{mKB}$), $P_{MB}$ ($P_{mMB}$), $P_{AB}$ ($P_{mAB}$), $P_{LB}$ ($P_{mLB}$), substance-specific (metabolite-specific) partition coefficients richly perfused organs to blood, kidney to blood, moderately perfused tissues to blood, adipose tissue to blood, and liver to blood; $Q_{alv}$, alveolar ventilation; $Q_{card}$, cardiac output (equals the blood flow through the lung); $Q_R$, $Q_K$, $Q_M$, $Q_A$, and $Q_L$, blood flows through the richly perfused organs, the kidneys, the moderately perfused tissues, the adipose tissue, and the liver; $V_R$, $V_K$, $V_M$, $V_A$, and $V_L$, volumes of the richly perfused organs, kidneys, moderately perfused tissues, adipose tissue, and liver

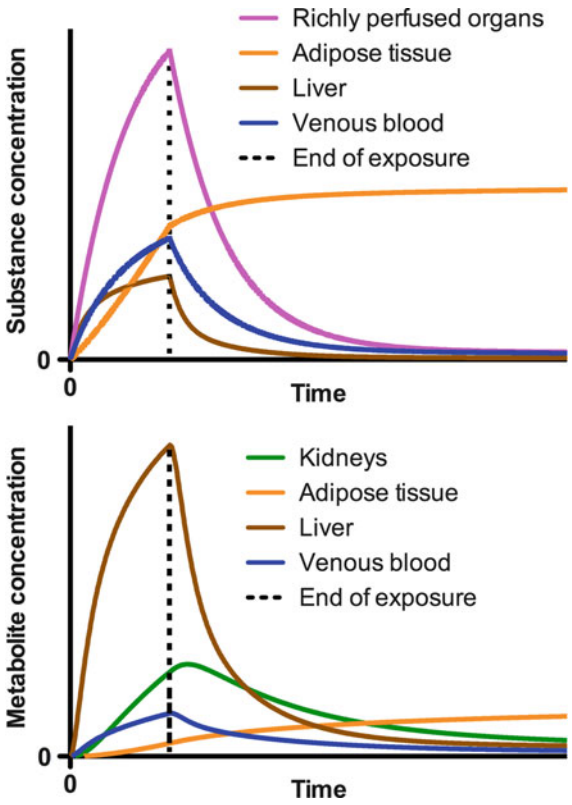



**Fig. 4** Predicted time courses of an inhaled lipophilic gaseous substance and its amphiphilic, nonvolatile metabolite in selected tissues of a mammalian organism, generated by means of the physiological toxicokinetic model described in Fig. 3. The exposure duration is modeled to be too short to reach steady state. During exposure, the substance is first distributed in the richly perfused organs. The accumulation in the poorly perfused adipose tissue takes place more slowly and is still continuing when the substance concentrations in the blood, liver, and richly perfused organs are already declining due to the end of exposure. The metabolite picture is similar, with the exception that the concentration of the metabolite is high in the liver, the organ in which it is formed. Most of the metabolite peaks are reached after the end of exposure, the latest one in the adipose tissue. The final decreases of substance and metabolite concentrations in the adipose tissue are not shown

## Recommended Reading

Bisswanger H (2008) Enzyme kinetics, 2nd edn. Wiley-VCH, Weinheim

Filser JG, Bogdanffy MS [guest editors] (2003) Application of physiological-toxicokinetic modelling to health hazard assessment of chemical substances. Toxicol Lett 138(1/2):1–184

Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Marcel Dekker, New York

Reddy M, Yang RS, Andersen ME, Clewell HJ III (2005) Physiologically based pharmacokinetic modeling: science and applications. Wiley, Hoboken

Ritschel WA, Kearns GL (2004) Handbook of basic pharmacokinetics – including clinical applications, 6th edn. American Pharmacists Association, Washington DC

# Exposure Analysis for Indoor Contaminants

Gerhard Volland

## Contents

Objectives and Purpose for the Determination of Volatile and Semi-volatile Organic Compounds in Indoor Air ..... 278
Screening Methods ..... 280
Sampling ..... 280
Determination of Organic Pollutants in Indoor Air ..... 281
Limits and Errors of the Determination of Organic Indoor Air Pollutants ..... 283
Influence of Emission Characteristics and Ventilation ..... 284
The Effect of Absorbing Effects (Sinks) ..... 285
Conclusion ..... 286
References ..... 287

**Abstract**

Due to changes in living and working habits, most individuals spend about 80–90 % of their time in public and private indoor environments. Those offer a broad diversity of pollution situations. Several hundred of very volatile, volatile, semi-volatile, and particular organic compounds (VVOCs, VOCs, SVOCs, and POMs) can be detected in indoor air. Emitting from construction materials (e.g., floorings, paints, furniture, joints), consumer products (electrical and electronic devices) as well as cleaning products, they are one of the determining factors for indoor air quality (IAQ). The wide variety of pollutants, exposure levels, differences in sensitivity as well as different cultural habits and ways of living complicate the assessment of risk. In a variety of reports, indoor

G. Volland
Schwäbisch Gmünd, Germany
e-mail: gerhard.volland@web.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_50, © Springer-Verlag Berlin Heidelberg 2014

air pollutants have been associated with health problems. Therefore, it must be of special interest to obtain reliable data to assess the IAQ. The basis for reliable and comparable data is given by a series of international and national standards for the sampling and determination of volatile (VOC) and semi-volatile (SVOC) organic compounds in indoor air.

## Objectives and Purpose for the Determination of Volatile and Semi-volatile Organic Compounds in Indoor Air

Indoor air, as dynamic system, is generally characterized by a wide variety of organic pollutants in differing concentrations (Salthammer 1999; Uhde and Salthammer 2007; Seifert 2002; Edwards et al. 2005; Schleibinger et al. 2001; Hofmann and Plieninger 2008). **Measurements of indoor air pollution are carried out for different reasons.** However in most cases, occupants **complain about poor air quality**, which often is correlated with complaints about odor and/or unspecific health problems such as headache, sleeplessness, lack of concentration, and fatigue (WHO 2000, 2010; COMEAP 2004; Anses 2011; EPA). Based on the results of a first measurement or a previous survey of a building, it might be necessary to **determine if a specified limit or guideline value is maintained**. Last but not least, the effectiveness of a remedial treatment has to be proven. A special task is to correlate observed or suspected effects on occupant health with indoor pollution. Due to these different questions, **individual sampling and determination strategies have to be applied**. Complaints from occupants in public buildings (e.g., offices, schools and kindergartens) are often characterized/accompanied by the presence of different complaints and differing health problems. As normally, only few information on possible pollutants and their sources exists, these cases regularly require an extended search for the possible causes of the complaints. In general, it is advisable to use questionnaires to obtain a systematic record of complaints as well as a systematic record of the affected building (e.g., ISO/DIS 16000-32 – Investigations of constructions and pollutants and other injurious factors – inspection). Based on the obtained information, an individual sampling strategy has to be developed. In the ISO Guides 16000-1 (general aspects), and 16000-5 (volatile organic compounds – VOCs), the general rules for different sampling strategies are specified. Besides these general aspects, ISO Guide 1600-2 and 1600-12 describe the sampling strategies for formaldehyde and polychlorinated Biphenyls, Dibenzo-dioxins, and furans (PCB, PCDD/F), respectively. In cases of complaints about "bad" indoor air, it is often useful to know the average level and the range of the concentration of organic pollutants in indoor air (Krause et al. 1991; Schleibinger et al. 2001; Hofmann and Plieninger 2008).

In this context, it must be noted that due to regulations and technical progress, new building and consumer products have been implemented on the market. These products are in general characterized by lower emission rates and the substitution of critical ingredients, e.g., solvents. This influences the average composition of

**Table 1** Development of the average concentrations of typical volatile organic compounds in indoor air (median) within the period from 1985 to 2008 in indoor air in Germany

| Compound | 1985/1986 (Krause et al. 1991) | 1999 (Schleibinger et al. 2001) | 2006 (Hofmann and Plieninger 2008) |
|---|---|---|---|
| | Median in µg/m$^3$ | | |
| Toluene | 62 | 28 | 12 |
| Sum of C9 aromatic hydrocarbons | 23 | 8 | 10 |
| 1-butanol | <1 | 27 | 11 |
| Limonene | 13 | 8 | 6 |
| Formaldehyde | 55 | 38 | 32 |
| Hexanal | <1 | 34 | 21 |

**Table 2** Indoor air guideline values for selected indoor air pollutants in different countries compared with the median in indoor air in Germany

| Organic Compound | Median (P50) indoor air Germany (Hofmann and Plieninger 2008) | WHO (2010) | USA (chRELs) (EPA 2007; OEHHA 2012) | Germany (recommended concentration RW I) (UBA 2007) | France (Anses 2012) | UK (COMEAP 2004) |
|---|---|---|---|---|---|---|
| | Concentration in µg/m$^3$ | | | | | |
| Formaldehyde | 32,5 | 100 (30 min) | < 20 (EPA) 9 (OEHHA) | 120 (30 min) | 10 | 100 (30 min) |
| Naphthalene | 1 | 10 | 9 | 2 | – | – |
| Toluene | 1 | – | 300 | 300 | – | – |
| Trichlorethene | 1 | – | 600 | – | 20 | – |
| Tetrachlorethene | 1 | 250 | – | – | 250 | – |

indoor air. The reduction of the average concentration of aromatic hydrocarbons in indoor air is an example for this development (see Table 1).

Based on first investigations, often the question occurs whether threshold or guideline values are exceeded in the indoor air. Besides formaldehyde, **most threshold or guideline values for VOC and SVOC are long-term guidelines**, regarding an **average concentration** in indoor air over a longer period (e.g., annual average). Depending on the definition of the guideline value, short-term sampling and/or long-term sampling methods have to be applied. Guideline values for indoor air quality are published by the World Health Organisation (WHO 2000, 2010), for the United States of America (EPA 2007, OEHHA 2012), France (Anses 2011), United Kingdom (COMEAP 2004), and Germany (Umweltbundesamt 2007). Table 2 gives an overview of existing guideline values in different countries.

## Screening Methods

Besides the given low concentrations of organic pollutants in indoor air, which normally prevents the use of screening methods, these methods just detected a "sum" of volatile compounds in indoor air. In principle, gas chromatography with flame ionization detector (FID), photo-ionization detector (PID), and photo-acoustic sensor (PAS) is applicable for screening methods. These methods may give a quick overview of possible indoor air pollution. In practice, however, these results often show substantial deviations from the real concentration of VOC in indoor air. Generally not suitable are commercially available short-term tubes used for air examination at the workplace in the range of workplace-related limit values. For special indoor air pollutants, e.g., formaldehyde, commercial enzyme-based screening systems (e.g., "Bio-Check") are available and suitable for pretesting.

## Sampling

Generally, the sampling strategy has to be adapted to the individual case. Sampling of indoor air should be carried out **at room temperatures between 19 °C and 24 °C** and a **relative humidity in the range of 30 % and 70 %** (comfort level see ISO 7730). Besides that, other **important parameters** like the **ventilation**, the nature of the **sources**, and the type of indoor environment have to be paid attention in choosing the conditions for sampling indoor air. Two basic sampling systems are applicable for the determination of VOC and SVOC in indoor air. Active short-term sampling is characterized by drawing the air through a defined absorption systems, e.g., char coal, polyurethane foam, or different sorts of silica gel.

The short-term sampling strategy for formaldehyde and VOC, for example, in natural ventilated rooms demands intensive ventilation as a first step. After this, ventilation doors and windows have to be closed for about 8 h (preferably over night). The sampling starts after this period (preferably next morning) without further ventilation (see ISO 16000-2 and ISO 16000-5). The sampling volume varies according to sampling method and the interesting compound between 1 and 10 l (thermal desorption and gas chromatography/mass spectrometry) up to 400 $m^3$ (determination of dioxins and furans). The hourly sampling rate should be less than 10 % of the room volume or less than 10 % of the ventilation rate (see ISO 16000-1). The disadvantage of the short-term sampling technique is that the result only represents the composition of indoor air during the sampling time. Normal differences in indoor air, effected by different air exchange rates or temperature-influenced changes in the emission rate, are not detected by active sampling methods. To detect these influences, a series of sampling are necessary.

To determine the average concentration of an indoor air pollutant, long-term sampling strategies should to be applied. If passive sampling systems for the interesting pollutants are available, this method offers the chance to determine the average indoor air concentration. Passive sampling systems are available and tested for aldehydes (see ISO 16000-4) and VOC (see ISO 16017-2 and EN 14412).

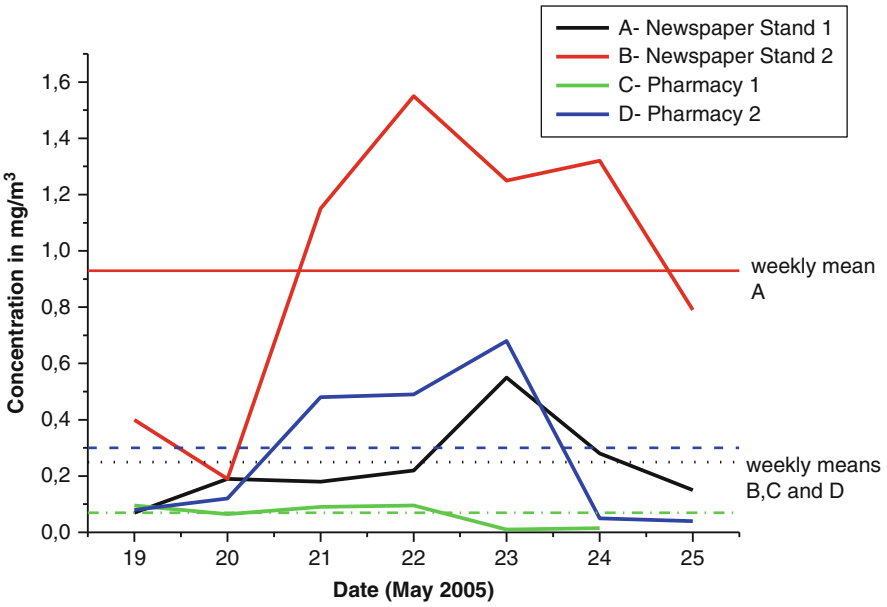



**Fig. 1** Weekly trend of toluene concentration in various indoor environments (Bruno et al. 2008)

For long-term measurements, generally no preconditioning is necessary. Sampling is carried out under actual living conditions. Occupants maintain their usual ventilation habits. It is recommendable to request and document these habits before the sampling starts. Deviations during the sampling period should be documented as well. For long-term measurements in offices, schools, and Kindergartens, it is recommendable to determine the room temperature, the humidity, and especially the concentration of $CO_2$ continuously parallel to the sampling. Disadvantage of the passive sampling method is that concentration peaks and concentration gradients are not detectable. Figure 1 gives an example of the results for toluene in indoor air in different indoor environments obtained by active short-term and passive long-term sampling method (Bruno et al. 2008).

Aspects of the sampling and measurement strategy as well as examples and limitations of the different sampling techniques are given in the ISO standards 16000-1, -2, -5, and 12 and ISO 16017-1 and -2

## Determination of Organic Pollutants in Indoor Air

After sampling volatile and semi-volatile compounds in indoor air on solid phase sorbents, several detection methods are applicable. For **VOC and SVOC,**

**Table 3** Selected dioxin-like PCB – an example for the accuracy of the determination of toxic semi-volatile organic compounds in indoor air (Volland 2006)

| | Laboratory A | | Laboratory B |
|---|---|---|---|
| | Sorbent PUF Method EPA TO-4A GC/MS (HR) | | Sorbent PUF Method ISO 16000–14 GC/MS (LR) |
| PCB congener | Arithmetic mean ($n = 4$) | Standard deviation (abs.) | Separate operation |
| | Concentration in ng/m$^3$ | | |
| PCB 77 | 0.68 | 0.05 | 0.67 |
| PCB 118 | 12.7 | 1.52 | 17.00 |
| PCB 156 | 2.60 | 0.14 | 2.46 |
| PCB 167 | 1.0 | 0.11 | 1.34 |
| WHO-TE (max.) in pg/m$^3$ | 5.0 | 0.59 | 5.4 |

**chromatography/mass spectrometry (GC/MS low and high resolution)** combined with either thermal desorption or solvent extraction is an appropriate method. ISO 16017-1 gives an overview of suitable sorbents tubes and the related accuracy. For common concentrations of VOC in indoor air, the standard deviation using thermal desorption and GC/MS is in the range of 1–5 % (see ISO 16017-1). Using char coal sorbent tubes combined with solvent extraction, comparable standard deviation can be obtained (see VDI 2001-2). For semi-volatile organic compounds like biocides or polychlorinated biphenyls, absorbed on polyurethane foam (PUF), standard deviations in the range between 10 % and 25 % are frequent (see Table 3).

Formaldehyde and other **carbonyl compounds (aldehydes and ketones)** can be absorbed on **silica gel cartridges coated with 2,4-dinitrophenylhydrazine (DNPH).** The formed DNPH derivatives are analyzed utilizing high performance liquid chromatography (see ISO 16000-3). The average random error (standard deviation for duplicate samples) for the determination of formaldehyde in indoor air is about 12 % (see ISO 16000-3). This proves that the available methods for sampling and detection of organic compounds in indoor provide sufficiently accurate results.

Detailed information for the determination of VOC and SVOC is given with ISO 16000-3, -4, -6, -13, -14, –31; ISO 16017-1 and -2; EN 14412; VDI 2100; VDI 2464, VDI 3498 and EPA Compendium Methods for the Determination of Air pollutants in indoor air and EPA Compendium Methods for the Determination of Toxic Organic Compounds in Ambient Air (EPA 1990, 1999)

The detection sensitivity of the applied methods ranges from few pg/m$^3$ (e.g., PCDD/F method ISO 16000-14) to several hundred micrograms/m$^3$ for the detection of organic pollutants.

**Table 4** Examples of the concentration range of phthalate-based softeners [Di-ethylhexyl-phthalate (DEHP)] and organo-phosphorous-based flame retardants [Tris-chloro-ethylphosphate (TCEP)] in indoor air and house dust in German schools (Hansen et al. 2001; Volland et al. 2010)

| Softener (DEHP) (Volland et al. 2010) | | | |
|---|---|---|---|
| | Living area and sleeping rooms | Classrooms | Community area (e.g., dining room) |
| House dust | Range and arithmetic mean (Ø) in mg/kg | | |
| Boarding school A | 300–2,300 | 500–2,600 | <50 |
| | Ø 1,200 | Ø 1,880 | |
| Boarding school B | 110–740 | 350–575 | <50 |
| | Ø 400 | Ø 450 | |
| Indoor air | Range and arithmetic mean (Ø) in µg/m$^3$ | | |
| Boarding school A | <0.1–0.57 | n.d. | <0.1 |
| Boarding school B | 0.1 | 0.1 | <0.1 |
| Flame retardant (TCEP) (Hansen et al. 2001) | | | |
| House dust | | Range in mg/kg | |
| School C | Nonexistent in this building | 320–530 | Nonexistent in this building |
| School D | | 770–2,190 | |
| School E | | 410–1,450 | |
| Indoor air in µg/m$^3$ | | Range in µg/m$^3$ | |
| School C | Nonexistent in this building | 0.36–0.43 | Nonexistent in this building |
| School D | | 1.2–3.9 | |
| School E | | 0.3–2.0 | |

## Limits and Errors of the Determination of Organic Indoor Air Pollutants

Despite of existing guidelines and standards for sampling and determination of organic pollutants in indoor air, very often, differing results for the same building are reported. Indoor air is a dynamic system, and thus, the concentration of organic pollutants in indoor air is influenced by a set of parameters. Besides the wide range of the contamination within a building (see Table 4), those differences are mainly caused by three parameters.

**Strength and emission characteristic of the source:** Depending on the room temperature, the temperature of the building element, and the air humidity, the emission characteristics of the sources change.

**Ventilation:** In most cases, the concentration of volatile organic pollutants in ambient air is significantly lower than in indoor air. Thus, different ventilation rates influence the obtained results. Depending on the tightness of a building envelope, the wind speed influences the ventilation even if the windows are closed.

**Absorbing effects and secondary sources:** Depending on the vapor pressure, organic compounds can absorb at the surfaces of, e.g., walls, floors as well as on dust particles (see Table 3). In particular, SVOCs generate relevant secondary sources based on absorbing effects.

## Influence of Emission Characteristics and Ventilation

The **emission rate** of VOC and SVOC is commonly determined by the diffusion potential and the **vapor pressure** of the organic compound. Figure 2 points out how different vapour pressures resp. boiling points of solvents in coatings influence the emission characteristics of volatile compounds (Zellweger et al. 1997). In general, VOC with boiling points between 60 °C and 150 °C will lead to short and high concentration of VOC in indoor air immediately after application of the product. The emission of products containing organic compounds (e.g., solvents) with high boiling points (>180 °C) resp. low vapor pressure is characterized by long-term emission of those compounds combined with a low concentration in indoor air. Regarding the influence of the temperature, it is obvious that **higher temperatures** will **increase the emission rate**. The concentration of dioxin-like PCB in indoor air in buildings with PCB coated ceiling slabs, for example, increases from 3.5 up to 13.6 pg WHO/TE/$m^3$ (Volland et al. 2006). At the first glance, normally, the room temperature is taken in account. Due to **structural conditions of a building**, parts of this building may have **different temperatures**. Depending on the season and the actual weather conditions, the temperature of these building materials ranges from 10 °C to 60 °C. Longer periods of sunshine, for example, will increase the temperature of building components. Experience shows that this effect is relevant for components containing indoor air pollutants. Influences caused by different temperatures of the building itself can be shown, regarding the season of sampling. Figure 3 gives an example of the influence of the room temperature and the season, when sampling was conducted.

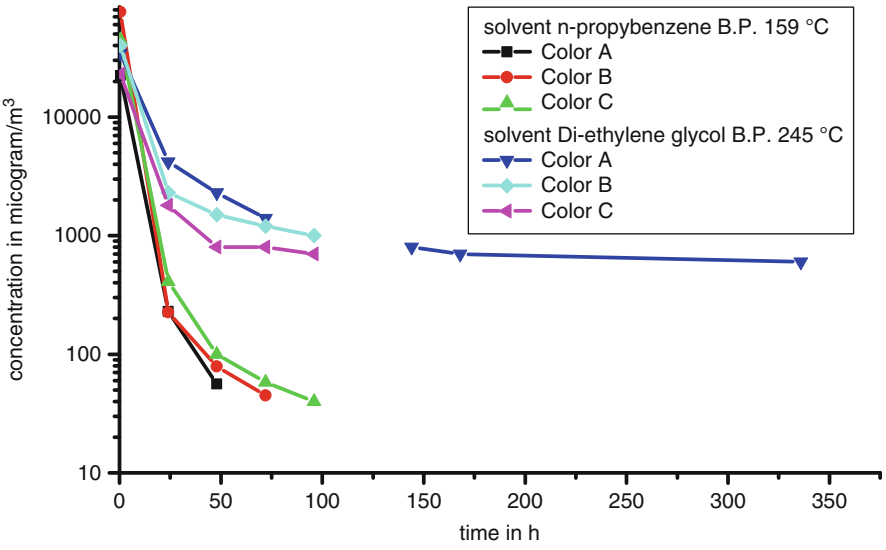



**Fig. 2** Development of the concentration of n-proply-benzene and Diethylene glycol in test chamber air within the period after application of different products (Zellweger et al. 1997)

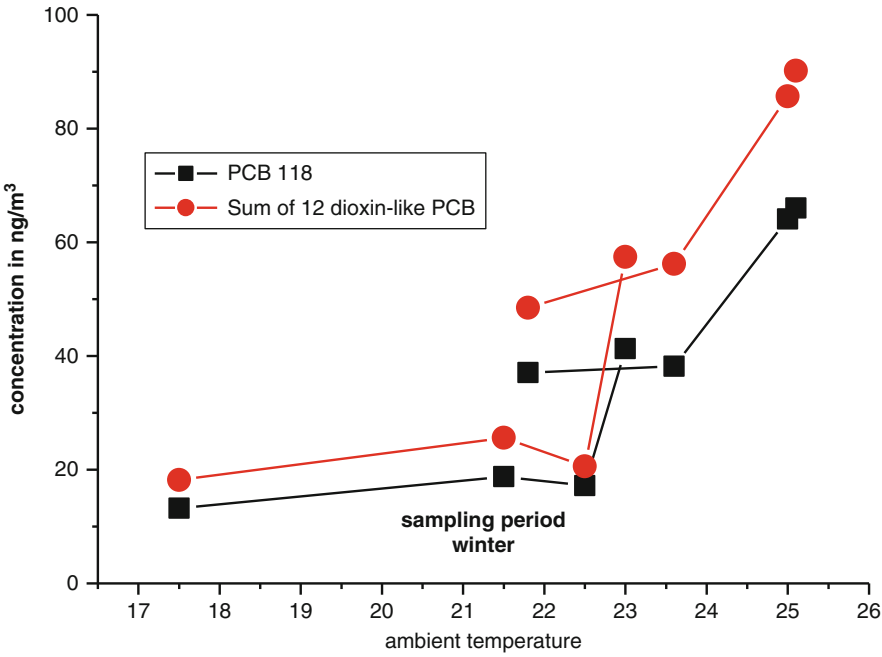



**Fig. 3** Influence of room temperature and season on the concentration of PCB 118 and the sum of 12 dioxin-like PCB in indoor air (Volland et al. 2006)

Depending on the definition of a long-term guideline value, the interesting average concentration of indoor air pollutants also depends on the ventilation. The effects of sampling carried out according to ISO 16000-1 (see short-term sampling above) or when sampling is carried out during actual living conditions are shown in Fig. 4. In most cases, the PCB concentration obtained during actual living conditions is less than results obtained by sampling according to ISO 16000-1.

The emission characteristic of steam volatile organic compounds like aldehydes is additionally influenced by the humidity of the indoor air. Increasing humidity increases the emission. Figure 5 shows the influence of room temperature, air humidity, and ventilation for formaldehyde in indoor air.

## The Effect of Absorbing Effects (Sinks)

Due to specific physical characteristics of indoor air pollutants, longer lasting indoor air contamination leads to relevant concentration on secondary areas in the room. One of the consequences is that these contaminated areas affect as a secondary source. Besides walls and floors, house dust is an important sink. Table 4 shows the contamination of house dust in buildings contaminated with softeners or flame retardants. Both sources, secondary contaminated building materials and contaminated house dust, may raise the indoor air concentration.

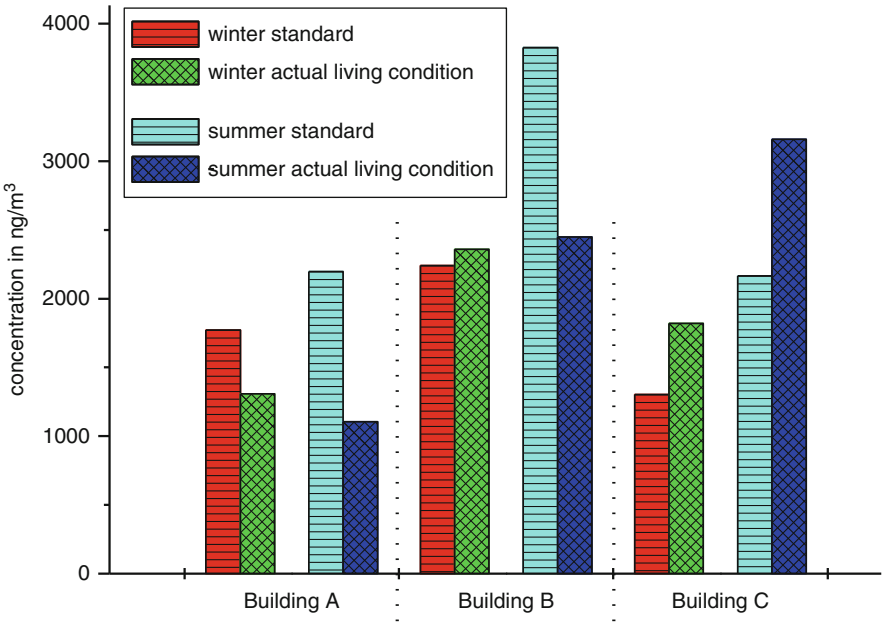



**Fig. 4** Comparison of the concentration of PCB (sum of 6) in indoor air depending on sampling according to ISO 16000-1 (short time sampling) and sampling under actual living conditions, regarding the season, when sampling was carried out (Volland et al. 2006)

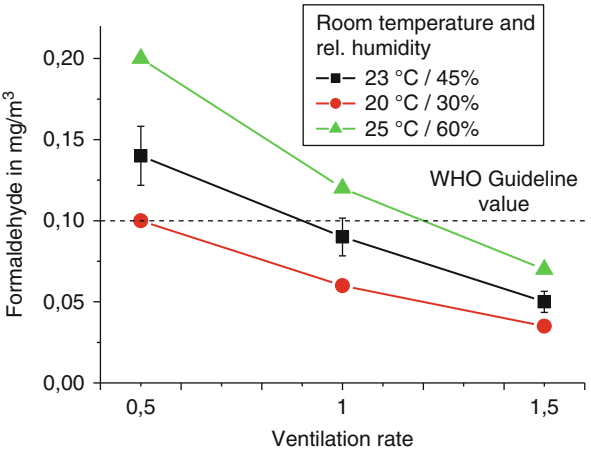



**Fig. 5** Influence of room temperature, air humidity and ventilation rate on the indoor air concentration of formaldehyde (calculated based on results reported by ISO 16000-3)

## Conclusion

The given examples illustrate the influence of the indoor environment (temperature, humidity, season, ventilation) as well as the specific physical characteristics of indoor

air pollutants on obtained measurement results. In the most cases, the methods to determine organic pollutants in indoor air deliver accurate and comparable results. For determination methods of VOC and SVOC in indoor air, if sampling is carried out under comparable conditions, the **usual accuracy is in the range of 10–30 %**.

The complex correlation of the given structural situation in a building and the variety of the indoor climate normally overlaps the analytical errors of the determination step. The sampling strategy, the time of sampling as well as the parameters influencing the emission rate of the source are dominating the quality of results measuring the indoor air quality. Reported results without detailed information about the sampling condition cannot be assessed.

**Higher differences** between two measurement results are mostly **caused by differences of relevant parameters in the room during sampling**. These differences in indoor environment can cause deviations of more than 100 %. Thus, the assessment of results of the determination of VOC and SVOC in indoor air without the knowledge of the conditions during the sampling is fairly impossible. In the praxis often an exceeding of a guideline value is detected based on one single measurement. If the guideline value is based on a long-term average concentration, the **state-of-the art bans an assessment based on a single measurement**.

## References

Bekanntmachungen des Umweltbundesamtes – Beurteilung von Innenraumluftkontaminationen mittels Referenz- und Richtwerten Handreichung der Ad-hoc-Arbeitsgruppe der Innenraumlufthygiene-Kommission des Umweltbundesamtes und der Obersten Landesgesundheitsbehörden Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 2007 50:990–1005

Anses (Agence national de scurite sanitaire alimentation, environment, travail) Proposition de valeurs guides de qualite D'air interieur – Avis de l'Anses Raport d'expertise collective Avril 2011 Edition scientifique. http://www.anses.fr/ET/PPN8F48.htm

Bruno P, Caselli G, de Gennaro G, Iacobellis S, Tutiono M (2008) Monitoring of volatile organic compounds in non-residental environments. Indoor Air 18:250–256

COMEAP (Committee on the Medical Effects of Air Pollutants) (2004) Guidance on the effects on health of indoor air pollutants. http://www.comeap.org.uk/documents/reports/85-indoor-air-pollutants.html

Edwards RD, Schweizer C, Jantunen M, Lai HK, Bayer-Oglesby L, Katsouyanni K, Nieuwenhuijsen M, Saarela K, Sram R, Künzli N (2005) Personal exposure to VOC in the upper end of the distribution-relationships to indoor, outdoor and workplace concentrations. Atmos Environ 39:2299–2307

EPA (U.S. Environmental Protection Agency) Indoor air pollution – an introduction for health professionals. http://www.epa.gov/iaq/pubs/hpguide.html

EPA (United States Environmental Protection Agency) (2007) The testing of indoor air quality section 01 81 09. http://www.epa.gov/rtp/campus/environmental/018109.pdf

EPA compendium of methods for the determination of air pollutants in indoor air – U.S. EPA PB 90–200288 (1990) volume 1 and 2 [IP-1B VOC Solid Adsorbent Tubes; IP 6 Formaldehyde

and other Aldehydes; IP-7 B(a)P and other PAH; IP-8 Organochlorine Pesticides] – [Indoor Air Monitoring Methods – California Air Resources Board]. http://www.arb.ca.gov/research/indoor/methods.htm

EPA compendium of methods for the determination of toxic organic compounds in ambient air – Second edition – EPA/625/IR-96/0-10b January 1999 – [TO-1 VOC – Tenax; TO 2 VOC Caron molecular sieve; TO-4A Pesticides, PCB; TO-5 Aldehydes and Ketones; TO-8 Phenol and Cresols; TO 9A Polyhaloginated Dioxins and Furans; TO 10A Pesticides, PCB; TO-11A Formaldehyde; TO 13A Polyaromatic Hydrocarbons; TO 17 VOC – sorbent tubes]. http://www.epa.gov/ttnamti1/files/ambient/airtox/tocomp99.pdf

Hansen D, Volland G, Krause G, Zöltzer D (2001) Bestimmung und Vorkommen von phosphororganischen Verbindungen in Hausstaub und Raumluft. Gefahrstoffe- Reinhaltung der Luft 61:S13–S17

Hofmann H, Plieninger P (2008) Bereitstellung einer Datenbank zum Vorkommen von flüchtigen organischen Verbindungen in der Raumluft WaBoLu Hefte 05/08. Umweltbundesamt Dessau

Krause C et al (1991) Umweltsurvey Band IIIC Wohn-Innenraum: Raumluft WaBoLu Hefte 4/1991. Institut für Wasser-, Boden- und Lufthygiene des Bundesgesundheitsamtes, Berlin

OEHHA (Office of Environmental Health Hazard Assessment) (2012) Acute, 8-hour and chronic reference levels (chRELs), February 2012. http://oehha.ca.gov/air/allrels.html

Salthammer T (ed) (1999) Organic indoor air pollutants – occurance, measurement, evaluation. Wiley VCH, Weinheim (Federal Republic of Germany). ISBN 3-527-29622

Schleibinger H, Hott U, Marchl D, Braun P, Plieninger P, Rüden H (2001) VOC-Konzentrationen in Innenräumen des Großraums Berlin im Zeitraum von 1988 bis 1999 Gefahrstoffe Reinhaltung der Luft 61(1/2):26–38

Seifert B (2002) Indoor air 2002 9th intenational conference on indoor air quality and climate – monterey 2002

Uhde E, Salthammer T (2007) Impact of reaction products from building materials and furnishings on indoor air quality – a review of recent advances in indoor chemistry. Atmos Environ 41:3111–3128

Volland G, Neuwirth A, Hansen D, Krause G, Zöltzer D (2006) Dioxinähnliche polychlorierte Biphenyle (PCB) und polychlorierte Dibenzodioxine/Furane (PCDD/F) in Raumluft und Hausstaub –Einfluss PCB-belasteter Bauteile in Abhängigkeit von baulichen und klimatischen Randbedingungen. Gefahrstoffe – Reinhaltung der Luft 66 (3):99–106

Volland G, Gabrio T, Wodarz R, Hansen D, Mann V, Hildenbrand S (2010) Einfluss von Di(2-ethylhexyl)Phthalat (DEHP) in Hausstaub und Innenraumluft auf die tägliche DEHP-Aufnahme Gefahrstoffe Reinhaltung der Luft 70 (3 März):83–88

WHO (World Health Organisation Regional Office for Europe) (2000) Air quality guidelines for Europe, 2nd ed. WHO regional publications european series no 91. ISBN 1358 3

WHO (World Health Organisation Regional Office for Europe) (2010) Air quality guidelines for indoor air quality – selected pollutants – WHO European Centre for Environment and Health, Bonn Office. ISBN 978 02 890 02134

Zellweger C, Hill M, Gehrig R, Hofer P (1997) EMPA Forschungsbericht Schadstoffemissionsverhalten von Baustoffen Schweiz

# Exposure Scenarios in Toxicology

Gerhard Heinemeyer

## Contents

Structure of Exposure Scenarios ..... 290
Use Scenario: Characterization of the Source and the Use of the Substance in a Product ..... 292
Categories of Use ..... 292
Disposition Scenario: Release, Distribution, and Disappearance ..... 294
Route of Exposure: Inhalation ..... 294
Typical Scenarios of Inhalation Exposure ..... 295
Route of Exposure: Dermal ..... 296
Typical Scenarios: Dermal Exposure ..... 296
Oral Path of Exposure ..... 297
Typical Scenarios ..... 297
Behavior Scenario ..... 299
Time Budgets ..... 299
Particular Age-Related Behaviors ..... 300
Combination of Scenarios ..... 300
Distribution-Based (Probabilistic) Exposure Assessment ..... 301
References ..... 302
Recommended Reading ..... 304
Resources ..... 304

**Abstract**

Exposure is defined as the "concentration or the amount of a particular agent that reaches a target organism, system, or (sub)population in a specific frequency for a defined duration" (WHO/IPCS 2004). Exposure is normally characterized by means of exposure scenarios. The information from the exposure scenario is used for building up an exposure model. Exposure models can be understood as a translation of an exposure scenario to a mathematical algorithm to yield

G. Heinemeyer
Federal Institute for Risk Assessment, Berlin, Germany
e-mail: Gerhard.heinemeyer@bfr.bund.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_51, © Springer-Verlag Berlin Heidelberg 2014

a qualitative and a quantitative estimate of exposure. Exposure can be understood as dose estimation, by the oral, dermal, or inhalation route.

Exposure assessment is based on three basic elements: (i) the exposure scenario, (ii) the exposure model, and (iii) the exposure parameters (WHO/IPCS 2005). The basic characterization of the exposure is made by the exposure scenario (ES). The ES describes the circumstances of the exposure, covering all situations and corresponding information needed to perform an exposure estimate. The WHO (2004) defines the term exposure scenario as "a combination of facts, assumptions, and interferences that define a discrete situation where potential exposures may occur. These may include the source, the exposed population, the time frame of exposure, micoenvironment(s), and the activities. Scenarios are often created to aid exposure assessors in estimating exposure." This definition should be used as a basic concept for exposure estimation.

Since 2006, an additional definition of exposure scenario must be taken into consideration regarding to the European Chemicals Regulation (REACH; European Commisson 2006). In the regulation, the exposure scenario is defined as ". . .the set of conditions that describe how the substance is manufactured or used during its life-cycle and how the manufacturer or importer controls, or recommends others to control, exposures of humans and the environment."

This chapter is explaining the exposure scenario on the basis of the WHO definition, with hints of the particularities of the REACH regulation.

Similarly to drug treatment, an exposure estimate can be understood as the dose of a contaminant or hazardous substance that can be taken in by an individual or a population.

## Structure of Exposure Scenarios

Exposure scenarios describe the complex characteristics of the external exposure from any substance that can be released from a variety of sources, e.g., the environment, consumer products, food, and other sources. This resulting external "dose" will be systemically absorbed and results in the toxicologically relevant "internal" exposure. The characterization of the exposure scenario describing external exposure should be divided into "subscenarios" to be combined with each other yielding the complete scenario.

*In the REACH regulation (European Commission 2006), the scenario contains basically the same information. However, the exposure scenario also contains information about measures that reduce the exposure to an extent that will not exceed the DNEL. If, for example, in an exposure calculation, the DNEL is exceeded, the registrant must reduce it by risk management measures (Bruinen de Bruin et al. 2007) [Registrant: The company that prepares the chemical safety report for notification to ECHA (European Chemicals Agency)]. Examples for RMM are reduction of the concentration of a substance in a product, hindrance of migration of a substance from an article, or release reduction by special dispensers. Non-exceedance of the DNEL indicates that a product is safe.*

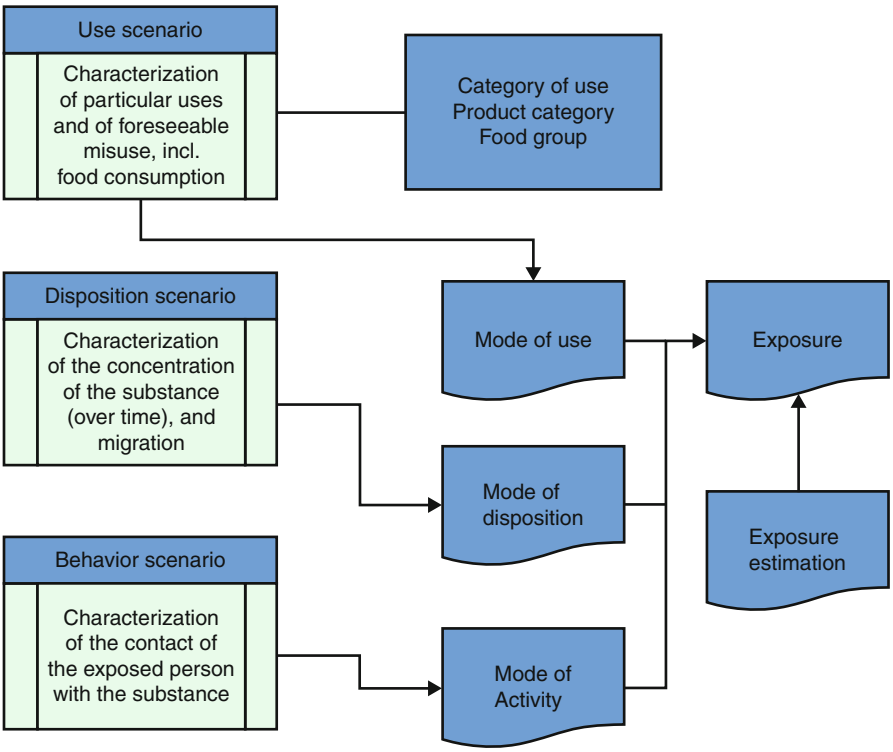



**Fig. 1** The different types of exposure scenarios and their interaction

Three subscenarios (see Fig. 1) should be considered when describing an exposure scenario, as shown by the following simple example:

*Use scenario*: *Characterization of the source in contact and amount of a substance that will be potentially released*

**Example**

A certain household cleaner (use category) containing a substance in a particular concentration will be applied to a bigger area (e.g., the ground of a room). The amount of substance has the potential of release from that source.

*Disposition scenario*: *Characterization of release, distribution, and disappearance of a substance in the environment*

**Example**

The substance, due to its vapor pressure and molecular weight will be released and evaporated to room air to yield a certain concentration. The concentration will increase and continue over time and can be inhaled by persons in that room.

Due to air exchange, the concentrations in room air will decrease. Substances having low volatility will be distributed mostly via the house dust path.

*Behavior scenario*: *Description of the exposed population and the characteristics of use of products and articles, consumption of food*, etc.

**Example**

The exposed person stays in that room for a certain time and will inhale the air. The time an exposed person is spending in a room may account for, e.g., 4 h.

### Use Scenario: Characterization of the Source and the Use of the Substance in a Product

This subscenario is used to characterize the source of the substance and the amount that is released during the use of the product. The limitations of these processes are determined by the product itself that contains the substance, its physicochemical properties, its concentration, and the mode of use.

## Categories of Use

A substance may appear as an ingredient in many different products and product types (Heinemeyer and Hahn 2005). An approach that characterizes product use categories can therefore be very helpful to identify the sources of substances. Product categories have been used on the national and international level. Some of the documents became "official" due to their use in technical guidances (ECHA 2010) or from use and recommendations by international agencies (EFSA 2009) and organizations (WHO 2005). Therefore they have some standardizing character, although the details are differing. Major importance is due to the guidance documents and classifications used in international databases, such as the *industrial categories* and *product and article categories* described in the ECHA guidance R12 *use descriptor system* (ECHA 2010) (Table 1).

Other classification systems have been published by the EIS-Chemrisks framework developed by the EU-JRC (European Commission 2003). Also, poison centers around the world are using product use classification systems for documentation of cases and to prepare annual reports. In most of the classification systems, a differentiation is made according to the use of the products, e.g., paints, household cleaners, pesticides, cosmetics, and others. Due to these documentations, it can be checked how close exposure scenarios are close to reality (Heinemeyer and Hahn 2005). The identification of use of a substance and the description of manufacturing and the use process is an important part in defining exposure scenarios under REACH (van Engelen et al. 2007; Heinemeyer 2008).

**Table 1** Important sources of information on classification systems to characterize exposure scenarios

| Reference | Editor | Remarks |
|---|---|---|
| AUH report | Behörde für Arbeit, Gesundheit und Soziales, Hamburg. Ausschuss für Umwelthygiene der AGLMB | Food intake data from the national survey 1985–1989 |
| Bundeslebensmittel-schlüssel | BVL (2012a) und Max-Rubner Institut (2012) | Nutrient database with food category system, national, Germany |
| Food contamination surveys | For example, BVL (2012), EFSA (2009) | |
| EFSA concise food consumption database | EFSA (2012) | A collection of national food consumption data due to harmonized food grouping |
| EFCOSUM report | Efcosum Consortium (2001), Brussard et al. (2002) | Report from an EU research project |
| LanguaL | Møller and Ireland (2010) | |
| EIS-Chemrisks | EU Commission, Joint Research Centre, Ispra | Project report and database EIS-Chemrisks |
| GEMS food | WHO (2012) | Worldwide classification system for foods |
| ECHA technical guidance document R12 | European chemicals agency (2010) | Compilation of different product and article categories and product use classification for REACH |
| EU commission | Technical guidance document 2003 | |
| General factsheet | RIVM; Bremmer et al. (2006) | Collection of exposure defaults and assumptions |
| Paint products factsheet | RIVM, Bremmer and van Engelen (2007) | Collection of model parameters for paints |
| Pest control products factsheet | RIVM, Bremmer et al. (2006a) | Collection of model parameters for pesticides |
| ECETOC TRA | European centre for ecotoxicology and toxicology of chemicals, several versions (2012) | Guidance document and tools for targeted exposure assessment |
| Annual reports of poisonings reported due to chemical law | Federal Institute for Risk Assessment (2011) | Product classification developed on national levels in cooperation with poison centers |
| INTOX | WHO (2012) | Classification developed for poison center annual reports |

The development of classification systems available for foods is more advanced than the others mentioned above. Food classification is used since longer times for systematic characterization and for exposure assessments. National food consumption surveys normally are using food classifications. The EU the European Food Safety Agency has introduced a harmonized food classification characterization in its *Comprehensive Food Consumption Database* (EFSA 2012), which comprises data on food consumption from nearly all EU member states. In Germany the *Bundeslebensmittelschlüssel* (BVL 2012a; Max-Rubner-Institut 2012b) is used to

classify food. The Max-Rubner Institute is responsible to maintain this classification system up to date which is close to the *LanguaL* (Møller and Ireland 2010). The latter combines a fixed three-level thesaurus with relational and dynamic tables, so-called facets. Product/use categories can be transferred and expressed as subscenarios on different levels of aggregation to apply a standardized approach (use model) with respective model parameters (model variables/exposure factors).

## Disposition Scenario: Release, Distribution, and Disappearance

As in pharmacokinetics, the disposition scenario describes the appearance, distribution, and disappearance of a substance in an environment. The disposition scenario includes:

1. A description of the concentration of a substance in the product and its release, by migration, evaporation, or emission.
2. The distribution of the substance in the environment of contacting it, as described in Fig. 2. Substances can be bound to particles, e.g., house dust, but also distributed in the gas phase.
3. The disappearance of the substance from the microenvironment

Source, (micro)environment, and substance characteristics are limiting the release of the substance. In combination with the use, the route of exposure will be oral, dermal, or by inhalation.

## Route of Exposure: Inhalation

The scenario characterizing the exposure by inhalation normally describes the concentration – time course of a volatile substance in the indoor air, either in one or multiple rooms. The concentration can be used for comparison with toxic concentrations.

It is recommended to use the concentration in air to estimate the uptake of a substance via the lungs to systemic circulation. Internal exposure evaluation enables risk assessors to estimate total body burden, e.g., in children or other particular populations. To perform these estimates, the respiratory volumes per time and pulmonary absorption rates are needed.

In addition to inhalation of substances in its gas phase, the inhalation of small particles should be also taken into account. Dust is a vehicle for nonvolatile substances that can be adsorbed and desorbed from the particles, absorbed through the alveoles, and thus enter the human body.

Exposure factors needed to estimate exposure from inhalation

- Concentration of the substance in room air
- Concentration of the substance in fine dust particles
- Migration rates (release rate per time)
- Vapor pressure
- Molecular mass
- Density

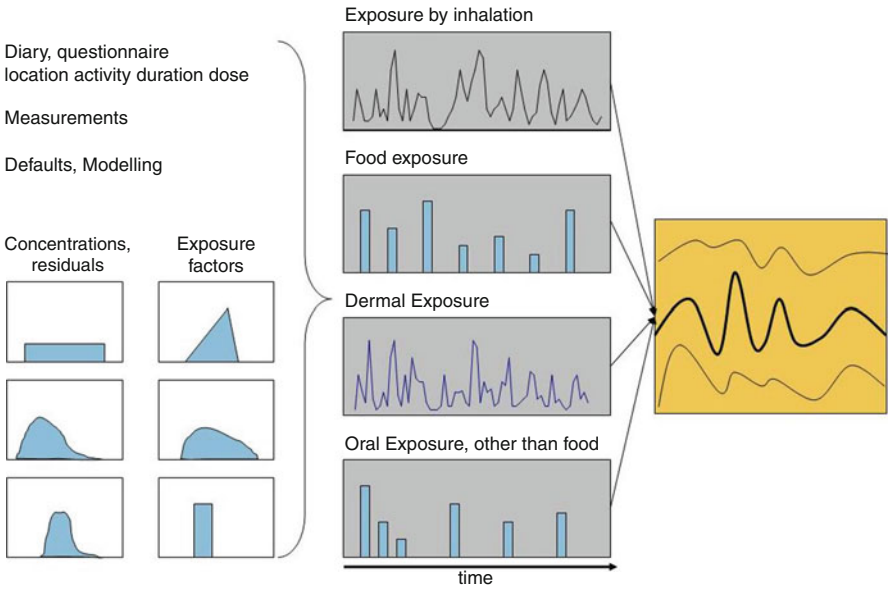



**Fig. 2** Theoretical contribution of different paths to the total exposure

- Product amount used in the application
- Concentration of the substance in the product
- Duration of the application
- Room volume
- Air exchange rate

## Typical Scenarios of Inhalation Exposure

1. Use of volatile substance, e.g., solvents in paints, laquers, or cleaners
   A certain amount of a product (e.g., a paint or cleaner) will be applied to a surface. A volatile substance will be evaporated and produces indoor room concentrations. The substance distributes in the room and disappears after some time, according to the air exchange rate. This type of scenario has been considered, e.g., in the computer tool ConsExpo (RIVM 2012) and the wall paint emission model published by the US EPA (2001).
2. Emission from solid bodies
   A constant amount will be evaporated over a longer time period from, e.g., furniture and textiles. This may lead to constant (steady state) concentrations of the substance in indoor air. The extent of this concentration depends on the air exchange rate, temperature, and other factors, e.g., whether the substance can be adsorbed to particles. This scenario may be applicable for exposures from inhalation due to solvent contaminated residual wastes.

3. Inhalation of dust
   Dust inhalation represents a special form of exposure by inhalation, where the substance is adsorbed to inhalable fine particles in the microenvironment. By this pathway, they can enter the lungs and alveoles. After desorption from the particles, the substance can be absorbed to the systemic circulation. Sometimes, they will remain in the alveolar cells and lead to local effects. The concentrations in the dust cannot be estimated and have to measured.

The example shown below represents a so-called *worst case* estimation of exposure for a child (bodyweight (BW) 10 kg). This estimate is characterized by its conservatism, taking low body weight (5th percentile), a respiratory volume (RV) that considers (partly) activity *and* rest, as well a maximal contact time (CT) and a high pulmonary absorption rate (RPA; 100 %).

Example "worst case" estimation of exposure by inhalation

- Concentration in room air (estimated or measured, C) – 10 μg/$m^3$
- Body weight (BW) – 8.1 kg
- Respiratory volume per time (RV) – 2.9 $m^3$/day
- Contact time (TC) – 1 day
- Pulmonal absorption rate (RPA) – 1
- inhalation exposure (absorbed amount) [C*RV*RPA*TC/BW] – 3.5 μg/kg/day

## Route of Exposure: Dermal

The dermal exposure estimation characterizes the amount of a substance which is on the skin and can be absorbed through the skin.

### Typical Scenarios: Dermal Exposure

1. Use of cosmetic products
   A product will be applied to skin; one or more substances in the product can be absorbed through the skin. In dermal exposure assessment, products that can remain on skin (non-rinse) will be differentiated from those that will be removed by washing (rinse off).
2. Use of household cleaners
   The hands will be shortly put into the water that contains the washing product. Substances in that diluted product can be adsorbed to and remain on skin and may be dermally absorbed. When taking a bath, the whole body surface will be exposed.
3. Dermal exposure via air
   Volatile substances in the air can become into contact with the skin and are dermally absorbed. Normally, the extent of this exposure is small.
4. Wearing textiles and contact with leisure and hobby products
   Direct contact of substances with the skin from textiles or leisure and hobby products is possible from migration to the skin. The exposure surface is the part of skin that is covered by the textile or contacting the leisure and hobby product.

5. Contact with pets

Ingredients from, e.g., pesticides used for domestic animals to treat against pest may lead to dermal contact when touching pets. Children may have oral exposure after licking hands (mouthing behavior) after touching the animals.

A basic rule for estimating dermal exposure has been described in the EU technical guidance document for existing chemicals and has been taken over by ECETOC (2005) as well as in the ECHA technical guidance document (2010). The *amount* (AM) that can lead to exposure can be estimated from the *area* (A) of exposure times am estimated *thickness of the layer* (TL) of 0.01 cm and from the *concentration* (C) of the substance in the *product* (AM = A*TL*C). In some documents, additional *absorption rates* given as percentages are used. However, it must be considered that dermal absorption is a time-dependent process. Taking percentages as rates can lead to errors and should only be applied as a default assumption, e.g., a *worst case concept* for 100 % of absorption. For short contact times (e.g., shortly applied cosmetics), correction factors have been introduced that reduces the absorption rate. In general, values from 1 – (10) – 50 % are used as default assumptions, with different justifications, depending on the purpose of the evaluation. For some substances, absorption constants and coefficients have been derived, due to lipid solubility (octanol/water coefficient) and molecular weight. Respective models have been established by Wilschut et al. (1995) and have been integrated into the ConsExpo tool.

Exposure factors needed to estimate dermal exposure

- Exposed skin area (e.g., 840 $cm^2$ for hands)
- (Theoretical) thickness of layer (0.01 cm; mixtures; 0.001: articles)
- Concentration of substance in the product
- Migration rates of the substance (measured)
- Absorption coefficient (derived by model evaluation), alternative: absorption rates (worst case, percentages)

## Oral Path of Exposure

Oral exposure characterizes the oral intake of a substance by mouth and the amount that is absorbed in the gastrointestinal tract. Oral intake is possible with food, drinking water, house dust, the mouthing behavior, and some personal care products (e.g., tooth paste). House dust and related paths are particularly important in small children. In general oral exposure estimation requires knowledge of the concentration of the substance in and the amount of the medium that is taken in.

### Typical Scenarios

1. Intake of food and drinking water

A number of different sources have to be distinguished to estimate the dietary exposure to contaminants in the food chain, food additives, process contaminants,

substances in food packaging, and bacterial toxins and metabolic products. Process contaminants, e.g., acrylamide or MCPD, (3-Chlor-1,2-propandiol) can be formed during heating of foods.

Dietary exposure estimation is normally performed by modeling concentration data in the food and the respective food consumption data. Concentrations in food can be obtained from, e.g., market control measurements. However, as these data are risk oriented (there is a reason for expecting high concentrations), systematic and representative evaluations of concentrations in food are more adequate to study dietary exposure in a population. Such data are available from, e.g., the German food monitoring system (BVL 2012). The European Food Safety Agency is establishing a system to regularly collect data of concentrations of substances in food, collected from the member states (EFSA 2011). Due to the immense number of samples needed to describe concentrations in food, approaches have been developed to reduce numbers of sample by, e.g., pooling, for example, by the concept of total diet study (TDS).

The identification of food consumption data normally is performed by means of questionnaire studies. On the national levels, food consumption surveys have been performed in many countries, for example, the Nationale Verzehrsstudie II (Max-Rubner-Institut 2012a) in Germany. There are several methodological approaches by which consumption studies can be performed (24-h recall, dietary history, food frequency study, diary studies, with and without weighing the food). It should be mentioned that theses study types have advantages and disadvantages for the particular questions asked in risk assessment, e.g., acute or chronic hazards.

Normally, to perform food consumption studies, foods will be characterized by a food basket that contains $> 90$ % of all foods eaten. The particular foods should be classified by a systematic food group classification system (see respective chapter).

Food exposure estimation is in general performed for the general population and normal food consumers (eaters), by taking concentration and consumption data describing a central tendency (means, medians). To describe high consumers, EFSA has proposed to identify those foods that have the highest contribution to exposure and exchange the means by 95th percentiles.

2. Ingestion of substances via the house dust and soil path

House dust and soil represent an important vehicle for nonvolatile substances. House dust consists of particles from several sources, e.g., soil dust, and from pollution. It contains a lot of different materials, e.g., plant pollen, mites, human and animal skin cells, fibers, soil, and vapors. Substances migrate from the different materials (textiles, floor coverings, furniture, etc.) and, after release due to mechanical or thermic influence, adsorb to house dust. Partly, bigger particles may become a part of dust themselves.

The daily intake of house dust is unknown. Extrapolations from soil dust intake studies are normally used to estimate exposure from house dust intake. The intake of soil has been identified by means of tracer studies, taking substances that are poorly absorbed in the gut and comparing the concentrations measured in the stool with those in the soil. The AUH report (1995) recommends to take an estimate of 16 mg (median) and 110 mg (95th percentile) as standard values for house dust intake. The US EPA (2009) employs 60 mg per day as an estimate for central

tendency. The extrapolation of soil to house dust may introduce uncertainties into the assessment; overestimation of exposure by house dust should be assumed.

Exposure factors needed to estimate oral exposure

- Concentrations of the substance in food and drinking water
- Consumption values for the food or drinking water, preferably related to individual body weight
- Weight
- Concentrations of substances in house dust
- Default – values of house dust intake

## Behavior Scenario

Many exposed people are limiting their exposure by themselves and by their particular behavior. Studying the behavior in certain populations is essential and plays an increasing role in exposure assessment. While in the use scenario, the instructions of use will govern the scenario characterization, the behavior scenario influences the variability of the uses in a population. The behavior scenario characterizes how exposed persons act and handle the products. Two different types can be distinguished: (i) the active exposure where a person actively uses a product and (ii) the passive exposure where the exposed is a bystander. The major difference between active and passive exposure is that the active person may be closer to the source of exposure. An older version (3.0) of the ConsExpo tool is using a fictive room volume that is considerably smaller than the room to consider that situation. The indirect exposure via the environment is a particular form of passive exposure. From this perspective, eating food is passive exposure as well as being in a room and inhaling a substance that is released from furniture, while painting that furniture is active exposure.

Active and passive exposure can also be differentiated in terms of the degree of activity having impact for, e.g., exposure by inhalation. For example, the respiratory volume over time can vary from 15 $m^2$/day (at rest) up to 100 $m^3$/day (heavy work). This may lead to considerable variability in the exposure estimate and thus having impact for the risk characterization. When estimating exposure from inhalation, it is appropriate to assume a well-balanced ratio of activity and resting times.

### Time Budgets

As an important element of behavior scenarios, time budgets characterize the contact times of an exposed person. In case of exposure by inhalation, this is the time a person is staying in the room where the exposure takes place. Small children have normally longer contact times as adults because they may stay at home for longer time while adults are at work, outside, or at other business. This will change with school age. It is therefore of great importance to relate the time budgets to age. Data sources for time budget are, e.g., the US-EPA exposure factors handbook, the AUH (1995) report, and the RIVM general factsheet (Bremmer et al. 2006).

## Particular Age-Related Behaviors

Behavior scenarios can be used to characterize important differences between adults and children. For example, the ingestion of soil and house dust may account for an important amount of oral exposure in small children. This occurs primarily in the toddlers, by crawling on the ground, as well as in the kindergarten, becoming less importance in the school age. Children frequently put their hand into the mouth, which is called the *mouthing behavior*. The latter has particular importance for exposure from insecticides after treatment of pets against insects (lies, flies). Migrating substances from toys may also be relevant for mouthing. Therefore, migration rates are very important to estimate exposure. The mouthing time may vary over a big range (Groot et al. 1998; Juberg et al. 2001; Smith and Norris 2003). House dust evaluations represent an essential part of exposure assessment in children.

Exposure factors needed to characterize a behavior scenario

- Duration of stay
- Frequency of staying
- Air ventilation
- Activities of "daily life"
- Exposure as active user or bystander
- Hand to mouth activities

### Anthropometric Data

Exposure estimation needs anthropometric data that characterize the exposed person or population. Estimation of exposure by inhalation needs, according to the exposure scenario and the respective model, data about respiration rates and the lung surface. Dermal exposure evaluation requires information about body surfaces. However, estimation results are normally related to body weight. Relation to body surface is more appropriate, because body surface is correlating better with the extracellular fluid. Many substances distribute into body water, and there is also correlation between body surface and the basic metabolic rate. This is in particular of relevance when comparing results in children and adults.

Most important anthropometric data

- Body surface and parts of body surface, e.g., hands and arms
- Body height
- Body weight
- Respiration time volume and related to activity
- Lung surface

## Combination of Scenarios

The scheme in Fig. 2 shows how use and disposition scenarios can be combined to yield the entire exposure. All possible sources and paths have to be taken into account which may result in very complex scenarios. The estimation is performed

by separated estimations of the particular pathways with subsequent summation. Possible correlations of exposure paths must be taken into account. Also, summarizing exposure results should only be made for central tendency estimations. Results from individual conservative estimations, e.g., by using 95th percentiles, should not be summarized. Consideration of worst case estimates must be performed very carefully, possibly by addition of one conservative estimate with other averages. The European Food Safety Agency has proposed to take the 95th percentiles of exposures contributing most to exposure, exchange them with the averages, and sum all up.

## Distribution-Based (Probabilistic) Exposure Assessment

Exposure factors can be characterized as single and fixed values (deterministic approach) or as distributions (probabilistic approach). Therefore, every deterministic value represents a certain value from the distribution. The bounds of the distribution may represent conservative estimations. In many exposure calculations, arbitrary high values are used, in order to end up with an overestimation, without knowing the real situation. Such approaches are often lacking from reality and cannot be called *worst case*. From this reason, it is appropriate to use distributions and their statistical descriptors as a basis for exposure estimations. It is therefore recommended to check whether or not the used value can be matched with other representative values. Well characterized distributions should be used for exposure estimations. This approach will be facilitated considerably by use of modern computer tools. The total range and variability of the individual distributions will be weighted out and ends up with a distribution as result.

Probabilistic exposure modeling can be used as an alternative that considers the variability and uncertainty of the assessment. Distributions are characterized by (i) variability and (ii) uncertainty. Variability is characterizing the natural variation of parameters, while the uncertainty is determined by the lack of knowledge, which is often depending on data quality. For example, the body weight in the population participating in, e.g., the German food consumption study is described mostly by variability, because it is based on a representative sample from the entire population. On the other hand, the basis of data characterizing, e.g., concentrations of substances in products or food is often very poor. Therefore, these data must be considered uncertain.

Probabilistic models are formed by taking a similar general algorithm in the model but characterizing the model variables (parameters) as distributions. If the distributions are appropriately formed, i.e., the data basis is sufficient large and the values are representative for a population, the probabilistic distributions are describing the variability of the parameters. The less the number of data is and their representativeness, the more will distributions represent a mixture of variability and increasing degree of uncertainty.

### Importance of Exposure Assessment

Exposure assessment represents, besides hazard identification, the second pillar that is needed for risk characterization. The margin between the quantitative estimate exposure and the N(L)OAEL) is characterizing the risk (risk characterization). It is called the margin of exposure (MOE), in earlier times the **m**argin of safety (MOS), but both are meaning the same. The larger the MOS/MOE is, the more can the probability of risk be denied. A concern for risk is assumed if the exposure is exceeding the NOAEL. Risk can also expressed as a ratio of the exposure dose and the NOAEL, which should be lower than 1. Uncertainty factors are used in this formula to consider uncertainties, e.g., the lack of knowledge of the intraindividual and interindividual variation between animals and humans.

In the REACH regulation, the DNEL will be used instead of the NOAEL (compare the resp. chapter).

For these reasons it is of great importance to estimate the exposure as exact as possible. Estimates taking exposure scenarios and models are having sometimes considerable uncertainties, leading to partly extreme ranges of the exposure estimates which depend on the exactness of the description of the exposure scenario. It is essential to describe the exposure parameters as exactly as possible. The approach of using worst case scenarios is leading to overestimations, resulting from rough models or taking defaults or other conservative values as model parameters. Due to the precautional principle, there is an intention to overestimate the exposure; it should, however, not result in unrealistic results. Distribution-based (probabilistic) modeling can be taken as an appropriate alternative because it considers the range of exposure parameters and reveals a distribution of exposure. Taking distribution allows to consider extremes that characterize the skewness of a distribution. 95th and higher percentiles are therefore appropriate descriptors of *reasonable worst case* assumptions and estimates and thus reflect "reality."

Measurements can be taken into account for exposure estimations, if they are representative for the population of interest. On the other hand, they are showing a *shot* of a particular event or situation which can hardly be transferred to a general scenario. Measurements available for, e.g., contaminants in food, in house dust, and indoor air should therefore be given attention, but they are not necessarily representative for the scenario of interest. Although there is a lot of data available for some substances, they often lack from representativity and thus can be used for risk assessment only with great caution.

## References

AUH, Ausschuss für Umwelthygiene (1995) Arbeitsgemeinschaft der Leitenden Medizinalbeamtinnen und beamten der Länder, Standards zur Expositionsabschätzung. BAGS Hamburg, 2. Aufl., 2000

Bremmer HJ, Prud'homme de Lodder LCH, van Engelen JGM (2006) General Fact Sheet Limiting conditions and reliability, ventilation, room size, body surface area. RIVM report 320104002

Bremmer HJ, Blom WM, van Hoeven-Arentzen PH, Prud'homme de Lodder LCH, van Raaij MTM, Straetmans EHFM, van MP, van Engelen JGM (2006a) Pest Control Products Fact Sheet. To assess the risks for the consumer. RIVM Report 320005002

Bremmer HJ, van Engelen JGM (2007) Paint products fact sheet, to assess the risks for the consumer. RIVM report 320104008

Bruinen de Bruin Y, Hakkinen PB, Lahaniatis M, Papameletiou D, Del Pozo C, Reina V, Van Engelen J, Heinemeyer G, Viso AC, Rodriguez C, Jantunen M (2007) Risk management measures for chemicals in consumer products: documentation, assessment, and communication across the supply chain. J Expos Sci Environ Epidemiol 17(Suppl 1):S55–66

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (2012) Aufgaben im Bereich Lebensmittel. http://www.bvl.bund.de/DE/01_Lebensmittel/lm_node.html. Last date of access 8 April 2013

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (2012a) Bundeslebensmittelschlüssel, http://www.bvl.bund.de/DE/01_Lebensmittel/04_AntragstellerUnternehmen/09_BLS/lm_ bls_node.html Last date of access 8 April 2013

ECETOC (2012) Targeted risk assessment tool TRA version 3, http://www.ecetoc.org/tra

EFSA (European Food Safety Authority; (2009) General principles for the collection of national food consumption data in the view of a pan-European dietary survey. The EFSA J 27(12):1435–1486

EFSA (European Food Safety Authority) (2012) The EFSA comprehensive european food consumption database. http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm. Last date of access 8 April 2013

European chemicals agency, ECHA (2010) Guidance on information requirements and chemical safety assessment. Guidance volumes A – D, R12 and R15. http://echa.europa.eu/web/guest/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment. Last date of access 8 April 2013

European Commission, Institute for Health and Consumer Protection European; Chemicals Bureau (2003) EU Exposure assessment toolbox, http://web.jrc.ec.europa.eu/eis-chemrisks/toolbox.cfm. Last date of access 8 April 2013

European Commission (2006) Regulation (EC) No 1907/2006 of the European Parliament and of the council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Official Journal of the European Union L 396/1, 30.12.2006

Groot ME, Lekkerkerk MC, Steenbekkers LPA (1998) Mouthing behavior in young children: an observational study. Agricultural University Wageningen, Wageningen

Heinemeyer G, Hahn A (2005) Use of product databases for risk assessment purposes. Toxicol Appl Pharmacol 207(2 Suppl):636–44

Heinemeyer G (2008) Concepts of exposure analysis for consumer risk assessment. Exper Toxicol Pathol 60:207–213

Juberg DR, Alfano K, Coughlin RJ, Thompson KM (2001) An observational study of object mouthing behavior by young children. Pediatrics 107:135–142

Max-Rubner-Institut (2012a) Nationale Verzehrsstudie II, www.was-esse-ich. Last data of access 48 April 2013

Max-Rubner-Institut (2012b) Bundeslebensmittelschlüssel. Http://www.mri.bund.de/service/datenbanken/bundeslebensmittelschluessel.html. Last date of access 8 April 2013

Møller A., Ireland J (2010) LanguaL 2010 – The LanguaL Thesaurus. EuroFIR Nexus Technical Report D1.13. Danish Food Information, 2011

RIVM (2012) ConsExpo 5.0 beta, or 4.1. http://www.rivm.nl/en/Library/Scientific/Models/Download_page_for_ConsExpo_software. Last date of access 8 April 2013

Smith SA, Norris B (2003) Reducing the risk of cooking hazards: Mouthing behaviour of children aged 1 month to 5 years. Inj Contr Saf Promot 10(3):145–154

US-EPA Office of Pollution Prevention and Toxics, Washington, DC, and, National Paint and Coatings Association, Washington, DC (2001) Wall paint emission model. http://www.epa.gov/oppt/exposure/pubs/wpemman.pdf. Last date of access 8 April 2013
U.S. EPA. Highlights of the child-specific exposure factors handbook (Final Report). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-08/135, 2009
Van Engelen JGM, Heinemeyer G, Rodriguez C (2007) Consumer exposure scenarios: development, challenges and possible solutions. J Expos Sci Environ Epidemiol 17:S26–S33
WHO/IPCS (World Health Organization, International Programme of Chemical Safety) (2004) IPCS Risk assessment terminology. Part 2. IPCS Glossary of key exposure assessment terminology. IPCS harmonization project document Nr. 1, Geneva, ISBN 93 4 156 267 6
WHO/IPCS (World Health Organization, International Programme of Chemical Safety) (2005) Principles of characterizing and applying human exposure models. IPCS harmonization project document Nr. 3, Geneva, ISBN 92 4 165311 7
Wilschut A, ten Berge WF, Robinson PJ, McKone TE (1995) Estimation of skin permeation. The validation of five mathematical skin permeation models. Chemosphere 30:1275–1295

## Recommended Reading

Brussaard JH, Johansson L, Kearney J; EFCOSUM Group (2002) Rationale and methods of the EFCOSUM project. Eur J Clin Nutr 56(Suppl 2): S 4–7
EFCOSUM group (2001) European Food Consumption Survey Method, Final report TNO report V3766
Federal Institute for Risk Assessment (2011) Ärztliche Meldungen bei Vergiftungen http://www.bfr.bund.de/cm/350/aerztliche-mitteilungen-bei-vergiftungen-2010.pdf. Last date of access 8 April 2013

## Resources

EFSA (European Food Safety Authority) (2010) GUIDANCE OF EFSA. Standard sample description for food and feed. EFSA Journal 8 (1):1457 http://www.efsa.europa.eu/en/efsajournal/doc/1457.pdf. Last date of access 8 April 2013
European Commission, Institute for Health and Consumer Protection European; Chemicals Bureau (2003) Technical guidance document on risk assessment in support of commission directive 93/67/EEC on risk assessment for new notified substances commission regulation (EC) No 1488/94 on Risk Assessment for existing substances, Ispra, Italy, EUR 20418 EN/1
WHO (Last reviewed/updated 16 August 2012) Global environment monitoring system - food contamination monitoring and assessment programme (GEMS/Food) http://www.who.int/foodsafety/chem/gems/en/. Last date of access 8 April 2013
WHO (2013, last download) The IPCS INTOX Programme. The INTOX Data Management System and harmonized data collection, http://www.who.int/ipcs/poisons/package/en/index.html

# Quality Criteria for Primary Literature in Toxicology

Klaus Schneider, Agnieszka Kinsner-Ovaskainen, and Martin Hassauer

## Contents

Data and Data Sources ..... 305
Evaluation of the Reliability of Studies ..... 306
Plausibility of Study Design and Results ..... 307
Plausibility of Results in Relation to the Overall Knowledge on a Given Substance ..... 307
Weighting of Borderline Cases ..... 308
Conclusions ..... 308
References ..... 308
Resources ..... 309

**Abstract**

Toxicological evaluations are based on information derived from scientific studies. Use of high quality of data is crucial for that purpose. Definition of evaluation criteria allows for an examination of the quality of toxicological studies.

## Data and Data Sources

The OECD Test Guidelines and European Council Regulation (EC) 440/2008 for the testing of chemicals (see "Resources") provide standardized, internationally agreed test method procedures used by government, industry, and independent

K. Schneider (✉) • M. Hassauer
Research and Advisory Institute for Hazardous Substances, FoBiG, Freiburg, Germany
e-mail: klaus.schneider@fobig.de; martin.hassauer@fobig.de

A. Kinsner-Ovaskainen
European Commission DG Joint Research Centre, Institute for Health and Consumer Protection, Ispra (Varese), Italy
e-mail: agnieszka.kinsner@jrc.ec.europa.eu

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_53, 

laboratories to determine the safety/toxicity of chemicals and chemical mixtures, including pesticides and industrial chemicals. Standards of *Good Laboratory Practice* (GLP) set up rules for an adequate conduct of chemical nonclinical safety tests to ensure uniformity, consistency, reliability, reproducibility, and quality of the experimental procedures and data reporting.

However, despite these standardization efforts, a large number of toxicological studies which can be found in the published literature have not been performed according to recent guidelines and/or GLP standards. This holds especially true for older studies and for scientific investigations by academic research groups aimed at answering specific questions.

Studies published in scientific journals (*primary literature*) are one of the main sources of information on toxicological properties of chemical compounds. The peer-review process should in principle implicate high quality standards of the published data. Depending on the regulatory area, non-published data from industry study reports constitute another important source of information for toxicological evaluations. Recent industry studies are generally performed according to the current guidelines and under GLP conditions and are therefore important data sources.

*Secondary literature* (scientific reviews or evaluations and reports from authorities or scientific organizations, which are increasingly available via Internet portals such as eChemPortal, see "Resources") allows for a quick data overview and may help identifying the relevant key studies. Nevertheless, the final evaluation of a certain substance should be performed on the basis of primary literature and original studies.

## Evaluation of the Reliability of Studies

Comprehensive reporting of methodological details and results is crucial for a high *reliability* of the data in any toxicological study. Klimisch et al. (1997) proposed a system for categorizing the *quality* of experimental toxicological and ecotoxicological studies, which is now widely used in several regulatory schemes (e.g., the EU's REACH Regulation). In this system, studies are assigned one of the four categories as presented in Table 1.

In order to conclude on a specific reliability category of published in vivo studies, the following criteria concerning the documentation of methodology and presentation of data should be assessed (summarized from Klimisch et al. 1997):

- Information on experimental animals (species, strain, gender, numbers, age)
- Information on the test substance (identity, purity, composition, source)
- Information on route of administration, dosage, and test conditions (e.g., methods for analytical verification of test concentrations)
- Information on performed examinations (endpoints investigated) and description of methods used (including statistical analysis)
- Description of observed effects and lesions
- Corresponding data of control group or historical controls of the laboratory
- Description of dose–response relationship, if applicable.

**Table 1** *Klimisch categories* for assessing reliability of toxicological studies

| Klimisch category | Explanation |
|---|---|
| 1 | Reliable without restriction |
| 2 | Reliable with restriction |
| 3 | Not reliable |
| 4 | Not assignable |

Nevertheless, to judge on the reliability of studies, especially in borderline cases, is often difficult and may be influenced by personal views and experiences and biased by circumstances. In order to support a harmonized and transparent evaluation of reliability of published in vivo and in vitro toxicological studies, a tool ("ToxRTool", see "Resources") has been developed, which provides more detailed criteria for assigning Klimisch categories and a way for transparent documentation of this evaluation (Schneider et al. 2009). Hulzebos et al. (2010) proposed a generalized scheme to assess adequacy of (eco)toxicological studies under REACH, which was defined to comprise reliability of the data, the validity of the methods used, and the regulatory need of the data.

## Plausibility of Study Design and Results

Besides reliability, further considerations are necessary to judge on the suitability of a study to be used in a substance safety evaluation. The comprehensive reporting of experimental designs and procedures for data evaluation allows for the examination of the consistency of the observations in relation to following aspects:

- Is the chosen design of the study (e.g., animal strain, cell line, route of administration, methods for statistical evaluation) appropriate for the question to be answered?
- Are the study results mechanistically plausible?
- Is the interpretation presented by the authors supported by the study results?

## Plausibility of Results in Relation to the Overall Knowledge on a Given Substance

A final evaluation of a toxicological study should take into consideration how the presented results are related to the already existing knowledge on this substance. Contradictions between different studies have to be discussed taking into account possible explanations (e.g., differences in study design, animal strains, exposure route etc.). Only then, a final conclusion on the adequacy of the study results for risk assessment purposes can be drawn.

## Weighting of Borderline Cases

Toxicological evaluations and subsequent regulatory decisions on a given substance should be based on the entirety of available data, in accordance to the concept of *Evidence-Based Toxicology* (see "Resources").

In cases where a toxicological study does not fulfill all criteria of good quality, one should judge whether the gain in knowledge predominates the uncertainty of this additional information or vice versa. As an example, should a study of restricted quality, which points out a certain risk that has not been identified up to now, be disregarded (if the observation is plausible in the context of the entire knowledge on the substance)? This can only be decided on a case-by-case basis, bearing in mind that any decision taken will ultimately have regulatory consequences.

A *weight-of-evidence approach* can be used to integrate available information from various sources, which individually cannot be considered sufficient but in its entirety is adequate to draw conclusions. This weighting process requires a lot of experience in the evaluation of study design and results. However, albeit being a quantitative method for combining evidence in support of a hypothesis, it is based on opinions that are influenced by individual expert's knowledge and personal backgrounds, which might lead to divergent decisions. Therefore, the decision-making process with respect to the study evaluation should be presented in a transparent manner.

## Conclusions

Specified criteria for a formal evaluation of the reliability of toxicological studies have been developed and are well established. A comprehensive evaluation of quality of a primary source and its reliability for safety evaluation focuses on a consistent study design and the comprehensive documentation of methods and results. Furthermore, the internal plausibility of study design (e.g., appropriateness of the study design to address a specific question) as well as the plausibility of observations in view of existing information on the investigated substance should be evaluated to judge on the adequacy of study results for risk assessment. In equivocal cases, it is necessary to take decision on a case-by-case basis, judge whether the gain in knowledge from a study of restricted quality is superimposed (or not) by its uncertainty. This process of decision-making has to be presented in a transparent and reproducible way.

## References

Hulzebos E, Gunnarsdottir S, Rila J-P, Dang Z, Rorije E (2010) An integrated assessment scheme for assessing the adequacy of (eco)toxicological data under REACH. Toxicol Lett 198:255–262

Klimisch HJ, Andreae M, Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5

Schneider K, Schwarz M, Burkholder I, Kopp-Schneider A, Edler L, Kinsner-Ovaskainen A, Hartung T, Hoffmann S (2009) ToxRTool, a new tool to assess the reliability of toxicological data. Toxicol Lett 189:138–144

## Resources

Council Regulation (EC) 440/2008 of 30 May 2008 on test methods. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:142:0001:0739:en:PDF

ECHA Guidance on information requirements and chemical safety assessment Chapter R.4: Evaluation of available information, ECHA-2011-G-13-EN, December 2011, European Chemicals Agency. http://echa.europa.eu/documents/10162/13643/information_requirements_r4_en.pdf

eChemPortal. http://www.echemportal.org/

Evidence-based toxicology. http://www.ebtox.com/

OECD Test Guidelines. http://www.oecd.org/document/12/0,3746,en_2649_37465_48704140_1_1_1_37465,00.html

ToxRTool. http://ecvam.jrc.it/page_pdf.cfm?voce=s&idvoce=110

# Data-Mining in Toxicology

Inge Mangelsdorf

## Contents

Simple Searches ..... 312
Using Online Search Engines ..... 312
Portals of Publishing Companies ..... 313
Assessment by a National or International Committee ..... 313
Review Articles ..... 314
Factual Databases ..... 314
Comprehensive Data Searches and Analyses ..... 314
Bibliographical Databases ..... 314
Selection of the Database ..... 315
Searching Strategies ..... 316
Reference Management ..... 317
Recommended Reading ..... 318
Resources ..... 318

**Abstract**

Literature searches are necessary to find answers to many toxicological issues. Fortunately, today we are no longer reliant on time-consuming searches in reference books, but can make use of the Internet as an important tool for gathering information. A lot of information including complete substance assessments is easily available and free of charge. Because of the large variety of possible data sources, however, literature searches are nevertheless difficult to undertake and in addition can take a lot of time. Depending on the particular issue of research, different searching strategies should be used.

I. Mangelsdorf
Department Chemical Risk Assessment, Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany
e-mail: inge.mangelsdorf@item.fraunhofer.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_54, 

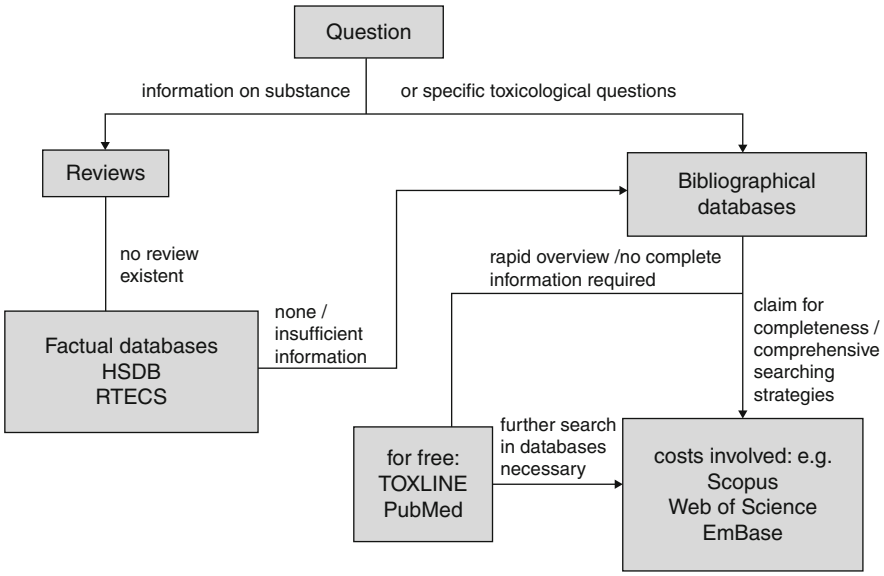



**Fig. 1** Overview of possible data searching methods

Figure 1 gives an overview of different searching methods. The individual steps will be described in the following.

## Simple Searches

Simple data searches will normally draw upon the available secondary literature. The term *secondary literature* refers to surveys of data from original literature. They provide a critical review of the published literature and, ideally, present it in a comprehensible manner. However, errors in the analysis of the data cannot be excluded. Furthermore, important details necessary to assess the meaningfulness of the data are often missing. The publication date indicates whether the reviewed data are up to date. In many cases, the text also includes information about the date and scope of the performed literature search.

### Using Online Search Engines

The easiest way of performing a data search is by searching in the Internet. Proven search engines include Google, Yahoo, Bing, and Google Scholar. If adequate search terms are entered, relevant Internet sites can often be found.

Two issues of the journal "Toxicology" have been dedicated to Internet addresses in the field of toxicology. They furnish links and descriptions of numerous institutions worldwide that provide toxicological information. However, some of the addresses may no longer be up to date.

## Portals of Publishing Companies

Many publishing companies meanwhile offer their journals and books online. As a result, on their homepages they provide the possibility to systematically search for specific literature. Good online search tools for journals and e-books are offered, for example, by SpringerLink, ScienceDirect, and Wiley Online Library. For the downloading of articles, a fee has to be paid in most cases.

## Assessment by a National or International Committee

If the assessment of a particular substance is of interest, reports by expert committees can be helpful. The problem is that there exists no up-to-date collection of all reports available. A search in bibliographical databases, for example, in TOXLINE (see below), will find a number of documents. The most comprehensive collection is offered by the OECD eChemPortal. It includes, for example, information and reports from the search portal INCHEM of the International Programme on Chemical Safety (IPCS) but also reports of the World Health Organization (WHO), the International Agency for Research on Cancer (IARC), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). It furthermore provides access to reports of the Unites States Environmental Protection Agency (US-EPA), datasets of the International Uniform Chemical Information Database (IUCLID), and the registration dossiers submitted to the European Chemicals Agency (ECHA) under the European program for reevaluation of existing chemicals (REACH). Reports of the United States National Toxicology Program (NTP) and the US Agency for Toxic Substances and Disease Registry (ATSDR) can be searched via the respective homepages of these organizations. Reports on chemicals that are consumer-relevant (e.g., fragrances, preservatives) can be found via the homepage of the Scientific Committee on Consumer Products (SCCP) of the European Union. Other important sources of information are documents justifying occupational exposure limits. Besides the German documents in this regard (MAK), also those of the English Health and Safety Executive (HSE), the Dutch Expert Committee on Occupational Standards (DECOS), the US Occupational Safety and Health Administration (OSHA), American Conference of Industrial Hygienists (ACGIH), and National Institute for Occupational Safety and Health (NIOSH) as well as the European Scientific Committee on Occupational Exposure Limits (SCOEL) and the Nordic Expert Group (NEG) are worth mentioning.

### Review Articles

Furthermore, there are journals publishing exclusively review articles, such as Critical Reviews in Toxicology. These articles will be found by searching through the relevant bibliographical databases (see below).

### Factual Databases

A good overview can also be obtained by querying factual databases. As the name indicates, these databases – in contrast to bibliographical databases (see below) – include the relevant information about a substance and also provide references to the literature that was used as source of this information. TOXNET via its homepage offers access to a variety of databases free of charge, including TOXLINE (see below), *CCRIS* (carcinogenicity data from single-case studies), and *HSDB* (collection of referenced data derived from a core set of books, reports, and other literature). Another frequently used factual database is *RTECS*, whose current datasets can meanwhile be accessed free of charge via the homepage of the NIOSH Pocket Guide to Chemical Hazards. Its disadvantage compared to the other above mentioned factual databases is that data entries are not being verified, so that erroneous numerical data are not uncommon here.

## Comprehensive Data Searches and Analyses

The search becomes a lot more complicated, if no assessments by expert committees and no review articles are available, or if preparation of such an assessment is actually the reason for your search. In this case, a comprehensive search in bibliographical databases is necessary.

### Bibliographical Databases

Bibliographical databases, as the name indicates, provide the bibliographic information by which an article published in a journal (p*rimary literature*) or book can be found. In addition, most of these databases include also the abstracts of the publications.

Searches in bibliographical databases will yield journal articles but also book chapters, doctoral theses, and reports by research institutions. In toxicology in particular, it is important to find also the so-called gray literature, that is, papers that have not been published in journals. The TOXLINE database, for example, includes information about unpublished studies from the chemicals industry that can be ordered from a special information service.

**Table 1** Number of hits for the substance 2-butoxyethanol in the databases TOXLINE, PubMed, Web of Science, and Scopus and number of overlaps

| | PubMed | TOXLINE | WoS | Scopus |
|---|---|---|---|---|
| PubMed | **234** | 158 | 116 | 155 |
| TOXLINE | 158 | **761** | 91 | 124 |
| WoS | 116 | 91 | **610** | 93 |
| Scopus | 155 | 124 | 93 | **641** |

1,681 hits overall, 75 hits common in the 4 databases

## Selection of the Database

Important databases include TOXLINE, MEDLINE, EMBASE, Chemical Abstracts (CA), BIOSIS, Web of Science, and Scopus. Furthermore, there are a few smaller databases tailored to specific subject areas. The databases differ in their thematic orientation. MEDLINE and EMBASE, for example, are focused on medicine, BIOSIS on biology, CA on chemistry, and TOXLINE on toxicology (including ecotoxicology and analytics). But there are also geographical differences. For example, European publications are taken into consideration much more in EMBASE than in MEDLINE.

For some years already, free access to TOXLINE and MEDLINE (PubMed) has been offered in the Internet via the US National Library of Medicine (NLM). As the search and query options are limited there, these are not suitable though for complicated issues. However, both databases can also be queried at a charge (e.g., via the providers DIMDI and STN). The search and query options are much better there, but access is limited to trained staff.

The most comprehensive database is TOXLINE. About 50–75 % of all literature that can be searched for in the large databases can be found here.

For a very thorough and comprehensive search, therefore, several databases have to be queried. The problem in this case, however, is that most articles will be found more than once. Table 1 shows that for the example of 2-butoxyethanol (CAS: 111-76-2) 761, citations were found in the TOXLINE database and that 124 of these will also be found, for example, in the Scopus database.

This is due to the fact that the different databases partly access the same publications. The major commercial database providers, therefore, offer the possibility to eliminate duplicates across different databases. Because of the more professional searching possibilities and the possibility to eliminate duplicates, it is recommendable to make use of an online database provider for comprehensive searches. The cost of a complex search, however, may then easily amount to several thousand euros. Alternatively, you can perform your own check for duplicates using so-called reference management systems (see below). Reference Manager, Citavi, and EndNote are only a few, and there are many others available.

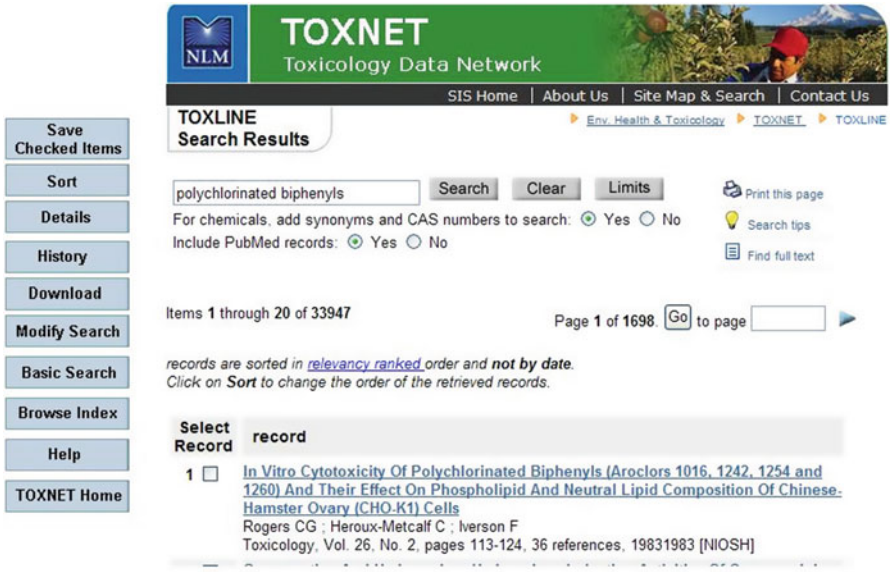



**Fig. 2** Search for polychlorinated biphenyls in TOXLINE

The search results depend on the queried substance. For poorly investigated substances, it may be that not a single citation is found, whereas for well-investigated substances such as polychlorinated biphenyls, there may be well over 30,000 hits in existing literature (see Fig. 2).

## Searching Strategies

Searches in factual databases are easy to perform, as they address a particular substance. Searches in bibliographical databases are more complicated, and you have to distinguish between a search for a particular substance name and a search for certain toxicological effects in this case. A chemical substance can be queried preferably via its CAS number, which unambiguously identifies the substance. CAS numbers can be queried in all of the above described databases except Web of Science. A small number of additional documents can be found by using synonyms, but many databases already include synonyms automatically (see Fig. 3). On the other hand, this may also result in related substances being found that are not actually being queried.

A search for toxicological effects is performed using specific search terms. Some databases (e.g., MEDLINE) offer structured lists of search terms (thesauri) that make it possible to search also for superordinate and subordinate terms. Because of the database structure and the large number of sub-databases, TOXLINE does not include this possibility. The database provider DIMDI offers a very helpful tool in this context, in the form of comprehensive search term lists in “preprocessed

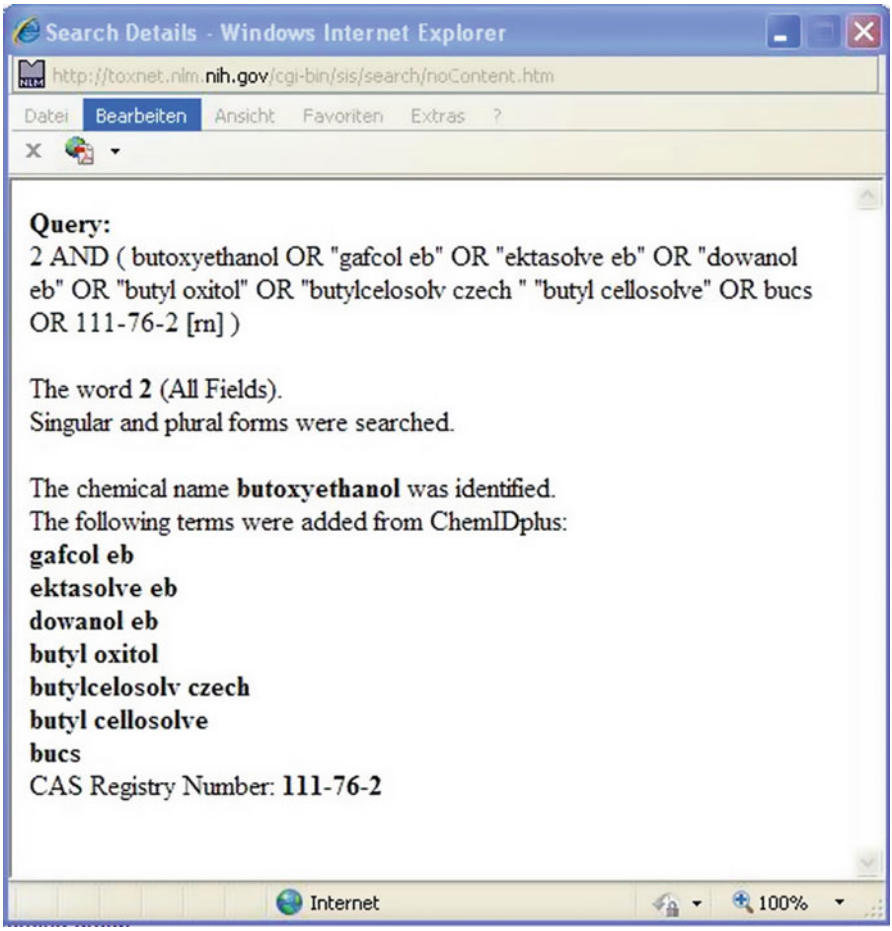



**Fig. 3** Search details of the 2-butoxyethanol (CAS 111-76-2) query in TOXLINE

searches" (pps). Searches of this kind, however, often return a multitude of hits that are not really useful. The general rule is as follows: The more specific the search terms, the more appropriate the identified articles, but also the greater the risk of missing important publications. This means that the sensitivity of a search is adversely affected by high specificity, and vice versa.

## Reference Management

Helpful tools for managing large amounts of literature are commercially available software packages such as "EndNote," "Reference Manager," "Citavi," and "Faust." Most of these programs store not only bibliographic information but also abstracts and key words. They allow systematic searches for specific search terms.

Reference lists can be automatically generated in different formats. The import of data from the individual databases into reference management systems is state of the art now. This saves a lot of writing; however, the quality of the data is not the same with all databases, and if data have been imported from different databases, some manual postediting is in most cases necessary.

## Recommended Reading

GDCh-Advisory committee on existing chemicals (BUA), numerous reports. S. Hirzel, Stuttgart
Greim H (2002) Gesundheitsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten. Wiley-VCH, Weinheim
Ludl H, Schöpe K, Mangelsdorf I (1996) Searching for information on toxicological data of chemical substances in selected bibliographic databases – selection of essential databases for toxicological researches. Chemosphere 32:867–880
Ludl H, Schöpe K, Mangelsdorf I (1995) Searching for information on chemical substances in selected biomedical bibliographic databases. Chemosphere 31:2611–2628
Wexler P (2001) Toxicology, 157(1–2):1–164, Wexler P (2002) Toxicology, 173(1–2):1–189; both issues exclusively include articles about internet addresses for toxicology

## Resources

Databases and database providers:
***Free of charge:***
Databases:
EC - ESIS: http://esis.jrc.ec.europa.eu/
HSDB: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
Medline: http://www.ncbi.nlm.nih.gov/sites/entrez?holding=idefiblib
RTECS: http://www.cdc.gov/niosh/npg/npgdrtec.html
TOXLINE: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?TOXLINE
Database portals:
IPCS INCHEM: http://www.inchem.org/
OECD eChemPortal: http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en
***Available at a charge:***
Databases:
DIALOG: http://www.dialog.com
DIMDI: http://www.dimdi.de
Scifinder (CAplus): http://www.cas.org/products/scifinder
STN: http://www.stn-international.de
Web of Knowledge (Web of Science): http://wokinfo.com/
Portals of publishing companies:
Elsevier (ScienceDirect): http://www.sciencedirect.com/science/browse
Springer (SpringerLink): http://www.springerlink.com/
Wiley (Online Library): http://onlinelibrary.wiley.com/
Organizations:
ATSDR: http://www.atsdr.cdc.gov/toxprofiles/index.asp
http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
JECFA: http://www.who.int/foodsafety/chem/jecfa/publications/en/index.html
NTP: http://ntp-server.niehs.nih.gov/?objectid=7DA86165-BDB5-82 F8-F7E4FB36737253D5

SCCP: http://ec.europa.eu/health/scientific_committees/consumer_safety/sccnfp/index_en.htm
US EPA: http://www.epa.gov/
WHO: http://www.who.int/ipcs/en/
Statements explaining occupational exposure limits:
ACIGH: http://www.acgih.org/home.htm
DECOS: http://www.gezondheidsraad.nl/en/about-us/council/committees-standing-committees/commissie-gezondheid-en-beroepsmatige-blootstelling-
HSE: http://www.hse.gov.uk/
MAK: http://onlinelibrary.wiley.com/book/10.1002/3527600418/topics
NEG: http://www.av.se/arkiv/neg/the_neg/
NIOSH: http://www.cdc.gov/niosh/
OSHA: http://www.osha.gov/
SCOEL: http://ec.europa.eu/social/main.jsp?catId=148&langId=en&intPageId=684

# Principles of Analytical Chemistry for Toxicology

Jürgen Durner and David C. Watts

## Contents

History ..... 323
The Aim and Means of Analytical Chemistry ..... 324
Important Techniques of Pre-analysis ..... 329
Solid-Phase Extraction ..... 330
Liquid-Liquid Extraction ..... 331
Solid-Phase Microextraction ..... 331
Separation Techniques ..... 333
  Chromatography ..... 333
  HPLC: Including Coupled Techniques ..... 334
  LC-MS (Thermospray, Fast Atom Bombardment, Particle Beam) ..... 337
  LC-MALDI-TOF ..... 339
  GC: Including Coupled Techniques ..... 341
  Headspace GC ..... 342
  GC-MS ..... 342
  Thin-Layer Chromatography (TLC) ..... 343
  Ion-Exchange Chromatography (IEC/IEX) ..... 344
Electrophoresis ..... 345
Atomic Spectroscopy ..... 346
Atomic Absorption Spectroscopy (AAS) ..... 347
Inductively Coupled Plasma Mass Spectrometry (ICP-MS) ..... 349
Selective Analytical Chemistry ..... 350
  Sensor Techniques ..... 350
  Immunoassays ..... 351
References ..... 353

J. Durner (✉)
Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-University of Munich, Munich, Germany
e-mail: juergen.durner@med.uni-muenchen.de

D.C. Watts
School of Dentistry and Photon Science Institute, University of Manchester, Manchester, UK
e-mail: david.watts@manchester.ac.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_91, © Springer-Verlag Berlin Heidelberg 2014

**Abstract**

In the first section, an historical summary of analytical chemistry is presented. In ancient history, one function of an analytical chemist was to confirm the identity of noble metals, especially gold. In later times, important inventions and their discoverer were named. Now, in the twenty-first century, analytical chemistry is an interdisciplinary scientific field.

Next, the aim and means of analytical chemistry are discussed. For analytical tasks, the chemist has over 6,000 experimental procedures (including subspecifications) available. The most important procedures are summarized. Moreover analytical problems, such as analyte(s) from complex matrixes, and the necessary purification as well as determination steps are discussed. Quantification measures, such as parts per trillion, are considered. The three analytical phases (pre-analysis, analysis, post-analysis) are presented, and recently developed analytical procedures such as "Lab on a chip" and the "omics" sciences are introduced.

In the section "Pre-analysis" different techniques of sample preparation prior to analytical measurement are described. Apart from classic methods, such as crushing and homogenization, extraction techniques such as solid-phase extraction, liquid-liquid extraction, and solid-phase microextraction are reviewed.

The analytical section is divided into three parts, plus subparts: (i) separation techniques are presented followed by (ii) atomic spectroscopy and (iii) selective analytical chemistry. Each (sub)part begins with a short historical overview. For separation techniques, first the principles of chromatography are described followed by the principles of electrophoresis and capillary electrophoresis. The chromatographic and atomic spectroscopy classifications and techniques are not presented in isolation, as in many analytical textbooks. They are described along with associated coupled techniques.

Such coupled techniques are liquid chromatography (LC), gas chromatography (GC), thin-layer chromatography, and ion-exchange chromatography (IEC). LC is often coupled with mass spectrometry (MS, including different ionization techniques such as Thermospray, Fast Atom Bombardment, Particle Beam) or matrix-assisted laser desorption/ionization time-of flight-mass spectrometry (MALDI-TOF-MS). GC is also often coupled with MS. Moreover, derivatization techniques and Headspace GC are presented.

In the case of atomic spectroscopy, atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are presented in more detail. In the section "selective analytical chemistry," sensor techniques with ion-selective electrodes and the principles of immunoassays are described. These techniques are primarily for routine and fast analysis of known components in a sample. In most cases the sample preparation steps are easy and rapid compared to, say, the sample preparation steps for gas chromatography.

## History

As an applied science, the history of analytical chemistry dates back to ancient history. Initially, analytical chemistry was a regulatory method to detect forgery of noble metals (Volke 2004). This was important because noble metals, especially gold, had an important function in the monetary system. In the early study of alchemy, which aimed to transform base metals to gold, analytical chemistry was necessary to check for any success.

Analytical chemistry is as old as chemistry itself because after any preparation steps, the result has to be verified. Normally, the desired molecule or compound was extracted, distilled, or precipitated from the reaction solution. In a further step, the separated component reacted with a second component to yield a substance that was then characterized by some distinctive physicochemical attribute. These could include its color, its melting or boiling point, its solubility in a series of solvent media, its smell, its optical activity, or its refractive index. Further quantitative analysis was achieved by gravimetric or titrimetric measurements. Many of the greatest discoveries in chemistry could fairly be described as classic examples of successful analyses, including the discovery of oxygen, the halogens, and several other elements. Discovering a new chemical element was regarded as the highest and most prestigious achievement possible for an academic chemist.

Parallel to the development of various chemical synthetic methods, special techniques were developed in the field of analytical chemistry. About 1660, R. Boyle (1627–1962) used litmus for the detection of acids and alkaline solutions. A. Lavoisier (1743–1794) investigated the composition of water (previously it was believed that water was an element) and published the law of conservation of mass. About 1800, J. Dalton (1766–1844) published his atomic theory and the law of multiple proportions (Dalton's law); A. Avogadro (1776–1856) published his theory of gases. In 1817, J. Gay-Lussac (1778–1850) presented a volumetric procedure to determine the amount of silver in solution. He also accelerated the development of titrimetry.

In the nineteenth century, analysis became a recognized subdomain of chemistry. J. Berzelius (1779–1848) was one of its famous representatives (qualitative analysis, law of definite proportions, chemical notation, discovery of elements, etc.). In 1821–1822, the German scientist C. H. Pfaff (1773–1852) published his two-volume book "Handbuch der analytischen Chemie für Chemiker, Staatsärzte, Apotheker, Oekonomen und Bergwerks Kundige" ("Handbook of Analytical Chemistry for Chemist, Physician, Pharmacist, Economists and Mining Engineers" (Pfaff 1821; Pfaff 1822)). In 1861, R. Bunsen (1811–1899) and G. Kirchhoff (1824–1887) developed emission spectroscopy. In 1898, M. Curie (1867–1934) and her husband Pierre (1859–1906) discovered the elements polonium and radium. In 1894, the chemist W. Ostwald (1853–1932) published his book "Die wissenschaftlichen Grundlagen der analytischen Chemie" ("The Scientific Basics of Analytical Chemistry" (Ostwald 1894)). He explained many phenomena

seen in analytical chemistry by the newly developing physical chemistry. Thus, analytical chemistry has been jointly responsible for many central contributions to our understanding of nature (e.g., the existence of the various elements, gas theory, stoichiometry, atomic theory, the law of mass action, nuclear fission).

In the twentieth century, with the knowledge transferred from other scientific areas, especially physics and engineering, new methods such as chromatography and spectroscopy were applied. About 1920, instrumental methods were introduced into analytical chemistry to support the classic methods of precipitation, extraction, and distillation. Several Nobel Prizes were awarded in the field of analytical chemistry, including W. Ostwald (1909, catalysis, chemical equilibria, and reaction velocities), F. Pregl (1923, quantitative organic microanalysis), A. Tiselius (1948, electrophoresis and adsorption in analytical chemistry, especially in the identification of blood serum proteins), A. Martin and R. Synge (1952, partition chromatography), J. Heyrovský (1959, polarography), R. Ernst (1991, Fourier Transform Nuclear Magnetic Resonance (NMR) spectroscopy), and J. Fenn and K. Tanaka (2002, both for their work in mass spectrometric analyses of biological macromolecules) as well as K. Wütherich (for his development of NMR spectroscopy for determining the three-dimensional structure of biological macromolecules in solution). Further aspects of the history of analytical chemistry are presented in the book by F. Szabadvary (Szabadvary 1966).

Analytical chemistry thus developed as an interdisciplinary scientific field spanning physics, biology, gene technology, toxicology, material sciences, engineering sciences, informatics, and (forensic) medicine.

## The Aim and Means of Analytical Chemistry

C. R. Fresenius stated in his classic *Introduction to Qualitative Chemical Analysis* (Fresenius 1866):

> Chemical analysis is based directly on general chemistry, and it cannot be practiced without a knowledge thereof. At the same time it must be regarded as one of the fundamental pillars upon which the entire scientific edifice rests; for analysis is of almost equal importance with respect to all the branches of chemistry, the theoretical as well as the applied, and its usefulness to doctors, pharmacists, mineralogists, enlightened farmers, technologists, and others requires no discussion.

Analytical chemistry (often also called analysis – the word is a transcription of the ancient Greek "αναλυσις," meaning "resolution") penetrates all areas of live and working (Fig. 1). It is normally divided into different subspecies, depending on the field of application, such as environmental analysis, biological analysis, geological sciences, online process analysis, food analysis, and instrumental analysis as well as forensic science and materials characterization. Analytical chemistry is not only concerned with trace analysis but also analysis of bulk substances or ingredients, as in the food industry.

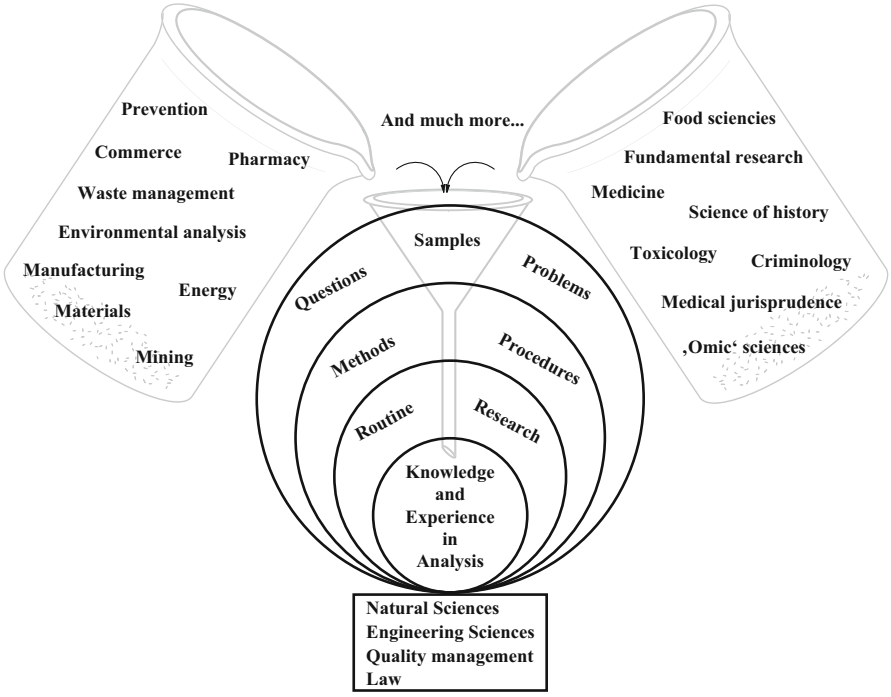



**Fig. 1** Challenges for the analytical chemistry. Samples from all areas are brought to the analyzer. With his spectrum of methods and – last but not least – his experience and knowledge, he analyzes the samples. Moreover, the analyst has to take into account current laws, quality management standards, and new technical developments in his field (Modified after Tölg et al. (2000))

In more general terms, analytical chemistry is concerned with methods to determine the chemical composition of different samples, including trace or bulk analysis. Basic issues in analytical chemistry are sensitivity, selectivity, and accuracy. The speed of determination is now especially important in clinical chemistry and forensic science. The analytical chemist is normally confronted with two questions: (1) What substances or chemical groups are present in the sample (*qualitative* chemical analysis, molecular structure analysis)? and (2) What is their content in the sample (*quantitative* chemical analysis)? In most cases, it is unnecessary to know the full chemical composition of the sample but only the most important compounds of interest.

In practice, qualifying and quantifying an analyte in a complex sample becomes an exercise in problem solving. The sample can be regarded as the sum of two parts: The *matrix* and the *analyte*(*s*). To be efficient and effective, an analytical chemist must know the appropriate tools to tackle a wide variety of problems. Therefore, analytical chemistry requires a broad education in chemical and physical concepts. Advanced separation and spectroscopic techniques, as well as data analysis ("chemometrics"), play an important role in this field.

For the analytical task, over 6,000 experimental procedures including subspecifications are available. A selection of the most important methods is listed in Table 1 (Durner 2010).

To isolate the analyte from a complex matrix, two further questions arise for the chemical analyst in practice:

1. Which steps of purification and isolation are necessary for the determination (qualitative and/or quantitative) of the analyte(s)?
2. Which method of determination is suitable for my analyte(s)?

To accomplish these goals, a sample is prepared by traditional methods like dissolution, homogenization, extraction, filtration, evaporation, separation, and chemical derivatization as well as newer methods like solid-phase extraction. Traditional and new methods are often combined to reduce the number of (time-consuming) preparation steps, and the degree of automation is increasing. The achievements of analytical chemistry, especially in inorganic or organic trace analysis, can be shown by the amounts that can now be detected. To emphasize what **ppm** (parts per million), **ppb** (parts per billion), **ppt** (parts per trillion), or **ppq** (part per quadrillion) mean, we can use mass or volume units:

ppm is comparable to mg/kg, μg/g, or mL/$m^3$, μL/L
ppb means μg/kg, pg/g, or μL/$m^3$, nL/L
ppt is the same as ng/kg, pg/g, or nL/$m^3$, nL/L
ppq means pg/kg, fg/g, or pL/$m^3$, fL/L

For such trace analysis, as well as for normal analysis, quality management plays a very important role. To guarantee cross-border equivalence in the field of analytical chemistry, international rules for laboratory working such as "good laboratory practice (GLP)" or European and International Standards like EN ISO 17025 were introduced.

In analytical chemistry, three phases can be distinguished:

1. Pre-analysis
2. Analysis
3. Post-analysis

The term "pre-analysis" or "pre-analytical phase" encompasses all the administrative and functional factors and processes that occur prior to laboratory analysis. These include preparation, isolation, work-up by centrifugation, storage, and transport. The term "analysis" or "analytical phase" covers taking aliquots and the general preparation of an analytical sample, the analysis itself, and acquisition of the appropriate data value(s). In this phase, precision, accuracy, detection limit, method specificity, analytical sensitivity, and statistical quality control play an important rule. The term "post-analysis" or "post-analytical phase" covers the analytical assessment of analytical results and the recorded set of definitive findings. Keywords in this context are plausibility, trend analysis, abnormal values, status assessment, diagnostic sensitivity, diagnostic specificity, and predicted value (O'Kane et al. 2008).

A few years ago a new technology was introduced which has started to change classical laboratory chemistry in some areas. It uses "Lab on a chip" devices that have the size of a credit card or even a fingernail. A "lab on a chip" allows

**Table 1** Selection of the most important methods in analytical chemistry (Durner 2010)

| | |
|---|---|
| **Spectrometry** | |
| Absorption spectroscopy/photometry | Nephelometry/immunonephelometry |
| UV-/VIS-/NIR-/IR-spectroscopy | Turbidimetry/immunoturbidimetry |
| Atomic absorption spectroscopy (AAS) | Atomic emission spectroscopy (AES) |
| Atomic fluorescence spectroscopy (AFS) | Flame emission spectroscopy |
| Nuclear magnetic resonance (NMR) spectroscopy | Inductively coupled plasma mass spectrometry (ICP-MS) |
| Luminescence spectroscopy: bioluminescence measurement, chemiluminescence, fluorescence, time-resolved fluorescence, fluorescence polarization, and phosphorescence spectroscopy | Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) |
| **Ligand assays** | |
| Enzyme immunoassay | Fluorescence polarization enzyme immunoassay |
| Fluorescence immunoassay | Radioimmunoassay |
| Immunoblot (Western blot) | Receptor assay |
| Luminescence and electrochemiluminescence immunoassay (CLIA/ECLIA) | |
| **Chromatography** | |
| Thin-layer chromatography (TLC) | Gas chromatography (GC) and GC-MS |
| Liquid chromatography (LC) | High-performance liquid chromatography (HPLC) and HPLC-MS |
| **Electrophoresis** | |
| Zone electrophoresis: cellulose acetate electrophoresis | Rocket electrophoresis |
| Immunoeleetrophoresis/immunofixation | Isotachophoresis |
| Isoelectric focusing | Capillary electrophoresis |
| Pulsed-field gel electrophoresis | Counterimmune electrophoresis (countercurrent electrophoresis) |

(*continued*)

**Table 1** (continued)

| | |
|---|---|
| **Blood cell counts** | |
| Impedance measurement | Immunophenotyping of hematopoietic cells (flow cytometry) |
| Flow cytometric cell count determination with cytometric or cytochemical-cytometric cell classification particle property determination with automated processing (particle counting and particle size determination of blood cells) | |
| **Electrochemical studies** | |
| Amperometry: $O_2$ partial pressure (Clark electrode) | Coulometry |
| Potentiometry: pH, ion-selective electrodes | Voltammetry |
| **Molecular biological methods** | |
| (Real-time) polymerase chain reaction (PCR) | Fluorescence in situ hybridization(FISH) |
| Southern Blot | Evidence for single-nucleotide polymorphisms (SNPs) with, e.g., restriction fragment length polymorphisms (RFLPs), fluorescence resonance energy transfer (FRET) probes, density gradient gel electrophoresis (DGGE), denaturing HPLC (DHPLC) |
| **Additional procedures** | |
| Aggregometry | Areometry |
| Filtration (adsorption filtration, membrane filtration, ultrafiltration) | X-ray diffraction |
| Immunohistochemistry | Coagulometry |
| Microscopy (light and dark field, fluorescence, and phase-contrast microscopy) | Osmometry: cryoscopy, vapor pressure osmometry |
| Qualitative studies with visual evaluation (e.g., osmotic erythrocyte resistance) | Sedimentation studies (erythrocyte sedimentation rate) |
| Reflectometry/carrier-bonded methods of analysis | Radioactivity measurement |
| Rheology, viscosimetry | Centrifugation: analytical ultracentrifugation, density gradient centrifugation |

determination of multiple analytes without further matrix purification. Fixed capture molecules on the chip sensor array bind molecules, such as nucleic acids or blood proteins, from the fluid matrix (a few femtoliters of which are normally sufficient). The sensor array may incorporate electrochemical sensors that recognize the binding of a substance by the change in potential or current flow. As this chip technology is derived from the field of electronics, the chips are rather expensive. Therefore, inexpensive alternatives, such as paper-based "chips," are in development (Deisingh and Thompson 2004; Palchetti and Mascini 2008; Yehya and Wael 2010; Trietsch et al. 2011).

This new micro-fluidic technology is important for medical analysis as well as for basic "omics" sciences (proteomics, metabolomics, etc.). This technology has been praised as offering "devices suitable for every purpose" to find the proverbial "needle in the haystack" (Suter-Dick and Singer 2008; Singh et al. 2010; Saleem and Reddy 2011; Schneider and Orchard 2011).

## Important Techniques of Pre-analysis

For the (trace) analysis of different substances from complex matrices, special treatments are necessary to determine the analyte(s) with sensitive analytical methods (Peters and Remane 2012). This field of pre-analytics involves different techniques of sample taking and especially different possibilities of sample preparation prior to the analytical measurement (Persoon et al. 2006). Examples of pre-analytical methods, for "working up" a sample, are crushing, homogenization, solubilizing, chemical exploration, and extraction techniques. In toxicology, extraction techniques play an important role. Some long-established extraction techniques are based on the principle of two non-mixable phases in close contact (Hennion 2000). An overview of common extraction techniques is shown in Table 2.

**Table 2** Overview of widely used extraction techniques (Modified (Gey 2008))

| Phase 1 | Phase 2 | Extraction technique |
|---|---|---|
| Solid | Liquid | Solvent extraction techniques, e.g., Soxhlet extraction, ultrasonic- or microwave-assisted extraction, accelerated solvent extraction |
| Solid | Supercritical | Supercritical fluid extraction |
| Solid | Gas | Gas extraction techniques, e.g., Headspace extraction |
| Fluid | Solid | Adsorptive extraction techniques like solid-phase extraction (SPE), solid-phase microextraction (SPME), or adsorptive microextraction techniques as well as dispersive extraction techniques |
| Liquid | Liquid | Liquid-liquid extraction techniques, e.g., liquid-phase extraction, liquid-phase microextraction (incl. hollow fiber techniques), ion pair extraction |
| Liquid | Gas | Purge and Trap techniques (PT techniques) |
| Gas | Liquid | SPME |
| Gas | Solid | SPME |

## Solid-Phase Extraction

Solid-phase extraction (SPE) is a chromatographic technique known for over 60 years (Liska 2000). It is a physical extraction process to enrich, isolate, and/or clean up the analyte(s) from a complex liquid matrix onto a stationary phase from the SPE material. This technique can be very effective, even when the solutes are present in extremely dilute concentrations (e.g., ppb). The extraction tube is usually packed with an appropriate bonded phase that is reproducible in activity, selectivity, and retention properties. In the first step, an adsorption on the solid phase takes place that means that the interaction of the analyte(s) and the solid state is stronger compared to the liquid phase. In the second step, an extraction from the solid phase takes place. In other words, the interaction of the analyte(s) and the liquid phase is now stronger compared to the solid phase. Therefore, it is possible to retain and enrich the analyte(s) in the first step and to elute it in the second step by rejecting the matrix. Because it is widely used, the SPE technique is discussed in more detail. The principal setup of an SPE tube is shown in Fig. 2. The I.D. of a tube is in the range 2–10 mm, and it is 2–4 cm long and is usually made from an inert polymer or, occasionally, from stainless steel or other materials. Normally the first step in using SPE tubes is conditioning. This means that the solid phase (sometimes also called the adsorbent) is pretreated with a (organic) solvent. This is necessary to activate the side chains from the solid phase to get a high and reproducible recovery. After conditioning, the sample can be placed. With the help of a vacuum, the sample is drawn through the solid phase of the tube. Afterwards, the solid phase is washed. In the next step, the solid phase is dried, and then the analyte(s) is eluted by a solvent or a series of solvents/solvent mixtures of successively increasing elution strength (an eluotropic series) (Fig. 3).

Effective separation by SPE depends primarily on the proper choice of sorbent and eluting solvents depending on the chemical and physical properties of the components in the sample. SPE tubes are available incorporating a wide variety of chemistries, adsorbents, and sizes. The most commonly used phases for the solid state are reversed phase, normal phase, ion exchange, and adsorption (Camel 2003; Zwir-Ferenc and Biziuk 2006; Majors 2010).

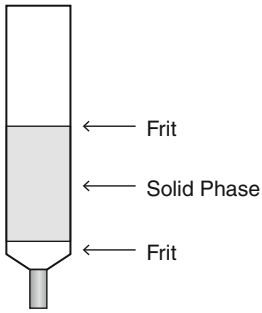



**Fig. 2** General setup of a solid-phase extraction (SPE) tube

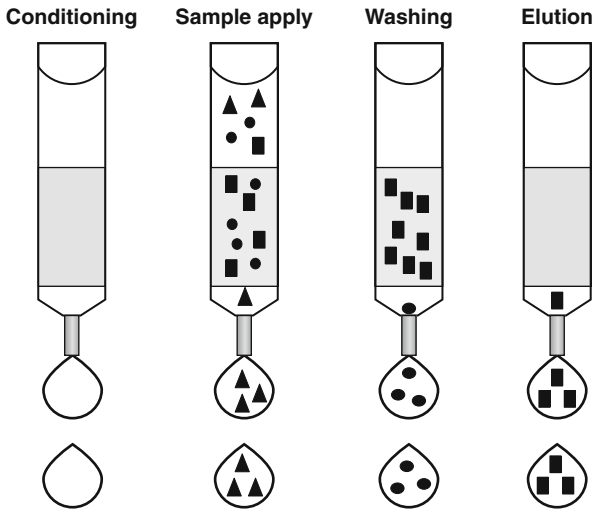



**Fig. 3** Working principle and general steps in using solid-phase extraction (SPE) tubes. The first step is conditioning, i.e., the cleaning and activating of the solid phase of SPE. The second step is normally the sample application. Only the desired analyte(s) should be adsorbed, and these can be enriched. The third step is a washing step, and in the fourth step, the analyte(s) is eluted

## Liquid-Liquid Extraction

Liquid-liquid extraction (LLE) is a tool for separating and isolating the favored analyte(s) of a liquid mixture by contacting it with a second, immiscible liquid in which one or more of the favored analytes are preferentially soluble. Normally one of the two phases is an organic phase, while the other is an aqueous phase. Under equilibrium conditions, the distribution of analyte(s) over the two phases is determined by a distribution law. In practice it is not always possible to find the optimum conditions that provide both high recovery and high purity of the analyte(s) in one extraction step. Therefore, it is not unusual that a second extraction procedure, with a different solvent or other extraction conditions (e.g., pH value), is necessary. Moreover, multiple extraction steps with the same solvent can also be required. In the case of a large extraction volume, the solvent must be evaporated to enrich the analyte(s) (Silvestre et al. 2009; Tedder 2009; Hii and Lee 2010; Testard et al. 2010; McConvey and Nancarrow 2011).

## Solid-Phase Microextraction

Solid-phase microextraction (SPME) is a common, solventless, fast, and field-compatible technique for extraction and concentration of volatile and semi-volatile analyte(s). It was invented in 1990 by Dr. J. Pawliszyn and colleagues. The physical basics are adsorption and desorption of the analyte(s) from a polymer-coated fused fiber (Pawliszyn 1997). In SPME, analyte(s) establishes equilibrium between the sample matrix, the headspace above the sample, and a polymer-coated fused fiber (SPME adsorptive layer; see Fig. 4). After the sampling period, during which extraction has ideally reached equilibrium,

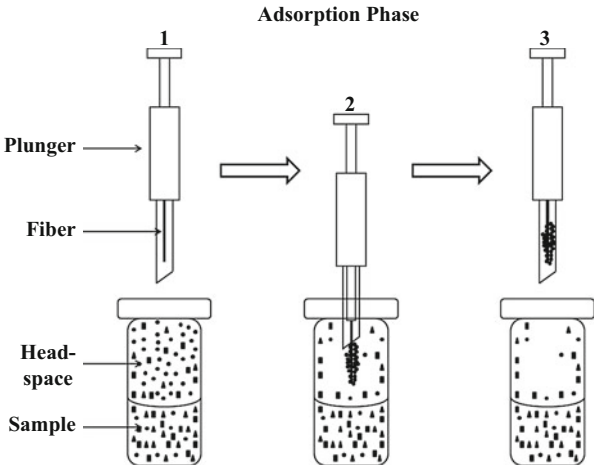



**Fig. 4** Adsorption phase: First, the SPME needle pierces the septum on the sample container. Second, the SPME fiber is incubated in with the analyte(s). Third, after incubation from minutes to hours, independent from, e.g., the concentration of the analyte(s), the SPME fiber is retracted and the needle is withdrawn

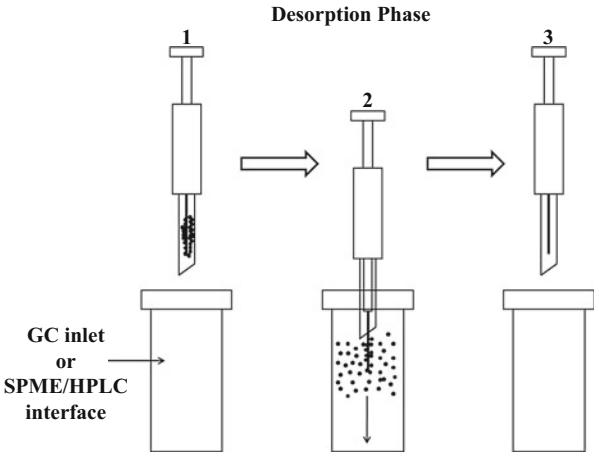



**Fig. 5** Desorption phase: First, the SPME needle pierces the GC inlet, or the needle is introduced into the SPME/HPLC interface. Second, the analyte(s) on the SPME fiber is desorbed, e.g., by heating the inlet. Third, the SPME fiber is retracted and the needle withdrawn

the adsorbed analyte(s) is/are transferred into an inlet system that desorbs the analyte(s) from the SPME adsorptive layer into a gas (for GC) or liquid (for LC) mobile phase (Fig. 5). Because analyte(s) is concentrated on the SPME fiber and are rapidly delivered to the column, minimum detection limits are improved and resolution are maintained. SPME provides linear responses for wide concentrations of analyte(s). By controlling the polarity and thickness of the coating on the fiber, maintaining consistent sampling time, and adjusting several other extraction parameters, an analyst can ensure highly consistent, quantifiable results from low concentrations of analyte(s) (Chen and Pawliszyn 2007). Sometimes a secondary

trapping and release of desorbed solutes after SPME is necessary when desorption from the SPME adsorption layer is too slow. This trapping and release can be accomplished using a discrete thermal trap or, in the case of column stationary-phase trapping, by injection onto a cold column and subsequently temperature programming for solute elution (Risticevic et al. 2009; Risticevic et al. 2010; Vuckovic et al. 2010; Duan et al. 2011).

## Separation Techniques

### Chromatography

Chromatographic methods have been used for a long time. In ancient Greece, Aristotle used alumina for the cleaning of seawater. In 1859, the German scientist F. Runge made experiments that were a precursor to paper chromatography (Runge capillary pictures). In 1901, the Russian botanist M. Tswett initially described the method to separate plant pigments such as chlorophyll and carotenes (Furr 2004). Since 1906, the term "chromatography" was used, derived from the Greek words "χρωμος, chromos" (color) and "γραφειν, graphein" (to write), meaning "color writing."

The physical bases of chromatography are the chemical and/or physical interactions of the analytes from the sample, present in the mobile phase, with the particles of a stationary phase – resulting in a temporal and spatial separation of the analytes (retention of the analytes, Fig. 6). The greater the affinity of the analyte to the stationary phase, the greater the delay period during chromatography. The separated analytes are detected at the exit of the column by a flow-through detector that measures their quantity. The result of the separation is a chromatogram (Fig. 7), where the signal intensity is shown as the ordinate and the retention time as the abscissa. The different retention times are characteristic for the substance. The height of the signal/peak, or the area under the signal/peak, can be used for quantification of the analytes' concentration (Guiochon and Trapp 2000).

One possible classification of chromatographic techniques considers the following points (see also Fig. 8):

(a) Selection of the separation distance, as in column or layer chromatography
(b) Selection of the phases: Normally, the mobile phase is liquid or gaseous and the stationary phase is solid or liquid. So four combinations can result: Liquid–solid and liquid-liquid as used in liquid chromatography (LC) and gaseous-solid and gaseous-liquid as used in gas chromatography (GC).
(c) Selection of the separation mechanisms, such as separation by adsorption, distribution, ion exchange, and cavity diffusion (molecular sieve chromatography, gel permeation chromatography)

In the following, important chromatographic techniques will be presented together with a widely used coupled technique: mass spectrometry.

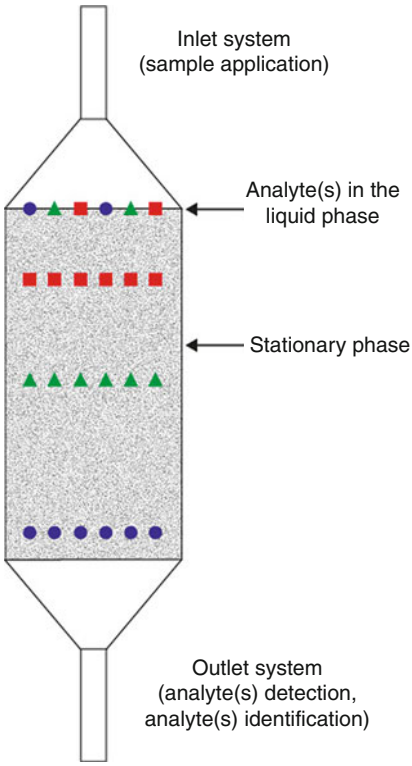



**Fig. 6** Principles of chromatography. The sample with the analyte(s) is applied to the chromatographic system. The analyte(s) in the mobile phase interacts with the stationary phase. Thereby, the separation takes place. After leaving the chromatographic system, the analyte(s) can be detected by different analyzing systems

## HPLC: Including Coupled Techniques

High-performance liquid chromatography (HPLC, formerly also called high *pressure* liquid chromatography) is a technique of liquid chromatography and a highly improved form of column chromatography. In comparison to traditionally chromatography, a solvent is not allowed to drip through the column under gravity; instead, it is forced through under high pressure – up to 400 atm. The HPLC instrument consists of a solvent reservoir, degasser, pump, injector, detector, integrator, and column, the last of which is a temperature-controlled oven. Advantages of HPLC techniques compared with traditional chromatography are:

– The analysis time is shorter.
– It is possible to use much smaller particles as the stationary phase in the column that gives a greater surface area for the interaction between the analytes and the stationary phase. In consequence, the separation of a multicomponent mixture is better, the reproduceability is higher, and the detection limit is lower.

Depending upon the interaction between the particles of the stationary phase and the analytes in the liquid phase, the following kinds of liquid chromatography are distinguishable:

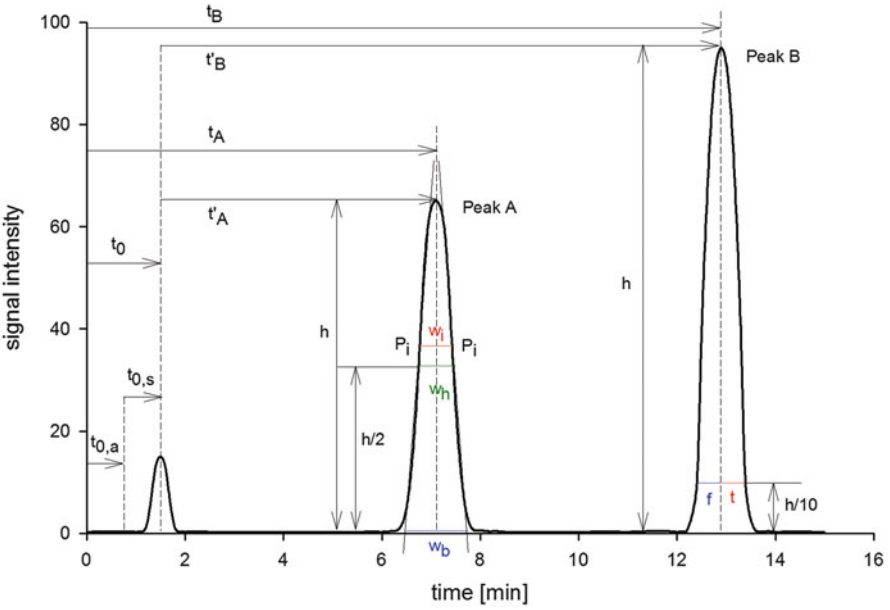



**Fig. 7** Example of a chromatogram. $\mathbf{t_{0,a}}$: Time delay of the apparatus. This is the time the eluent needs to reach the detection system from the injector. It should be as low as possible ($t_{0,a} \approx 0$); $\mathbf{t_{0,c}}$: Time delay of the column. This is the time the eluent needs to pass the column; $\mathbf{t_0}$: Dead time. This is the time the eluent needs to pass the distance from the injector through the column to the detector. $t_0 = t_{0,a} + t_{0,c} \approx t_{0,c}$; **t** Retention time of the components. This is the time required for the components to pass the distance from the injector through the column (including interaction) to the detector; $\mathbf{t'}$ Net retention time for a component. This is the time the component is in the stationary phase. $t' = t - t_0$; $\mathbf{k'}$ Retention factor (formerly called as capacity factor). k′ is a characteristic parameter of a component in the phase system. $k' = \frac{t'}{t_{0,c}} = \frac{(t-t_0)}{t_{0,c}} \approx \frac{(t-t_0)}{t_0}$; **T** Tailing factor. This is a measure for the symmetry of a peak. It is measured at 10 % peak height after the International Union of Pure and Applied Chemistry (IUPAC) or 5 % peak height after the United States Pharmacopeia (USP). $T = \frac{t}{f}$; α Separation factor (formerly called the selectivity). α characterizes the potential of a phase system to separate two components. In the numerator, the data of the later eluted substances are named. Therefore, α is ≥ 1. $\alpha = \frac{t'_B}{t'_A} = \frac{k'_B}{k'}$; **R** Resolution. Considering the width of a peak, it characterizes the separation of two neighboring peaks. $\Delta t_{B,A} = t_b - t_a$; $R = 2 \cdot \frac{\Delta t_{B,A}}{w_{b,A} + w_{b,B}} = 1,177 \cdot \frac{\Delta t_{B,A}}{w_{h,A} + w_{h,B}}$

- Exclusion chromatography
- Ion chromatography
- Adsorption chromatography
- Chromatography of optical isomers

Adsorption chromatography is widely used. Depending upon the stationary phase, "normal phase," and "reversed phase," chromatography can be distinguished. The stationary phase in normal phase chromatography is made of unmodified silica gel and in rare cases of $Al_2O_3$. Because of the polar character of the stationary phase, the components (eluents) of the mobile phase are nonpolar

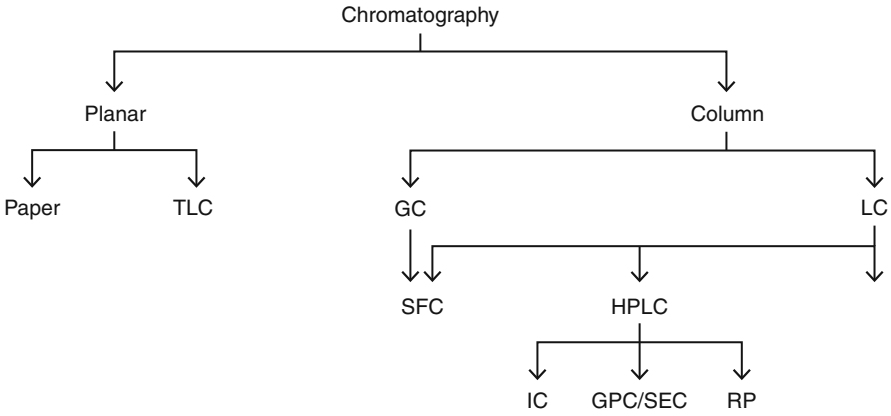



**Fig. 8** One possible classification of chromatographic techniques. *TLC* thin-layer chromatography, *GC* gas chromatography, *LC* liquid chromatography, *SFC* supercritical fluid chromatography, *HPLC* high-performance liquid chromatography, *IC* ion chromatography, *GPC* gel permeation chromatography, *SEC* size exclusion chromatography, *RP* reversed phase chromatography

like hexane. Normal phase chromatography is used in about 5–10 % of routine measurements.

In reversed phase chromatography, the stationary phase is more nonpolar (hydrophobic) compared to the mobile phase ("reversed phase"). The free silanol groups of the silica gel have been reacted with alkyl chlorosilane to form siloxane groups. One common stationary phase uses the n-octadecylsilane (OSD) modification of the silica gel. For reversed phase chromatography, mixtures of water, methanol, tetrahydrofuran (THF), or acetonitrile (ACN) are used as mobile phases. The most polar analytes of the sample will be eluted first because their interaction with the hydrophobic groups of the stationary phase is weak, whereas the most nonpolar analytes will be eluted at longer retention times because their interaction with the stationary phase is greater (whereas, in the case of normal phase chromatography, the elution sequence is inverted).

Another important factor in chromatography is the temperature. It influences the interaction between mobile and stationary phases as well as the viscosity of the liquid phase. For high reproducibility and robustness, it is important to use a constant temperature (Meyer 2010).

The composition of the liquid phase (often also called the elution phase) can be either constant or varying. The first case is named *isocratic elution* and the second case *gradient elution*. The gradients can be linear, concave, convex, or in steps. Besides binary gradients, also ternary or quaternary gradients can be used, involving three or four different elution media.

The correct flow rate of the liquid phase also depends on factors such as the internal diameter of the column. At high flow rates, the interaction of the analytes

with the stationary phase is insufficient. It is advisable to choose a constant velocity (that a linear flow pattern results) when attempting to reproduce chromatography results obtained with columns of differing internal diameters.

In HPLC the analytes can be detected using different detector types. The detectors are classified into two groups: The first group uses certain characteristics of the total elution flow (depending on the dissolved analytes therein) such as density, refractive index, and capacitance. The second group uses certain characteristics of the dissolved analytes such as UV absorption, fluorescence, and redox behavior. The choice of the detector type depends on the analytes. In some cases multiple detection systems are used. Typical detector types use the principles of UV/VIS, (FT)-IR, fluorescence, electrochemical, conductivity, refractive index detectors, evaporative light scattering detectors, and mass spectrometric detectors (LC-MS).

Many substances of current interest cannot be detected by HPLC because they do not contain the necessary chromophoric or fluorophoric groups. In this case, it is possible to add a chromophoric group by a derivatization reaction (the derivatization process is discussed below). Derivatization can be done in a pre-column mode (that means before analytical separation) or in post-column mode (after separation). Typical chromophoric groups for UV/VIS detection are, e.g., 4-dimethylaminoazobenzene-4′-sulfonyl chloride, 1-fluoro-2,4-dinitro-benzene (Sanger's reagent), 4-(dimethylamino)benzaldehyde (Ehrlich's reagent) and for fluorimetric detection, fluorescamine, 4-(dimethylamino-sulfonyl)-7-fluoro-2,1,3-benzoxadiazole, and 3,4-dihydro-3,4-dioxo-1-naphthalenesulfonic acid sodium salt (Folin's reagent). HPLC derivatization plays an important role in the determination of many pharmaceutical compounds (amino acids, antibiotics), in agrochemistry (proteins, peptides, toxins), in the environment (pollutants), and in food sciences (biogenic amines as indicators of protolytical process) (Gianotti et al. 2011; Kaushal et al. 2011; Polettini 2011; Milroy et al. 2012).

This chapter will only describe selected methods with practical use in the field of toxicology. Therefore, molecular spectroscopic techniques like mass spectrometry (MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) will be presented as coupled technique with the chromatographic methods and not separately, as in textbooks of analytical chemistry. Atomic spectroscopic techniques that are widely used in the field of toxicology are considered below.

## LC-MS (Thermospray, Fast Atom Bombardment, Particle Beam)

In 1898, the scientists E. Goldstein and W. Wien showed that a beam of positive ions is deflected by electric and magnetic fields. The development of mass spectrometry (MS) goes back to the work of J. J. Thomson in the year 1910. He demonstrated that the noble gas neon consists of two stable isotopes with mass 20 and 22. Fifty years later, this technique was used in organic chemistry for

structure determination, determination of the relative molecular mass, and for analysis of small amounts of a sample. The fundamental principle of MS is to produce ions from inorganic and organic substances (without destroying them by ionization). Major components of a mass spectrometer are the ion source, for generation of ions; the ion separation/mass selection fields; and the ion detectors of different types such as electron multipliers, Faraday cups, ion-to-photon detectors, or scintillation counters (Miller 2009).

## Ionization Techniques

The ionization of analytes can be realized thermally, with an electric field or by bombardment with electrons, ions, and photons. The resulting ions from the analytes can be single ions or clusters, ionized molecules, as well as fragments or associates from the ionized molecules. During thermospray ionization (TSI) the analytes within the liquid sample are contained in a capillary, with the end at a temperature of about 200 °C, under pressure in a heated atomizing chamber. Widely used solvents are $CH_3CN/H_2O$ or $CH_3OH/H_2O$, with an evaporable electrolyte additive such as 0.1 M $CH_3COONH_4$. As the liquid leaves the capillary, a nebula of fine drops is formed. Because of the high temperature in the atomizing chamber, the solution media vaporizes. Ions are formed, by the agency of the electrolyte additive, which reach the mass analyzer by a small leak (called a skimmer) in the atomizing chamber. Furthermore, an electric potential is maintained between the skimmer and the repeller (a further component of the atomizing chamber). One main advantage of this technique is that polar and thermolabile substances can attain the gas phase without a direct vaporization process. Problems can arise when the sample is insoluble in the solvent and/or only few solvents are suitable.

Fast Atom Bombardment (FAB, also called liquid secondary ion mass spectrometry (LSIMS)) belongs to the group of desorption methods. The analytes of the sample are dissolved in a thin, liquid, nonvolatile matrix (e.g., glycerol, 3-nitrobenzyl alcohol, thioglycerol, diethanolamine) that is placed onto a metal plate. The matrix is brought into the ion source and is bombarded with accelerated primary particles in the keV energy range. For this desorption process, inert gases like Xe or Ar are used. During this process, secondary ions are formed which can be accelerated, focused, and analyzed by common methods. Cluster ions from the liquid matrix are also desorbed and produce a chemical background that varies with the matrix used. The FAB technique is gentle and can therefore be used for analysis of proteins and peptides.

Particle Beam (also called monodisperse aerosol generator-based interface for liquid chromatography (MAGIC)): The solved analytes are separated with a chromatography column. At the end of the capillary, a helium flow, in combination with TSI or other pneumatic techniques, generates aerosols. The aerosol is sprayed (after focusing the particles into a beam by aerodynamic lenses) into the desolvation chamber where the solvent is vaporized by temperature and low pressure. A fraction of the vaporized particle beam is ionized and diffuses into the mass spectrometer (Smith et al. 2011).

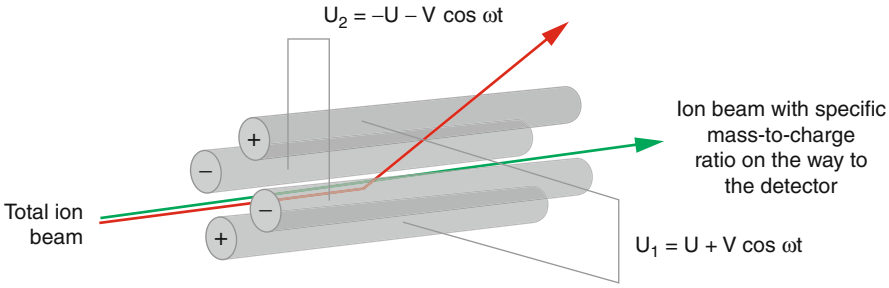



**Fig. 9** Scheme of a quadrupole used in quadrupole mass spectrometer. The quadrupole electromagnetic field is adjusted so that only a special mass-to-charge ratio of the total ion beam can pass through the quadrupole (stable ion path, *green arrow*) and enter the detector system. Ions with another mass-to-charge ratio cannot pass the detector (unstable ion path, *red arrow*). The potential on the quadrupole, and in consequence the electromagnetic field, is not static. It will change many times per second. Therefore, other mass-to-charge ratios reach a stable ion path and can traverse the quadrupole and thus reach the detector system

### Mass Spectrometer

The ions are separated by their mass-to-charge ratio and recorded with the aid of a detection system according to their mass and count frequency (qualitative/quantitative). To realize the separation of the ions, static or dynamic electric and magnetic field are used as well as differences in their time of flight. Sector field mass spectrometers and, more commonly, quadrupole mass spectrometer (see Fig. 9) are widely used (Gross 2011).

LC-MS systems are widely used as in the analysis of pesticides, mycotoxins, in the field of clinical chemistry and in forensic analysis (Gergov 2008; Maurer 2010; Botitsi et al. 2011; Roux et al. 2011; Shephard et al. 2011).

## LC-MALDI-TOF

MALDI-TOF-MS is one of the newer methods in the field of analytical chemistry. The technique was developed in the 1980s by M. Karas and F. Hillenkamp and also K. Tanaka et al. (Karas and Hillenkamp 1988; Tanaka et al. 1988). It is a discontinuous method that produces ions after exposure to a laser beam. Ion generation, acceleration, and mass analysis can be repeated in short time intervals. It is a suitable method for protein, peptide, oligonucleotide, synthetic polymer, and organic macromolecule measurements as well as for bacterial identification in clinical microbiology (Oeth et al. 2009; Vestal 2009; Li et al. 2010; Seng et al. 2010; Kafka et al. 2011). The sample is mixed with a matrix. This consists of low molecular mass organic substances such as all-*trans*-retinoic acid, 2,5-dihydroxybenzoic acid, 5-hydroxysalicylic acid, or 9-nitroanthracene which

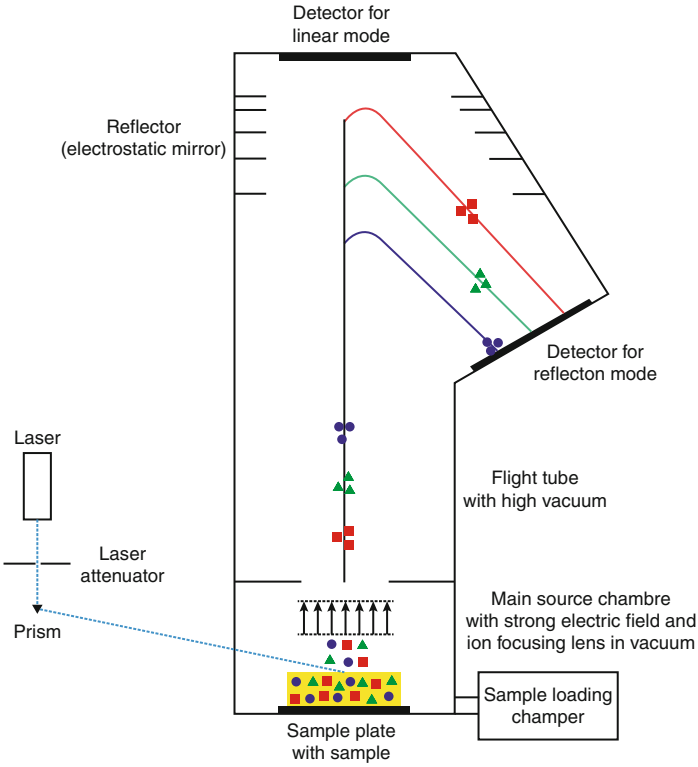



**Fig. 10** MALDI-TOF-MS schematic. A laser beam ionizes the analyte(s) from the sample plate which is accelerated in the electric field. In the linear mode, the analyte ions are separated by their mass-to-charge ratio (m/z) in the time-of-flight tube. The higher the molecular mass, the lower the velocity in the tube. During the flight, the analyte ions can be decomposed (post-source decay, *PSD*). In the linear mode, it is not possible to separate these PSD ions. By using the reflection mode, uncharged molecules pass the reflector, whereas charged ions will be deflected to a "V"-like path by different potentials in the reflector. Thereby, a further separation of ions takes place

have an absorption maximum at a laser wavelength of 337 nm. This mixture is presented on a sample plate. Then the solvent is evaporated, the plate inserted within the sample chamber of the MS and bombarded with the laser beam (discontinuous in the range of ns). Then an acceleration voltage is applied to accelerate the ions to the detector. The kinetic energy from each ion is equal. Their velocity depends on their mass-to-charge ratio. After detection, the mass of the ions can be determined by their time of flight. In some MALDI-TOF-MS, reflectors are inserted. A reflector generates a multilevel electric field. The ions are reflected from their trajectory and registered by a second detector. Using this technique it is possible to compensate for smaller kinetic energy differences from ions equal in mass (see Fig. 10). Faster ions plunge deeper into the electric field of the reflector

and stay there longer (Mamyrin 1994). By using this "trampoline effect," it is possible to reduce the mass resolution limit many times over.

## GC: Including Coupled Techniques

The chemists E. Cremer and F. Prior are among the most important pioneers in gas chromatography (quantitative adsorption-GC with mixtures of gases) (Bobleter 1990). They conceived this technique in 1944. A. Martin and A. James invented the GC detector in 1952. Gas Chromatography (GC) is suitable for chromatography involving the separation of gases or other substances that are vaporizable. The mobile phase is a gas. The stationary phase can be liquid (gas liquid chromatography, GLC) or solid (Gas solid or adsorption chromatography, GSC). For GLC thin films of a liquid are deposited on a solid particle. Together, this combination builds the stationary phase. It is important to use nonvolatile liquid substances like silicone oil, liquid paraffin, waxes, and polymeric esters. Materials for particles are glass, PTFE powder, diatomaceous earth, or alumina. In adsorption chromatography the molecules from the sample interact with the solid adsorbent. Typical materials used as adsorbent are aluminum oxide, silica gel, zeolites, or polyamide.

In analytical chemistry, capillary GC, sometimes also called High Resolution (HR)-GC, is often used. The capillary columns consist of amorphous sintered quartz (Fused Silica, FS columns) stabilized with a thin layer of polyimide. Two different types of capillary columns or Golay columns (named after the inventor) are used: the wall-coated open-tubular column (WCOT column) and the support-coated open-tubular column (SCOT column). A WCOT column can have a length from 5 to 200 m, an inner diameter of 0.1–0.5 mm, and a thin film of separation fluid (stationary phase) at the inner wall of 0.1–0.3 μm. SCOT columns have an impregnated support material instead of a thin film of fluid as the stationary phase. Special forms of SCOT columns are the porous-layer open-tubular columns (PLOT columns). In this case, the stationary phase consists of adsorption material such as aluminum oxide, silica gel, or a molecular sieve. For practical use, the column dimensions (length, diameter, film thickness) and the phase composition (such as 10 % phenyl polysiloxane) are of interest.

Only compounds with vapor pressures exceeding about $10^{-10}$ Torr can be analyzed by GC. Many compounds with lower pressures can be analyzed if they are chemically derivatized. Derivatization, in this context, is the process of chemically modifying a compound to produce another compound that has properties suitable for analysis using GC. In most cases, the volatility or the stability of the analytes as well as their chromatographic behavior requires improvement. In chemical terms, derivatization can eliminate polar groups such as OH, NH, COOH, $PO_4^{3-}$, or SH and therefore increase the volatility and thermal stability of the compound. With steroids and cholesterol, the detectability is increased. GC derivatization methods can be classified into four groups according to the reagents used and the reaction achieved: silylation, acylation, alkylation, and esterification.

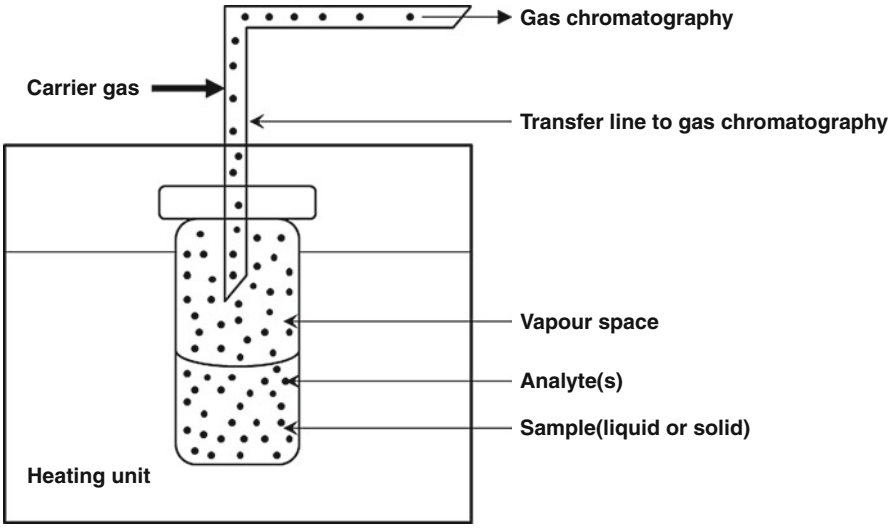



**Fig. 11** Scheme of a headspace sampler. A solid or liquid sample is applied to a heating unit (e.g., a water bath). After heating (and waiting), an equilibrium is formed between the analyte(s) in the sample and the analyte(s) in the vapor space (headspace). Aliquots from the headspace are transferred with a carrier gas to a gas chromatography instrument

## Headspace GC

Headspace GC analysis is a special technique for the detection of volatile analytes in the space over fluid or solid samples (see Fig. 11). The sample is put in a gas-tight vial with a septum and heated within the Headspace apparatus to a certain temperature. After establishing equilibrium between the sample and the space over the sample, an aliquot from the headspace is analyzed. In routine analytical use, the headspace technique has been applied to the detection of chlorinated hydrocarbon and other (organic) solvents in drinking water, oxbow lake, and wastewater. Moreover, the amount of unpolymerized monomers from acrylate-, isocyanate-, or styrene-based materials can be detected.

This technique has some parallels to the SPME technique. But in contrast to that technique, no adsorption material is used. For Headspace GC, it is necessary to have an aliquot from a sample to put inside the apparatus. The SPME technique, however, can be used outside the laboratory for onsite measurements.

## GC-MS

The great advantage of this combination is that GC can separate volatile and semi-volatile compounds with high resolution, although it cannot identify them. MS can provide detailed structural information on most analytes such that they can be exactly identified, but it cannot readily separate them. After the separation of the analytes by the chromatography column, a mass scan can be obtained. The mass

spectrogram of each component is a characteristic fingerprint. To identify a substance, the fingerprints can be compared with mass spectrograms from a database. To quantify the analyte(s), a special mode (selected ion monitoring, SIM) can be used. In this case, the mass spectrometer measures not all masses, but only a few analyte typical masses. In this case, the sensitivity is increased up to the pg or ng range (Watson and Sparkman 2008; Song and Marriott 2012).

GC-MS systems are used for the identification and quantization of volatile and semi-volatile (organic) compounds and for structural determination (in combination with other techniques) of unknown substances. Common applications are the quantization of pollutants in drinking and wastewater as well as quantization of drugs and their metabolites in blood and urine for pharmacological and forensic reasons. Some people want to know their blood level of amino acids or free fatty acids (FFAs). Of our experience FFA can be extracted by SPE from serum, derivatized in the injection system with trimethylsulfoniumhydroxide (TMSH) to the fatty acid methyl esters (FAMEs). The FAMEs are separated in the GC and identified (chain length, *cis*/*trans* configuration; position of the C–C double bond(s)) as well as quantified in the mass spectrometer. Moreover, GC-MS is used for the identification of unknown substances in waste dumps and for the identification of reaction products in synthetic steps and in quality management for the analysis of industrial products. For a suitable and effective derivatization reaction, some criteria should be considered before choosing the derivatizating reagent:

- High degree of derivatization achievable (90–100 %).
- The derivative is stable with respect to time.
- The derivative does not react/destroy the GC column.
- During the derivatization reaction, no rearrangements or structural alterations should occur.
- The derivatization reagent should not induce a loss of analytes during the reaction.

Typical chemical derivatization reagents are:

(a) For silylation, e.g., allyltrimethylsilane, N,O-bis(trimethylsilyl)trifluoroacetamide (both introduce a trimethylsilyl (TMS) group, which is the most popular and versatile silyl group).
(b) For acylation, e.g., trifluoroacetic anhydride, 1-(pentafluoropropionyl)imidazole.
(c) For alkylation, e.g., *N*,*N*-dimethylformamide dimethyl acetal, trimethyloxonium tetrafluoroborate.
(d) For esterification, e.g., boron trifluoride, methanol-HCl (Halket and Zaikin 2006; Rosenfeld 2010; Soederholm et al. 2010).

## Thin-Layer Chromatography (TLC)

The physical principle of thin-layer chromatography (TLC) is the movement by capillary forces of a liquid phase, usually an organic solvent, through a thin, uniform layer of solid phase, usually silica gel ($SiO_2$). The solid phase is held on a rigid or semirigid support, normally a glass, aluminum, or plastic sheet or plate.

The analytes of the sample are separated by the interaction between the mobile and stationary phases (Spangenberg et al. 2011).

Advantages of TLC, especially in the field of forensic analytics, are (Bele and Khale 2011):

- Reliable, rapid, and easy procedure (normal case)
- Relatively inexpensive
- Relatively simple in use
- Possibility of detecting upward of 700 different types of drugs, medications, and metabolites
- Validated as a diagnostic tool that holds up under the scrutiny of legal challenges, inside and outside the courtroom
- Combined with sample pretreatment (e.g., solvent extraction), TLC can be a powerful qualitative technique

It should be mentioned that the interpretation of TLC results is sometimes very difficult, especially when a number of drugs, medications, and metabolites are present.

TLC can detect, accurately, a large number of medically significant substances, such as anticonvulsants/antispasmodics (e.g., phenytoin, carbamazepine), antidepressants (e.g., amitriptyline, nortriptyline, sertraline), antihistamines (e.g., chlorpheniramine, diphenhydramine), anti-inflammatories (e.g., naproxen, ketoprofen, ibuprofen), anesthetics (e.g., lidocaine, procaine), decongestants/bronchodilators (e.g., ephedrine, theophylline), muscle relaxants (e.g., carisoprodol, meprobamate), narcotic analgesics (e.g., methadone, tramadol), sedatives (e.g., ketamine, imipramine), stimulants (e.g., methylphenidate, methylenedioxymethamphetamine (MDMA)), and miscellaneous (e.g., strychnine, verapamil, haloperidol) (Parmar et al. 2011; Tuzimski 2011; Shewiyo et al. 2012).

## Ion-Exchange Chromatography (IEC/IEX)

IEC is a distinctive kind of adsorption chromatography, which allows separation of ions. It has a special significance in the analysis of organic and inorganic ions such as phosphate ($PO_4^{3-}$) or sulfate ($SO_4^{2-}$). Ion separation is based on the charge and the size of each analyte ion itself as well as the counterions, the pH, and the ionic strength in the mobile phase and the type of exchange resin. The stationary phase is an ion-exchange resin. Ion-exchange resins are categorized as cation and anion exchangers. In both classes, strong and weak ion-exchange resins exist (see Table 3). Liquid-phase analyte ions are attracted via Coulombic forces to ions in the exchange resins (stationary phase). Elution of the analyte ions occurs by an exchange with an ion from the eluent (Inamuddin and Luqman 2012a, b).

IEC is used in many fields where small molecules/ions must be detected; for some molecules/ions it is the preferred method. Here are some examples: In the field of environmental analytics, it can be used for the detection of inorganic anions such as nitrate, nitrite, bromide, fluoride, and chloride or

**Table 3** Classification of ion-exchange resins and their chemical functional groups

| Ion-exchange resin | Functional group | |
|---|---|---|
| **Cationic exchanger** | | |
| Strong acid | Sulfonic acid | $–SO_3H$ |
| | Phosphoric acid | $–PO(OH)_2$ |
| Weak acid | Hydroxyl group | –OH |
| | Carboxyl group | –COOH |
| **Anionic exchanger** | | |
| Strong alkaline | Quaternary amine | $–N^+(CH_3)_3$ |
| | Diethylaminoethyl (DEAE) | $–(CH_2)_2\text{-}N^+H(C_2H_5)_2$ |
| Weak alkaline | Primary amine | $–NH_2$ |
| | Secondary amine | –NH– |
| | Tertiary amine | |
| | Chelating resins (aminophosphonic acid, iminodiacetic acid, thiols) | $NH_2CH_2PO(OH)_2$ |
| | | $NH(CH_2COOH)_2$ |
| | | –SH |

inorganic cations like Cr(VI), $Ni^{2+}$, and $Cu^{2+}$ or metals in complexes such as $Au(CN)^{2-}$ and $Au(CN)^{4-}$ from complex matrices. In the field of food analytics, organic molecules such as pyruvate, lactate, citrate, or amino acids can be measured (Karlsson and Hirsh 2011; Inamuddin and Luqman 2012a, b).

## Electrophoresis

The electro kinetic phenomenon was observed in 1807 by the German scientist F. Reuss at Moscow University (Reuss 1809). Electrophoresis, as known today, was first described in 1937 by the Swedish chemist A. Tiselius. The term derives from the Greek words "ηλεκτρου, electron" (electron) and "φορεσις, phoresis" (carrying) meaning "electric carried." The physical principles of electrophoresis are based on the motion of analytes (cells/particles/proteins/ substances) relative to a fluid under the influence of a spatially uniform electric field (see Fig. 12). The migration speed and in consequence the retention time of the analytes depend on their charge, mass, and size as well as the electrophoresis media and the strength of the electric field. The results are singular bands visualized on a gel, a foil, or an electropherogram (e.g., presentation of DNA sequencing). Modifications of electrophoresis are, for example, slab-gel electrophoresis and capillary electrophoresis (CE). CE (also known as capillary zone electrophoresis, CZE) uses – besides the normal electrophoresis conditions (conductive liquid medium under the influence of an electric field) – electroosmosis, as a further separation principle. If ions are in the solution medium, then an electroosmotic flow will be generated with the

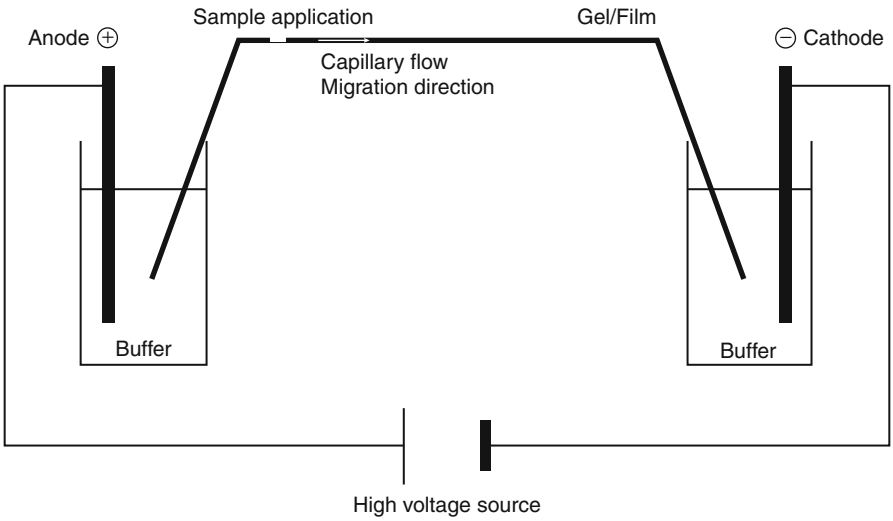



**Fig. 12** Principle of electrophoresis. The sample with the macromolecules is applied on a film/gel, e.g., a cellulose acetate gel. Then an electric field is applied. The charged molecules migrate towards the positive or negative pole according to their (opposite) charge (in the figure, the positive macromolecules migrate to the cathode). By passing through the film, the macromolecules are separated by their size, charge, and conformation. After a certain time, the electrophoresis is stopped and the macromolecules are stained (discontinuous working system). The gel is only for single use. In contrast to electrophoresis, capillary electrophoresis is a continuous working system. The separated macromolecules are detected by a UV detector, or a similar device

negatively charged analytes and the negatively charged ions moving to the anode. The positive ions in the medium as well as the analytes (independent of the charge) flow to the cathode. A short overview of the application fields and possibilities are given in (Simpson et al. 2008; Harrington et al. 2010; Pascali et al. 2012).

## Atomic Spectroscopy

Atomic spectroscopy embraces a set of spectro-analytical techniques for the qualitative and quantitative determination of chemical elements (Hywel Evans et al. 2012). J. Marci von Kronland described in 1648 the diffraction and the scattering of light in waterdrops. The first spectroscope, consisting of a lens, a prism, and a screen, to define a light beam, was developed by I. Newton in 1666. He showed that the white light from the Sun could be dispersed into a continuous series of colors (a light spectrum). In 1752, T. Melville discovered that putting different substances/salts in flames, and passing the light through a prism, leads to different spectra. He found that table salt generated a "bright yellow." In 1815, J. Fraunhofer discovered in the optical spectrum of the Sun dark features (absorption lines). In 1853, A. Angstrom analyzed the spectrum of hydrogen and obtained the first insight into atomic structure. In the years from 1855 to 1863, R. Bunsen and G. Kirchhoff systematically investigated

thousands of spectral lines (Burns 1975; Thomsen 2006). The first element discovered by spectral lines was helium. The first hint of the existence of helium was seen in 1868 by the astronomer J. Janssen. He saw a bright yellow line with a wavelength of 587.49 nm in the spectrum of the chromospheres of the Sun (Tayler 1995). The principles of quantum theory, inter-relating atomic structure with electromagnetic radiation, were initiated by M. Planck, about 1900.

The principle of all atomic spectroscopy techniques is based on the characteristic behavior of atoms/elements (but not chemical compounds) that under certain physical circumstances an *element-specific* electromagnetic emission or absorption takes place (Bings et al. 2010). This interaction corresponds to a change of the energy state of the outer electrons of each atom. For this reason the analyte elements in the sample have to be released from their compounds. Free atoms can be generated by atomization in a flame or plasma. Widely used atomic spectroscopy techniques are:

- Atomic absorption spectroscopy (AAS)
- Atomic emission spectroscopy (AES)
- Atomic fluorescence spectroscopy (AFS)
- Optical emission spectroscopy (OES)
- Inductively coupled plasma mass spectrometry (ICP-MS)
- X-ray fluorescence spectroscopy (XRF spectroscopy)

The interaction between the outer electrons of the atom and electromagnetic radiation can involve atomic absorption, atomic emission, and atomic fluorescence. Atomic absorption occurs when they absorb ultraviolet (UV) and/or visible light (VIS) radiation. The unabsorbed radiation is measured. The atoms reach an excited state (higher orbital) from the ground state. In the case of atomic emission, the excited electrons (e.g., after thermal or electronic excitation) revert to the ground state by emission of electromagnetic radiation. With fluorescence spectra, the atoms are excited with light or laser, and then light of a longer wavelength range is emitted and measured.

In *qualitative* atomic spectroscopy the characteristic lines (wavelengths) for each element are measured. In *quantitative* atomic spectroscopy, the intensity of the lines from each element is determined, and the amount of this element is calculated with the aid of a calibration line. The quantification relies on the Lambert-Beer law. With different atomic spectroscopy techniques, the following (most relevant) elements that can be measured are Ag, Al, As, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cs,Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Hg, Ho, In, Ir, K, La, Li, Lu, Mg, Mn, Mo, Na, Nb, Nd, Ni, Os, P, Pb, Pd, Pr, Pt, Rb, Re, Rh, Ru, S, Sb, Sc, Se, Si, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, V, W, X, Y, Yb, Zn, and Zr (Welz et al. 2005; Skoog et al. 2006).

## Atomic Absorption Spectroscopy (AAS)

This technique is based on the absorption of optical radiation by free atoms in the gaseous state. AAS can be used to determine over 70 different elements in solution

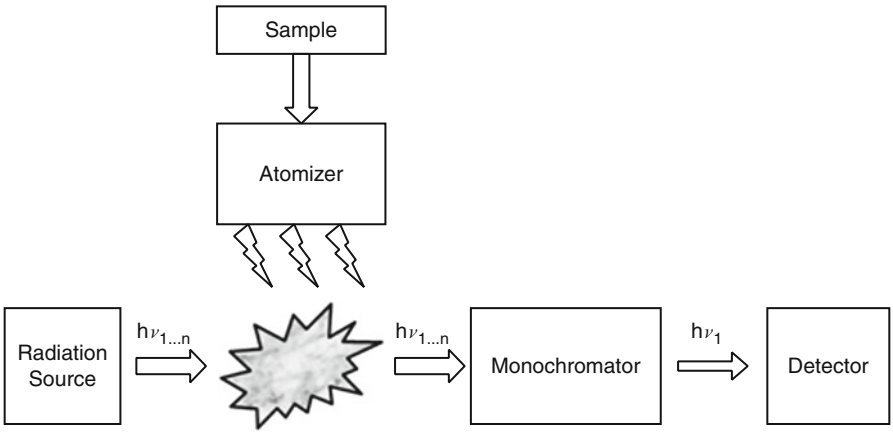



**Fig. 13** Schematic description of atomic absorption spectroscopy (AAS). The elements in the sample are atomized. The elements are activated by a radiation source. The emitted spectra from the elements are, after passing a monochromator, detected, multiplied, and evaluated

or in solid samples. AAS was developed in the 1950s by the team led by A. Walsh. The first stage of AAS requires atomization of the sample analytes in the atomizer. Then the atoms are irradiated by optical radiation. To obtain an exact element-specific wavelength for each element, the radiation has to pass a monochromator. The element-specific radiation is measured and the signal amplified within the detection system (see Fig. 13) (Welz and Sperling 2007).

Within the general technique of atomization, different atomizers are available: flame atomic absorption spectroscopy (FAAS), electrothermal atomic absorption spectroscopy (ETAAS), graphite-furnace atomic absorption spectroscopy (GFAAS), and cold-vapor atomic absorption spectroscopy (CVAAS). In the case of FAAS, a combination of a burning gas and an oxidation gas is used. The combination depends on the analyte elements (poorly vaporable elements like Mg, Ca, W need higher temperatures). Usual combinations include, e.g., acetylene (burning gas) and nitrous oxide ($N_2O$, oxidation gas), which reaches temperatures of about 3,200 K, or acetylene and oxygen, reaching temperatures of about 3,000 K. In the case of GFAAS, the liquid, solid, or gaseous sample can be analyzed directly. The sample is put in the graphite tube, and a temperature program is started leading to drying, pyrolysis, and atomization of the sample. As a final step, the graphite tube is cleaned at high temperature.

Different lamps are used as radiation sources. First, it is necessary to distinguish between two types of lamps: line source (LS) and continuum source (CS) lamps. LS lamps emit a single line spectrum. CS lamps emit continuous spectra. In classical AAS, CS lamps like deuterium hollow cathode lamps (HCL) were used for background compensation. Newer developments, such as high-resolution continuum source atomic absorption spectroscopy (HR-CS AAS), use CS lamps like xenon

compact-source arc discharge lamps. These provide a high radiation density and cover the complete spectral range from the near vacuum UV to the near infrared.

For LS AAS, normally HCL are used. HCL consist of a glass tube containing a cathode, an anode, and a buffer gas (usually a noble gas). The cathode is made from the element to be analyzed. The high voltage between the anode and cathode ionizes the buffer gas (a plasma is created). The gas ions are accelerated towards the cathode, sputtering off atoms from the cathode. The sputtered atoms from the cathode will be excited by collision with other particles in the plasma. By decaying to lower energy states, these excited atoms emit photons, which are used for identifying the element in the sample (Ataman 2008; Kumar et al. 2009; Karabegov 2011).

## Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

In the early 1980s, the commercialization of ICP-MS started. Today, many different ICP-MS instruments are commercially available, each with their own strengths and limitations. They all share similar components such as the nebulizer, spray chamber, plasma torch, interface, and detector, but can differ significantly in the design of the mass spectrometer and in particular the mass separation device (Nelms 2005).

ICP-MS is based on the ionization of sample elements in a plasma at about 5,000–10,000 K. Normally the plasma is produced by the interaction of an intense magnetic field (produced by radio-frequency radiation passing through a copper coil) on a tangential flow of gas (normally argon), flowing through a concentric quartz tube (torch) at about 15 L/min. This setup ionizes the gas and, when seeded with a source of electrons from a high-voltage spark, forms a very high temperature plasma discharge (~10,000 K) at the open end of the tube.

The sample, typically in liquid form, is pumped into the sample introduction system, which is made up of a spray chamber and nebulizer. It emerges as an aerosol and eventually passes – by way of a sample injector – into the base of the plasma. As it travels through the different heating zones of the plasma torch, it is dried, vaporized, atomized, and ionized. During this time, the sample is transformed from a liquid aerosol to solid particles, then into a gas. When it finally arrives at the analytical zone of the plasma, at approximately 6,000–7,000 K, it exists as excited atoms and ions, representing the elemental composition of the sample.

In the next step the ions are directed into the mass spectrometer via the interface region. The role of the interface is to transport the ions (and only the ions) from the plasma, which is at atmospheric pressure (760 Torr) to the mass spectrometer analyzer region at approximately $10^{-5}$ Torr. Moreover the interface has to reduce or eliminate the secondary discharge, which arises by capacitive coupling between the radio-frequency coil and the plasma. After the interface, the ion optic (a series of electrostatic lenses) focuses the ion beam towards the mass separation device, and it stops photons (that would otherwise increase the signal noise), particulates,

and neutral species from reaching the detection system. The most common types of mass separation devices are based on quadrupole, magnetic sector, time of flight, collision/reaction cells, and dynamic reaction cell technology. The basic principle of these different types of mass separation devices is to allow only analyte ions of a particular mass-to-charge ration (m/z) to pass the device and to fly to the detection system. Other particles such as matrix ions have to be filtered out. At last, the ion detector converts the ion beam into an electrical signal. Widely used are dynode detector systems, containing a series of metal dynodes along the length of the detector. In this case the ion beam impinges upon the first dynode and creates an electron beam, which attracts the next dynode. The process of electron multiplication starts.

One great advantage of this technique is its ability to carry out rapid multi-element determinations at low detection limits (ultra-trace level), especially enhancing the speed of analysis, and the isotopic capabilities (Aggarwal 2010; Butler et al. 2010).

## Selective Analytical Chemistry

### Sensor Techniques

The term sensor techniques subsumes different molecule measuring techniques. Biosensors are widely used with their special form of ion-selective electrodes (ISE), for detecting supramolecular interactions on interfaces. According to the International Union of Pure and Applied Chemistry (IUPAC), a biosensor is defined as "a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transduction element" (Turdean 2011). Biosensor techniques are based on direct measurement of a (biological) component with the aid of biorecognition phases, such as enzymes, antibodies (immunosensors), single-stranded DNA, or microorganisms (whole-cell-based biosensors). The analyte interacts with the biorecognition phase and produces a signal, e.g., a change in proton concentration; a release or uptake of gases like oxygen; a release or uptake of electrons; a light emission, absorption, or reflectance; a heat emission; and a mass change. For example, an antigen to be detected couples with an antibody. The antibody is directly coupled with a transducer, transforming the chemical signal into a measurement signal/measurable response. This type of system is quite general. Transducers can be electrodes based on an optical fiber, a piezoelectric crystal, electrochemical methods (potentiometric or amperometric systems), sonic methods, or a calorimetrical system (thermistor). The signal from the transducer is electronically processed, and the measurement value is displayed (see Fig. 14) (Farré and Barceló 2009).

One classification of biosensors is based on the detection system of the transducer. Biosensors are used in ecotoxicology (formaldehyde detection) and

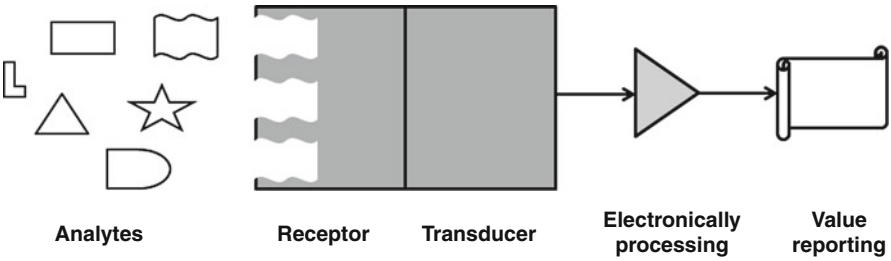



**Fig. 14** Principal construction of a biosensor. An analyte-specific detection system is used, which is coupled with a transducer. The transducer converts the chemical signal from the detection system into an electronic signal that is amplified and the value obtained is reported

environmental monitoring (pesticides, nitrites) as well as for breath tests, in food control measurements ("artificial nose" to determine freshness, aroma, odor), and military use, e.g., detection of nerve gas, chemical, or biological weapons.

ISE (ion-selective electrode) method is a form of potentiometry. That means a special form of electrochemical-based biosensors that determine the equilibrium cell voltage of galvanic cells. ISE measure the activity of a special analyte ion in a solution of different ions. This produces a potential that is proportional to the concentration of the analyte ion. ISE are used for measuring in brass, bronze, copper, lead, and cadmium baths as well as for the determination of ethylenediaminetetraacetate (EDTA) and citrate. Dependent on the ion-selective membrane, solid state and liquid membranes are distinguished. Solid state membranes can be based on glass membranes, single crystal membranes, or precipitation membranes. Liquid membranes can be based on ion carrier membranes or ion-exchange membranes (Gruendler 2007).

## Immunoassays

Immunoassays (IAs) are widely used laboratory methods for clinical and (forensic) toxicology diagnostics. IAs are useful for blood or serum therapeutic drug monitoring. They are also useful for serum and urine determinations of ethanol, medicines, drugs of abuse (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, ecstasy, methadone, opiates, phencyclidine, tricyclic antidepressants), and other toxins. The basic principle of the IAs is the detection of the analyte (in this case "the antigen") in a liquid phase by bonding with an antibody (antigen-antibody reaction, see Fig. 15). Normally, IAs are based on a competitive and cooperative interaction between the analyte (a hormone, a protein, a drug, or a hapten) to be determined and a labeled ligand, which is thus measurable, and an unlabeled ligand, both of which occupy the same binding site on the analyte. The labeling can be achieved with a fluorescent dye (FIA), a luminogen, a fluorophore, an enzyme (EIA), or a radioactive (RIA) substance. IAs are fast, sensitive, and accurate and permit determination of analytes in different kinds of (biological) fluids or

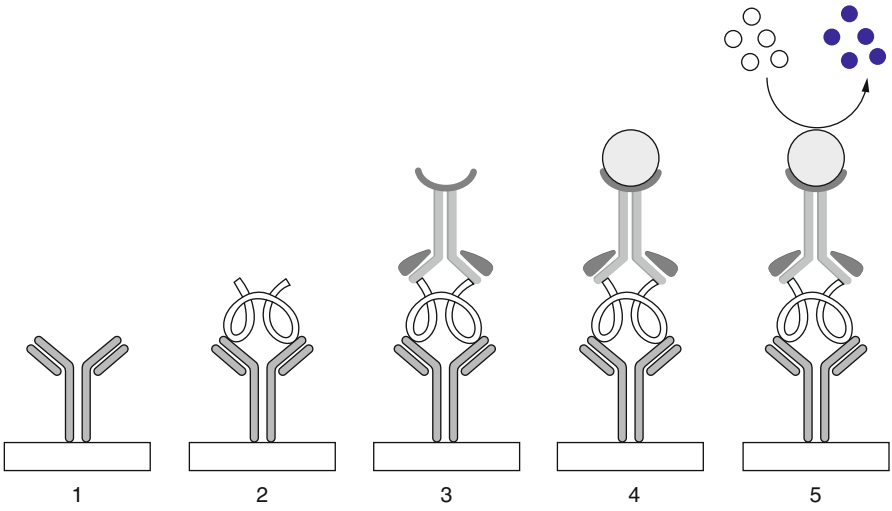



**Fig. 15** Principles of an enzyme-linked immunosorbent assay (ELISA). (1) Analyte-specific antigens are bound to the wall of a reaction vessel. (2) After adding the sample, the analyte binds to the analyte-specific antibody. (3) After washing, a second biotin-labeled antibody binds to the analyte. (4) After washing, a streptavidin-enzyme conjugate binds to the biotin-labeled antibody. (5) The streptavidin-enzyme conjugate catalyzes the formation of a chromogen from a colorless substance that is added

suspensions (Moody 2006; Chan et al. 2008; Durner 2010). One problem is cross-reactivity with additional matrix components, such as metabolites or structurally related substances.

IAs can be classified by different criteria. Widely used is the classification depending on their realization. In this case homogeneous IA scan be distinguished from heterogeneous. In contrast to homogeneous IAs, the unbound reactants are separated prior to measurement in heterogeneous IAs. In the case of heterogeneous IAs, two further types can be distinguished, a competitive IA and an immunometric IA, also known as "two-site" or sandwich IA. A very common form of sandwich IA is the enzyme-linked immunosorbent assay (ELISA). In ELISA test systems, the antigen in the liquid sample is captured by immobilized antibodies (e.g., on the wall of cavities in 96-well plates or on polystyrene globes). After washing steps, a second, enzyme-labeled antibody (e.g., with horse radish peroxidase, alkaline phosphatase, beta-galactosidase) against the antigen is added. Then a substrate is added which is converted to a chromogenic reaction product if the enzyme from the antibody (and therefore the analyte) is present in the reaction vessel. The concentration of the analyte can then be determined through absorption spectroscopy according to the Lambert-Beer law.

In general, homogeneous IAs are more amenable to full automation and thereby quicker throughput. Heterogeneous IAs are less susceptible to matrix interference and thereby more versatile with non-urine matrices.

Other common IAs are cassette or strip rapid tests like lateral flow immunoassays (LFA) (Christopher et al. 2005; Posthuma-Trumpie et al. 2009; Shi et al. 2010). Such tests are used in environmental analytics (water testing, pesticides, dust mite testing), food testing (genetically modified (gm) food, Escherichia coli, Salmonella strains), military analytic (germ warfare, explosives chemical warfare), veterinary analytics (feline cancer, BSE, canine heart worm), disease diagnostics (malaria, hepatitis B, tuberculosis), testing of sexual transmitted diseases (STDs, chlamydia, syphilis, HIV), fertility diagnostic (pregnancy, luteinizing hormone), or drug abuse (cocaine, cannabis, ecstasy).

**Acknowledgement** DCW gratefully acknowledges the support of the Alexander von Humboldt Foundation (Bonn, Germany) through provision of a Humboldt Research Award.

## References

Aggarwal SK (2010) Inductively coupled plasma mass spectrometry for trace analysis. In: Lahiri S (ed) Advanced trace analysis, 1st edn. Alpha Science International, Oxford, pp 71–92

Ataman OY (2008) Vapor generation and atom traps: atomic absorption spectrometry at the ng/L level. Spectrochim Acta, Part B 63B(8):825–834

Bele AA, Khale A (2011) An overview on thin layer chromatography. Int J Pharm Sci Res 2(2):256–267

Bings NH, Bogaerts A, Broekaert JAC (2010) Atomic spectroscopy: a review. Anal Chem (Washington, DC, United States) 82(12):4653–4681

Bobleter O (1990) Professor Erika Cremer – a pioneer in gas chromatography. Chromatographia 30(9):471–476

Botitsi HV, Garbis SD, Economou A, Tsipi DF (2011) Current mass spectrometry strategies for the analysis of pesticides and their metabolites in food and water matrices. Mass Spectrom Rev 30(5):907–939

Burns DT (1975) One hundred years of atomic spectroscopy, 1874–1974. Proc Anal Div Chem Soc 12(5):155–158

Butler OT, Cairns WRL, Cook JM, Davidson CM (2010) Atomic spectrometry update. Environmental analysis. J Anal At Spectrom 25(2):103–141

Camel V (2003) Solid-phase extraction. Compr Anal Chem 41:393–457

Chan CPY, Cheung YC, Renneberg R, Seydack M (2008) New trends in immunoassays. Adv Biochem Eng Biotechnol 109:123–154 (Biosensing for the 21st Century)

Chen Y, Pawliszyn J (2007) Theory of solid phase microextraction and its application in passive sampling. Compre Anal Chem 48:3–32

Christopher P, Robinson N, Shaw MK (2005) Antibody-label conjugates in lateral-flow assays. In: Wong RC (ed) Drugs of abuse, 1st edn. Springer, Heidelberg, pp 87–98

Deisingh AK, Thompson M (2004) Biosensors for the detection of bacteria. Can J Microbiol 50(2):69–77

Duan C, Shen Z, Wu D, Guan Y (2011) Recent developments in solid-phase microextraction for on-site sampling and sample preparation. Trends Anal Chem 30(10):1568–1574

Durner J (2010) Clinical chemistry: challenges for analytical chemistry and the nanosciences from medicine. Angew Chem Int Ed Engl 49(6):1026–1051

Farré M, Barceló D (2009) Biosensors for aquatic toxicology evaluation biosensors for environmental monitoring of aquatic systems. In: Barceló D, Hansen P-D (eds) Biosensors for environmental monitoring of aquatic systems: bioanalytical and chemical methods for endocrine disruptors. Springer, Berlin/Heidelberg, pp 115–160.

Fresenius CR (1866) Anleitung zur qualitativen chemischen analyse, 12th edn. Vieweg u. Sohn, Braunschweig
Furr HC (2004) Analysis of retinoids and carotenoids: problems resolved and unsolved. J Nutr 134(1):281S–285S
Gergov M (2008) Forensic screening with liquid chromatography-mass spectrometry. In: Roger S, Bogusz MJ (eds) Forensic science. Elsevier, Amsterdam, pp 491–511
Gey MH (2008) Instrumentelle analytik und bioanalytik, 2nd edn. Springer, Berlin/Heidelberg
Gianotti V, Polati S, Gosetti F, Gennaro MC (2011) HPLC in environmental analysis. Chromatogr Sci Ser 101:535–559 (Handbook of HPLC)
Gross J (2011) Mass spectrometry: a textbook, 2nd edn. Springer, Heidelberg
Gruendler P (2007) Chemical sensors: an introductrion for scientists and engineers, 1st edn. Springer, Heidelberg
Guiochon G, Trapp O (2000) Basic principles of chromatography. In: Wiley-VCH (ed) Ullmann's encyclopedia of industrial chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
Halket JM, Zaikin VG (2006) Derivatization in mass spectrometry-7. On-line derivatization/degradation. Eur J Mass Spectrom 12(1):1–13
Harrington CF, Vidler DS, Jenkins RO (2010) Analysis of organometal(loid) compounds in environmental and biological samples. Met Ions Life Sci 7:33–69
Hennion MC (2000) Sample handling strategies for the analysis of organic compounds in environmental water samples. Tech Instrum Anal Chem (Sample Handl Trace Anal Pollut) 21:3–71
Hii TM, Lee HK (2010) Liquid-liquid extraction in environmental analysis. In: Pawliszyn J, Heather L (eds) Handbook of sample preparation, 1st edn. Wiley, Hoboken, pp 39–51
Hywel Evans E, Palmer CD, Smith CMM (2012) Atomic spectrometry update. Advances in atomic spectrometry and related techniques. J Anal At Spectrom 27(6):909–927
Inamuddin M, Luqman M (2012a) Ion exchange technology I, 1st edn. Springer, Heidelberg
Inamuddin M, Luqman M (2012b) Ion exchange technology II, 1st edn. Springer, Heidelberg
Kafka AP, Kleffmann T, Rades T, McDowell A (2011) The application of MALDI TOF MS in biopharmaceutical research. Int J Pharm 417(1–2):70–82
Karabegov MA (2011) New developments in atomic absorption spectrometry. Meas Tech 53(10):1174–1181
Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular masses exceeding 10,000 Daltons. Anal Chem 60(20):2299–2301
Karlsson E, Hirsh I (2011) Ion exchange chromatography. Methods Biochem Anal (Protein Purif) 54:93–133
Kaushal R, Kaur N, Upadhyay A, Suri OP, Thakkar A (2011) High performance liquid chromatography detectors – a review. Int Res J Pharm 2(5):1–7
Kumar SA, Udas A, Ramanamurthy M (2009) Atomic absorption, emission and mass quantification in the elemental characterization of materials. Mater Sci Found 49–51:301–338
Li L, Mu Q, Zhang B, Yan B (2010) Analytical strategies for detecting nanoparticle-protein interactions. Analyst (Cambridge, United Kingdom) 135(7):1519–1530
Liska I (2000) Fifty years of solid-phase extraction in water analysis–historical development and overview. J Chromatogr A 885(1–2):3–16
Majors RE (2010) Solid-phase extraction. In: Pawliszyn J, Heather L (eds) Handbook of sample preparation. 1st edn. Wiley, Hoboken, Ltd, pp 53–79
Mamyrin BA (1994) Laser assisted reflectron time-of-flight mass spectrometry. Int J Mass Spectrom Ion Process 131:1–19
Maurer HH (2010) Analytical toxicology. EXS 100:317–337
McConvey IF, Nancarrow P (2011) Liquid-liquid extraction for process development in the pharmaceutical industry. RSC Drug Discov Ser (Pharm Process Dev) 9:209–237
Meyer V (2010) Practical high-performance liquid chromatography, 5th edn. Wiley, Hoboken

Miller JM (2009) Chromatography. In: Andrews DL (ed) Encyclopedia of applied spectroscopy. 1st edn. Wiley, Hoboken, pp 1055–1102

Milroy CS, Stevenson PG, Mnatasakyan M, Shalliker RA (2012) Multidimensional high-performance liquid chromatography. Chromatogr Sci Ser (Hyphenated Altern Methods Detect Chromatogr) 104:251–285

Moody DE (2006) Immunoassays in forensic toxicology. In: Mayers RA (ed) Encyclopedia of analytical chemistry. Wiley, Hoboken

Nelms S (2005) Inductively coupled plasma mass spectrometry handbook, 1st edn. Wiley-VCH, Weinheim

Oeth P, del Mistro G, Marnellos G, Shi T, van den Boom D (2009) Qualitative and quantitative genotyping using single base primer extension coupled with matrix-assisted laser desorption/ionization time-of-fight mass spectrometry (MassARRAY). Methods Mol Biol (Totowa, NJ, United States) (Single Nucleotide Polymorph) 578:307–343

O'Kane MJ, Lynch PLM, McGowan N (2008) The development of a system for the reporting, classification and grading of quality failures in the clinical biochemistry laboratory. Ann Clin Biochem 45(2):129–134

Ostwald W (1894) Die wissenschaftlichen Grundlagen der analytischen Chemie. Verlag Wilhelm Engelmann, Leipzig

Palchetti I, Mascini M (2008) Electroanalytical biosensors and their potential for food pathogen and toxin detection. Anal Bioanal Chem 391(2):455–471

Parmar KD, Patel NM, Patel PM (2011) A review: impurity profile study. J Pharm Res 4(8):2566–2569

Pascali JP, Bortolotti F, Tagliaro F (2012) Recent advances in the application of CE to forensic sciences, an update over years 2009–2011. Electrophoresis 33(1):117–126

Pawliszyn J (1997) Solid phase microextraction theory and practice, 1st edn. Wiley, Weinheim

Persoon TJ, Zaleski S, Frerichs J (2006) Improving preanalytic processes using the principles of lean production (Toyota production system). Am J Clin Pathol 125(1):16–25

Peters FT, Remane D (2012) Aspects of matrix effects in applications of liquid chromatography-mass spectrometry to forensic and clinical toxicology – a review. Anal Bioanal Chem 403(8):2155–2172

Pfaff CH (1821) Handbuch der analytischen chemie für chemiker, staatsärzte, apotheker, oekonomen und bergwerks kundige. J. F. Hammrich, Altona

Pfaff CH (1822) Handbuch der analytischen chemie für chemiker, staatsärzte, apotheker, oekonomen und bergwerks kundige. J. F. Hammrich, Altona

Polettini A (2011) HPLC in forensic sciences. Chromatogr Sci Ser 101:661–682 (Handbook of HPLC)

Posthuma-Trumpie GA, Korf J, van Amerongen A (2009) Lateral flow (immuno)assay: its strengths, weaknesses, opportunities and threats. A literature survey. Anal Bioanal Chem 393(2):569–582

Reuss FF (1809) Charge induced flow. Proc Imp Soc Natural Moscow 3:327–344

Risticevic S, Niri VH, Vuckovic D, Pawliszyn J (2009) Recent developments in solid-phase microextraction. Anal Bioanal Chem 393(3):781–795

Risticevic S, Vuckovic D, Pawliszyn J (2010) Solid-phase microextraction. In: Pawliszyn J, Heather L (eds) Handbook of sample preparation. 1st edn. Wiley, Hoboken, pp 81–101

Rosenfeld J (2010) Chemical derivatizations in analytical extractions. In: Pawliszyn J, Heather L (eds) Handbook of sample preparation. 1st edn. Hoboken: John Wiley & Sons, Ltd, pp 225–245

Roux A, Lison D, Junot C, Heilier J-F (2011) Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: a review. Clin Biochem 44(1):119–135

Saleem TSM, Reddy AE (2011) Omic technologies: an overview. BioChem: An Indian J 5(3):145–150

Schneider MV, Orchard S (2011) Omics technologies, data and bioinformatics principles. Methods Mol Biol (New York, NY, United States) (Bioinforma Omics Data) 719:3–30
Seng P, Rolain J-M, Fournier PE, La Scola B, Drancourt M, Raoult D (2010) MALDI-TOF-mass spectrometry applications in clinical microbiology. Future Microbiol 5(11):1733–1754
Shephard GS, Berthiller F, Burdaspal P, Crews C, Jonker MA, Krska R et al (2011) Developments in mycotoxin analysis: an update for 2009–2010. World Mycotoxin J 4(1):3–28
Shewiyo DH, Kaale E, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander HY (2012) HPTLC methods to assay active ingredients in pharmaceutical formulations: a review of the method development and validation steps. J Pharm Biomed Anal 66:11–23
Shi L, Wang X, Liu Y, Lu Y (2010) Research progress on immunoassays for food allergen. Fenxi Ceshi Xuebao 29(9):981–986
Silvestre CIC, Santos JLM, Lima JLFC, Zagatto EAG (2009) Liquid-liquid extraction in flow analysis: a critical review. Anal Chim Acta 652(1–2):54–65
Simpson SL Jr, Quirino JP, Terabe S (2008) On-line sample preconcentration in capillary electrophoresis. Fundamentals and applications. J Chromatogr A 1184(1–2):504–541
Singh S, Singhal Naveen K, Srivastava G, Singh MP (2010) Omics in mechanistic and predictive toxicology. Toxicol Mech Methods 20(7):355–362
Skoog D, Holler F, Crouch S (2006) Principles of instrumental analysis, 6th edn. Brooks Cole, Canada
Smith SA, Smith RW, Xia Y, Ouyang Z (2011) Introduction to mass spectrometry. In: Pramanik B, Lee MS, Chen G (eds) Characterization of impurities and degradants using mass spectrometry. 1st edn. Wiley, Hoboken, pp 3–57
Soederholm SL, Damm M, Kappe CO (2010) Microwave-assisted derivatization procedures for gas chromatography/mass spectrometry analysis. Mol Divers 14(4):869–888
Song SM, Marriott PJ (2012) Analytical aspects of modern gas chromatography: mass spectrometry. Chromatogr Sci Ser (Hyphenated Altern Methods Detect Chromatogr) 104:31–59
Spangenberg B, Poole CF, Weins C (2011) Quantitative thin-layer chromatography, 1st edn. Springer, Heidelberg
Suter-Dick L, Singer T (2008) Methods in toxicology: omics in toxicology. In: Greim H, Synder R (eds) Toxicology and risk assessment: a comprehensive introduction, 1st edn. Wiley, England, pp 437–449
Szabadvary F (1966) Geschichte der analytischen chemie. Vieweg und Sohn, Braunschweig
Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T et al (1988) Protein and polymer analyses up to m/z 100 000 by laser ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 2(8):151–153
Tayler RJ (1995) Helium in the universe. Contemp Phys 36(1):37–48
Tedder DW (2009) Liquid-liquid extraction. In: Albright LF (ed) Albright's chemical engineering handbook. 1st edn. New York: CRC Press, pp 709–735
Testard F, Zemb T, Bauduin P, Berthon L (2010) Third-phase formation in liquid/liquid extraction: a colloidal approach. Ion Exch Solvent Extract 19:381–428
Thomsen V (2006) A timeline of atomic spectroscopy. Spectroscopy (Duluth, MN, United States) 21(10):32–42
Tölg G, Günzler H, Williams A (2000) Analytical chemistry: purpose and procedures. In: Wiley-VCH (ed) Ullmann's encyclopedia of industrial chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
Trietsch SJ, Hankemeier T, van der Linden HJ (2011) Lab-on-a-chip technologies for massive parallel data generation in the life sciences: a review. Chemom Intell Lab Syst 108(1):64–75
Turdean GL (2011) Design and development of biosensors for the detection of heavy metal toxicity. Int J Electrochem
Tuzimski T (2011) Application of different modes of thin-layer chromatography and mass spectrometry for the separation and detection of large and small biomolecules. J Chromatogr A 1218(49):8799–8812

Vestal ML (2009) Modern MALDI time-of-flight mass spectrometry. J Mass Spectrom 44(3):303–317
Volke K (2004) Zu den anfängen der analytischen chemie: wider fälscher und betrüger. Chem unserer Zeit 38(4):268–275
Vuckovic D, Zhang X, Cudjoe E, Pawliszyn J (2010) Solid-phase microextraction in bioanalysis: New devices and directions. J Chromatogr A 1217(25):4041–4060
Watson JT, Sparkman OD (2008) Gas chromatography/mass spectrometry. In: Watson JT, Sparkman OD (eds) introduction to mass spectrometry: instrumentation, applications and strategies for data interpretation, 4th edn. Wiley, Hoboken, pp 571–638
Welz B, Sperling M (2007) Atomic absorption spectrometry. Wiley-VCH, Weinheim
Welz B, Becker-Ross H, Florek S, Heitmann U (2005) High-resolution continuum source AAS, 1st edn. Wiley-VCH, Weinheim
Yehya HG, Wael B (2010) Lab-on-a-chip: techniques, circuits, and biomedical applications, 1st edn. Artech House, Bosten/London
Zwir-Ferenc A, Biziuk M (2006) Solid phase extraction technique – trends, opportunities and applications. Pol J Environ Stud 15(5):677–690

# Benchmark Dose in Regulatory Toxicology

Lutz Edler

## Contents

Dose–Response Data for the Risk Assessment ..... 360
Dose–Response Modeling ..... 361
Benchmark Dose Approach ..... 362
Before Starting the BMD Approach ..... 364
  Select the BMR ..... 365
  Select the DRMs ..... 366
From BMD Modeling to a RP/PoD ..... 367
  Acceptable Models ..... 367
  Selection of the Model for the RP/PoD ..... 368
  Reporting the BMD Approach ..... 369
When Modeling More than One Data Set ..... 369
  Covariate Analysis ..... 370
  One Endpoint Only ..... 370
  Endpoint by Endpoint ..... 371
Data Selection for the BMD Analysis ..... 371
Use of the BMDL for Risk Characterization ..... 372
Illustrations ..... 373
References ..... 374

**Abstract**

The Benchmark-Dose (BMD) approach aims at determining an exposure level/dose corresponding to a predefined change in response (the Benchmark Response – BMR) – usually defined over background – and allows using all available dose–response (DR) information by fitting mathematical models to

L. Edler
Department of Biostatistics –C060, German Cancer Research Center (DKFZ), Heidelberg, Germany
e-mail: edler@dkfz.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_93, 

those data. The confidence interval of the BMD estimate accounts for the statistical uncertainty in the data and the lower (one-sided) confidence limit, denoted BMDL, is used as reference point (RP) or point-of-departure (PoD) for the characterization of the risk of hazardous compounds replacing the no–observed–adverse–effect level (NOAEL) when sufficient DR data are available. The concept, the requirements for application, and practical applications of the BMD approach are presented in this chapter.

## Dose–Response Data for the Risk Assessment

In basic as well as applied research for risk assessment, toxicity studies are performed to identify sources of hazards and risks for human health and for the human environment, including plant and animal health and other critical elements of the eco-system. This step identifies compounds and agents which constitute a hazard and risk. When, in a subsequent step, the hazards and risks such identified should be subject to risk management and control, often the same or extended toxicity studies must provide data for a quantitative risk assessment such that for defined scenarios of the exposure of vulnerable subjects or systems, the risk can also be quantified. More efficient for practical risk management and control than quantifying the risk for a given exposure is a sort of "inverse" assessment where for a given risk level, say $R^*$, and defined in a wider sense by the society, an exposure level, say $D^*$, is determined which assures with sufficient confidence that this risk level $R^*$ is not surpassed when exposure stays at or below the level $D^*$. Such exposure levels, or levels derived there from, have been used to establish so-called health-based guidance values (HBGV) to be implemented in risk management and regulation.

Dose–response (DR) data from human studies or DR studies on experimental animals can provide such data and have been used for decades in risk assessment. This chapter will focus on the assessment of risks of chemicals and compounds in food and diet which has been among the most intensively studied fields of quantitative risk assessment. The methods apply as well to the risk assessment of other consumer products but are different from the assessment of risks from radiation risk, pharmaceutical medicines, or technical origin where also the exposure is of a different quality.

Various approaches have been applied for the risk assessment of hazardous compounds in the past (see, e.g., Edler et al. 2002). Most of those intended to extrapolate from high to low doses (see also the chapter "► Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds" in this volume) usually over several orders of magnitude, resulting in risk descriptors of high uncertainty. The BMD approach does not claim to be without uncertainty but to investigate and describe the hazard and risk at a dose range where it can be characterized and to separate that from the extrapolation to doses of

human exposure. Therefore, it is more transparent than previous approaches. Common to all these approaches is, however, a thorough analysis of available dose–response data.

## Dose–Response Modeling

Quantitative risk assessment using DR data and aiming at the relationship between the effect $R^*$ and the dose $D^*$ must be based on a specific concept of DR modeling and methods of statistical inference which suit this concept. In general, the experimental data consist of two generic parts. On the one side, there are the doses chosen or observed by the investigator. Except in human studies where a continuum of exposure doses could prevail, one usually faces a set of doses, say $I$, denoted $d_1, d_2, \ldots, d_I$ and a control group denoted by the "dose" $d_0$, where the doses are ordered by their amount as $0 < d_1 < d_2 < \ldots < d_I$ and where $d_0 = 0$. On the other side are the responses of, say $n_i$, subjects exposed to dose $d_i$ which result in $n_i$ responses $Y_{ij}$ , $j = 1, \ldots, n_i$ , $i = 0,1, \ldots, I$. When considering tumor incidence in a carcinogenesis experiment (so-called quantal or dichotomous data), the response would be either $Y_{ij} = 1$ (for tumor) or $Y_{ij} = 0$ (for no tumor) in the jth animal of the ith dose group and the data are then summarized as $\{d_i, n_i, p_i), i = 0,1, \ldots I\}$ when $r_i$ denotes the number of tumors in dose group $i$ and $p_i = r_i/n_i$ the proportion of tumor bearing individuals. When considering quantitative or continuous data, the responses $Y_{ij}$ usually represent measurements of biologically relevant parameter values, ranging between the value $0$ and a maximum value (non-negative values) of that parameter observed in the experiment which varies from experiment to experiment. Obviously, the type of DR modeling depends decisively on the nature of the responses $Y_{ij}$ (see the chapter "► Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds"). This chapter will focus mostly on the BMD approach for *quantal data* which has been the most prominent type of data subject to BMD analyses for the risk assessment in the past and will sketch the application to quantitative data only. A short overview on the BMD approach with a generic figure explaining the main features has been given in chapter "► Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds".

Before starting a DR analysis of toxicity study, the risk assessor is advised to Identify the kinds of data available on dose and response, Select the response and dose metric for assessment, Present and discuss the data of the study, e.g., using graphical presentations, Discuss the results of such preliminary descriptive analyses in terms of quality of data, Available, and the assessment aims to be achieved or achievable in the view of his/her assessment problem. There have been ongoing discussions on the framework of the risk analysis within which DR modeling is embedded as one of the most relevant steps of risk assessment at the interface to risk characterization (see Renwick et al. 2003; Abt et al. 2010) which can be consulted.

## Benchmark Dose Approach

The BMD approach has been introduced by Crump (1984) after related discussions within US EPA and when the paradigm of risk assessment established by the US National Research Council (NRC 1983) as "A new method for determining Allowable Daily Intakes" with the explicate motivation for replacing the no–observed–effect–level (NOEL) approach because of the potential shortcoming of the NOEL and the no–observed–adverse–effect–level (NOAEL), see also Dourson et al. (1985) and Murell et al. (1998). These shortcomings have been reiterated and confirmed in numerous investigations and reviews since then (EPA (1995, 2012); Edler et al. (2002); IPCS (2009); EFSA (2009)) and are not repeated and discussed in this chapter. At the same time, the movement for the BMD approach has been a response to the problems which were faced by risk assessors when using mathematical models for the extrapolation to low doses and observing differences in the risk estimates and HBGV of several orders of magnitude depending on the extrapolation model/method used.

Therefore, the BMD approach was proposed that defines an exposure level or dose (the benchmark dose, BMD) which produces a small but measurable effect (usually over the background effect) that is large enough for not critically depending on the mathematical models used and which is small enough to represent a relevant adverse effect in the context of the data generated in the toxicity study. To account for the statistical variation of the toxicity data, the statistical lower one-sided confidence bound (mostly called confidence limit in the BMD context) of the BMD was calculated to be used as Reference Point (RP) or Point-of-Departure (PoD) for the risk characterization (EFSA 2005). That lower one-sided confidence limit is calculated for the statistical confidence level of 95 % and denoted as BMDL (benchmark dose lower confidence limit).

The effect over background is a critical value to be chosen during the risk assessment and is denoted as benchmark response (BMR) level. The BMDL can then be interpreted statistically as a dose level at which it can be assured (with 95 % confidence) that in the experimental context, the selected BMR is not exceeded. For example, when a BMR of 10 % is chosen as critical effect size of tumor incidence over background, the corresponding BMDL value is denoted as $BDML_{10}$ (where the subscribed 10 represents the BMR = 10 %,) and can be interpreted easily as that dose where the effect is likely smaller than 10 %. The term "likely" is precisely defined by the (one-sided) statistical confidence level which is usually set to 95 % confidence in toxicological and epidemiological research. It should be noted that the BMR is not defined as a change with regard to the *observed mean* background response, but with regard to the background response *predicted* by the fitted model. This distinction is important because, in general, the fitted curve does not hit the observed background response exactly such that simply adding the BMR to the observed background response will in general not provide the correct intersection with the dose–response at dose 0 which may confuse some readers when checking published results (see Fig. 1 in EFSA (2009)).

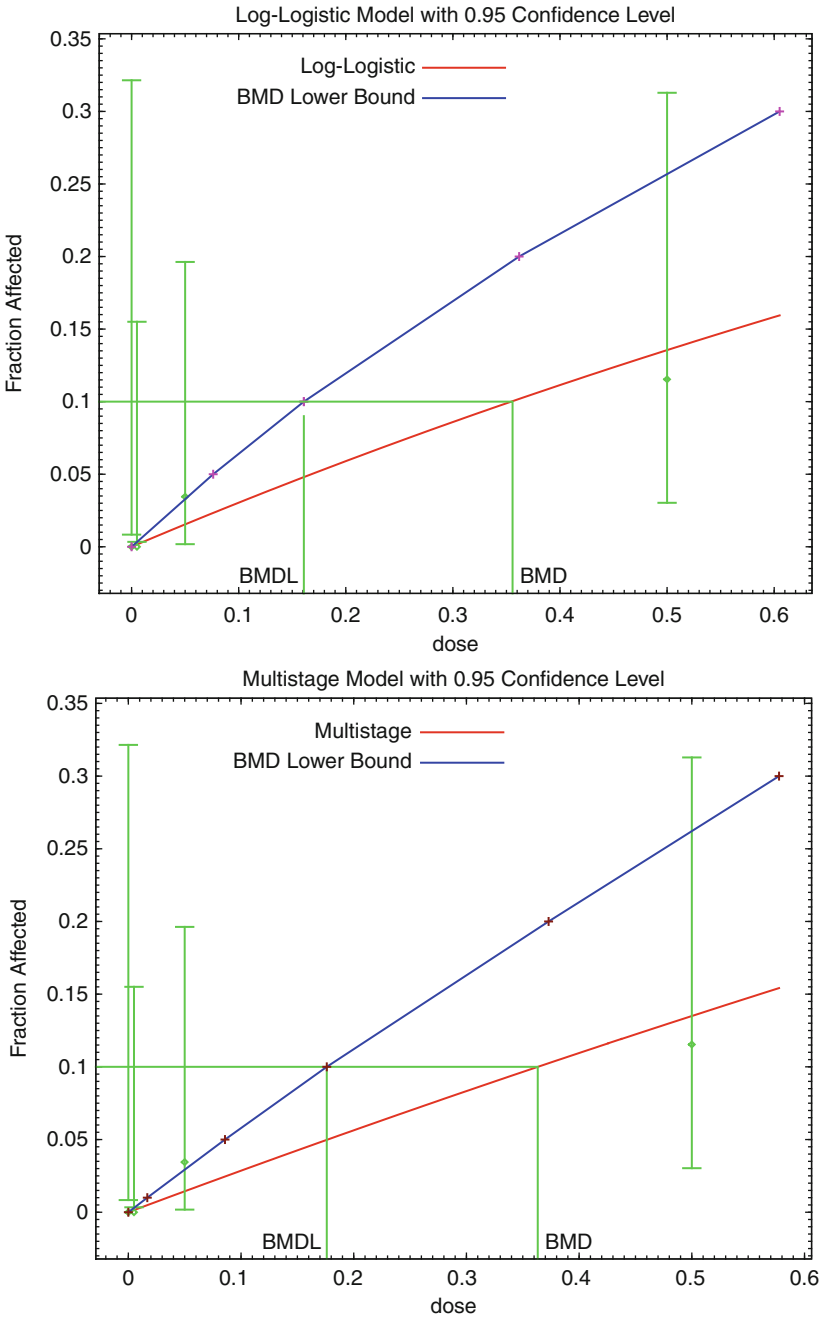



**Fig. 1** Graphs to determine the BMD. In each of the two graphs the fitted model curve (in BMDS software in red) is below the BMDL-curve (in BMDS software in blue). The underlying DR data are reported in Table 1

The BMD approach accounts for the statistical variability of the dose–response data by calculating the confidence bounds, an important difference compared to the NOEAL approach. Primarily, statistical uncertainty of the BMD is addressed through its confidence interval ranging from the one-sided lower bound (the BMDL) to the one-sided upper bound (the BMDU). With the default confidence level at 95 % (one-sided), the interval (BMDL, BMDU) is a two-sided confidence interval of the BMD at the level of 90 % which can also be used as a means to express the variability of the BMD. The BMDL–BMDU interval covers exclusively, but completely, the uncertainty of the underlying data. Properties of the design of a study, e.g., the choice of the number and location of dose levels and also the sample sizes of each dose level, are covered by the BMDL and BMDU only as far as the models fitted to these data allow for. Therefore, general design issues may add to the uncertainty of the BMD and as such also of the BMDL.

Furthermore, one should note that risk assessors, toxicologist, epidemiologists, and statisticians could not agree completely on the notion of *effect over background* or *risk over background*. However, when assessing cancer incidence, one has largely agreed to consider at first place the *extra risk* defined as the ratio $[R(d)-R(0)]/[1-R(0)]$ where $R(d)$ denotes the probability of the occurrence of cancer (usually) over lifetime at dose $d$ and where $R(0)$ denotes the respective background probability (at dose $d = 0$). Extra risk with that definition is now widely used as the default endpoint for risk assessment. Similarly, when continuous effects are analyzed, the relative effect size defined as the ratio $[Y(d)-Y(0)]/[Y(0)]$ is used where $Y(d)$ denotes the effect size at dose $d$ and where $Y(0)$ denotes the respective background effect size (at dose $d = 0$).

It has been already mentioned that the BMD approach controls the response level at the place BMDL. This is in contrast to the NOAEL where the response level at that NOAEL is not controlled and can be rather high, in particular when the sample size at the dose point of the NOAEL is small and such the precision of the response level estimated at the NOAEL is low. A second *advantage* of the BMD approach over the NOAEL is that the approach attempts to use all available dose–response information by fitting a mathematical model to the data. The NOAEL is locked at one dose level chosen when designing the toxicity study.

The so-called hybrid approach where originally available continuous response data are transformed to quantal data is not discussed in this document, see Gaylor & Slikker (1990) and Falk Filipsson et al. (2003).

## Before Starting the BMD Approach

When a data set, e.g., represented as a dose–response curve with response levels $Y_i$ at doses $d_i$, has been chosen to be appropriate for a BMD analysis, the specification of the relevant toxicological effects and the models to analyze that effect are decisions upfront to make the approach working at all, as indicated in Box 1.

**Box 1**
Essential steps for calculating a BMDL from one single DR data set/DR curve for one DR model
- Specification of a low but measurable response level, e.g., a 5 % or 10 % increase or decrease in response compared with the background response: BMR
- Fitting the dose–response model using statistical and computational methods including appropriate software
- Estimating the BMD and deriving the BMDL as two parameter/dose descriptors accounting for statistical criteria of model fit and robustness of modeling

The two most relevant prerequisites are the selection of the BMR and the dose–response models (DRMs) addressed next.

## Select the BMR

When analyzing DR data for a risk assessment project, the choice of the type and the size of BMR is one of most important but also most difficult decisions to be made at the very beginning which needs both toxicological and statistical reasoning. Toxicological expert knowledge should assess the chosen critical endpoint for possible sizes of changes over background and their biological (eventually also medical) and public health relevance.

Statistical expertise should inform on the practicability in view of the available DR data. These considerations would be related to a discussion of the *design of the toxicity study* and its influence on the modeling. This issue cannot be further detailed in this chapter except noting that precise statistical methods for defining optimal designs unfortunately do not exist at present for BMD modeling. However, it has been noted that studies with more dose levels and less animals per dose would be preferred over studies with only few dose groups and a large number of animals in each. Deficiencies in study design usually result in lowered BMDLs. This is in striking contrast to the behavior of the NOAEL which tends to increase with inferior designs and would such be less protective. Therefore, DR modeling using the BMD approach informs indirectly on the quality of the toxicity database. Some researchers have therefore suggested running the BMD analysis over all data of toxicity studies identified for risk assessment questions as a sort of screening for good quality DR data.

It has been advocated that the BMR should be set equal to a low but measurable response level reflecting an effect that is negligible or non-adverse. Obviously, a too low BMR would normally result in an extrapolation outside the range of the observed data and induce severe model dependence of the BMD and the BMDL. Therefore, a practically useful BMR may not be such small in practice.

For quantitative continuous data, a percentage change in the average magnitude of the response variable compared to the predicted background response would define the BMR (see the ratio $[Y(d)-Y(0)]/[Y(0)]$ above). In that case, EFSA (2009) recommended a BMR = 5 % (e.g., a 5 % decrease in red blood cells) as a default value if there would exist no toxicological reasons to deviate. In the case of quantal or dichotomous data (e.g., cancer incidence in animal studies), an extra risk BMR of 10 % has been established as default. Obviously, this choice is far from any acceptable human risk level which has been set in the past to range between $10^{-4}$ and $10^{-6}$, corresponding to BMRs between 0.01 % and 0.0001 %. However, setting the BMR much lower than 10 % would usually not comply with the sensitivity of most cancer bioassays. Exceptionally, for large carcinogenicity bioassays of more than thousand animals, a BMR = 1 % has been used. Therefore, the default value of BMR = 10 % for quantal data must be viewed as a compromise between measurability and relevance. Tendencies to define the BMR even higher, e.g., at response levels of 25 % and even 50 % could occasionally be reasonable for statistical grounds, but one should note that this need could be simply an indication for deficits in the study design and the BMD approach cannot compensate therefore. The approach has not been intended for high BMRs.

## Select the DRMs

Fortunately, for the risk assessors, a standard set of dose–response models for risk assessment evolved since the introduction and the methodological research of the BMD approach and those are also available in the two currently most used software packages BMDS (EPA 2012) and PROAST (http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST). Although tailored selection and definition of models can be an option in special cases, there has been some agreement among most modelers to consider those models in routine BMD analyses. Eight dose–response models have been recommended to apply for quantal/dichotomous data:

- Probit
- Log-Probit
- Logistic
- Log-logistic
- Weibull
- Multistage family (with the special case of a multistate cancer model)
- Quantal-Linear
- Gamma-Multihit

see (EFSA 2009; EPA 2012). This set is thought to be flexible enough to cover a wide range of dose–response relationships of tumor incidence. Two model families with members of different degree of complexity have been recommended for quantitative/continuous data

- Exponential family
- Hill family

(see Crump 2002; Slob 2002). Note that all these models and members of model families are defined by a structural form, which is the model equation, and the model parameters, which are allowed to range over a large range of values limited only by being monotonous and not including artifacts (such as negative incidences). The slope of a dose–response model is therefore always positive for quantal models, but can be positive or negative for quantitative models depending into on direction a biological parameter reacts in the organism when that is exposed to a toxic compound.

By imposing additional constraints on model parameters, one can restrict the possible range of modeling separately for each model. This is, in particular, an option in the BMDS software and has to be used with great care (check for default settings!).

An often used constraint excludes dose–response curves which have a steep (up to infinite slope) at the origin (i.e., at dose =0). Those curves can be excluded by restricting the slope at $d = 0$ to be not higher than a fixed value, say 1. However, it has also been argued that this option should be avoided and that the full range of model parameters should be allowed for each model. For the log-logistic Weibull and Gamma models, such constraints translate into constraints of the shape parameter, usually denoted by c, such that $c > 1$ in the software. It should be noted that so far, no criteria have been developed to guide risk assessors in the use of constraints. It has also been recommended to examine visually the shape of the fitted DR curve and check how the values of the parameters in a model, such as the BMD and the BMDL values, react during model fit. As a default, it is recommended not to constrain the model parameters as long as there are no convincing biological arguments. From a statistical point of view, keeping the space of the model parameters as wide as possible is fortunate anyway, since it reduces the chance that model parameters hit boundaries in the parameter space which needs computational expertise for appropriate interpretation.

## From BMD Modeling to a RP/PoD

When a data set has been modeled as described in Box 1, the outcome needs to be analyzed accounting for statistical properties of the model fit. Furthermore, its value for answering the toxicological questions must be expressed and the results in terms of BMD and BMDL values must be summarized in view of the quality and variety of DR data of the toxicity studies selected at the very beginning of the risk assessment.

### Acceptable Models

In principle, model fitting means finding those values of the model parameters that move the (statistically estimated) DR curve as closely as possible to the observed data points. This is easy when fitting a straight line to data where the model fit can truly be seen as a sort of trial and error exercise but can also be performed using an explicate mathematical formula bases on the Gaussian least square method.

The complexity comes when several parameters have to be found, when the model is nonlinear such as all the above listed models are, and when there is noise in the data which is difficult to grasp. When models are nested, such as the two families of the quantitative data and the multistate model family for quantal data, the log-likelihood criterion can be used to find the optimal model. Otherwise, determination of an optimal model is much more difficult, although the log-likelihood still bears valuable information which is used to assess model fit and yields a $p$-value of goodness-of-fit which is also part of the output of BMD software.

There is also a general rule by starting with a simple model (with few parameters), and then checking whether adding a parameter to the model results in a significant improvement of the fit and to account the goodness-of-fit against the number of model parameters needed.

A principle to resolve the problem of finding a best fitting model has been developed by EFSA (2009) by claiming that the BMD approach should not aim to find the single statistically best fitting models and its BMD/BMDL values but rather to identify all plausible models and their BMD/BMDL values that are compatible with the data. Therefore, it is required to find those models with an acceptable fit. A model from the set of the above defined models is marked as *acceptable* when the goodness-of-fit test results in a p-value greater than 0.05 when testing the fitted model against the so-called *full model*. The full model simply consists of the observed (mean) responses at each applied dose and it just "interpolates " the observed DR curve. Hence, the number of parameters equals the number of dose groups. If a model's fit is not significantly worse than that of the *full model*, then the model may be accepted. This kind of goodness-of-fit test is a "nonsignificance" test and therefore a large p-value, e.g., $p > 0.05$, indicates a good fit. In addition, an acceptable model should also reflect substantial dose–response information. Therefore, the model should be statistically significant ($p < 0.05$) different from the *reduced model* reflecting no DR relationship. Usually, the reduced model (also called null model) is a straight line parallel to the dose axis representing the mean response. In summary, the statistical fit of an acceptable model should be statistically significantly better than the reduced model ($p < 0.05$) and not significantly worse than the full model ($p > 0.05$). In cases where none of the models pass these tests, visual inspection of the data may show that some models still adequately describe the observed DR. In that case, the decision to accept a particular model needs to acknowledge the high level of uncertainty in the BMD and the BMDL value. It should be noted that the choice of the significance levels of 0.05 above is based more on convention than on statistical reasoning and should therefore be considered as a default value.

## Selection of the Model for the RP/PoD

The above described approach to consider all acceptable models from the fit of a data set has consequences when the BMD/BMDL values identified for those models are summarized to determine a RP. Having defined a range of acceptable models,

the model with lowest BMDL value has been proposed to represent the RP resulting from that DR analysis. Following this strategy, one may face data sets where an RP appears problematic, e.g.,

- When the BMDLs among models are very different and vary by several orders of magnitude including a BMDL = 0
- When the BMDL is substantially lower than the BMD, e.g., when the BMD/BMDL ratio is larger than 10, i.e., one order of magnitude and therefore, the BMDL is in the region of low-dose extrapolation

Such a situation would require again a visual inspection of the data and a qualitative comparison of the shape of the fitted curves with that expected from biological reasoning. Guidance of EFSA (2009) is such that BMDLs and BMD/BMDL ratios should not vary by more than one order of magnitude. Otherwise, further examination of the DR data is recommended and further measures may be taken, e.g., fitting constrained models, changing the size of the BMR, deleting high doses from the analysis. The overall quality of a study could be put on stake with the option to deny suitability of the data for risk assessment, see next section.

*Model Averaging* has been developed recently as an alternative to the selection of the minimum BMDL using Bayesian methods, see e.g., Wheeler and Bailer (2007). This approach combines the estimates of the different models, not distinguishing between acceptable and non-acceptable models, through a weighted average of the DR models where the weights reflect the relation of the fitted curves to the observed data. The method assumes that the true model is one of the models in the family of models being averaged and it reflects both the sampling variability and model uncertainty. It is expected to yield an RP higher than the lowest BMDL.

A *stepwise and decision tree based procedure* has been proposed by Davis et al. (2010) which is iterated in the recent guidance document of EPA (2012). This differs from the EFSA approach outlined above and uses an adaptive approach to find the best fitting model in contrast to the EFSA approach which is based on finding all models which are compatible with the DR data.

### Reporting the BMD Approach

A comprehensive scheme for reporting the outcome of a BMD analysis has been compiled in EFSA (2009) and iterated in EFSA (2011). Besides providing information on all endpoints, it is advisable to justify any decisions made during the BMD analysis, e.g., regarding model selection, and to include in the report information on data sets and studies that were not used. Published examples of BMD analysis discussed below may be contacted therefore.

## When Modeling More than One Data Set

When performing a risk assessment on a compound, one has to examine often more than one toxicity study and each study may report more than one DR data set for

a selected critical endpoint. Furthermore, a risk assessment may be based on more than just one endpoint, and therefore, a multiplicity of data sets and studies may prevail for a set of critical/pivotal endpoints for the risk characterization. In that situation, the determination of a RP may follow a stepwise process accounting for (i) the selected critical responses, (ii) the selected studies where those endpoints have been investigated, and (iii) the study data sets available from each study.

## Covariate Analysis

Before considering this hierarchy of data in detail, one should mention that in contrast to other approaches, the BMD approach is suitable for analyzing the effects of covariates, such as sex, exposure duration, or even co-exposure to another chemical, see EFSA (2009). For example, the factor sex can be included as a covariate in the DR analysis, and in this way, it can be assessed if males and females show statistically significantly different DRs, e.g., using PROAST software (http://www.rivm.nl/en/Documents_and_publications/Scientific/Models/PROAST; see also Slob, 2002). When the DRs for the levels of males and females or other subpopulations are found to have a similar form, the DR data from both sexes can be combined. Such a combined BMD analysis would then be based on a larger sample size than when males and females would be analyzed separately. Therefore, a covariate adjusted BMD anlysis could result in a more appropriate RP value than a BMD analyis where each data set related to the specific covariate is analyzed separately and when the resulting BMDL values must be summarized to determine a "joint" RP. Likewise even DR data from different studies with similar characteristics could be combined, with study as a covariate, again making use of all data available in one evaluation. Note that a covariate adjusted BMD analysis should not be based on statistical reasons only but accompanied by thorough biological and toxicological reasons allowing for the combination of the data sets in the case of the chosen endpoint of response, see also next sections.

## One Endpoint Only

When several data sets are available for a single endpoint from one toxicity study from varied experimental conditions, e.g., different dosing schedules, different species, and/or for both sexes, one would at first analyze each data set separately as described above and derive the RP (e.g., the lowest BMDL) for each. Next one may investigate which of the DR data sets could be combined and subject to a covariate-adjusted evaluation. Therefore, the size of the response, the shape of the observed DR curves, and the biological background would be investigated. Such combinations using covariate adjusted BMD analyses could reduce the number of BMDL values for that single endpoint. But even then several BMDL values

may coexist from different data sets and in order to establish a HBGV a RP (e.g., the lowest BMDL or the mean) must be determined from the available ones.

When several studies are available with data sets for the same endpoint, one may again examine whether these data sets can be combined for a comprehensive BMD analysis with study and or source of data within a study as a covariate. A joint RP for establishing a HBGV could be determined using a procedure similar to that described above for several data sets available for one study.

When the data sets are too heterogeneous, e.g., when originating from different studies performed at different sites and times and, in particular when performed under different designs, one may decide against combining and choose as RP the lowest BMDL obtained from all acceptable models of all data sets available.

### Endpoint by Endpoint

When more than one response has been identified as relevant, the BMD analysis would ideally be performed at first endpoint by endpoint, starting with the most relevant for the risk assessment. That could be an endpoint with highest toxic potency and likely the lowest BMD and BMDL values. For example, when the compound would be identified as a genotoxic carcinogen, the BMD approach would be restricted to carcinoma incidence for the risk characterization and the margin of exposure (MoE) approach would be applied (see chapter "► Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds"). Nevertheless, one may even for genotoxic carcinogens be interested also in safety margins for other endpoints, e.g., for a neurological endpoint, and perform further BMD analysis of respective DR data, when available. That evaluation would be judged as secondary, and it may suffice just to tabulate the BMDL values and the RPs determined from these analyses.

Otherwise, when several endpoints are judged as equally relevant, one would proceed in the same way for each of them and determine a set of endpoint specific RPs. Pragmatically, one may define a study BMDL by taking the lowest RP in a conservative approach. Alternatively, one would keep the specific RPs separately and assess them together with their uncertainties in a comprehensive risk characterization step.

## Data Selection for the BMD Analysis

At the very beginning of planning a BMD analysis for risk assessment is the step of the problem formulation and the selection of data. ILSI Europe has hosted recently an Expert Group on this subject with results and recommendations under progress for publication (ILSI to appear). This work addresses very general issues including the human relevance of tumor data and an extended analysis of the uncertainty

comprising also statistical issues. Regarding specific issues related to the BMD approach, it was realized among others that:

- The observed shape of the DR curve alone is not a sufficient criterion for selecting DR data since the biological relevance of effects, e.g., a deviation from monotonicity, must be weighed against the statistical significance of model fitting. Statistical model selection does not overrule available biological DR information.
- Attempts to qualify data through prescreening DR data for their suitability for the BMD approach may not be without arbitrariness due to the multiplicity of testing and the absence of statistical rules of how to set the significance level for a sequence of goodness-of-fit tests.
- Testing for the presence of a DR relationship, e.g., using a trend test, could be misleading, when all effects remain below the primary chosen level of the BMR.
- An in-depth examination of the utility of studies is recommended before a DR analysis is performed.

It was also emphasized that suitability of data for the BMD approach should not be separated from the quality of a toxicity study itself. General criteria for rating the quality of toxicity studies have been proposed by Klimisch et al. (1997) and were further specified recently by Schneider et al. (2009). When selecting DR data, one should consider, in particular, species, strain, and sex differences in sensitivity to the test substance in target organs.

Most relevant for the size and precision of the BMD and BMDL are number and range of dose levels. Preferably, one would use individual weight data for the analysis to calculate the BMD and BMDL in the relevant units, e.g., in mg per kg b.w. per day.

Generally, agreed criteria to judge the quality of a study for a BMD analysis are difficult to establish and, at present, not available. However, exisiting quality standards regarding the test material used in the bioassays of the studies and guidance available for the conduct and reporting of bioassays (including, e.g., genetic origin, housing and health status of the animals) should not be different for data used for the BMD analysis than for any other regulatory purposes, see therefore a fortcoming paper of an ILSI Expert group (ILSI to appear).

## Use of the BMDL for Risk Characterization

The use of the BMDL as RP/PoD depends on the nature of the critical effect and the mode of action of this or related toxicity endpoints identified to be used for risk characterization of a specified compound, see chapter "► Extrapolation-Procedures for Carcinogenic and Noncarcinogenic Compounds". When characterizing the risk of compounds which are both genotoxic and carcinogenic and for which the MOE approach would be the most appropriate approach, the $BDML_{10}$ is used as RP serving as the numerator of the MOE defined as the ratio of exposure over RP. When establishing an HBGV (e.g., an ADI or TDI), uncertainty factors (UFs) are applied to the BMDL in the same as for the NOAEL. Additional uncertainty factors,

beyond those regularly applied, are not necessary. However, when data are sparse and modeling is not robust or when a larger than the default BMR value is chosen to adjust for the specific shape of a DR curve, an additional UF may be necessary.

DR data from observational epidemiological studies may differ from typical animal toxicity data, e.g., not falling into a small number of dose groups, not including an unexposed control group; however, the BMD approach still applies, but a more careful check for the appropriateness of the data is indicated and the influences of confounders on the DR relationship should be addressed and, if possible, modeled.

## Illustrations

Examples and illustrations on the application and the outcome of a BMD analysis have been reported already in Crump (1984). The guidance documents on the BMDS software developed by the US-EPA (EPA 2012) and the guidance document of the EFSA on the benchmark-dose approach (EFSA 2009) exhibit additional examples both for quantal and for quantitative data. The summary from a tutorial held by EFSA in 2010 EFSA (2011) provides details on how to use the BMDS and the PROAST software with application of the approach to quantal data available for melamine (EFSA 2010) and for a more complex set of quantitative data on an OP ester which is analyzed in detail using PROAST and where also covariates are considered.

Opinions of EFSA on heavy metals and mycotoxins demonstrate the applicability and use of the BMD approach at several occasions and for a variety of DR data, even in cases where the approach reaches its limits. The opinion on nivalenol (EFSA 2013b) used the PROAST software to calculate BMDLs for quantitative white blood cell counts from a 90-days rat study combining the DR data of male and female rats to establish a tolerable daily intake of 1.2 μg nivalenol/kg b.w. per day.

A typical reporting of BMD results can be found in the opinion of the EFSA on sterigmatocystin (EFSA 2013a) where a simple data set on the incidence of hemangiosarcomas in rats was analyzed to obtain guidance for the generation of future exposure data to be used to complete the risk characterization of this compound which remained incomplete due to the lack of exposure data. In this case, rats were dosed at 0, 0.005, 0.05, and 0.5 mg sterigmatocystin/kg b.w. per day and exhibited tumor incidences of 0/11, 0/27, 1/29, and 3/26, respectively. Although these data were rather sparse, a BMDL = 0.16 mg sterigmatocystin/kg b.w. per day could be calculated, see Table 1. All dichotomous models satisfied the log-likelihood goodness-of-fit acceptability criteria when compared to the null model and the full model (Fig. 1). The $BMD_{10}$ ranged between 0.36 and 0.50 mg/kg b.w. per day and the $BMDL_{10}$ ranged between 0.16 and 0.34 mg/kg b.w. per day. The lowest $BMDL_{10}$ of 0.16 mg/kg b.w. per day was obtained for the log-logistic model, and this was only slightly smaller than that of the multistage model family.

**Table 1** BMD and BMDL values for the incidence of hemangiosarcomas reported by Maekawa et al. (1979) calculated for a BMR of 10 % for rats dosed at 0, 0.005, 0.05, and 0.5 mg sterigmatocystin/kg b.w. per day and the incidence 0/11, 0/27, 1/29, and 3/26, respectively

| Quantal data models | Restriction used? | No. of model parameters | Minus log-likelihood | Goodness of-fit *P*-value | Accepted (yes/no) $p > 0.05$ | $BMD_{10}$ (mg/kg b.w./d) | $BMDL_{10}$ (mg/kg b.w./d) |
|---|---|---|---|---|---|---|---|
| **Full model** | na | 4 | 13.65 | – | – | – | – |
| **Null (reduced) model** | – | 1 | 16.50 | – | – | – | – |
| **Probit** | na | 2 | 14.36 | 0.49 | **Yes** | 0.48 | 0.33 |
| **Log-probit** | None | 2 | 14.50 | 0.19 | **Yes** | 0.50 | 0.28 |
| **Logistic** | na | 2 | 14.40 | 0.48 | **Yes** | 0.49 | 0.34 |
| **Log-logistic** | None | 1 | 13.99 | 0.88 | **Yes** | 0.36 | 0.16 |
| **Quantal-linear**[a] | na | 1 | 14.03 | 0.86 | **Yes** | 0.36 | 0.18 |
| **Multistage cancer**[a] | na | 1 | 14.03 | 0.86 | **Yes** | 0.36 | 0.18 |
| **Multistage**[a] | None | 1 | 14.03 | 0.86 | **Yes** | 0.36 | 0.18 |
| **Weibull**[a] | None | 1 | 14.03 | 0.86 | **Yes** | 0.36 | 0.18 |
| **Gamma**[a] | None | 1 | 14.03 | 0.86 | **Yes** | 0.36 | 0.18 |

*b.w.* body weight, *BMD* benchmark dose, $BMDL_{10}$ 95 % lower confidence limit of the benchmark dose with BMR = 10 % extra risk, *na* not applicable

[a]the three versions of the multistage model and the Weibull and Gamma model fitted the data with the same model

## References

Abt E, Rodricks JV, Levy JI, Zeise L, Burke TA (2010) Science and decisions: advancing risk assessment. Risk Anal 30:1028–1036

Crump K (1984) A new method for determining allowable daily intakes. Fundam Appl Toxicol 4:854–871

Crump K (2002) Critical issues in benchmark calculations from continuous data. Crit Rev Toxicol 32:133–153

Davis JA, Gift JS, Jay Zhao Q (2010) Introduction to benchmark dose methods and U.S. EPA's benchmark dose software (BMDS) version 2.1.1. Toxicol Appl Pharmacol 254:181–191

Dourson ML, Hertzberg RC, Hartung R, Blackburn K (1985) Novel approaches for the estimation of acceptable daily intake. Toxicol Ind Health 1:23–41

Edler L, Poirier K, Dourson M, Kleiner J, Mileson B, Nordmann H, Renwick A, Slob W, Walton K, Würtzen G (2002) Mathematical modelling and quantitative methods. Food Chem Toxicol 40:283–326

EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. Available under http://www.efsa.europa.eu/en/efsajournal/doc/282.pdf

EFSA (2009) Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. EFSA J 1150:1–72

EFSA (2010) Scientific opinion on melamine in food and feed. EFSA J 8(4):1573

EFSA (2011) Use of BMDS and PROAST software packages by EFSA Scientific Panels and Units for applying the Benchmark Dose (BMD) approach in risk assessment. EN-113. p 190

EFSA (2013a) Scientific opinion on the risk for public and animal health related to the presence of sterigmatocystin in food and feed. EFSA J 11(6):3254, 81. doi:10.2903/j.efsa.2013.3254
EFSA (2013b) Scientific opinion onrisks for animal and public health related to the presence of nivalenol in food and feed. EFSA J 11(6):3262, 119. doi:10.2903/j.efsa.2013.3262
EPA (1995) The use of the benchmark dose method in health risk assessment. U.S. Environmental Protection Agency, Washington, DC 20460, Report EPA/630/R-94/007
EPA (2012) Benchmark Dose Technical Guidance. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC 20460, EPA/100/R-12/001
Falk Filipsson A, Sand S, Nilsson J (2003) The benchmark dose method – review of available models, and recommendations for application in health risk assessment. Crit Rev Toxicol 33:505–542
Gaylor DW, Slikker W Jr (1990) Risk assessment for neurotoxic effects. Neurotoxicology 11:211–218
ILSI (to appear) Selection of appropriate tumour data sets for Benchmark Dose Modelling (BMD) and derivation of a Margin of Exposure (MoE) for substances that are genotoxic and carcinogenic: Considerations of biological relevance of tumour type, data quality and uncertainty assessment
IPCS (2009) Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. A joint publication of the Food and Agriculture Organization of the United Nations and the World Health Organization. WHO Press, Geneva
Klimisch H-J, Andreae M, Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5
Maekawa A, Kajiwara T, Odashima S, Kurata H (1979) Hepatic changes in male ACI/N rats on low dietary levels of sterigmatocystin. Gann 70:777–781
Murrell JA, Portier CJ, Morris RW (1998) Characterizing does-response I. Critical assessment of the benchmark dose concept. Risk Anal 18:13–26
NRC (1983) Risk assessment in the federal government: managing the process. National academy of sciences. National Academy Press, Washington, DC
Renwick A, Barlow SM, Herz-Picottio I, Boobis AR, Dybing E, Edler L, Eisenbrand G, Greig JB, Kleiner J, Lambe J, Müller DJG, Smith MR, Tritscher A, Tuijtelaars S, van den Brandt PA, Walker R, Kroes R (2003) Risk characterization of chemicals in food and diet. Food Chem Toxicol 41:1211–1271
Schneider K, Schwarz M, Burkholder I, Kopp-Schneider A, Edler L, Kinsner-Ovaskainenc A, Hartung T, Hoffmann S (2009) "ToxRTool", a new tool to assess the reliability of toxicological data. Toxicol Lett 189:138–144
Slob W (2002) Dose–response modelling of continuous endpoints. Toxicol Sci 66:298–312
Wheeler MW, Bailer AJ (2007) Properties of model-averaged BMDLs: a study of model averaging in dichotomous response risk estimation. Risk Anal 27:659–670

# Uncertainty Analysis in Exposure Assessment. Relevance for Regulatory Toxicology

Michael Schümann, Özkaynak Haluk, and Alexandre Zenié

## Contents

Introduction ..... 378
Development of a Regulatory Status of Uncertainty Analysis ..... 379
Importance of Uncertainty Analysis ..... 379
Rationale for Characterizing Uncertainty in Exposure Assessment ..... 381
Definition of Assessment Objectives ..... 381
Sources of Uncertainty in Exposure Assessment ..... 382
Scenario Uncertainty ..... 383
Model Uncertainty ..... 383
Parameter Uncertainty ..... 386
Evaluating the Total Impact of Uncertainty ..... 388
Model Evaluation and Data Calibration ..... 388
Sensitivity Analysis ..... 390
Interpretation of Uncertainty Characterization Results ..... 392
References ..... 393

**Abstract**

Exposure assessment is a core step in the risk regulation process. There are many potential sources of uncertainty in exposure assessment, such as inadequate scenarios, insecurity about the ambient substance concentrations, analytical

M. Schümann (✉)
Department of Health and Consumer Protection, Hamburg, Germany
e-mail: michael.schuemann@bgv.hamburg.de; Michael.Schuemann@hamburg.de

Ö. Haluk
U.S. Environmental Protection Agency (EPA), Durham, NC, USA
e-mail: ozkaynak.haluk@epa.gov

A. Zenié
Joint Research Centre, European Commission, Ispra, Italy
e-mail: alexandre.zenie@ec.europa.eu

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_111, © Springer-Verlag Berlin Heidelberg 2014

errors, or unsuitable population parameters. It is essential for the exposure assessor to identify these uncertainty sources qualitatively and quantitatively, and to communicate the results with the partners of the risk management process.

## Introduction

Communicating the results of an exposure assessment that is based on model assumptions and numerical estimates is demanding; communicating the inherent uncertainties at the same time makes the task complex. An exposure analysis relies on information on the concentrations of a pollutant in an exposure media, on the circumstances and the human behavior and the activities that result in contact and exposure, as well as on the transfer rates from the exposure media to the individual. Exposure increases the internal dose when the agent is transferred into and taken up by the body. Any exposure assessment includes assumptions with respect to appropriate exposure scenarios, in relation to the models that should reflect the selected exposure scenarios and with regard to the type and quality of available data that characterizes the exposure conditions described for a population or a subgroup of concern. Risks cannot be reliably estimated if exposures and their uncertainties are not properly characterized and, if necessary, sufficiently quantified (IPCS/WHO 2008). Any risk quantification relies on good measurement or appropriate estimates of influential variables. Since valid exposure assessment is a core element in risk assessment, any inherent uncertainty will influence the quality of an assessment.

Knowledge of the exposure is a basic prerequisite for risk characterization and risk management strategies. The role of exposure assessment is to provide information about the distribution of expected magnitude of exposure, the source, the route of exposure, and the individuals who are exposed. Uncertainty in risk assessment is defined by IPCS/WHO (2004) as "imperfect knowledge concerning the present or future state of an organism, system, or (sub)population under consideration." The evaluation of uncertainty has qualitative, quantitative descriptive and prognostic aspects.

Variability and heterogeneity refer to the natural variations in the environment, exposure paths, and susceptibility of subpopulations. They should be seen as inherent characteristics, which cannot be controlled by the exposure assessor or the decision makers. Variability and heterogeneity cannot be reduced by collecting more information, only by a stepwise selection of more homogeneous subgroups (stratification of analysis).

Uncertainty in exposure assessment refers to lack of knowledge regarding the true value of quantities describing real or expected exposure. Any uncertain information used in the exposure estimation process will lower the confidence into the validity of exposure assessment results.

## Development of a Regulatory Status of Uncertainty Analysis

Within the last decade, the documentation of uncertainty as a necessary part of any exposure and risk assessment has become mandatory for accepted chemical safety dossiers in the United States of America and Europe (US EPA 2001, EU 2003; EFSA 2006; ECHA 2008a, b, 2012a, b). Other countries have adopted the approaches (e.g., MEP 2012). Several conditions must be fulfilled to assure the quality of an exposure assessment.

A key document that is used as a framework in all regulatory approaches is the IPCS/WHO (2008) guidance document. The structure of this chapter follows the outline and the guiding principles described there. The terminology is mainly in accordance with IPCS/WHO (2004, 2008), specific terms in the context of REACH are described in ECHA (2008a).

Uncertainty analysis plays a central role in risk communication too. It might clarify the question which confidence should be given to the risk assessment results in total and how the reported results might be evaluated in relation to the residual uncertainties. Since the objective of any exposure assessment in a regulatory context is contributing reliable information to the process of decision making, all sources and all consequences of existing variability, heterogeneity, and uncertainty should be identified. Uncertainty analysis increases the transparency about the state of knowledge, about inherent assumptions, and about the data quality that influences the results of an assessment.

The ICPS/WHO (2008) document includes ten recommendations, which summarize the experience that uncertainty analysis is a necessary part of each step of exposure analysis. These steps will be explained.

## Importance of Uncertainty Analysis

(IPCS/WHO 1) Uncertainty analysis should be an integral part of exposure assessment.

Although this is formulated very general, it is the most demanding. It means that all steps from the definition of the scope and the selection of the target variables up to the summary report of the assessment should be evaluated and (at least) the main results of an uncertainty analysis must be part of the documentation.

Uncertainty analysis should display which information might be assumed to be sufficiently reliable and which should be used with caution. Furthermore, an uncertainty analysis might clarify which steps and actions might be taken to reduce the level of uncertainty. A sensitivity analysis as part of uncertainty analysis might additionally contribute important information for the risk management process: It will clarify which model variables (influence factors) have a high impact on the overall exposure estimate and which are of high (or minor) importance for possible exposure reduction measures. A comparative evaluation of the costs, the time,

and the necessary efforts for an increase in quality of the exposure assessment on one side and the expected information gain for risk the management on the other side might be the result of such an evaluation.

An exposure and risk assessment should be organized as a stepwise process (tiered approach) that starts with a simplified approach (e.g., with simplified scenarios, simple models, and/or with defaults for reasonable some upper-bound estimates for all model variables). Such screening approaches will mostly overestimate the real population exposure, since they are based on conservative assumptions in terms of influencing factors. However, they have the advantage to be simple and not to require detailed information. If no consumer risk is identified with the screening methodology, it is not necessary to use more sophisticated calculation tools (EFSA 2007). If the documentation of inherent uncertainties does not indicate restrictions with respect to the interpretation of results, even a simplified analysis might be useful for an early management decision. But, such simplified approaches should generate valid upper-bound-estimates of possible exposure for the population under consideration with a low degree of inherent uncertainty.

> (IPCS/WHO 2) The level of detail of the uncertainty analysis should be based on a tiered approach and consistent with the overall scope and purpose of the exposure and risk assessment.

If the quality assessment points to relevant limitations and if the results indicate uncertain, but relevant results, an iterative refinement of the scenarios, of the models, and of the data basis will be necessary. Under these circumstances, a refinement would be required to achieve a higher quality of the results.

A simplified upper-bound exposure assessment together with an uncertainty analysis has a high value in risk communication: The management might use the preliminary results as a first and timely, but uncertain estimate. The risk management might furthermore describe the ongoing and planned steps to clarify the exposure situation. In these situations, the exposure assessors will have a justification for a time and resource binding refinement of the exposure assessment on a higher tier (level).

> (IPCS/WHO 10) Communication of the results of exposure assessment uncertainties to the different stakeholders should reflect the different needs of the audiences in a transparent and understandable manner.

Communicating uncertain information in parallel with a description of the inherent problems, joined by a statement about necessary or ongoing steps to reduce uncertainty, might have a higher degree of perceived accuracy and credibility than waiting for complete information. Giving no information to the risk management or the public is communication too. If necessary, decisions for controlling existing risks might be made on a provisional basis, subject to verification or revision. It is the responsibility of the exposure (and risk) assessment experts to explain the inherent uncertainties of an expertise. Since the audiences may differ with respect to knowledge in the field, interests, and demands, the task of risk communication, in general, will be difficult.

A detailed analysis of uncertainties will support the risk communication process with respect to the demands, arising questions, and general requirements.

## Rationale for Characterizing Uncertainty in Exposure Assessment

The evaluation of human health risks requires information about the pollutant (e.g., emission rates, physical and chemical characterization of the substances, rates of degradation, and transformation), environmental concentrations, sources and pathways of exposure, and exposure/dose–response data. Information about each of these assessment elements might be limited. The identification of critical gaps in knowledge (scenarios and models) and data quality will be supported by an evaluation of uncertainties.

> (IPCS/WHO 3) Sources of uncertainty and variability should be systematically identified and evaluated in the exposure assessment.

## Definition of Assessment Objectives

The assessment objectives should be clearly defined. Which information is of most interest has to be decided together with the risk management by the risk assessor prior to any exposure analysis. Within the first phase of an assessment, the reduction of language-based uncertainty should be seen as a communication target. Precision of language is often overlooked as a source of uncertainty in this phase of the assessment; it can result in misunderstanding, lost efforts, and time. In general, exposure assessment should provide information about the nature of the source(s) and route(s) of exposure as well as information about the individuals who are exposed. Two different purposes for exposure assessment might be distinguished: (a) to assess the safety of legal limits (pre-regulatory dietary exposure assessment), or (b) to assess the actual exposure situation of a population or a specific subgroup (post-regulatory exposure assessment).

For regulatory purposes, a major question that should be answered is "Do the results indicate exposure higher than a predefined critical limit?" This requires a comparison with, e.g., TDI, ADI, PTWI values. The unit of exposure, in concentration [mg substance per kg body weight for a given time scale] or in intensity/frequency (maximal daily, average daily of long-term exposure), should be defined in advance. If the results indicate, even in parts, a "higher or near the evaluation level"-answer, then ranges of the input variables that produce high exposure, subgroups with high exposure and/or specific sources and pathways should be identified. This requires qualitative evaluation, quantitative ranking of inputs, and a discrimination of the importance among different influence factors. By this, input variables (and their inherent variance) that do not contribute to critical results could be separated from those influence factors (variables) that contribute mostly to high exposure conditions. Any uncertainty in the included exposure scenarios, about the appropriate models and about the parameters

applied, might influence the results. It is the task of the exposure assessor to clarify the magnitude and the direction of the influence that possible errors and uncertainties might have.

Typical questions of the management and the public which require an uncertainty analysis (Saltelli et al. 2004) are as follows: (a) How confident are you in the results? (b) How much will the results change if your basic (input) data is slightly wrong or will change over time, over regions, over subgroups? (c) Which impact will a change of input data and assumptions have? (d) Which of the uncertain input factors is most important in determining the output? (e) Which factor should we start with to reduce the uncertainty of results?

An emerging challenge is how to quantify variability and uncertainty in integrated assessments over the source-to-exposure-to uptake continuum. Since many scientific fields are tangled, any exposure assessment process should be seen as an interdisciplinary approach.

## Sources of Uncertainty in Exposure Assessment

The IPCS/WHO (2008) harmonization document calls for an analysis and full description for characterizing uncertainty using qualitative as well as quantitative approaches. Although inconsistencies in the application and methodology of uncertainty analysis might be seen, comparing the recommendations of different organizations, some common elements should be highlighted – these include qualitative and quantitative approaches.

> (IPCS/WHO 7) Uncertainty analyses for exposure assessment should be documented fully and systematically in a transparent manner, including both qualitative and quantitative aspects pertaining to data, methods, scenarios, inputs, models, outputs, sensitivity analysis and interpretation of results.

The level of uncertainty that is contributed by the selection of scenarios, the conceptual and mathematical model applied, and the choice of parameters should be documented. A qualitative evaluation should include the appraisal of the current knowledge base. Controversial sources of uncertainty should be referred to and a (qualitative) evaluation of inherent subjectivity of choices for each of the controversial sources should be presented.

> (IPCS/WHO 5) Data, expert judgement or both should be used to inform the specification of uncertainties for scenarios, models and model parameters.

If different scientific approaches are available, then evidence and plausibility, the scientific support, and the consistency of methods and data should be considered. The robustness of results using different assumptions and models (choice space) should be checked. A full uncertainty analysis might offer a framework to facilitate and promote a qualitative consideration of the impact that uncertainties might have on the exposure assessment's results.

## Scenario Uncertainty

The scenarios should describe how people may be exposed to substances by emission, by ambient air concentration, during manufacture, during industrial, professional, and consumer use of products as well as during the service life of articles and products. In principle, scenarios do not reflect one specific local situation, but have the objective to be representative of either mean, typical, or most sensitive situations in a region for a defined population.

ECHA (2008) proposed some rules for considering exposure scenarios: If the intended use of a chemical is known, as it is assumed in ECHA regulations, then a detailed description of all resulting exposure scenarios is required. The type and the number of exposure scenarios depend on how the substance is used in a predicable manner. Attributes that trigger the description of exposure scenarios are the sector of use (SU), the product category (PC), the article category (AC) together with the environmental release category (ERC). For exposure in occupational settings, the process category (PROC) should characterize production- and application-related characteristics. For consumer exposure, the product categories are defined in ECHA's Guidance R.12 (2008), describing the scope of exposure scenarios. Uncertainties might arise, (a) if the identified uses are not consistent with other sources of information, if (b) identified uses are not covered by exposure scenarios, or (c) if operational conditions do not seem to be sufficient realistic.

Within REACH documentation, an exposure scenario describes within a chemical safety report (CSR) how the manufacturer or importer controls, or recommends downstream users to control the exposure of humans and the environment to the substance in order to ensure its safe use. The variability in consumer behavior and the recognition of possible multiple exposures to the same substances from different products should be taken into account in the consumer exposure setting. Additional information on scenario description and assessment methods are available in the ECHA (2012a, b) guidance documents which includes some practical examples.

Exposure events might differ over age (e.g., due to behavior, consumption, sources), sex/gender (e.g., with respect to behavior like using cosmetics, product usage), and region (e.g., by nutritional habits, environmental conditions). With respect to age, a lifestage-specific dosimetry might impact the temporal resolution required for exposure assessment.

## Model Uncertainty

Any mathematical model corresponding to an exposure scenario should reflect the dependencies of the degree of exposure in relation to all influential factors. The identification and description of relevant exposure scenarios is an important prerequisite for any assessment. An exposure assessment should provide full

information about the origin of the model together with a detailed description of the model and its validation status. This includes all formula(s) and a description of all variables. The set of variables needs a definition with respect to content and units. A list of all parameters that represent the exposure factors distributions of the population under concern should be part of the documentation. A sensitivity analysis is useful for providing insight regarding model verification and regarding the robustness of models. Any uncertainty that is related to the exposure scenarios will propagate to the exposure model and will influence the uncertainty of results. The general structure of exposure models includes for each route and pathway (oral, dermal, inhalation) and all exposure sources information about the contact or intake frequency, about the amount of transfer per contact/intake/uptake as well as information about the concentration of the substance per item unit (e.g., mg MeHg/kg fish fresh weight; μg $NO_2/m^3$ air). All intake-related variables are defined for specific time intervals.

$$\frac{Intake}{Time} = \sum^{All\_Items} \frac{Frequency_{Item}}{Time} * Intake_{Event} * Concentration_{Item} * Transfer$$

At least one variable for a transfer factor is necessary. If the concentration of the substance of interest is changing during preparation (e.g., peeling) or cooking/frying or if concentration data is only available for whole food concentration, then a transfer factor should describe the rate of change in concentration. If the internal (ingested/absorbed) dose is the target variable, then the transfer factor must include sufficient information about the absorption via ingestion, inhalation, or the dermal route. The absorbed dose of the agent or its metabolite is also known as uptake. In this situation, a transfer rate for the intake to uptake relationship (e.g., an absorption rate) has to be selected. The intake by each pathway (oral, dermal, inhalation) is a sum over all contact items (sources) considered as relevant. All sources of exposure (e.g., food items, contact material, product application, indoor and outdoor emission) must be considered.

It should be noted that variance, measurement errors, and uncertainty of each element in the calculation propagate in a factorial manner (multiplication) for each item. The uncertainty of each item (source of exposure) is dependent on the quality of information of all elements in this model equation.

The errors $\varepsilon_i$ of each source-related intake estimate, describing the total of uncertainty for this item (e.g., the average MeHg intake per day by tuna consumption), will increase the total error in a multiplicative manner. The measured (or estimated) value of each parameter is a composite of the true value $x_i$ and an error $\varepsilon_i$, the latter dependent on the uncertainty of each variable (e.g., frequency of consumption, amount eaten per meal, MeHg concentration in fresh tuna, preparation factor, or absorption rate). The type of error linkage might be additive ($V_i = x_i + \varepsilon_i$, e.g., measurement error) or *multiplicative* ($V_i = x_i * \varepsilon_i$, e.g., dilution error).

**Total error of intake estimate** $_{\text{item i}} = \varepsilon_i \sim \varepsilon_{\text{frequency}} * \varepsilon_{\text{amount}} * \varepsilon_{\text{concentration}} * \varepsilon_{\text{transfer}}$

The error of the intake estimate is the multiplicative combination of all errors. Any systematic shift or error in exposure frequency, of the amount consumed, or in substance concentration will result in an error of the exposure estimate. The sum of substance intake over all items (exposure sources) per pathway might include many partial calculations (e.g., with varying consumption of different fish/food species with varying substance concentrations). Each variable might have a different quality for each exposure source. An exposure assessment integrates all the information into one result. In consequence, the uncertainty assessment gains complexity. At least a basic evaluation of possible error sources is necessary to avoid wrong or distorted estimates.

The lack of quality might be a result of the model selection too. Describing an average exposure (per day, per week, per month) will require statistical information about average contacts, average frequencies and amounts of use, consumption, ingestion, or inhalation together with information about the substance concentration over time. A model that is describing exposure in an event-based manner requires information about more details (e.g., the number of hand-to-mouth-contacts for toddlers per time unit, the contamination of the contact environment over a certain period, the substance transfer by hand-to-surface-contact and by hand-to-mouth-transfer). In consequence, the timescale of the model and the information about the variables should be in accordance with the timescale of the target variable.

Exposure models might describe different periods of time: The temporal scale for estimating exposure (and dose) depends on the scope of assessment, these could be peak doses (aRfD), exposures occurring over a very short period of time (e.g., minutes), time weighted averages, or exposure per day (e.g., for ADI, TDI, RfD comparison) or doses per week (e.g., for PTWI comparison).

The errors and uncertainties of the path-related intake estimates $\varepsilon_{\text{total exposure}}$ will *add up* over all pathways. In general, the contribution of each path to the total exposure and an evaluation of inherent uncertainty per pathway are recommended.

**Error of path estimate** $\varepsilon_{\textbf{total exposure}} = \varepsilon_{\textbf{total exposure}} = \varepsilon_{\textbf{oral}} + \varepsilon_{\textbf{dermal}} + \varepsilon_{\textbf{inhalation}}$

The magnitude of exposure is in general reported as an approximation of a risk-related numerical value, the total exposure divided by the body weight (as a proxy for the distribution volume). By this, the exposure estimate and the regulatory values, e.g., for the TDI, ADI, PTWI, are reported in unified units [μg substance/kg body weight per time unit]. But, the step of dividing exposure by body weight introduces some additional uncertainty: (a) body weights show variation, (b) the intake (e.g., water and food consumption) might be correlated to the body weight, (c) the relation between intake (e.g., breathing volume) and age might show non-linearities, and (d) the relationship between nominator (exposure) and denominator (body weight and time scale used) might be modulated by other influential factors (e.g., level of activity, cultural and nutrition habits). All these relations might result in a lack of independence of the estimates, used as parameters. If these influences result in systematic over- or underestimation, then correlation and dependency between variables of the model must be included.

(IPCS/WHO 4) The presence or absence of moderate to strong dependencies between model inputs is to be discussed and appropriately accounted for in the analysis.

Good modeling approaches use sensitivity analysis as a companion tool to identify possible errors (e.g., by evaluation of predictions of the model results against known data as a model calibration). Sensitivity analysis might demonstrate possible impact of dependencies (e.g., described by correlation between the input variables).

Model evaluation requires an interdisciplinary approach since the information aggregated in exposure models stem from separate scientific disciplines: data about source contaminations, about exposure related behavior (e.g., consumption data, use of products) and about nonchemical influence factors (e.g., age-related body weight, body surface), about transfer and absorption factors. It is the multidisciplinary task of exposure assessors to link and evaluate the information. An involvement of different scientists might help to evaluate sub-models and data sources adequately.

## Parameter Uncertainty

As a starting point for a (deterministic) exposure assessment in general, default values (single-value-estimates) are used. These defaults should correspond to a description of the central tendency (mean, median of the parameter distribution representing the target population) or should stand for an upper-bound-estimate (e.g., reasonable-most-exposed (RME) in general described by 95 % distribution coverage of the particular variable). If the assessors intend to produce conservative estimates of exposure, a combination of RME values for variables in the nominator (e.g., consumption per day, concentration) and lower-bound-estimates (e.g., 5 % quantiles) of the denominator (e.g., body weight) should be used for calculation. The Scientific Committee of the EFSA (2007) recommends that each panel should review whether this requirement is satisfied by the assumptions and default values that they used previously. Treating the most significant uncertainties at each refinement step (higher tiers) progressively should refine the characterization of uncertainty about the likelihood of exceeding health-based guidance values. This should be done by evaluating the variability and the uncertainty in an integrated assessment.

A description of uncertainty in parameters by error bands might be given as (a) symmetric confidence intervals (e.g., standard deviation), (b) defined quantile ranges, or (c) as an asymmetric confidence band [$CI_{\text{lower bound}}$, $CI_{\text{upper bound}}$] for skewed distributions.

### Uncertainty in Measurement

Ideally, any exposure measurement would be free of random error and should not be influenced by any systematic error. The higher the quality of a measurement instrument with respect to accuracy and precision, the lower will be the uncertainty of a measurement. Random error is associated with the fact that repeated

measurement in general will provide different measured values although the attributes of the object are assumed to be constant over time. The term "random error" describes the unpredictability of the deviances in a series of measures. Random error of a model parameter restricts the reliability of the model results in relation to its influence on the output (see section "Sensitivity Analysis"). If a numerical estimate of the random error is available (e.g., by repeated measurement), the quantitative impact of random errors on the exposure results might be evaluated.

In contrast, systematic errors generate shifts on the measurement scale of model parameters. They might depend on external influence factors (e.g., differences over measurement instruments, over observers, over laboratory standards, and in relation to conditions of measurement and sampling). The degree of confidence about the absence of systematic error is described in general in a qualitative manner. If the direction of a systematic error is known, but not its magnitude, then the impact on the results might be estimated only in a qualitative manner (e.g., by the expected direction of a systematic over- or underestimation). If a systematic error might be described by numerical boundaries, then the range of a possible quantitative impact on the results might be estimated too. The resulting one-dimensional uncertainty interval might describe the range of "true" value(s) of the outcome. For a detailed description and discussion of dealing with uncertainty in measurement, we refer to the discussion of standards for measurement (e.g., NIST 2011).

### Selection of Data Sources for Model Parameters (Exposure Factors)

Numerical default values (e.g., reference values) for exposure parameters are obtained using various approaches (e.g., expert judgment, opinion of committees, statistical analysis) and different sources (e.g., survey data, consumer panels, market observation). The statement of the former EPA-administrator William Ruckelshaus (1984) points to the problem that default values too need empirical justification, not only tradition: "First, we must insist on risk calculations being expressed as distributions of estimates and not as magical numbers that can be manipulated without regard to what they really mean. We must try to display more realistic estimates of risk to show a range of probabilities."

Within the last years, several countries have reported National Exposure Factor handbooks (e.g., US EPA 2011). Beside reporting default values (e.g., median, mean, upper quantiles), the documents include information about the parameters: (a) statistical descriptives including variability, (b) the cumulative distribution, and (c) in parts, the type of underlying distribution. In general, a stratification for age and sex, and, if necessary, heterogeneity and ethnic groups is included. Statistical uncertainties of estimates resulting from restricted sample size are in parts reported for single-value-estimates (defaults). By this, conducting statistical uncertainty analysis using default values and confidence intervals is possible. The uncertainty of exposure estimates, due to the statistical "random" errors in the combination of parameters, might be estimated too, e.g., by Monte Carlo simulation. Using the exposure factors (and variability indicators) published on a national level will result in general in an accepted state-of-the-art assessment, reporting additionally an uncertainty assessment in a dossier.

Uncertainties inherent in parameter values for exposure factors can be classified as sampling and non-sampling errors. Sampling errors arise from limited sample sizes in relation to the population size under consideration. The magnitude of this error is a function of the variability of the measured attribute and sample size.

More general problems might occur if exposure magnitude should be estimated for specific periods of the life span (child development, pregnancy, occupation). The age stratification of exposure factor handbook is restricted. Especially for developmental studies, any changes in the exposure media, with respect to the sources and the pathways over the life stage, should be considered. Each developmental stage requires the selection of specific scenarios, models, and appropriate age-related parameters – and a specific uncertainty evaluation.

## Evaluating the Total Impact of Uncertainty

The objective of a full characterization of uncertainty of an exposure assessment includes transparency, the identification of key sources of uncertainty, and an evaluation of the consequences of limited information in the decision. A systematic qualitative characterization of the sources of uncertainty is encouraged, as it provides the appropriate degree of confidence in outcome and associated recommendations. This section provides a short overview of concepts and methods that might be useful for reading assessments and for the evaluation in parallel to preparing an exposure assessment (IPCS/WHO 2008).

A simple documentation scheme for identified uncertainties is proposed (Table 1). Each row contains aspects that might contribute to the overall uncertainty of exposure assessment results. The column headers should be a guide for the identification of the mayor uncertainties: the appraisal of the knowledge base, the lack of scientific consensus, and existing controversial expert positions. For each element of the exposure assessment, a classification should be assigned and written down in the empty fields. IPCS/WHO (2008) recommends the terms (no, medium, high or NA="not applicable") for the quality and uncertainty assignments. EFSA proposed a ranking using two "+" and "−" signs, indicating the direction and the magnitude of uncertainty for each subject of consideration. Short verbal descriptions of relevant uncertainty aspects for each cell of the table will support the transparency of the analysis.

## Model Evaluation and Data Calibration

The promise given by an exposure assessment is that the estimated results would approximately reflect the real exposure situation for a defined population. According to the classification of exposure assessment methods by the NAS (1991), data and model-based exposure assessment belongs to the class of indirect measurement. They utilize existing (secondary) data on chemical concentration, frequency, strength, and duration of contact, without doing any specific measurement of the outcome variable.

**Table 1** Modified version of the IPCS/WHO (2008) evaluation scheme

| Sources of uncertainty | Characteristics of uncertainty | | |
|---|---|---|---|
| | Overall level of uncertainty | Appraisal of the knowledge base | Subjectivity of choices |
| Scope/assessment objectives | *>free text fields>* | | |
| Scenarios | | | |
| Conceptual model | | | |
| Mathematical model | | | |
| Parameters | | | |
| **Result(s)** | | | |

In contrast, a point-of-contact approach involves measurements of chemical concentrations at the point where exposure occurs to assess the outcome variable. These measure concentration close to the interface between the person and the environment, e.g., by personal samplers, by personal protocols, or duplicates of dietary intake. If the time interval of contact is recorded, the average exposure per time unit might be calculated. This type of exposure estimate requires data from environmental samplers (e.g., measuring pollutants in indoor or outdoor), information of the individual's characteristics (e.g., breathing rates), time-budget in different environmental media like indoors, outdoors, in cars. An example from Payne-Sturges (2004) shows for instance that personal sampler-based exposures measure higher values than exposure calculations for indoor VOC exposure based on exposure factors. Personal monitoring might reflect the variance of exposure conditions better than exposure calculation.

Since the target variables of an exposure assessment should reflect the uptake of a substance in relation to body weight (distribution volume), the most appropriate information for comparison exposure estimates stems from biomonitoring studies. For example, Xue and Zartarian (2010) studied the intake of inorganic arsenic in the general US population with the objective to compare exposure model predictions with observed biomonitoring data. The goal was to quantify the distribution of total dietary arsenic exposure. Comparing model predictions with observed data, the evaluation was conducted via comparing exposure and dose-modeling predictions against duplicate diet data and biomarker measurements, respectively, for the same individuals. The distribution of the modeled exposure (biomonitoring with pharmacokinetic dose estimation) and the distribution of estimates of exposure matched well with the distribution of the duplicate diet estimates.

The use of biomarkers of exposure may provide a more detailed and less biased estimate of substance uptake and distribution than any indirect methods. But this requires full information about the distribution in the body and metabolism of the substance. The linkage of biomonitoring data to specific sources requires again exposure models. Only few evaluation studies have analyzed the predictive quality of exposure assessment for human biomonitoring data in detail.

## Sensitivity Analysis

Sensitivity analysis is used to determine how different values of an input (independent) variable will impact a particular output (dependent) variable under a given set of assumptions (Saltelli et al. 2004).

> IPSC/WHO 6) Sensitivity analysis should be an integral component of the uncertainty analysis in order to identify key sources of variability, uncertainty or both and to aid in iterative refinement of the exposure model.

If risk managers like to consider the impacts of alternative regulatory or risk management choices, then sensitivity analysis is inevitable. Sensitivity analysis techniques are used to assess key sources of variability and uncertainty. The identification of model variables which are not controllable by the risk management (e.g., breathing rates, body surface area, body weight) will inform about the limiting conditions that might not be changed by regulation, control, or advice. Those variables that are manageable might be selected to control exposure in a predictable manner. Up to this step, sensitivity analysis is a qualitative exercise.

Causal inference about the impact of exposure sources and pathways are based on the strength of scientific evidence of an exposure model. Uncertainty concerning causal analyses must be characterized qualitatively. A qualitative judgment of the overall uncertainty should be accompanied by a list of major sources of uncertainty and a quantification of the expected influence of variation of the parameters on the results. Variables that might not be modified might have a high impact on the outcome (breathing rates, water consumption). Using, for instance, physiologically based pharmacokinetic (PBPK) models to predict the dose of a chemical substance or metabolite will result in a strong dependency of many model parameters (especially the organ weights) to body weights. The identification of all variables that have a high influence on the target variable requires quantitative analysis.

Building a ranked list that describes the influence of the input variables on the target variables requires statistical analysis. The goal is to quantify the degree of influence of the input variables variance on the variance of the target variable. An analysis of all possible outcomes for all ranges of the input variables (variability), together with a consideration of inherent quantitative (and numerical expressed qualitative) uncertainties, is a scientific task that will call for an involvement of mathematical, statistical, and exposure science expertise. This has to be considered if the models include many pathways and sources. If the global exposure model contains several sub-models for influence factors, uncertainty evaluation should be conducted by scientists from different faculties.

### Quantitative Sensitivity Analysis: Identification of Key Sources of Exposure, Uncertainty, and Variability

Since the efforts for a statistical sensitivity analysis should be balanced with respect to cost and time versus the expected gain of information, any uncertainty analysis should start with a screening step, which uses defaults for all parameters, evaluating the change of outcome by stepwise changing these values.

The impact of variability of a variable might be controlled by a parameter-wise alternation of central tendency default to an upper-bound-estimates (e.g., the 95 % or 5 % quantiles). This procedure gives an overview about the 95 % ranges of an influential variable, keeping all other influences on the mean. In a similar manner, the impact of statistical uncertainty might be controlled by a parameter-wise alternation of central tendency and/or upper-bound-estimates using the confidence intervals of these values. This describes the degree of uncertainty due to statistical reasons about the stability of estimates. Range-based estimates might be used for this calculation too. Changing only one input parameter while keeping all other values constant is strongly recommended at the screening level. A diagram, ranking the variables by the outcome change, might illustrate the relative importance of each input variable.

The main advantage of these One-At-A-time approaches is the fact that the resulting changes in the model outcome are directly related to the change of input. These methods are simplified approaches for gaining information about the slope of change (mathematically the local partial derivate) at a given point in a multidimensional problem. An evaluation of results based on a One-At-A-Time approach is in general understandable for risk managers and the public. But, it describes only the effects of variability or uncertainty for selected values, possible interactions between variables are ignored. The behavior of the model might deviate if all variables show variation (and uncertainty) in a multivariate setting with dependencies and interaction. The identification of those input variables that have a strong influence on the variance of the target variables will indicate to variables with a high potential for exposure control.

Taking into account the combined effects of many input variables requires data sampling plans similar to experiments or simulation analysis. Looking at the variance impact is mostly done by Monte Carlo (MC) simulation. Software tools like Crystal Ball®, @Risk® and Analytica®, JRC/SimLab® (see Reference List), for instance, support the necessary calculations. If relevant association exists between the input variables, information about the correlation (covariance) structure should be used in the simulation model. Technically, a MC simulation consists of random combinations of random variates following the distribution of each input variable. Repeating these random choices many times, the distribution of the exposure variable will represent the set of all possible combinations of input variables, constraint by the distribution of input. If uncertainty is included into the simulation, the analysis emerges from a one-dimensional variance propagation model into a two-dimensional analysis.

Using MC simulations, the dependency of the output values on the input variability might be evaluated by variance based approaches. Typical methods are as follows: (a) drawing scattergrams for visual inspection of dependency, (b) rank correlation calculation describing the ordinal degree of "the more/less of input, the more/less of exposure." Ranking the correlation coefficients by the degree of association gives information that illustrates the positive and negative impact. (c) Calculation of a regression model with the input variables as independent and the exposure estimate as the dependent variable allows an integrative view.

Using the standardized regression coefficients allows a direct comparison, if the dependency is sufficiently linear and the input indicates low intercorrelation between variables.

Introducing quantitative estimates of uncertainty into a variation-based model results in a calculation that consists of a (in general additive) mixture of variation and uncertainty. Uncertainty and variance compounds need to be represented by different variables within the model. They should be used as different terms in the (rank) correlation and regression calculations too. The combined effect might be evaluated. But, this approach presumes not only a lot of information about the set of variables, it requires good statistical knowledge. In practice, a full sensitivity analysis including variability and uncertainty components is rarely done. In these cases, if necessary due to model complexity or safety requirements, even more elaborated mathematical methods might be appropriate.

If raw data sets from representative samples of the population (e.g., collected as national surveys) are available, then the original data set might be used as a calculation basis for exposure estimation. Using the individual consumption frequencies, the individual amounts eaten/used together with the individual anthropometric data (e.g., body weights), only the substance concentration distribution needs to be simulated according to the information about the type of distribution. The calculation results in a population-based estimate of exposure. This approach avoids the problems of data dependency, correlation, and interaction and reduces data and model uncertainty. The techniques for a sensitivity analysis are the same as described above.

An approach of stratifying for homogeneous subgroups (e.g., age, sex, region, nutritional habits) will reduce the variability within each stratum (subgroup) but will keep the variation over the groups. Stratification rules should be guided by attributes that are reasonably linked to exposure (e.g., age, sex, region, behavior). Differences in behavior (e.g., typical activities, consumption habits, product usage) might provide an indication for such a classification. This might be done by exclusion of the exposure sources, e.g., "fish intake" in the model (model differentiation) or by assigning "zero mass" to the fish intake variable according to the rate of nonconsumers. Alternatively, the scope of the assessment might be changed, developing a model tailored to the "exposed fish-eater group."

If an uncertainty assessment identifies important uncertainties in relation to knowledge about appropriate data, this should be seen as a prioritizing argument for additional data collection or research. By this, it justifies a higher tier analysis and further iteration (Recommendation 2 of the IPCS/WHO 2008).

## Interpretation of Uncertainty Characterization Results

Exposure assessment is based on scenarios, models as wells as sufficient data about all influential exposure factors. The result of an exposure assessment is a prognosis about the expected level of exposure or the resulting body burden. Direct methods of exposure assessment, such as personal sampling, duplicate

studies, and human biomonitoring, provide information on a measurement level. In consequence, exposure assessors and risk managers should balance the reasons for using prognostic techniques instead of direct exposure measurement methods. The main advantage of using exposure models over direct measurement is cost and time.

A prerequisite for exposure analysis is that the state of knowledge about all the different influence factors is sufficient and that existing knowledge might be translated into an exposure model. The assessor should keep in mind, why an assessment was required, which problems and which questions have triggered the request. A full uncertainty assessment avoids losing credibility. Critical questions about the validity of the exposure assessment (accuracy, precision of prediction, validity, and objectivity) should be expected in the course of risk communication – and anticipated by an uncertainty analysis.

> (WHO/IPSC 8) The uncertainty analysis should be subject to an evaluation process that may include peer review, model comparison, quality assurance or comparison with relevant data or independent observations.

The guiding principle eight of the IPCS/WHO document (2008) is related mainly to the questions, whether the exposure assessment is valid in the sense of scientific sound quality and if it provides answers that are resistant to critical questions. Identification of uncertainty does not restrict the quality of the assessment, although it might restrict the utility of an exposure assessment for regulatory or prevention-directed purposes. A documentation of information about what is known, what is reasonable to expect, and what needs clarification might have a high impact on the risk management process.

> (WHO IPCS 9) Where appropriate to an assessment objective, exposure assessments should be iteratively refined over time to incorporate new data, information and methods to better characterize uncertainty and variability.

Where the level of uncertainty is too high, only doing additional research, collecting more information, and/or obtaining better exposure measurements will change the situation.

## References

@Risk®: Risk analysis using Monte Carlo simulation. Palisade Corp. http://www.palisade.com

Analytica® Lumina Decision System. http://www.lumina.com

Crystal Ball®. www.oracle.com

ECHA (2008a) European Chemicals Agency (ECHA). Guidance on information requirements and chemical safety assessment. Chapter R.20: Table of terms and abbreviation. European Chemicals Agency, Helsinki. http://echa.europa.eu/documents/10162/13632/information_requirements_r20_en.pdf

ECHA (2008b) Guidance on information requirements and chemical safety assessment. Part D: Exposure scenario building (version 1.1, May 2008). European Chemicals Agency, Helsinki/Finland

ECHA (2012a) Guidance on information requirements and chemical safety assessment. Chapter R.15: Consumer exposure estimation. European Chemicals Agency, Helsinki. http://echa.europa.eu/documents/10162/13632/information_requirements_r15_en.pdf

ECHA (2012b) Practical guide 13: how downstream users can handle exposure scenarios. ECHA-12-G-04-EN. European Chemicals Agency, Helsinki/Finland

EFSA (2006) Guidance of the Scientific Committee on a request from EFSA related to uncertainties in dietary exposure assessment. The EFSA Journal, 438: 1–54. http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_uncertainty%20exp_en.pdf

EFSA (2007) Reasoned opinion on the potential chronic and acute risk to consumers' health arising from proposed temporary EU MRLs. European Food Safety Authority, Parma, 15 Mar 2007. http://www.efsa.europa.eu/en/efsajournal/pub/32r.htm

EFSA Scientific Committee (2007) Opinion of the scientific committee related to uncertainties in dietary exposure assessment. European Food Safety Authority, Parma. http://www.efsa.europa.eu/en/efsajournal/pub/438.htm

EU (2003) Technical guidance document on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances, Commission Regulation (EC) No 1488/94 on risk assessment for existing substances and Directive 98/8/EC of the European Parliament and of the council concerning the placing of biocidal products on the market. Part I. Ispra, European Union, European Chemicals Bureau, Joint Research Centre, Institute for Health and Consumer Protection (EUR 20418 EN/1. http://ecb.jrc.it/tgdoc)

IPCS/WHO (2004) IPCS risk assessment terminology. Part 2: IPCS glossary of key exposure assessment terminology. World Health Organization, International Programme on Chemical Safety, Geneva, Switzerland

IPCS/WHO (2008) Uncertainty and data quality in exposure assessment. World Health Organization, Geneva, Switzerland

JRC (2012) Simlab 2.2, a framework for sensitivity and uncertainty analysis. Joint Research Centre of the European Commission, Ispra/Italy. http://ipsc.jrc.ec.europa.eu/?id=756

MEP (2012) The guidelines for the risk assessment of chemicals, China. Ministry of Environmental Protection (MEP). Translated by REACH24H Consulting Group. http://www.reach24h.com/CN/download/Ebook7_The_Guidelines_for_Risk_Assessment_of_Chemicals.pdf

NIST (2011) Uncertainty in measurement results – guidelines for the expression of uncertainty in measurement. The NIST reference on constants, units, and uncertainty. Update 2011. National Institute of Standards and Technology

Payne-Sturges DC, Burke TA, Breysse P, Diener-West M, Buckley TJ (2004) Personal exposure meets risk assessment: a comparison of measured and modeled exposures and risks in an urban community. Environ Health Perspect 112(5):589–598

Ruckelshaus WD (1984) Risk in a free society. ELI, Environ Law Rep, 14 ELR 10190

Saltelli A, Tarantola S, Campolongo F, Ratto M (2004) Sensitivity analysis in practice: a guide to assessing scientific models. Wiley, Chichester

US EPA (2001) Office of pesticide programs general principles for performing aggregate exposure and risk assessments. http://www.epa.gov/oppfead1/trac/science/aggregate.pdf

US EPA (2006) Data quality assessment: a reviewer's guide. EPA QA/G-9R. Office of Environmental Information, Washington, DC. http://www.epa.gov/quality/qs-docs/g9r-final.pdf

US EPA (2011) Exposure factors handbook: 2011 edition (Final). U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-09/052F. http://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=236252

Xue J, Zartarian V, Wang SW, Liu SV, Georgopoulos P (2010) Probabilistic modeling of dietary arsenic exposure and dose and evaluation with 2003–2004 NHANES data. Environ Health Perspect 118(3):345–350

# Part III

# Paradigms used in Risk Evaluation

Paradigms are constellations of opinions, ratings, and methodologies, which underlie the approaches to interpretation of data by a scientific community. Although one might expect that consistent opinions prevail in a standard-setting profession, this is not always the case in regulatory toxicology. On the contrary, diverging views on scientific interpretations and societal evaluations are fairly frequent. This can lead to fundamental differences in approaches to standard setting. For example, some regulating sectors strive for quantitative risk assessment for carcinogenicity, while others explicitly prefer a qualitative approach. The regulatory toxicologist must know the paradigms that underlie a specific argument. A successful regulation depends, among other factors, on the ability of the toxicologist to work within such conflicting paradigms and to seek to reconcile the different approaches.

**Toxicological Paradigms**

When in a specific scenario the substance concentration is so low that no adverse effect is measurable but a risk can still not be excluded, then risk assessment has to rely on assumptions. We consider here assumptions related to the mode of action of carcinogens, to combination effects, and to the different biology in sensitive persons. All these paradigms and conceptual models serve the final goal to achieve safety.

**Evaluation Paradigms**

Evaluation paradigms are influenced by the way of thinking and cultural and social settings. Depending on how these are incorporated in a regulation, seemingly contradictory regulations may coexist. The potential for conflict due to different evaluation paradigms can be exemplified as follows: Let us assume that a harmful substance such as PCB is regularly found in human fat due to its enrichment in the food chain. In such a case, one could argue that from a hygienic and toxicological point of view, any additional exposure and intake must be absolutely avoided. On the other hand, one can argue that in view of the variability of the PCB-background levels between individuals, a small additional burden may be considered as irrelevant. There is a surprisingly large number of such conflicts, mainly relating to protection strategies at low doses.

# Do Carcinogens Have a Threshold Dose? Pro and Contra

Bernd Kaina, Adam D. Thomas, and Jan G. Hengstler

## Contents

Introduction ..... 398
Tumor Promoters ..... 398
Factors Modifying Carcinogenesis ..... 399
Further Examples of Genotoxic Carcinogens ..... 402
Conclusions ..... 404
References ..... 405

**Abstract**

With sound understanding of biological concepts, the notion of threshold effect levels has grown in acceptance especially for electrophile-induced mutations. However, mutagenesis is one part of the exposure-to-tumor process in chemical carcinogenesis. In the following chapter, we postulate diverse protective mechanisms that may contribute to no-effect thresholds in chemical carcinogenesis. Key mechanisms contributing to threshold doses are carcinogen detoxification and DNA repair. Elimination of cells harboring premutagenic DNA lesions by apoptosis and other cell death pathways and reduced proliferation rates within tissues may minimize mutation rates and therefore, contribute to threshold dose effects.

B. Kaina (✉) • A.D. Thomas
Department of Toxicology, University Medical Center, Mainz, Germany
e-mail: kaina@uni-mainz.de; thomasad@uni-mainz.de

J.G. Hengstler
Leibniz Research Centre for Working Environment and Human Factors at the Technical University of Dortmund (IfADo), Dortmund, Germany
e-mail: hengstler@ifado.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_55, 

## Introduction

One of the most significant questions in the field of regulatory toxicology pertains to the concept of threshold dose: do genotoxic carcinogens have a no-effect threshold? The practical ramifications of this question are immense. If there is no evidence of a threshold dose, then limiting exposure to such chemicals is essential. Conversely, if effect pertains to be threshold, then exposure limitation becomes an unnecessary burden. The current paradigm assumes that genotoxic carcinogens do not have a threshold dose whereas tumor promoters and non-genotoxic carcinogens do. Recent evidence for direct genotoxins has challenged this assumption. There is considerable need for sound understanding of cellular defense mechanisms to substantiate the no-effect levels observed for potent genotoxins, which is necessary for their acceptance of non-linearity in risk assessment.

The question of whether absolute thresholds exist for genotoxic carcinogens cannot be experimentally determined because potential low-dose effects are masked by inherent biological variation. Additionally, due to the complexity of the multi-target model of carcinogenesis, the theoretical deduction of a no-effect threshold is difficult. Thus, *practical* thresholds are *inferred* through experimentally determined dose–response relationships for each end point of the carcinogenic process. Therefore, the term no-*observed*-effect level (NOEL) is used. Increasing evidence reports nonlinear even "exponential" curves for long-term carcinogenicity bioassays in rodents (Waddel et al. 2006). Increased cell proliferation through regenerative hyperplasia at higher doses may potentiate the mutagenicity and carcinogenicity of adducts that remain "dormant" at lower doses, where cell proliferation is comparatively slow (Schulte-Herman et al. 1980). Suffice to say, the question of a *true* null effect at low dose will require extensive work.

## Tumor Promoters

It is generally assumed that the non-genotoxic mode of carcinogenicity of tumor promoters comes from their ability to modulate signaling pathways, which can lead to stimulation of cellular proliferation or inhibition of apoptotic cell death (Blumberg and Boutwell 1980). Incidentally, there are suggestions that proliferation is stimulated by tumor promoters specifically in cells that were initiated by a genotoxic carcinogen. An example is provided by c-Ha-ras-mutated skin keratinocytes in the two-stage mouse skin cancer model (Parkinson 1985). It is conceivable that genotoxic effects arise indirectly from the promoters mode of action. For example, the tumor promoter phorbol-12-myristate-13-acetate (TPA) is non-DNA reactive. The promoting effect of TPA is the consequence of interaction with protein kinase C (PKC) and the resulting alteration of signaling pathways under its control. However, TPA indirectly induces DNA damage by releasing intracellular DNA-reactive oxygen radicals. This DNA damage mode of action (promotion I) may have a mutagenesis threshold dependent upon the concentration of intracellular radical scavengers (Seager et al. 2012).

Additionally, the observations of Lutz et al. (1996) indicate that tumor promoters may well be characterized by a threshold dose. Thresholds are dependent upon the mode of action and whether this threshold is due primarily to protection against reactive oxygen-induced damage or through lack of mitogenesis at low doses is unclear. Other tumor promoters stimulate cell division through binding to and activating cell surface receptors. According to the pharmacological receptor concepts, a specific amount of activated receptor is required to activate a signal pathway to elicit a biological effect. For example, a certain amount of epithelial growth factor (EGF) is required to activate sufficient receptors to have an impact on cell proliferation and promotion of initiated cells. Therefore, to postulate existence of a NOEL seems reasonable.

## Factors Modifying Carcinogenesis

The hypothesized cytoprotective mechanisms that can theoretically result in a no-observed threshold are summarized in Fig. 1. The following passages report on the arguments concerning the involvement of each mechanism in no-effect thresholds through the carcinogenic process.

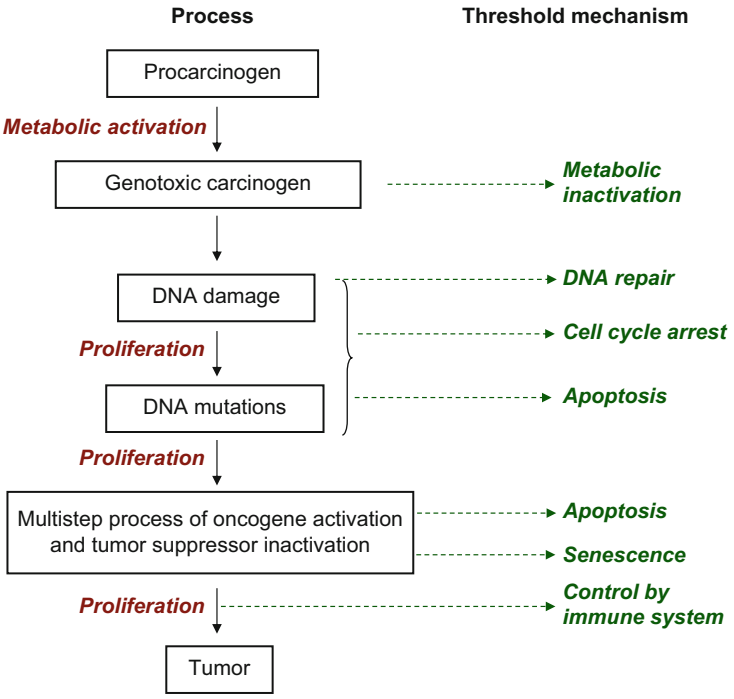



**Fig. 1** Multistep process of carcinogenesis and factors that possibly determine thresholds

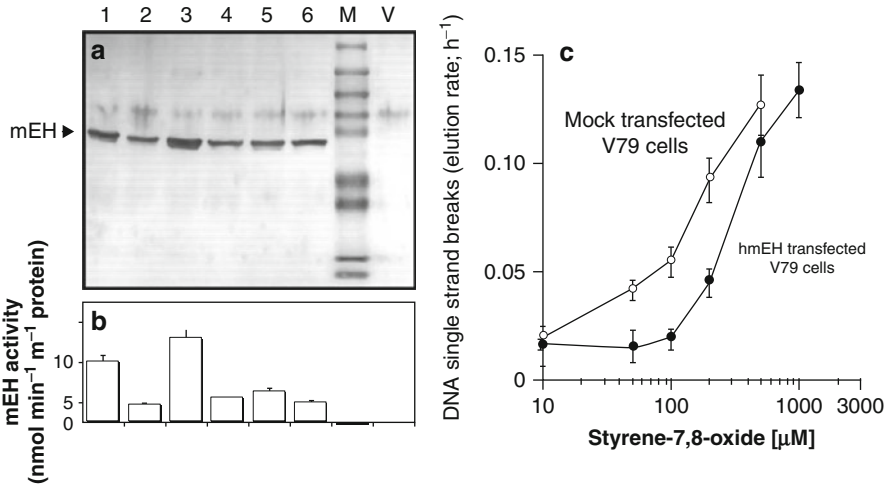



**Fig. 2** Expression of recombinant microsomal epoxide hydrolase (*mEH*) protects V79 cells from styrene-7,8-oxide-induced DNA damage. (**a**) Western blot analysis of mEH-transfected (lane 1–6) and mock-transfected (lane V) V79 cells. (**b**) Corresponding mEH activity. (**c**) DNA breaks induced in V79 wild-type cells and V79 cells stably transfected with mEH, induced by styrene-7,8-oxide. Clone no. 3 from (**a**) was used for this experiment. Expression of mEH induced a "practical threshold" up to concentrations of 100 μM (From Herrerro et al. 1997)

**Carcinogen Activation**. An important part of the genotoxic effect of many carcinogens is their metabolic activation through enzymes such as cytochrome P450 monooxygenases. The importance of activating enzymes, for provoking a carcinogenic threshold, is observed in the cynomolgus ape. Due to a deficiency in cytochrome P4501A2, cynomolgus apes are resistant to the carcinogenic effects of particular heterocyclic aromatic amines. However, assuming first-order kinetics, it seems likely that even at the lowest dose, each molecule of (pro-) carcinogen could be activated into a carcinogenic metabolite. Saturation of such pathways has only been observed at high dose, thus rendering a low-dose threshold unlikely.

**Carcinogen Detoxification**. A typical example is the impact of the microsomal epoxide hydrolase (mEH) on styroloxide-imparted genotoxicity (Fig. 2). Chinese hamster fibroblasts (V79) constitutively express low mEH activity and are, therefore, not able to detoxify styroloxide efficiently. Consequently, a threshold following styroloxide exposure was not observed in this cell line (Herrerro et al. 1997). Following transfection and expression of human mEH, V79 cells displayed a threshold (Fig. 2; in a linear plot a hockey stick curve). In human cells, the involvement of a radical scavenger, glutathione, has been implicated in a no-effect threshold upon treatment with hydrogen peroxide (Seager et al. 2012). On the other hand, theoretical examinations render a model unlikely in which detoxifying enzymes work "perfectly." In fact, it is likely that some molecules of a carcinogen escape detoxification and thus induce DNA damage at low doses.

Thus, we assume that detoxifying enzymes can cause a "practical," but not genuine no-effect threshold.

**DNA Repair**. DNA repair mechanisms are potentially the main causes of no-effect thresholds. In this context, we assume that DNA repair of premutagenic adducts prior to replication is free from errors. Of note is the protection offered by the suicide repair protein $O^6$-methylguanine-DNA methyltransferase (MGMT) in the repair of the critical premutagenic adducts $O^6$-methylguanine ($O^6$MeG) and $O^4$-methylthymine ($O^4$MeT) and thus, in the prevention of point mutations. MGMT is also very efficient in protecting against MNU and chloroethylnitrosourea-induced skin cancer formation by blocking the tumor initiation but not the tumor promotion step (Becker et al. 2003). Lack of MGMT renders mice highly susceptible to colon cancer formation induced by azoxymethane (AOM), an $O^6$-methylating agent (Ochiai et al. 2001; Wirtz et al. 2010). It is reasonable to suggest a hypothetical threshold in colon cancer following methylating agent exposure in MGMT proficient mice.

Recent data has shown the importance of MGMT-mediated repair of $O^6$MeG in the no-effect level of MNU-induced point mutations in lymphoma cells (Johnson et al. 2012; Thomas et al. 2013). Once this protection has been removed, the threshold dose is reduced (Zair et al. 2011).

We wish to stress the point that induction of DNA repair genes or repair activity would allow the cell to tolerate a higher dose of chemical and should potentiate a no-effect threshold. This was first shown in *E. coli*, in which MNNG treatment induces the expression of the *ada* gene, thereby equipping the cell with significantly more Ada proteins (alkyltransferases) as part of the "adaptive response." Upon future exposures, adapted cells tolerated a higher exposure without an increase in mutation frequency (Lindahl et al. 1988). While the *MGMT* gene has been shown to be inducible in rodent hepatocytes, it is still unclear if such an induction system is present in human cells (Fritz et al. 1991).

In human cells, the nucleotide excision repair (NER) genes *XPC* and *DDB2* are upregulated downstream of p53 stabilization, whereas the products of *XPF* and *XPG* NER genes are upregulated via the transcription factor AP-1 (Christmann et al. 2006) following a low dose of ultraviolet light (UV). This induction protected the cells against a second, "challenging" dose of UV and thus, provides an example of a genuine adaptive response in mammalian cells (Tomicic et al. 2011). Similar upregulation was also observed for apurinic endonuclease as part of the adaptive response to oxidative stress (Ramana et al. 1998). On the contrary, there are indications that increased repair activity does not necessarily confer greater resistance to cell death and mutation. DNA mismatch repair (MMR), base excision repair (BER), and NER are complex pathways involving the coordinated effect of sequentially working enzymes. Overexpression of only one enzyme in the pathway can lead to unbalanced DNA repair, conferring a hypermutable phenotype, which was shown for cells overexpressing *N*-methylpurine-DNA glycosylase (MPG), a primary glycosylase in the BER pathway that removes *N*-alkylpurines from DNA (Coquerelle et al. 1995).

Additionally, many repair polymerases particularly involved in translesion synthesis (TLS) are known to be error prone and induce mutations as they bypass bulky lesions to prevent replication fork stalling. Theoretically, genotoxic tolerance could be enhanced from a lower expression of these polymerases, providing the adduct was successfully removed from DNA by other high-fidelity mechanisms such as NER. It is likely that the effect of reducing these polymerases would be minimal since mammalian cells have very low basal levels.

It could be said that post-replicative DNA repair of base mispairs could contribute to a no-effect level. However, in the example of post-replicative MMR processing of $O^6$MeG-thymine mispairs, the outcome can be unpredictable. In this case, although thymine is removed, it is reintroduced due to the miscoding potential of $O^6$MeG. An ensuing futile cycle eventually leads to DNA strand breaks that are toxic to the cell. This mechanism is thought to remove cells from the cell pool, which harbor mutagenic $O^6$MeG adducts, which has in fact been suggested to occur in colon carcinogenesis induced by azoxymethane (Wirtz et al. 2010). $O^6$MeG is a highly mutagenic, clastogenic and recombinogenic lesion (Kaina et al. 1993). Therefore, MMR-driven cell killing may be considered a causal factor for causing a no-effect threshold for gene mutations, clastogenicity, and cancer formation.

**Apoptosis**. The process of programmed cell death (apoptosis) is generally seen as a process to remove mutated cells. The existence of "sensor mechanisms" is postulated to trigger the apoptotic pathway in response to gross DNA damage. Currently, there is considerable impetus to elucidate the sensor mechanisms. $O^6$MeG provides us with a useful example: it is highly mutagenic, pre-carcinogenic, and a potent trigger of apoptosis. Signaling studies have shown that the MMR proteins MSH2 and MSH6 initiate apoptosis through a variety of pathways upon recognition of $O^6$MeG-thymine mispairs (Quiros et al. 2010). It is often speculated that only severely damaged cells are removed from the population through cytotoxicity occurring at high doses. At low doses, however, with comparatively low levels of DNA damage, the trigger for apoptosis may be insufficient and therefore, apoptosis may not play a role in low-dose thresholds.

**Immune System**. The immune system is equipped with cells to recognize and eliminate tumor cells. The system involves dendritic cells that may represent tumor cell antigens, cytotoxic T cells, neutrophils and macrophages that respond upon activation with a cytotoxic ROS burst. The question remains if tumor cells are targeted following initiation but prior to phenotypic transformation. It can only be speculated that a no-effect threshold would be dependent upon a perfectly functioning immune system, which becomes saturated due to an increased demand for removal of tumor cells at higher concentrations where more cells are initiated. The role of immunity against genotoxin-initiated cancer cells in thresholds has not yet been addressed experimentally.

## Further Examples of Genotoxic Carcinogens

The following examples outline the heterogeneity of the dose–response relationship among direct acting genotoxins. For most genotoxic carcinogens, the linear

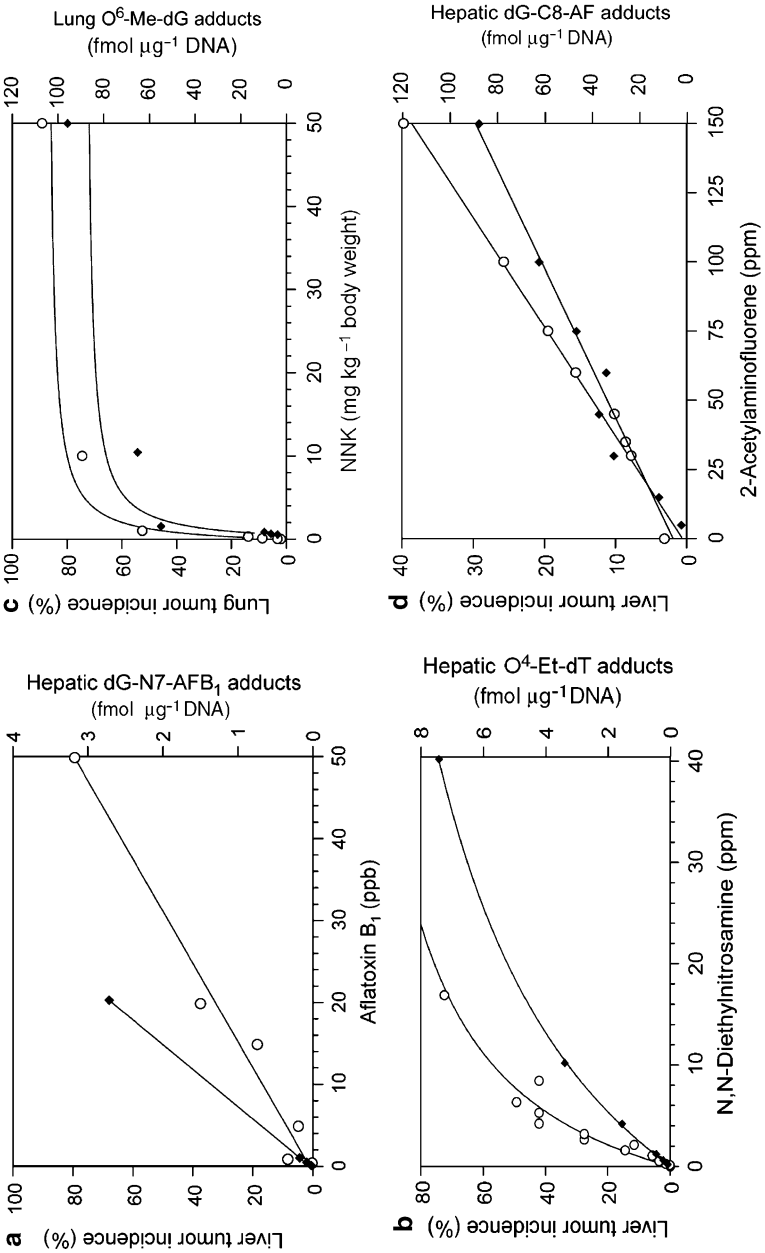



**Fig. 3** Induction of tumors (o) and DNA adducts (◆) induced by different carcinogens as a function of dose. (**a**) Incidence of liver tumors and aflatoxin B1 adducts in rat livers. (**b**) Induction of liver tumors in rats by DEN. Incidence of liver tumors (o) and the DNA adduct $O^4$-ethylthymine (◆) is shown as a function of dose. (**c**) Incidence of lung tumors (o) and levels of $O^6$-methylguanine (◆) in rats after administration of NNK. (**d**) Incidence of liver tumors (o) and DNA adducts (◆) after administration of 2-acetylaminofluorene to BALB/*c* mice (Data are from publications compiled in Hengstler et al. 2003)

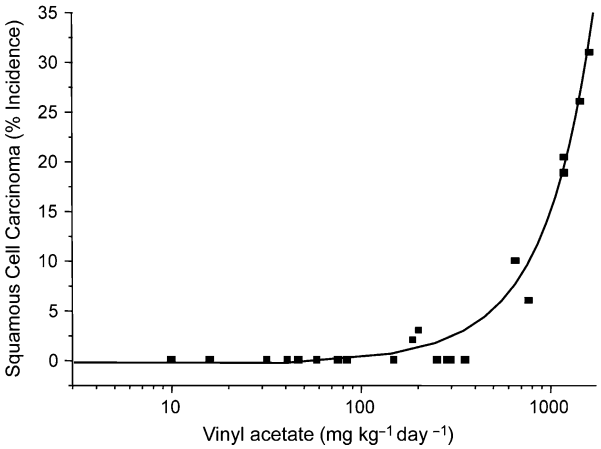



**Fig. 4** Incidence of squamous cell carcinoma as a function of vinyl acetate dose in rodents (From Hengstler et al. 2003)

assumption holds true. These include aflatoxin (B1), diethylnitrosamine (DEN), and the tobacco-specific carcinogen 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Figure 3a–c shows their dose–response relationship for tumorigenesis and the amount of adducts involved. The largest tumorigenesis research was performed with 2-aminoacetylfluorene (2-AAF). In a large study (24,000 mice), researchers were unable to prove a threshold for the induction of liver tumors (Littlefield et al. 1980) (Fig. 3d).

Despite these definitive examples, we should not assume the generalizability of the linear model for all genotoxins given recent and ever-increasing support for non-linearity. For example, tumor induction by vinyl acetate has a no-effect level of $<$100 mg/kg/day (Fig. 4). The underlying mechanism has been exhaustively discussed (Hengstler et al. 2003).

## Conclusions

A practical no-observed-effect level could occur with genotoxic carcinogens. Effective detoxification, scavenging mechanisms and lack of activation could prevent DNA interaction. Critical DNA adducts are subject of error-free repair, which might be considered a major mechanism provoking a no-effect threshold. Furthermore, elimination of damaged cells by apoptosis and premalignant cells by the immune system may further reduce the level of risk. The concept of a threshold is theoretically plausible at each requisite step involved in chemical carcinogenesis. Manipulating the intracellular levels of metabolizing enzymes and upregulation of MGMT and other repair enzymes in a balanced fashion may potentiate the existence of a threshold. Conversely, due to the complexity of the DNA repair and damage response pathways, altering expression of effector proteins may unbalance the repair process and abrogate a threshold by promoting genomic instability and mutagenesis. The processes discussed here have been implicated as the casual event in nonlinear dose–response relationships. Suffice to say, whether the observed

thresholds are a true null effect remains to be seen. Mechanistic investigations are ongoing to discover the biological relevance of low-dose exposures. Such studies are mandatory in the acceptance of no-effect thresholds.

## References


Becker K, Gregel C, Fricke C, Komitowski D, Dosch J, Kaina B (2003) DNA repair protein MGMT protects against *N*-methyl-*N*-nitrosourea-induced conversion of benign into malignant tumors. Carcinogenesis 24(3):541–546

Blumberg PM, Boutwell RK (1980) In vitro studies on the mode of action of the phorbol esters, potent tumor promoters: Part 1. Crit Rev Toxicol 8(2):153–197

Christmann M, Tomicic MT, Origer J, Aasland D, Kaina B (2006) c-Fos is required for excision repair of UV-light induced DNA lesions by triggering the re-synthesis of XPF. Nucleic Acids Res 34(22):6530–6539

Coquerelle T, Dosch J, Kaina B (1995) Overexpression of *N*-methylpurine-DNA glycosylase in Chinese hamster ovary cells renders them more sensitive to the production of chromosomal aberrations by methylating agents – a case of imbalanced DNA repair. Mutat Res/DNA Repair 336(1):9–17

Fritz G, Tano K, Mitra S, Kaina B (1991) Inducibility of the DNA repair gene encoding O6-methylguanine-DNA methyltransferase in mammalian cells by DNA-damaging treatments. Mol Cell Biol 11(9):4660–4668

Hengstler JG, Bogdanffy MS, Bolt HM, Oesch F (2003) Challenging dogma; thresholds for genotoxic carcinogens? The case of vinyl acetate. Annu Rev Pharmacol Toxicol 43:485–520

Herrerro ME, Arnd M, Hengstler JG, Oesch F (1997) Recombinant expression of human microsomal epoxide hydrolase protects V79 Chinese hamster cells from styrene oxide, but not from ethylene oxide-included DNA strand breaks. Environ Mol Mutagen 30:429–439

Johnson GE, Zair ZM, Bodger OG, Lewis PD, Rees BJ, Verma JR, Thomas AD, Doak SH, Jenkins GJS (2012) Investigating mechanisms for non-linear genotoxic responses, and analysing their effects in binary combination. Gene Environ 34(4):179–185

Kaina B, Fritz G, Coquerelle T (1993) Contribution $O^6$-alkylguanine and *N*-alkyl-purines to the formation of sister chromatid exchanges, chromosomal aberrations and gene mutations: new insights gained from studies of genetically engineered mammalian cell lines. Environ Mol Mutagen 22:283–292

Lindahl T, Sedgwick B, Sekiguchi M, Nakabeppu Y (1988) Regulation and Expression of the Adaptive Response to Alkylating Agents. Annu Rev Biochem 57:133–157

Littlefield NA, Farmer JH, Gaylor DW, Sheldon WG (1980) Effects of dose and time in a long-term, low-dose carcinogenic study. J Environ Pathol Toxicol 3(3):17–34

Lutz WK, Beland PE, Candrian R, Fekete T, Fischer WH (1996) Dose-time response in mouse skin tumor induction by 7, 12-dimethylbenz[a]anthracene and 12-O-tetradecanoyl-phorbol-13-acetate. Regul Toxicol Pharmacol 23(1 Pt 1):44–48

Ochiai M, Ubagai T, Kawamori T, Imai H, Sugimura T, Nakagama H (2001) High susceptibility of Scid mice to colon carcinogenesis induced by azoxymethane indicates a possible caretaker role for DNA-dependent protein kinase. Carcinogenesis 22(9):1551–1555

Parkinson EK (1985) Defective responses of transformed keratinocytes to terminal differentiation stimuli. Their role in epidermal tumour promotion by phorbol esters and by deep skin wounding. Br J Cancer 52(4):479–493

Quiros S, Roos WP, Kaina B (2010) Processing of O6-methylguanine into DNA double-strand breaks requires two rounds of replication whereas apoptosis is also induced in subsequent cell cycles. Cell Cycle 9(1):168–178

Ramana CV, Boldogh I, Izumi T, Mitra S (1998) Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. Proc Natl Acad Sci 95(9):5061–5066

Schulte-Herman R, Hoffman V, Parzefall W, Kallenbach M, Gerhard A, Schuppler J (1980) Adaptive response of rat liver to the gestagen and antiandrogen cyproterone acetate and other inducers, II, Induction and growth. Chem Biol Interact 31:287–300

Seager AL, Shah UK, Mikhail JM, Nelson BC, Marquis BJ, Doak SH, Johnson GE, Griffiths SM, Carmichael PL, Scott SJ, Scott AD, Jenkins GJ (2012) Pro-oxidant induced DNA damage in human lymphoblastoid cells: homeostatic mechanisms of genotoxic tolerance. Toxicol Sci 128(2):387–397

Thomas AD, Jenkins GJ, Kaina B, Bodger OG, Tomaszowski KH, Lewis PD, Doak SH, Johnson GE (2013) Influence of DNA repair on nonlinear dose-responses for mutation. Toxicol Sci 132(1):87–95

Tomicic MT, Reischmann P, Rasenberger B, Meise R, Kaina B, Christmann M (2011) Delayed c-Fos activation in human cells triggers XPF induction and an adaptive response to UVC-induced DNA damage and cytotoxicity. Cell Mol Life Sci 68:1785–1798

Waddel WJ, Fukishima S, Williams GM (2006) Concordance of thresholds for carcinogenicity of *N*-nitrosodiethylamine. Arch Toxicol 8:305–309

Wirtz S, Nagel G, Eshkind L, Neurath MF, Samson LD, Kaina B (2010) Both base excision repair and O6-methylguanine-DNA methyltransferase protect against methylation-induced colon carcinogenesis. Carcinogenesis 31:2111–2117

Zair ZM, Jenkins GJS, Doak SH, Singh R, Brown K, Johnson GE (2011) *N*-methylpurine DNA glycosylase plays a pivotal role in the threshold response of ethyl methanesulfonate–induced chromosome damage. Toxicol Sci 119(2):346–358

# Single Compounds Versus Combination Effects in Toxicology

Thomas R. H. Büch, Eva A. M. Schäfer, John H. Duffus, and Thomas Gudermann

## Contents

Basic Considerations ..... 408
Basic Aspects of the Scientific Evaluation and Regulatory Specifications of Safety Values for Combined Substance Exposures ..... 410
References ..... 410
Recommended Reading ..... 411

**Abstract**

Effective protection against harmful effects of toxic substance mixtures requires the ability to assess the combined risk potential of the various constituents. The biological impact of chemical mixtures may arise from independent, additive, synergistic, or antagonistic effects of the single constituents. Mathematical models may be used to characterize these effects. In most cases, models act on

Based on the Chapter 3.1.2 of Hermann M. Bolt in the German Edition of "Regulatory Toxicology"

T.R.H. Büch
Rudolf-Boehm Institute of Pharmacology and Toxicology, Clinical Pharmacology, University of Leipzig, Leipzig, Germany
e-mail: thomas.buech@medizin.uni-leipzig.de

E.A.M. Schäfer
Walther-Straub Institute of Pharmacology and Toxicology, Ludwig-Maximilian University Munich, Munich, Germany
e-mail: eva.schaefer@lrz.uni-muenchen.de

J.H. Duffus
The Edinburgh Centre of Toxicology, Edinburgh, Scotland, UK
e-mail: j.h.duffus@blueyonder.co.uk

T. Gudermann (✉)
Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität München, Munich, Germany
e-mail: thomas.gudermann@lrz.uni-muenchen.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_56, 

the assumption of independent effects or unknown mechanisms of action. However, a mechanistic understanding of interactions among mixture constituents, if available, is the best basis for quantitative predictions of the consequences of co-exposure to different stressors.

## Basic Considerations

It has long been known that co-exposure to different substances may trigger effects other than the simple summation of the effects caused by the individual constituents. The supra-multiplicative increase in the risk of esophageal cancer by co-exposure to tobacco smoke and ethanol is a well-known example. Nevertheless, most risk assessments performed by regulatory boards like the US Environmental Protection Agency (EPA), the US Food and Drug Administration (FDA), the European Chemicals Agency (ECHA), or the European Food Safety Authority (EFSA) have focused on single substances.

In the following chapter, the possible modes of interaction of substance mixtures and the general mechanisms of interactions are described. Furthermore, formulae to quantify the combined risk of co-exposure to multiple substances are depicted.

Multiple substances acting simultaneously on the human or animal organism in general result in four fundamental possibilities concerning their toxic effects:

1. The individual substances affect each other neither directly nor indirectly and exert completely independent effects on the exposed organism (**independent effects, no interference**). Thus, the constituents of the mixture act as if each one was the sole substance in the body.
2. The effect of a substance is attenuated by the presence of a second substance (**antagonism**) as depicted in Fig. 1. One utilizes this phenomenon in the therapy of intoxications. The mechanisms behind antagonistic effects are physical, chemical, or biological processes.
3. The effects of a substance mixture correspond to the sum of the effects of the single constituents leading to **dose additivity** (Fig. 1).
4. The effect of a substance can be increased by another substance. This process results in more-than-additive effects and is known as **synergy** (Fig. 1).

In scenario (1) the risk assessment of the substance mixture relies on the analysis of the single compounds. In scenarios (2)–(4) a mechanistic understanding of the interaction is desirable for risk evaluation although this is often not available. In principle, three types of interactions can lead to antagonistic, additive, or synergistic effects of combined substances:

– The single compounds of the mixture can react with each other (**agent-to-agent interaction**) prior to incorporation or after ingestion in the body. An example of a reaction occurring outside the body in air is the formation of ozone and peroxyacyl nitrates as so-called secondary pollutants, following the interaction of hydrocarbons with nitrogen oxides (NOx) in the presence of ultraviolet light.

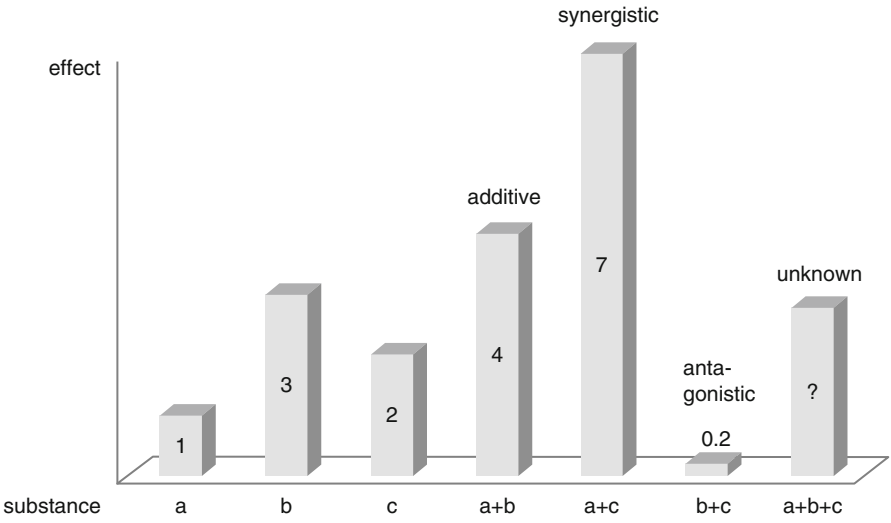



**Fig. 1** Combination effects of three compounds (a, b, c) with identical end point regarding the effect (Bolt 2004)

Agent-to-agent interactions occurring within the body have rarely been identified in mixture toxicology. However, this phenomenon is commonly used to neutralize drugs or toxic substances. One example is the binding of excessive muscle relaxants of the aminosteroid type by modified γ-cyclodextrin (sugammadex) or the neutralization of digoxin by application of digoxin-specific antibody fragments.

– A much more common mechanism in mixture toxicology occurs when constituents of the mixture display **toxicokinetic** interactions. These may lead to altered concentrations of substances in target organs by effects on the elimination or distribution. An illustrative example of a toxicokinetic interaction is the consumption of ayahuasca, a hallucinogenic mixture of *Banisteriopsis caapi* vine with *Psychotria* shrubs. The latter ingredient contains serotonergic dimethyltryptamine, and the former contains a monoamine oxidase inhibitor preventing the inactivation of dimethyltryptamine.
– Another very important mechanism of altered toxicity of substance combinations relies on **toxicodynamic** interactions. This means that the effect of a substance on a target structure is altered by another substance that in turn modifies the susceptibility of the target structure. Many well-established examples for toxicodynamic interactions are related to the field of carcinogenesis. For instance, the methylation of DNA by many DNA-methylating carcinogens like methylnitrosourea or dimethyl nitrosamines can be repaired by $O^6$-methylguanine-DNA methyltransferase (MGMT). Thus, co-exposure to inhibitors of MGMT substantially increases the DNA damaging and carcinogenic effects of these methylating agents.

## Basic Aspects of the Scientific Evaluation and Regulatory Specifications of Safety Values for Combined Substance Exposures

The regulatory recommendations for risk assessment of substance mixtures suggest the use of empirical toxicity data if available. Unfortunately, the experimental investigation of substance interactions using a full factorial design (every combination is tested) is limited by the high number of permutations to be tested. Thus, to test the interaction of two substances using three different concentrations of each compound leads to $3^2 = 9$ combinations, whereas the testing of five substances leads already to $3^5 = 243$ combinations. However, most toxicologically relevant mixtures contain far more constituents. For example, cigarette smoke contains over 5,000 compounds, and among them are more than 30 identified carcinogens.

In the case that no toxicity data for the substance mixture are available (which is the normal situation), the regulatory recommendations suggest combining the toxicity data of single constituents in an additive manner. As a rule of thumb, additive effects are most likely in mixtures of compounds with similar modes of action (especially with identical molecular target structures), whereas independent, antagonistic, or synergistic effects may appear if the substances have different modes of action.

To quantify additive effects of substance mixtures, the calculation of the Hazard Index is an appropriate approach. The Hazard Index results from the concentration, $C$, of the individual substance in the mixture and the reference dose ($RfD$, for ingestion or transdermal uptake) or the reference concentration ($RfC$, for inhaled exposures). The $RfD$ (or $RfC$) value is defined as the highest dose or concentration of an individual constituent that, as an independent exposure, does not produce harmful effects.

$$\textbf{Hazard Index} = \frac{C1}{RfD1} + \frac{C2}{RfD2} + \frac{Cn}{RfDn} = \sum_{i=1}^{n} HQ_i$$

**Hazard Index**: a value $< 1$ indicates that exposure is unlikely to be harmful

$HQ_i$ = Hazard quotient of each individual substance

Since the Hazard Index is based on the assumption of an additive mode of action, its application leads to an overestimation of the real risk of the substance mixture if antagonistic effects occur. Vice versa, the Hazard Index will underestimate the risk of a mixture if synergistic effects play a role. An established application of the Hazard Index is in evaluating the risk from exposure to hazardous air pollutants.

## References

Bolt HM (2004) Einzelstoffe versus Kombinationswirkungen [german]. In: Schwenk M, Reichl FX (eds) RegulatorischeToxikologie, Ch 3.1.2. Springer, Berlin, pp 262–267

## Recommended Reading

Callahan MA, Sexton K (2007) If cumulative risk assessment is the answer, what is the question? Environ Health Perspect 115:799–806

Pöch G (1993) Combined effects of drugs and toxic agents. Springer, Wien/New York

Sexton K (2012) Cumulative risk assessment: an overview of methodological approaches for evaluating combined health effects from exposure to multiple environmental stressors. Int J Environ Res Public Health 9:370–390

Sexton K, Hattis D (2007) Assessing cumulative health risks from exposure to environmental mixtures – three fundamental questions. Environ Health Perspect 115:825–832

Yang RSH (1994) Toxicology of chemical mixtures: case studies, mechanisms, and novel approaches. Academic, San Diego

# Biomolecules Versus Smaller Chemicals in Toxicology

Manfred Ruthsatz, Carlo Chiavaroli, and Emmanuelle Voisin

## Contents

Definitions ..... 414
The Global Regulatory Environment ..... 414
Recent Changes in the Regulatory Environment for First-in-Man Studies ..... 416
Comparison Between the Development of Biologics and New Chemical Entities ..... 416
Pharmacology ..... 418
Pharmacokinetics and Toxicokinetics ..... 418
Toxicology ..... 419
Immunogenicity and Immunotoxicity ..... 420
Impact of Manufacturing and Formulation Changes on the Development Process ..... 421
Conclusion and Recommendation ..... 421
References ..... 422
Peer-Reviewed Articles ..... 422
Regulatory Documents ..... 422

**Abstract**

For many life-science professionals, biological products represent the cutting edge of medical research and are the smartest means to target and treat a variety of disease and conditions for which the current treatments are still unsatisfactory.

Disclaimer: Any *views and opinions expressed in this article are those of the authors and do not necessarily reflect* those of any institutions the authors are associated with.

M. Ruthsatz (✉)
Nestlé Health Science, Vevey, Switzerland
e-mail: manfredruthsatz@aol.com

C. Chiavaroli
Voisin Consulting Life Sciences, Lausanne, Switzerland

E. Voisin
Voisin Consulting Life Sciences, Boulogne, France

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_57, 

In contrast to new chemical entities (NCE), most biologics are complex mixtures that are not easily identified or fully characterized. Nevertheless, due to the rapid development of biotechnology in the last three decades, the number of approved biological drugs is increasing at a faster rate than it is the case for new chemical entities. Biological drugs may be highly specific for a target, are more heat sensitive and susceptible to microbial contamination, and are likely antigenic. Thus, the quality and security testing of biologics is becoming increasingly important. This chapter compares the regulatory environment relevant for biological drugs, with a typical "case-by-case" development program versus NCEs, which are generally developed according to a more standard "classical" manner.

## Definitions

In the ICH S6 international guideline (USA, Europe, Japan), biotechnology-derived pharmaceuticals (biopharmaceuticals, biologicals, or more simply biologics) are defined as products derived from characterized cells through the use of various expression systems including bacteria, yeast, insect, plant, and mammalian cells. The active substances include proteins and peptides, their derivatives, and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology including production by transgenic plants and animals. Conversely, a NCE (new chemical entity) can be defined as a novel drug substance obtained by chemical change or synthesis and not yet approved for the prevention or treatment of human diseases.

## The Global Regulatory Environment

In the USA, the Center for Biologics Biopharmaceuticals Evaluation and Research (CBER) at the US Food and Drug Administration (FDA) is responsible for the evaluation of biologics (such as vaccines, blood products, monoclonal antibodies, antitoxins, allergenic extracts, venoms, gene therapy products). The Center for Drug Evaluation and Research (CDER) evaluates synthetic drugs, as well as antisense molecules, small synthetic peptides, and recombinant hormones (Schwieterman 2006).

In the EU, all human medicines derived from biotechnology and other high-tech processes are evaluated by the European Medicines Agency (EMA) via the centralized procedure (see Notice to Applicants 2005, Vol. 2A).

In order to circumvent regional discrepancies, the International Conference of Harmonization (ICH) has contributed to a significant global standardization of test conditions and regulatory approval of drugs for quality (ICH Q guidelines), safety (ICH S guidelines), and efficacy (ICH E guidelines). The ICH guidelines currently applicable for nonclinical development (ICH S guidelines) of drugs are listed below.

| | |
|---|---|
| M3(R2) | Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals (2009) |
| S1A | The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals (1995) |
| S1B | Testing for Carcinogenicity of Pharmaceuticals (1997) |
| S1C(R2) | Dose Selection for Carcinogenicity Studies of Pharmaceuticals (2008) |
| S2(R1) | Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (2011). It now replaces and combines the former ICH S2A and S2B guidelines |
| S3A | Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (1994) |
| S3B | Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies (1994) |
| S4A | Duration of Chronic Toxicity Testing in Animals (Rodent and Non-rodent Toxicity Testing, 1998) |
| S5(R2) | Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (1993, amended in 2000) |
| ***S6(R1)*** | ***Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*** *(1997, amended in 2011)* |
| S7A | Safety Pharmacology Studies for Human Pharmaceuticals (2000) |
| S7B | Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals (2005) |
| S8 | Immunotoxicity Studies for Human Pharmaceuticals (2005) |
| S9 | Nonclinical Evaluation for Anticancer Pharmaceuticals (2009) |

In contrast with the development of NCEs, the list comprises a unique guideline (ICH S6) to address the regulatory environment for the nonclinical development of all biologics (see also Baumann 2009 for a "foundation review" on nonclinical development of biologics). It is crucial to follow the recommendations of ICH S6 to achieve the three main goals of nonclinical safety evaluation which are to identify (1) an initial safe dose and subsequent dose escalation schemes in humans, (2) potential target organs for toxicity and for the study of whether such toxicity is reversible, and (3) safety parameters for clinical monitoring. Complying with the recommendations of ICH S6 may, however, still be insufficient to fully predict life-threatening adverse events in man, as discussed below.

## Recent Changes in the Regulatory Environment for First-in-Man Studies

**Case:** Biologics are purposely engineered to target specific receptors, and thus the border between desired and controlled pharmacological responses and unwanted toxicological effects is often blurred. In 2006, the press (Suntharalingam 2006) reported that failure to select a safe starting dose in humans at the early clinical stage with the CD28 superagonist monoclonal antibody TGN1412 led to a tragedy. To avoid such a life-threatening disaster in first-in-man studies in the future, a well-thought planning as well as a scientifically sound preclinical testing strategy for pharmaceuticals appears very important, as required in the EMA guideline which was issued shortly after the TGN1412 trial (EMEA/CHMP/SWP/294648/2007). This guideline somehow supersedes the previous US 2005 Guidance for Industry "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers."

Since 2007, both the NOAEL dose (No-Observed Adverse Effect Level, which is related to the "toxicological" effects of a drug) and MABEL dose (Minimum Anticipated Biological Effect Level, which reflects rather the "pharmacological" effect of the drug) should be determined by the Sponsor. The lower of these two doses should be considered for selecting the starting dose in humans. Even if the European guideline was implemented for both NCEs and biologics, the acquired experience demonstrates that the MABEL dose should be especially considered and receptor occupancy at this dose calculated when the drug under development is a biological superagonist molecule and when its mechanism of action suggests it can lead to uncontrolled enzymatic or cytokine cascade reactions. The test program to be performed when developing such biologics should be adapted ("case by case") to the properties of the product in development and may be fundamentally different from the toxicological and more "conventional" or "classical" program designed to develop small molecules (see Table 1).

## Comparison Between the Development of Biologics and New Chemical Entities

For both NCEs and biologics, the intended pharmacological target is a main factor for deciding which test systems should be selected for the nonclinical development of the drug under investigation. The Sponsor should justify the relevance of the animal species to humans taking into account the target, its structural homology, its distribution, the signal transduction pathways, and the nature of pharmacological effects. As a general rule, safety evaluation programs should only include the use of

**Table 1** Summary of the main differences in requirements to be considered for the nonclinical development of biologics versus new chemical entities

| Nonclinical studies (guidelines) | Biologics (ICH S6 applies) | New chemical entities |
|---|---|---|
| **Pharmacology** | | |
| Primary pharmacodynamics (ICH M3R2) | Yes, in vitro and in vivo, in at least a relevant species/model (case by case: humanized model if necessary) | Yes, in appropriate in vitro and in vivo models |
| Secondary pharmacodynamics (ICH M3R2) | If relevant, in appropriate in vitro and in vivo models | If relevant, in appropriate in vitro and in vivo models |
| Safety pharmacology (ICH M3R2, ICH 7A & B) | Yes (CNS, cardiovascular and respiratory systems, other systems if necessary) | Yes (CNS, cardiovascular and respiratory systems, other systems if necessary) |
| **Pharmacokinetics and toxicokinetics** | | |
| Analytical methods and validation reports EMEA/CHMP/EWP/192217/2009 | Yes | Yes |
| Absorption (ICH S3A & B) | Yes | Yes |
| Distribution (ICH S3A & B) | Yes | Yes |
| Metabolism (ICH S3A & B) CDER guidance: safety testing of drug metabolites (2008) | No (degradations in small peptides single amino acids) | Yes. If metabolite represents $> 10$ % of parent compound |
| Excretion (ICH S3A & B) | Yes | Yes |
| **Toxicology** | | |
| Single-dose toxicity (ICH M3R2) | Yes (to generate useful data for repeated-dose toxicity) | Yes, however no need to reach $LD_{50}$ anymore (two non-rodent species) |
| Repeated-dose toxicity (ICH M3R2, ICH S4A) | Yes (only in relevant species) | Yes (two species, rodent and non-rodent) |
| Genotoxicity ICH S2(R1) | No | Yes (in vitro and in vivo) |
| Carcinogenicity (ICH S1A, B, and C) | Generally not necessary | Yes (except for anticancer agents, ICH S9) |
| Reproductive and developmental toxicity (ICH S5R2) | Yes, the program could be done in a single species | Yes (two species, rodent and rabbit) |
| Local tolerance (ICH M3R2, CPMP/SWP/2145/00) | Yes, stand-alone study usually not necessary | Yes, stand-alone study usually not necessary |
| **Other toxicity studies** | | |
| Other toxicity studies (ICH S8) | Yes, immunogenicity and immunotoxicity assessments | Yes, immunotoxicity assessment only |

relevant species, and indeed toxicity studies in nonrelevant species may be misleading and are discouraged by the regulatory authorities. A relevant species is one in which the test material is pharmacologically active, and thus knowledge of receptor/epitope distribution provides generally an understanding of potential

in vivo toxicity of biologics. "Case-by-case" cross-reactivity evaluation, in vitro and/or in vivo, by immunochemical or functional tests between species and organs/tissues/cells should be performed because they are crucial for the selection of the relevant test system. This would optimize the evaluation of toxicity arising from the binding to the epitope and any unintentional tissue cross-reactivity. An animal species which does not express the desired epitope may still be of some relevance for assessing toxicity if comparable unintentional tissue cross-reactivity to humans is demonstrated.

In contrast to the key toxicological and pharmacokinetic activities, it is acknowledged that these studies do not need to be (fully) Good Laboratory Practice (GLP) compliant.

## Pharmacology

For both NCEs and biologics, the demonstrated pharmacodynamics (PD) characteristics of a drug under development in relevant animal model(s) will be considered as the nonclinical proof of concept.

Safety pharmacology studies are GLP (ICH S7A&B) and need to include assessment of effects on vital functions (cardiovascular, central nervous system [CNS], and respiratory systems) to investigate undesirable effects of a substance and its metabolites on physiological functions based on exposure at low, medium, and high doses. For some products, the evaluation of safety pharmacology endpoints can be conducted as part of toxicology and/or pharmacodynamics studies.

## Pharmacokinetics and Toxicokinetics

Guidelines ICH S3A and B require a comprehensive knowledge of the absorption, distribution, metabolism, and excretion (ADME) in view of the interpretation of pharmacology and toxicology studies. Measurement of drug concentrations (PK determinations) in biological matrices is an important aspect of medicinal product development for both NCEs and biologics. Tissue distribution studies are essential, especially in relation to potential sites of action. For NCEs, the nonclinical characterization of human metabolites is only warranted when these metabolites are observed at exposures greater than 10 % of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies (CDER Guidance 2008: Safety Testing of Drug Metabolites). Such studies should be conducted to support phase 3 clinical trials. In contrast, the expected consequence of metabolism of biologics is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood, and thus classical biotransformation studies as performed for pharmaceuticals are not needed.

Toxicokinetics (TK, see ICH S3A) is defined as the generation of pharmacokinetic data, either as an integral component in the conduct of nonclinical toxicity studies or in specifically designed supportive studies, in order to assess systemic exposure. Due to its integration into toxicity testing and its bridging character between nonclinical and clinical studies, the focus is primarily on the interpretation of toxicity tests and not on characterizing the basic PK parameters of the substance studied. Both TK and PK determinations should be performed using validated analytical methods used for sample analysis (EMEA/CHMP/EWP/192217/2009, see also Swann 2011 for FDA considerations).

## Toxicology

For NCEs, repeated toxicity studies in two species are normally required. The studies should be designed to reflect the intended clinical use (duration and frequency of administration, clinical route of administration) and take into account the therapeutic indication. Frequency of administration is based on PD, PK, and toxicological profile. Dose levels include a low (pharmacological), an intermediate, and a high (potentially toxic) dose. A control group should also always be included. ICH S4 requires treatment durations in non-rodents (9 months) and rodents (6 months) to enable long-term administration to humans (> 6 months). For biologics, however, it is not rare to note that the pivotal toxicity program can be performed in a single species only (i.e., cynomolgus monkey). If it appears that no relevant species exists, the use of transgenic animals expressing the human receptor or the use of homologous proteins should be considered. The information gained from the use of a transgenic animal model expressing the human receptor is optimized when the interaction of the product and the humanized receptor has similar physiological consequences to those expected in humans.

For genotoxicity, ICH S2R1 states that an assay for gene mutation is sufficient to support single-dose clinical trials with NCEs, whereas a complete battery of tests for genotoxicity should be completed before initiation of phase 2 trials. In contrast, genotoxicity studies are not applicable to biologics and therefore are not required (see ICH S6), because peptides or protein substances would probably not interact with chromosomal material.

Conditions relevant for the carcinogenicity testing of NCEs are discussed in ICH S1A. In general carcinogenicity studies should be conducted to support the marketing application and thus logically launched during phase 3. However, for pharmaceuticals developed to treat certain serious diseases, in order to speed up the development process, it is possible to discuss the timing with the agencies and conduct carcinogenicity studies post-approval. The basic scheme is one long-term rodent carcinogenicity study and one other study both supported with TK data (e.g., in vivo tests). As mentioned in ICH S6, standard carcinogenicity bioassays are generally inappropriate for biologics. However, product-specific assessment of carcinogenic potential may still be needed depending upon the duration of clinical dosing, patient population, and/or biological activity of the product (e.g., growth factors, immunosuppressive agents).

Reproduction toxicity studies should be conducted as is appropriate for the population to be exposed (ICH S5R2). The goal is to reveal any effect of the product on mammalian reproduction. The combination of studies selected should allow exposure of mature adults (toxicology study) and all stages of development from conception to sexual maturity. Observation should be done from conception in one generation through conception in the following generation (complete life cycle). For biologics, reproductive toxicity studies should also be conducted in accordance with the principles outlined in ICH S5R2. One species can be sufficient to address effects on embryo-fetal development.

For both NCEs and biologics, the evaluation of local tolerance (ICH M3R2) by the intended clinical route of administration is performed as part of the general toxicity studies. Stand-alone studies are generally not recommended.

## Immunogenicity and Immunotoxicity

In contrast to NCEs, many biologics intended for human are immunogenic in animals. The immunogenicity of biologics can cause hypersensitivity responses, anaphylaxis, and infusion reactions (Rosenberg 2003). Antidrug antibody (ADA) responses could affect the efficacy and/or safety of protein therapeutics and/or complicate interpretation of the toxicity, pharmacokinetic, and pharmacodynamic data. It is also known that particular glycosylation patterns might be immunogenic and some protein aggregates might trigger immunogenicity. Animal models are increasingly used to study immunogenicity of therapeutic proteins. They are employed as predictive tools to assess immunogenicity during drug development and have become vital in studying the mechanisms underlying immunogenicity of therapeutic proteins. However, the use of animal models needs critical evaluation (Brinks 2011). Because of species differences, the predictive value of these models is limited.

It is widely acknowledged that biologics often reveal their real immunotoxicity potential for humans only during clinical studies. The predictive value of animal studies and traditional in vitro screens is thus questionable. Despite these limitations, antibody levels associated with administration of biologics should be measured during repeated-dose toxicity studies. Antibody responses should be characterized (titer, number of responding animals, neutralizing or non-neutralizing), and their appearance should be correlated with any pharmacological and/or toxicological changes. Specifically, the effects of antibody formation on PK/PD parameters, incidence and/or severity of adverse effects, complement activation, or the emergence of new toxic effects should be considered when interpreting the data. Possible pathological changes related to immune complex formation and deposition should be evaluated. In summary, as stated in the ICH S8 guideline, immunotoxicity studies are mandatory for NCEs and biologics using standard toxicity studies and additional immunotoxicity studies as needed. Additional immunotoxicity studies should be completed before exposure of a large population of patients in phase 3.

## Impact of Manufacturing and Formulation Changes on the Development Process

The performance of safety bridging strategies within batches of the same biological produced at different scales is a key element to master in order to obtain clinical trial and marketing authorization. The use of cells of human, animal, or even plant origin for the production of biologics is subject to potential contamination. A change in manufacturing process and/or of formulation of the product represents a potential risk for patients (such as immunosuppression, immunostimulation, hypersensitivity, and autoimmunity). Particular attention must be paid to the characterization, purity, and stability of the starting materials, as well as the presence of aggregates. Products should be tested for viral safety (ICH Q 5 A (R1) Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin) and genetic stability (ICH Q 5 B Quality of Biotechnological Products: Analysis of the Expression Construct in Cell Lines Used for Production of r-DNA Derived Protein Products). A European guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials has been recently adopted (EMA/CHMP/BWP/534898/2008).

The production process must provide relatively large amounts of test material. The degree of comparability of the test material from batch to batch in the development program requires an early validation of the production and testing methods as well as the precise definition of the product specifications (EMEA/CHMP/BMWP/101695/2006). An early well-designed bridging strategy in terms of upscale process is preferable to a subsequent full test program.

## Conclusion and Recommendation

Biologics can provide more innovative, effective, and targeted therapies for numerous diseases than NCEs. In order to detect any potential toxicity of these promising products, the determination of the safe dosage at the start of clinical studies and the establishment of dose–response relationships, a rationale "case-by-case" nonclinical testing strategy, should be put in place taking into account not only ICH S6R1 (Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals) but also all other guidelines listed in this chapter. Biologics differ in many aspects from the more conventional NCE drugs, because of their species- and tissue-specific characteristics and their immunogenicity potential due to their particular nature and complex mode of production.

In order to avoid critical issues at the time of marketing authorization application, we strongly advise any drug developer to request a scientific advice meeting with a regulatory agency to discuss the relevance of the nonclinical development program they intend to perform.

## References

### Peer-Reviewed Articles

Baumann A (2009) Nonclinical development of biopharmaceuticals. Drug Discov Today 23–24:1112–1122

Brinks V (2011) Immunogenicity of therapeutic proteins: the use of animal models. Pharm Res 28:2379–2385

Rosenberg AS (2003) Immunogenicity of biological therapeutics: a hierarchy of concerns. Dev Biol (Basel) 112:15–21

Schwieterman WD (2006) Regulating biopharmaceuticals under CDER versus CBER: an insider's perspective. Drug Discov Today 19–20:945–951

Suntharalingam G (2006) Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. New Engl J Med 355(10):1018–1028

Swann PG (2011) Regulatory considerations for development of bioanalytical assays for biotechnology products. Bioanalysis 6:597–603

### Regulatory Documents

EMEA/CHMP/BMWP/101695/2006: Guideline on comparability of biotechnology-derived medicinal products after a change in the manufacturing process non-clinical and clinical issues

EMA/CHMP/BWP/534898/2008: Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials

EMEA/CHMP/SWP/294648/2007: Guideline on strategies to identify and mitigate risks for first-inhuman clinical trials with investigational medicinal products

EMEA/CHMP/EWP/192217/2009: Guideline on bioanalytical method validation

ICH guidelines: all the guidelines cited in this document are available for download at: http://www.ich.org/products/guidelines.html

Notice to Applicants Volume 2A (2005) Procedures for marketing authorization. Can be downloaded from http://ec.europa.eu/health/documents/eudralex/vol-2/index_en.htm

US Guidance for Industry (2005) "Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers"

US Guidance for Industry (2008) Safety testing of drug metabolites

# Sensitive Humans Versus Average Persons in Toxicology

Alexander Eckhardt

## Contents

Introduction ..... 423
Genetic Polymorphism ..... 424
Additional Parameters ..... 425
Cytochrome P450 and Toxicity ..... 426
Susceptibility in Children ..... 428
  Physiological Differences ..... 428
Increased Susceptibility in Fetuses, Babies, and Children ..... 429
Regulatory Considerations ..... 430
References ..... 430

**Abstract**

The sensitivity of human beings against toxic insults or pharmaceuticals varies considerably. This fact has various reasons, e.g., health status, age, body weight, as well as genetic background. An increased sensitivity against a noxious substance can have severe or even lethal consequences. Therefore, it is compulsory for toxicologists to take these differences into account when establishing limits for toxic compounds.

## Introduction

The genetic background of human beings from all over the globe varies quite a lot. In addition, varying environmental conditions and cultural habits can influence the susceptibility against a substance either of natural origin or manmade. On the first

A. Eckhardt
Toxicology of Drinking Water and Swimming Pool Water, Federal Environment Agency, Bad Elster, Germany
e-mail: Alexander.Eckhardt@uba.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_58, 

glimpse these differences do not appear to be a problem, but from a toxicological and/or pharmacological point of view, matters are different. Besides accidents and (attempted) suicides, there is usually no toxicological data based on human exposure against high doses. We thus have to rely on animal testing. A group of animals used for testing is usually well defined, regarding genetic background, age, and body weight. The problems which may arise when the data acquired are transferred to humans are based on the fact that humans are considered a good deal more genetically diverse than laboratory animals. Defining an "average person" is already impossible in a small population, let alone on a worldwide scale. Therefore, safety margins big enough to include those rare people with a very high sensitivity against a certain compound have to be set.

This chapter deals with the different reasons for diverging susceptibility in humans. Probably the most important reason is genetic polymorphisms, followed by parameters like age, health status, and body weight. The effect of cytochrome polymorphism on toxicity is described, followed by a special focus on children.

## Genetic Polymorphism

The effects of genetic polymorphisms vary greatly depending on the enzyme concerned. It is common knowledge that many adult Asians (~80 %) have developed lactose intolerance. This might be undesirable for the respective person, but it is usually not life threatening. Things are getting more complicated when we look at cytochromes, for example. This big class of enzymes encompasses at least 17 families (Lin and Lu 2001), which in turn can be divided into two major groups: cytochromes that are responsible for synthesizing compounds like steroids and fatty acids as well as enzymes dealing with xenobiotics. Among those xenobiotics are toxic compounds like aryl hydrocarbons, e.g., from tobacco smoke as well as pharmaceuticals. Pharmaceuticals are mainly metabolized by three cytochrome families: CYP1, CYP2, and CYP3, with the latter two accounting for about 50 % of a person's hepatic cytochrome makeup. Only two of almost 60 known isoforms seem to be well preserved: CYP1A1 and CYP2E1. The reason might be that the former is part of the cell cycle, and the latter is part of gluconeogenesis.

Genetic variations can account for differences in drug uptake, metabolism, and drug-receptor interaction. Each of these differences can lead to adverse drug effects. The estimate that more than 10 % of the admissions to internal medicine departments in Swedish hospitals are due to adverse drug effects gives an idea about the importance of the knowledge of polymorphisms (Mjörndal et al. 1999). Even more alarming is the US assessment that about 100,000 deaths annually are caused by adverse drug effects (Ingelman-Sundberg 2001). An overview of possible effects of gene mutations in cytochromes can be seen in Fig. 1.

One prominent example for genetic polymorphisms is CYP2D6, which metabolizes a variety of pharmaceuticals like antidepressants, antihypertensive drugs, and antiarrhythmics. At least five different variants are known, with variant CYP2D6*17 having a frequency of about one third in people of African origin, whereas it is

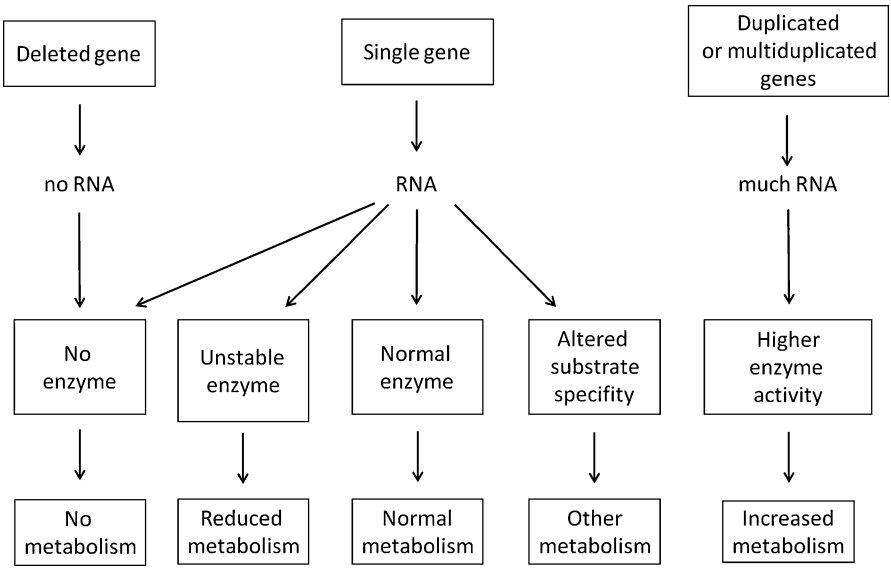



**Fig. 1** Possible reasons for differences in cytochrome activity in different subjects (Ingelman-Sundberg 2001, redrawn)

almost nonexistent in Caucasians (Ingelman-Sundberg 2001). Since this variation results in reduced substrate affinity, severe adverse drug effects have to be considered before prescription. One of the pharmaceuticals affected is the neuroleptic perphenazine, with variations of about tenfold in a patient's serum, when comparing standard to poor metabolizers. CYP2D6 is also known for being a cytochrome encoded by multiple, i.e., up to 13, gene copies. Consequently the individual concerned can be a very rapid metabolizer, which would result in only limited effects of conventional drug dosing regimens. Different plasma concentrations of a drug given at four intervals as expected by standard, rapid, or slow metabolizers are shown in Fig. 2. The differences in CYP-regulated metabolism between two individuals can amount up to 100-fold for a single drug.

There are also polymorphisms of the steroidogenic cytochromes. But due to their severely debilitating or even fatal effects, they are generally regarded as genetic defects (Guengerich 2002).

## Additional Parameters

Variations in the plasma level of the same pharmaceutical of up to 1,000-fold between two persons with identical body weight have not only genetic reasons (Ingelman-Sundberg 2001). In addition, age and (patho)physiological, nutritional, and environmental effects have to be taken into account. One class of xenobiotics

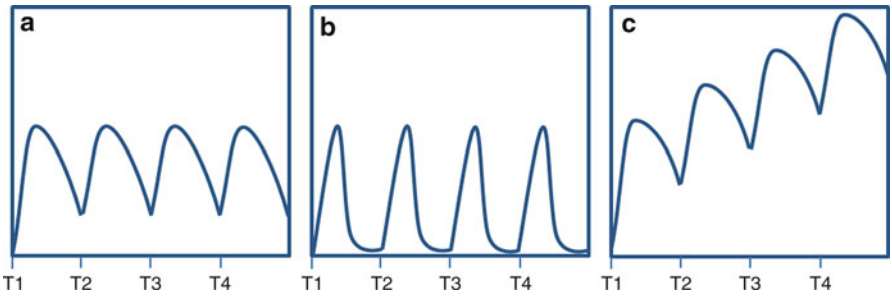



**Fig. 2** Effects of different metabolic rates on the plasma level of a compound: normal metabolizer (**a**), rapid metabolizer (**b**), or slow metabolizer (**c**)

that is known to increase cytochrome activity (induction) is polyaromatic hydrocarbons (PAHs). The source of the PAHs can be rather diverse: it could be the 8 oz steak from the barbeque, the cigarette, or the chemical plant in the neighborhood. All this results in an uptake of PAHs, which in turn induce CYP1A activity. Contrary to lab animals used in pharmaceutical testing, the diet of humans is very diverse, as are possible effects induced by food. Ethanol is another common inducing agent. Therefore, finding the reason for variations between two persons is very complicated and sometimes a virtually impossible task.

## Cytochrome P450 and Toxicity

The main task of CYPs is to oxidize xenobiotics. Hydrophilic groups such as hydroxyl groups are added to the original substance involving a chemical activation step. This usually increases water solubility, favors renal clearance, and leads to detoxification. Alas, this strategy has a drawback: adding one or more hydroxyl groups to a substance can also result in a destructive reactivity of the intermediates. Due to its lipophilic properties, benzo[a]pyrene has to be subjected to several activation steps by CYPs. Since benzo[a]pyrene can be metabolized by several cytochromes and subsequently by sulfatases, several different metabolites are formed. One of the metabolites formed is 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (Fig. 3). The resulting epoxy group is very reactive and can easily bind to amino groups of proteins or, more dangerous, DNA. Although the epoxide can be eliminated via glutathione transferases, DNA adducts can also be formed.

Depending on the activity of the enzymes involved in this reaction, the amount of DNA adducts formed can vary widely. With more adducts formed, the risks of unrepaired or inaccurately repaired DNA damage increases. Once the damage is done, there are three alternatives on the cellular level: apoptosis, correct repair, or inaccurate repair, resulting in mutation and possibly cancer (Fig. 4). Although deadly for a single cell, apoptosis is very useful for the organism as a whole, since the damage will not be inherited to daughter cells, thereby eliminating the risk of cancer.

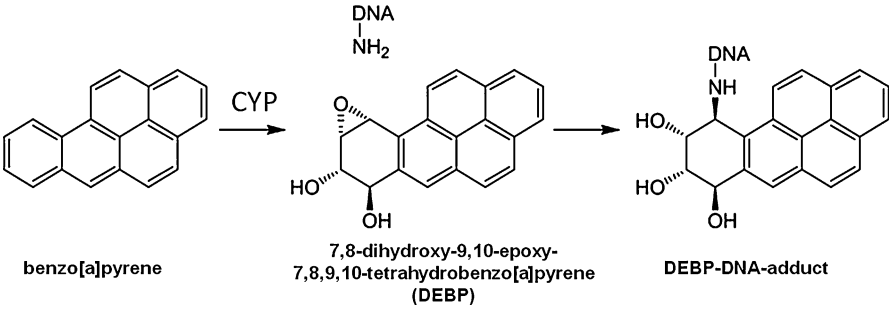



**Fig. 3** Activation of benzo[a]pyrene by adding four hydroxyl groups and formation of DNA adduct (http://www.chemgapedia.de/vsengine/media/width/713/height/190/vsc/de/ch/4/cm/funktgruppen/bilder/benzpyren_dna.svg.jpg)

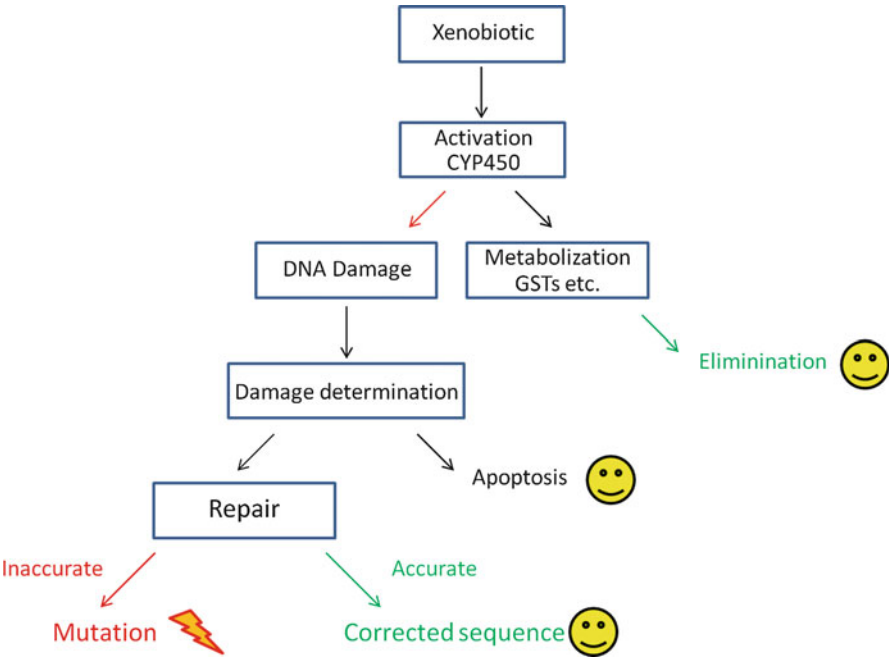



**Fig. 4** Possible pathway after activation of a xenobiotic by CYP

Polymorphisms in cytochromes can result in severe consequences when pharmaceuticals are not metabolized as expected by the physician. This can result in very different plasma levels as shown in Fig. 2. Antibiotics, like erythromycin, are metabolized by CYP3A4. In a worst-case scenario, too much CYP3A4 can lead to septic shock, because the bioavailability of the antibiotic is insufficient. A second problem arising from rapid metabolism can be an indirect effect. Acetaminophen also called paracetamol, for example, is metabolized by CYP2E1. It is known

**Table 1** Interindividual variability of cytochrome activity (Data from Lin and Lu 2001; Pelkonen et al. 2008)

| P450 enzyme | Variability | Substrate | Inhibitor | Inducer |
|---|---|---|---|---|
| **CYP1A2** | 20-fold | Caffeine | Ciprofloxacin | Smoking |
| **CYP2A6** | >50-fold | Nicotine | Pilocarpine | Phenobarbital |
| **CYP2B6** | >50-fold | Nicotine | 17-α-ethynylestradiol | Phenobarbital |
| **CYP2D6** | >50-fold | Codeine | Fluoxetine | ? |
| **CYP2E1** | 12-fold | Ethanol, acetaminophen | Pyridines | Ethanol (!) |
| **CYP3A4** | 8-fold | Testosterone | Grapefruit juice | Phenobarbital |

that the interindividual concentration of CYP2E1 can vary by a factor of 12. Too much CYP2E1 can lead to an accumulation of N-acetylbenzoquinoneimine, a major product in the metabolism of paracetamol. This accumulation on the other hand can result in irreversible hepatic necrosis, when the liver cannot provide enough glutathione for conjugation of N-acetylbenzoquinoneimine. Table 1 gives an overview of cytochromes, their variability, substrates, inhibitors, and inducers.

Since cytochromes are, in contrast to most other enzymes, inducible, the interplay between an enzyme and its substrate(s) becomes even more complex. Inhibition or induction can be triggered by other pharmaceuticals like fluoxetine, phenobarbital, or 17-α-ethynylestradiol. Not only medications can cause additional variations in CYP activity, but also dietary products are known to influence cytochrome activity. A very effective inhibitor of several cytochromes is grapefruit juice. The inhibition of CYP3A4, CYP1A2, CYP2C9, and CYP2D6 was observed after treating liver microsomes with grapefruit juice. Due to the fact that at least four different CYPs are inhibited by the juice, a broad spectrum of pharmaceuticals is affected: calcium channel blockers, immunosuppressives, as well as sedatives. Cytochrome inhibition can increase the bioavailability of these pharmaceuticals up to fivefold, e.g., for the calcium antagonist felodipine (Bailey et al. 1991). Although oranges are closely related to grapefruits, these effects are not seen after consumption of orange juice (Tassaneeyakul et al. 2000).

## Susceptibility in Children

### Physiological Differences

It is well justified to assume that there are biological reasons why children and especially newborns can be more sensitive towards a comparable toxicological stress than adults. Compared to adults, their consumption of food, water, and oxygen is increased in relation to their body weight. Moreover children are on average physically much more active. Consequently their exposure to environmental stress is also elevated. What makes matters worse is the fact that especially in the first 6 months, the ability to metabolize xenobiotics may not be fully developed.

## Increased Susceptibility in Fetuses, Babies, and Children

It is common knowledge that exposure against certain substances results in damage selectively in children. Examples are smoking, alcohol consumption, or uptake of pharmaceuticals which lead to neurotoxic or teratogenic effects or developmental retardation.

Less is known about intoxication of children with chemicals, like pesticides or food additives that are used only in restricted applications. Children are more susceptible against high acute doses of the pesticide chlorpyrifos but, on the other hand, less or as sensitive as adults against repeated low-dose exposure. Although many persistent organic compounds have been banned, and their prevalence is reduced, neurotoxins, like methylmercury, that damage the developing brain of children are still on the agenda. Lead is of special concern in children for two reasons. Firstly, the adsorption in a child's gastrointestinal tract is higher than in an adult, and secondly, the central nervous system of children is four times more susceptible than that of an adult. Therefore, the main focus is on neurotoxic effects when it comes to discussing tolerable lead concentrations.

There is only limited evidence from literature that low doses of easily excreted substance are more toxic to children than to adults. Damage through alcohol or cyanoses induced by nitrate/nitrite from private wells are known examples.

According to current knowledge about ontogenic development of human metabolism, children up to the age of 6 months are generally more susceptible against toxic insults than adults. This is caused by the fact that transformation and elimination is slower, which in turn results in higher plasma levels of many chemicals and pharmaceuticals.

The metabolic capacity for dealing with many xenobiotics is already established prior to or at the time of birth, but the capacity is smaller and the enzyme patterns can be different. At the age of 6 months, the metabolism of children is developed well enough that there are usually no important variations in the toxic susceptibility compared to adults. Nevertheless, the susceptibility of organs like brain, bones, and hormonal system can remain different until sexual maturity. Children can also be less susceptible to chemicals or pharmaceuticals when certain receptors or final metabolic capacities are not yet fully developed.

Due to age specific behavior, oral exposure to chemicals is increased in children. Since especially small children are trying to put almost everything they can grab a hold on into their mouth, households and public playgrounds have to be taken into account as additional sources of exposure. The risk is dependent on a child's susceptibility and the exposure conditions. Provided that the chemical exposure remains below the threshold above which detoxification mechanisms of the organism are overstrained and toxic effects are triggered, the hazard is the same as for an adult. During the first half year, when the human organism is generally most vulnerable, the exposure to chemicals is, also because of limited mobility, rather low. Therefore, exposure to environmental risks can be considered lower than in older children.

## Regulatory Considerations

Children of all ages are still in the process of maturation. This has to be taken into account when assessing the risk of substances that impair the development of organs when children could be exposed against them. Based on this, the US-EPA considers the implementation of an extra safety factor of 10 when the data on a compound does provide reliable information about a toxicological threshold in children.

Moreover there are additional regulations for the special protection of children, e.g., in regulations concerning food and toys (Diätverordnung, Spielzeugverordnung). In accordance with the WHO "Guidelines for Drinking-Water Quality" (WHO 2011), some parameters of the German drinking-water directive (Trinkwasserverordnung), like the concentration of nitrate and copper, were set to meet the requirements of children.

## References

Bailey DG, Spence JD, Munoz C, Arnold JMO (1991) Interaction of citrus juices with felodipine and nifedipine. Lancet 337:268–269

Guengerich FP (2002) Update information on human P450s. Drug Metab Rev 34(1 and 2):7–15

Ingelman-Sundberg M (2001) Genetic susceptibility to adverse effects of drugs and environmental toxicants: the role of the CYP family of enzymes. Mutat Res 482:11–19

Lin JH, Lu AYH (2001) Interindividual variability in inhibition and induction of cytochrome P450 enzymes. Annu Rev Pharmacol Toxicol 41:535–567

Mjörndal et al (1999) Abstract, EPACT, Jerusalem

Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H (2008) Inhibition and induction of human cytochrome P450 enzymes: current status. Arch Toxicol 82:667–715

Tassaneeyakul W, Guo L-Q, Fukuda K, Ohta T, Yamazoe Y (2000) Inhibition selectivity of grapefruit juice components on human cytochromes P450. Arch Biochem Biophys 378(2):356–363

WHO (2011) Guidelines for drinking-water quality, 4th edn. WHO, Geneva

# Extrapolation Factors and Safety Factors in Toxicology

Rainer Konietzka, Klaus Schneider, and Leonard Ritter

## Contents

Introduction ..... 432
Nomenclature ..... 432
Extrapolations ..... 434
  Duration Extrapolation ..... 435
  Extrapolation from LOAEL to NAEL ..... 435
  Interspecies Extrapolation ..... 435
  Intraspecies Extrapolation ..... 436
Consideration of Qualitative Aspects ..... 437
Application Framework ..... 437
References ..... 438
  Resources ..... 438

**Abstract**

Only rarely is sufficient toxicological knowledge available on a risk of interest. In cases where toxicological data are incomplete for a specific *quantitative risk assessment*, the assessment may also draw on general scientific knowledge gained from experience with other chemical substances. However, this approach of *extrapolation*, using *default factors* based on empirical evidence, is not without controversy.

R. Konietzka (✉)
Federal Environment Agency, Dessau-Roßlau, Germany
e-mail: rainer.konietzka@uba.de

K. Schneider
Research and Advisory Institute for Hazardous Substances, FoBiG, Freiburg, Germany
e-mail: klaus.schneider@fobig.de

L. Ritter
School of Environmental Sciences, University of Guelph, Bovey Bldg, Canada
e-mail: lritter@uoguelph.ca

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_59, 

## Introduction

Suitable human data for the relevant risk group is frequently absent when performing quantitative risk assessment. Risk assessment is therefore often based on test animal data which need to be evaluated with regard to the risk group in question. Such an assessment and estimation was first performed in the USA as early as 1954 based on defined principles. Also since the 1950s, bodies of the World Health Organization (WHO), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the Joint FAO/WHO Meeting on Pesticide Reviews (JMPR) have set ADI values (see chapters "► Health-Based Threshold ADI Versus MOS in Toxicology" and "► Limit Values and Guideline Values in Regulatory Toxicology") for food additives and pesticide residues in food as 1/100 of the NOAEL (see chapter "► Dose-Response Analysis, Identification of Threshold Levels for Chemicals") in experimental animals.

This factor-based assessment with the help of conventions has been the subject of discussions again and again since then. On the one hand, it is rejected as unscientific, while on the other hand, attempts have been made at the same time to justify the factor physiologically or empirically. For this purpose the overall factor was subdivided into individual factors. For example, the WHO (1999) typically defines, in purely formal terms, the traditional intraspecies factor of 10 as a quantity made up of 3.2 for toxicokinetic variability times 3.2 for toxicodynamic variability (=10), without further justifying the size of the factor. The WHO (1999) applied a similar approach in the case of the interspecies factor (4.0 for toxicokinetics times 2.5 for toxicodynamics, see Table 3).

Depending on the regulatory context (e.g., occupational safety and health or public health), different factors must be chosen with regard to the relevant population at risk which the risk assessment proposes to protect (healthy workers or the general population) and different exposure durations. The following focuses on public health aspects.

## Nomenclature

In general, a distinction can be drawn between two types of assessment steps: physiologically/empirically based assessment steps and assessment steps that cannot be scientifically or empirically validated:

1. Physiologically/empirically based assessments use toxicological data and *extrapolations* to an expected level on the basis of those data (e.g., lowering of the NOAEL to extend the test period to lifelong exposure). This quantitative assessment should include a comprehensive interpretation of empirical data.
2. Additionally, qualitative aspects, not based on the same data, are considered in order to safeguard against uncertainties (e.g., consideration of an incomplete database to safeguard against potential, untested effects).

**Table 1** Subdivisions of extrapolations and qualitative aspects in quantitative risk assessment

| Physiologically/empirically based extrapolations | Qualitative aspects |
|---|---|
| Duration extrapolation | Data quality/completeness of database |
| LOAEL/NAEL extrapolation | Type/severity of effects |
| Interspecies extrapolation | Grounds for suspicion |
| Intraspecies extrapolation | |

**Table 2** Default factors and underlying assumptions for equipotent extrapolation (scaling) of data from animal experiments to humans

| | Assumed body weight of experimental animal[a] | | | Factors (caloric demand scaling) | | |
|---|---|---|---|---|---|---|
| | UBA, Germany[b] | US EPA[c] | ECHA[d] | UBA, Germany | US EPA | ECHA |
| **Mouse/ human** | 30 g | 30 g | 30 g | 7 | 7 | 7 |
| **Rat/ human** | 350 g (Fischer) and 430 g (Sprague-Dawley) | 250 g | 250 g | 4 | 4 | 4 |
| **Hamster** | – | – | 110 g | – | – | 5 |
| **Guinea pig** | – | 500 g | 800 g | – | 3 | 3 |
| **Rabbit/ human** | – | 2.5 kg | 3.8 kg | – | 2 | 2.1 |
| **Monkey/ human** | 0.3 kg (marmoset) and 10.0 kg (rhesus) | – | 4 kg | 4 and 2 | – | 2 |
| **Dog/ human** | 12.0 kg (beagle) | – | 18 kg | 2 | – | 1,4 |

[a]Body weight assumed for humans: 70 kg
[b]Kalberlah and Schneider (1998)
[c]US Environmental Protection Agency: EPA/630/P-02/002F, December 2002, final report
[d]European Chemicals Agency: ECHA guidance on information requirements and chemical safety assessment, Chapter R.8, Version: 2, 2010

Table 1 provides an overview of how extrapolations and qualitative aspects are subdivided.

According to its "Guidance document for use of data in dose/concentration-response assessment," the WHO distinguishes between the terms "adjustment factor" for chemical-specific factors and "*uncertainty factor*" for default factors. The WHO applies these factors to account for both *uncertainties* and *variabilities*. The US Environmental Protection Agency (US EPA) also uses the term "uncertainty factors" for its assessments but also applies "empirically derived scaling factors" as addressed in Table 2. A possible reason for replacing the previously common term "safety factor" with "uncertainty factor" may have been to avoid conveying the (wrong) impression of absolute safety. The European Chemicals Agency (ECHA) uses the term "assessment factor" for factor-based "extrapolations." In Germany, the terms "extrapolation factor" or "safety factor" are applied in keeping with the assessment steps described above.

**Table 3** Comparison of default factors used by different organizations in quantitative risk assessment (systemic effects)

| Factor for | US EPA[a] | WHO[b] | ECHA[c] | UBA, Germany[d] |
|---|---|---|---|---|
| **LOAEL/ NAEL** | 10 alternatively BMD[e] | Up to 10 alternatively BMD | Preferred BMD | Preferred BMD if not possible: up to 10 |
| **Intraspecies variance** | 10, reduced to 3 if based on data from susceptible subgroups | Up to 10 ($3.16 \times 3.16$ for toxicokinetics and toxicodynamics) | 10 | 10 (toxicokinetics and toxicodynamics, not quantifiable) |
| **Interspecies variance** | 10 toxicodynamic component $10^{0.5}$ (≈3) | Up to 10 ($4.0 \times 2.5$ for toxicokinetics and toxicodynamics) | Factor for allometric scaling (Table 2)<br>Remaining diff. 2.5 | Allometric scaling (Table 2) remaining differences: 2–3 |
| **Subchronic to chronic** | 10 | 1–100 | 2 (Geometric mean) | 10 (≈75 percentile) |
| **Additional safety** | Modifying factor > 0–10 | | 1 | 1 |
| **Combination** | Multiplicative[f] (RfC: max. 3,000 RfD: max. 10 000) | Multiplicative | Multiplicative | Multiplicative (max. 3,000) |

[a]US Environmental Protection Agency: EPA/630/P-02/002 F, December 2002, Final Report
[b]World Health Organization: Environmental health criteria 170 (IPCS) 1994
[c]European Chemicals Agency: ECHA guidance on information requirements and chemical safety assessment, Chapter R.8, Version: 2, 2010
[d]Kalberlah and Schneider 1998
[e]*BMD* benchmark dose
[f]*RfC* reference concentration, *RfD* reference dose

## Extrapolations

The following extrapolations may have to be performed in quantitative assessment:

- Duration extrapolation (e.g., from subchronic to chronic exposure)
  - Extrapolation from an available LOAEL (lowest observed adverse effect level) to a NAEL (no-adverse-effect level) as the desired level of protection (see chapter "▶ Dose-Response Analysis, Identification of Threshold Levels for Chemicals")
- *Interspecies extrapolation* (from experimental animal to human)
- *Intraspecies extrapolation* (from groups with average susceptibility to groups with increased susceptibility)

Such extrapolations are a component of most assessment concepts. Standard or default values of up to 10 are usually invoked for these extrapolation steps (see Table 3), which are described in more detail below; these default values should,

however, be adjusted to reflect *substance-specific knowledge* as far as possible. The following rationales for applying these assessment steps and the following empirically derived quantifications, based on the analysis of relevant data on a large number of substances, have been under discussion:

## Duration Extrapolation

Duration extrapolation will be necessary if in a risk assessment designed to cover lifelong exposure (70 years), toxicological data are only available for short-term exposure, and it cannot be ruled out that the effect increases over time. An analysis of toxicological studies resulted in the following relations as compared to the traditional, commonly used default factor of 10 mentioned above.

For extrapolation **from subchronic to chronic**, a factor of 10 presumably covers a high percentile of the examined cases (substances). This means that for a large number of substances, this factor is sufficient to cover possible increases in effect over time. In terms of the geometric means, the analysis shows a factor of about two or three.

For extrapolation **from short term ("subacute") to chronic**, a duration extrapolation factor of 6 appears to be justified, based on the geometric mean. (Duration extrapolation from subacute to chronic is, however, uncommon in public health risk assessment.)

The factor resulting from such an analysis does not directly represent a measure of the actual dose-time relationship, but only describes the commonly observed dose-time relationship, since the design of the respective studies has a strong influence on the outcome (see chapter "► Examination of Acute and Chronic Toxicity").

## Extrapolation from LOAEL to NAEL

This extrapolation will be necessary in cases where a no-adverse-effect concentration is the desired level of protection and no dose without an effect was obtained in experimental studies.

The data on LOAEL/NAEL relations reported in the literature reflect the study-design-dependent spacing between doses more than the actual steepness of the dose-response curve. Results will vary depending on the conventions on which the underlying data are based. As an alternative to this extrapolation, benchmark procedures (benchmark dose approach, BMD) may be used (WHO, 2009).

## Interspecies Extrapolation

The toxicity of a substance is determined not only by its dose but also by anatomical features and physiological parameters. The relationship between these parameters

and body weight, which is used as dose reference, has been observed to follow certain laws (*allometric scaling*) which deviate from a simple linear correlation. Thus, the basal caloric demand of species of different sizes correlates with the results of toxicokinetic and toxicity (including toxicodynamics) studies in different species. Basal caloric demand correlates with body weight to the power of 0.75.

Consequently, the analysis of relevant available data leads to factors which are body weight dependent and therefore species specific (scaling factors) for equipotent extrapolation of data from animal experiments to humans (dose extrapolation based on basal caloric demand (or metabolic rate) scaling). The factors and underlying assumptions are shown in Table 2.

Assuming the allometric relation with basal caloric demand is valid, identical inhalation exposure concentrations must be considered to be equipotent in different species. Hence, no *extrapolation factor* is applied for this route, or the factor is 1 for all species comparisons.

Of course, individual substances may deviate from this "average situation," leading to higher or lower susceptibility of humans compared to that predicted by caloric demand scaling.

So, if the variability of substance data is to be accounted for, the factors have to be increased.

## Intraspecies Extrapolation

Sensitivity differences between individuals may be influenced by age, health status, gender, genetic factors (enzyme polymorphisms), or by their specific constitution and situation (weight, body mass index, gravidity).

The analysis of available data shows that the commonly used default factor of 10 is probably sufficient to protect a large part of a group of healthy adults, including with regard to potential toxicokinetics-related differences. There is considerable uncertainty when it comes to assessing the significance of genetic polymorphisms of xenobiotic-metabolizing enzymes. Data analyses suggest that, in fact, such polymorphisms may lead to large individual differences in internal exposure. When higher internal exposure due to polymorphisms occurs in subgroups with higher susceptibility, such as children, the sick, and the elderly, a factor of 10 may not adequately account for these differences, but this variability cannot yet be quantified.

A more in-depth analysis for the group "children" reveals a higher sensitivity, above that of the average healthy adult, of infants, and of newborn babies due to their still-incomplete capacity to excrete xenobiotics. The aforementioned default factor covers this deviation for the most part. In contrast, older children are not considered more sensitive compared to adults with respect to toxicokinetic differences. Phases during which sensitivity is particularly elevated occur in particular during the period of organ development (perinatal exposure) and rapid organ growth.

## Consideration of Qualitative Aspects

A distinction must be drawn between the extrapolations addressed above and the consideration of qualitative aspects. The need for this is often justified by qualitative risk assessment aspects. Their quantification requires subjective assessment steps which cannot be scientifically or empirically validated.

Such *safety factors* are derived to account, e.g.:

- Data quality (additional factor due to a poor database)
- The quality/severity of the observed effects (additional factor for particularly critical toxicological endpoints; see chapter "► Adverse Effects Versus Non-adverse Effects in Toxicology")
- Grounds for suspicion (additional factor for hitherto unquantifiable potential properties of a substance, e.g., suspected carcinogenicity)

Conventions on this have been defined by, e.g., the WHO and the US EPA (Table 3). The WHO applies a factor of up to 10 to account for suspected carcinogenicity, and the EPA gives a "modifying factor" of up to 10 in case of a poor database. Typically, these factors cannot be validated by data analysis.

## Application Framework

The assessment steps discussed here always constitute the attempt to incorporate into the assessment fundamental findings and standards on which no substance-specific knowledge exists. An overview of factors applied by different organizations, and their sizes, is provided in Table 3.

In the case of extrapolations, knowledge drawn from experience can provide justification both for each factor itself and for its quantification. The range this allows to be delimited, or default factors, should be refined as better knowledge becomes available. When in doubt, the decision should generally be in favor of the risk group to be protected. The consideration of better knowledge also means consideration of better alternative procedures. The replacement of LOAEL/NAEL extrapolation by benchmark procedures is a case in point. For interspecies extrapolation, for example, this means, in the first instance, use of a validated PBPK model (see chapter "► Toxicokinetic Models"); in the second instance, use of substance-specific data for species comparison; and in the third instance, an extrapolation based on metabolic rate scaling which also takes data variability into account. Similarly, in risk assessment, valid human data should always be preferred over data from animal experiments, which require additional extrapolation steps (e.g., for differences in susceptibility between species). Most of the existing extrapolation concepts combine the various subfactors by multiplication. However, this is only statistically correct if the individual factors are independent of each other. This is not necessarily the case. For example, when data from a large lifetime animal study are transferred to humans, the age component of sensitive groups of persons may already be covered, at least partially, by the study design. It seems appropriate

to limit the size of overall factors obtained by multiplication, for when above a certain level they express a data uncertainty which makes the performance of a quantitative risk assessment difficult to justify (Table 3). Here too, the use of better alternative procedures, such as probabilistic methods, should be considered where possible.

Regulatory toxicology is concerned essentially with predicting health effects and making decisions on the basis of limited data. In that sense, risk assessment outcomes contain uncertainties, due also to the use of extrapolations and factors, which are a problem intrinsic to this field. These uncertainties must be described clearly in order to characterize the reliability of a risk assessment, especially since that reliability is an important information item for risk management.

## References

Kalberlah F, Schneider K (1998) Quantification of extrapolation factors: final report of the research project No. 116 06 113 of the Federal Environmental Agency. Schriftenreihe der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Fb 797, Dortmund/Berlin

World Health Organization (WHO) (1994) Environmental health criteria 170: assessing human health risks of chemicals, derivation of guidance values for health-based exposure limits. IPCS, International Programme on Chemical Safety, World Health Organization, Geneva

World Health Organization (WHO) (1999) Environmental health criteria 210: principles for the assessment of risks to human health from exposure to chemicals. IPCS, International Programme on Chemical Safety, World Health Organization, Geneva

World Health Organization (WHO) (2009) Environmental health criteria 239: principles for modelling dose–response for the risk assessment of chemicals. IPCS, International Programme on Chemical Safety, World Health Organization, Geneva

## Resources

IPCS (2005) Guidance document for the use of data in development of chemical-specific adjustment factors (CSAF) for interspecies differences and human variability in dose/concentration response assessment. http://whqlibdoc.who.int/publications/2005/9241546786_eng.pdf

ECHA (2010) Guidance on information requirements and chemical safety assessment Chapter R.8: characterisation of dose [concentration]-response for human health, Version: 2. http://echa.europa.eu/de/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

U.S.-EPA (2002) A review of the reference dose and reference concentration processes. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/630/P-02/002F. http://www.epa.gov/raf/publications/review-reference-dose.htm

# Background Exposure Versus Additional Exposure in Human Biomonitoring

Hans-Günter Neumann

## Contents

Background Exposure and Additional Exposure ..... 440
Human Biomonitoring ..... 440
The HBM Concept ..... 440
Risk Assessment ..... 441
Uncertainty ..... 441
The Relative Risk ..... 441
The Reference Value ..... 442
Which Risk Should Be Considered Tolerable? ..... 443
Reference Values for the Workplace Environment ..... 444
Do Thresholds Play a Role? ..... 444
Reference Doses and Concentrations ..... 445
References ..... 447

**Abstract**

Due to the improved analytical methods, it is now possible to measure in man hazardous chemicals or their activation products in human blood and tissues. Biomarkers have been identified whose tissue level reflects the internal exposure resulting from all sources of uptake – including the contribution of endogenously occurring products (biological monitoring). One step further to risk assessment is the measurement of protein and DNA adducts (biochemical effect monitoring) which reflects the body burden with reactive metabolites. Applying these methods it turned out that in most cases not only the exposed but also not knowingly exposed controls had significant adduct levels. This raised questions about the existence of background exposures and their role for risk assessment.

H.-G. Neumann
Department of Toxicology, University of Würzburg, Würzburg, Germany
e-mail: hg-neumann@t-online.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_60, 

## Background Exposure and Additional Exposure

The concentration of hazardous chemicals or their metabolites in biological material are considered background if they are detected in populations without known exposure to the chemical. The background consists of various environmental sources and frequently also of compounds resulting from endogenous, physiological pathways, which means they are composed of avoidable and non-avoidable sources. Food and lifestyle predominantly contribute to background levels. Every biomonitoring result has to be assessed keeping a possible background in mind. The additional burden of a suspected compound should therefore be treated as an increment to background (Sugimura et al. 2000).

## Human Biomonitoring

Human biomonitoring is not anymore restricted to workplace control but increasingly also to assess suspected environmental exposures. It helps to establish health-based limit values. The Human Biomonitoring Commission of the German Environmental Protection Agency (Umweltbundesamt), Kommission Human-Biomonitoring (1996), has been commissioned this task since 1993. The idea is to define a concentration of a biomarker in a body medium below which health is not affected. Risk assessment may be based on a NOAEL (no observable adverse effect level) if the mode of action is reversible and the dose-effect relationship is not linear. HBM-1 and HBM-2 values (1996) can be established if sufficient toxicological information is available. If these are not the case, or tolerable exposures can, in principle, be defined – like with genotoxic carcinogens – additional exposures can be established in relation to such reference values.

## The HBM Concept

The HBM-1 value marks the concentration of a chemical in a body medium below which no harm is expected. If the concentration exceeds the HBM-1 value, further controls are necessary. The HBM-2 value indicates the limit above which an individual may suffer health effects. In this case, environmental follow-up is recommended and if possible measures should be initiated to reduce the exposure. The HBM-1 value is considered a check and control value, the HBM-2 value an interference or interaction point. It is not always possible to establish health-based limits, either because quantitative human data are not available or because data from animal experiments are not sufficient. With non-genotoxic chemicals, it is often difficult to agree which end point or which alterations have to be considered adverse. With carcinogens, it is in principle hard or impossible to identify a harmless exposure. With genotoxic carcinogens producing irreversible effects, the exposures should therefore be minimized, i.e., exposures should be kept as low as reasonably achievable (ALARA). A HBM-1 value cannot be established.

## Risk Assessment

Which criteria can be used to control the exposure of chemicals without a HBM value, particularly with carcinogens? How can we proceed from assessing the potential of a chemical to produce tumors to its potency, i.e., from qualitative to quantitative properties? Frequently, a tolerable risk is set at one additional tumor in a population of $10^5$–$10^6$. The "unit risk," i.e., the number of tumor cases associated with an exogenous exposure to the unit dose of 1 g/m$^3$, has often been used as an estimate of the absolute risk. The US EPA (US Environmental Protection Agency) presents the upper limit of the probability (95. percentile) with which tumors develop after a lifelong exposure to the unit dose.

## Uncertainty

The risk is usually derived from results obtained in animal experiments. To account for uncertainties three steps are connected using default factors. The risk observed with the high doses used in animal experiments must be extrapolated down to low doses, relevant in the human situation, assuming that the same mode of action works in both cases. Moreover, both the species specificity and individual susceptibility have to be accounted for. If, for instance, food has been found to be contaminated with a carcinogen, the public wants to know what it means. Based on available data, the risk is calculated and presented as additional cancer cases. Such values are extremely uncertain. An essential prerequisite which follows is that no estimate value should be given without the associated uncertainty. The US National Research Council suggested already in 1994 (National Research Council 1974) that for any single-point estimate, the sources for the calculation and the extent of uncertainty should be stated and the upper bound given. Since this requirement can mostly not be fulfilled, the unit risk is discussed very controversially.

The use of uncertainty factors, like 10 for dose uncertainty, 10 for species differences, and 10 for individual variability, remains most unsatisfying and does not meet the requirement of substance-specific evaluation. A fundamental objection against the unit-risk concept is that the result cannot be tested; it cannot be falsified. With a cancer rate of 20–25 % in the human population, it is impossible to prove an increment of 1:$10^5$–1:$10^6$. The use of default asset values, particularly more than one combined, is quite unsatisfying. It follows that the calculation of an absolute risk leads to uncertain and unrealistic results. Precise risk assessment seems to be impossible with the present scientific knowledge.

## The Relative Risk

Are there alternatives to regulate chemicals producing irreversible effects? An important step forward to control exposure was the development of biomonitoring procedures. Biomonitoring of effects is closer to the end point of decease than

biomonitoring of exposure. Biomonitoring of effects provides information about the biologically active dose and the individual susceptibility. The difference to the generally used risk assessment is that the end point of the assessment is not the population-based decease but the use of analytical data from an individual. The data represent the immediate situation of an individual human, without any defaults for the environmental situation or species differences or susceptibility. Although a biological active dose does not allow to calculate an absolute risk, it is possible to envisage the relative risk by comparing the actual value with reference data.

## The Reference Value

The Human Biomonitoring Commission of the German Environmental Agency (UBA) has defined reference values as follows: "The reference value for a chemical in a biological medium (for instance blood or urine) is a value which is derived from a number of respective measurements of a random sample of a defined population using a preset outlined statistical procedure." The reference value is a statistical value which describes a concentration of this compound in the body medium for this part of the population sample at the time of sampling. It is important to emphasize that the reference value is not related to any health effect.

According to the IUPAC directive, the reference value is statistically defined as the rounded 95 % percentile of the measured values within the 95 % confidence interval. Whether this arbitrarily chosen 95 % percentile makes sense depends on the frequency distribution of the measurements which has to be assessed from case to case. How appropriate the 95 % percentile is has to be evaluated from case to case based on the distribution curve. Reference values describe the actual situation of a certain subpopulation without any recognizable external exposure. Reference values may be applied to determine an extra exposure of individuals or population samples, and they are an important means to assess the known exposure at the time of sampling (background exposure). Reference values can be used to detect particular exposures of individuals or subpopulations by comparison. They are helpful in epidemiological studies of environmental stress.

To establish a reference value, it is necessary to characterize the reference population and to take care for possible confounding factors as precise as possible. UBA data from the environment survey, for instance, were considered a suitable base and have been used to quantify background exposures. The UBA Human Biomonitoring Commission has announced reference values for lead, cadmium, mercury, pentachlorophenol in body fluids; for polychlorobiphenyl and for polychlorinated biphenyls PCB 138, 153, 180, and the sum of these; as well as for ß-HCH (hexachlorocyclohexane) and HCB (hexachlorobenzene) in whole blood, blood plasma, and breast milk. Reference values are being used successfully in numerous scientific and environmental studies, even though the strong criteria of the definition have not been always applied.

## Which Risk Should Be Considered Tolerable?

The question which risk should be considered a tolerable risk cannot be answered with scientific arguments. Experts may look for the existence of background exposures. Eventually, the available information has to be evaluated (expert judgement). In this process it is important to consider existing background as well as interindividual variability and the quality of the available reference values, but the ultimate question is not how high is the absolute risk but to which extent does the respective dose contribute to the total body burden.

How can the concept of relative risk contribute to the problem of tolerance, for instance, with genotoxic chemicals? First of all, criteria have to be developed on how to define the limit for a tolerable risk. In this context, the German Commission (Senatskommission 2002) extended the classification of carcinogenic chemicals as a first step and introduced two new categories: 4 and 5. Before that, carcinogens were classified basically in three groups according to the strength of evidence: (1) sufficient evidence of carcinogenicity in humans, (2) evidence of carcinogenicity only in experimental animals, and (3) the evidence is inadequate in humans and inadequate or limited in experimental animals, but suspicious data exist. This system decided essentially about a carcinogenic potential, but not about – strong or weak – potency. With the new categories, the mode of action and the carcinogenic potency were introduced into the classification system. A carcinogenic chemical may either be non-genotoxic (4) or genotoxic (5), and members of both categories are characterized by having low carcinogenic potency. The new perspective is that the limit, i.e., a tolerable risk, is not expressed as an absolute value, but based on the contribution to risk. Low risk means the tolerable exposure does not contribute appreciably to cancer risk. The term "significantly" has been avoided at this point, because the value should not be understood as a statistical term. Instead it has been called "appreciably," which conforms with the German phrase "nicht nennenswerter Beitrag" (Neumann et al. 1997).

Biochemical end points exist which correlate with cancer risk and can be used as biomarkers. The experience with biochemical effect monitoring indicates that a contribution to risk could be called non-appreciable if an external exposure leads to an internal exposure and corresponding biochemical effects which are within the variability of the background of a reference population which is not knowingly exposed. If the level of a relevant biomarker lies within the range of the background level, a contribution to risk cannot be established.

Low-dose effects can be compared with unavoidable exposures and effects of structurally related compounds. The ALARA principle should still be observed, but reference values mean also a limit below which a limit value cannot reasonably be established. The concept includes the possibility that reference values have to be changed, for instance, if unknown sources are detected and contribute to the background or cleaning efforts were successful.

The tolerance criteria may be different depending on the effectiveness of the compound in question and on the quality of the available information.

The interindividual variability of biomarkers may be great. This may be due to uncontrolled external exposures, but it is likely that due to their toxicokinetic profile, individuals whose values are in the upper part of the distribution suffer a greater load than those in the lower part. Scientific criteria do not exist so far to handle this problem. Data have to be evaluated from case to case. A conservative criterium would be to use the reference value itself as a limit for the tolerance value. In case of less powerful compounds, an additional increment to the background level could be discussed. This could be treated less stringent with non-genotoxic than with genotoxic compounds.

## Reference Values for the Workplace Environment

As a first application of biochemical effect monitoring, technical guidelines have been established for hazardous chemicals (TRGS 710). To evaluate the analytical biomonitoring results, it has been recommended to consider the biological working place tolerance values (BAT values) from the MAK- and BAT-value list described in the TRGS 903. If such data are not available – BAT values for genotoxic chemicals from category 1–3 do not exist – the results may be compared with exposure equivalents of carcinogenic chemicals (EKA values) shown in the MAK- and BAT-value list or reference values from the general population, whether and to which extent workers are exposed. Moreover, biomonitoring is generally recommended if carcinogenic chemicals and germ cell mutagens are involved at the workplace. Since biomonitoring was introduced, background exposures have been detected in workers not exposed to such chemicals at the working place environment.

## Do Thresholds Play a Role?

A threshold is usually understood to separate an effective from an ineffective dose, although one may find in most dose–response relationships an exposure for a biological end point for which no strain can be seen (NOAEL). Even with ineffective exposures of compounds producing reversible effects, the search for the "ineffective threshold" is not any more adequate. Now it appears more appropriate to look for deviations of physiological balances, which are detectable below the NOAEL, and to assess how well the system adapts against strain and which degree of imbalance should be considered adverse.

The cell adapts to stress at the mitochondrial respiratory chain, for instance, with an increased synthesis of respiratory chain components. If that stress increases beyond a critical point, the cell is eliminated by apoptosis and substituted by a new one. Several signaling pathways are involved and may control a common end point, such as apoptosis. With this perspective, it is not reasonable to search for a concentration threshold of ineffectiveness. It is important to find out how much a certain stress affects the cellular energy balance, in other words, leads to

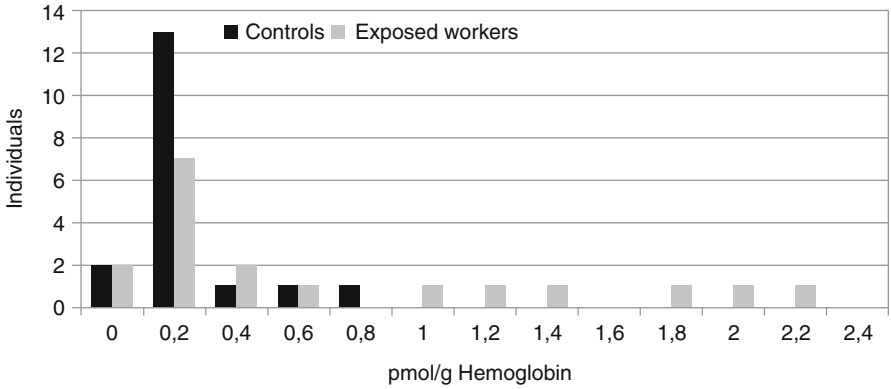



**Fig. 1** Distribution of hemoglobin adducts in 18 coke oven workers exposed to polycyclic nitroarenes at the workplace and 18 controls not known to be exposed. Five hemoglobin adducts were measured and the results added. Leading adduct levels for pyrolysis products were those from 1-nitropyrene and 2-nitrofluorene. Adduct levels were below the detection limit in two workers of both groups. Adducts were well measurable in all the other controls and were considered as background. Only 6 out of 18 workers, all of which were exposed at the same dirty workplace of the coke oven, had adduct levels above that background. This observation raises the question: what is the source of the background exposure? All pyrolysis products presumably contain nitroarenes, as well as polycyclic aromatic hydrocarbons (PAH) (Neumann et al. 1995)

proliferation. A threshold between effect and no effect cannot be defined for a chemical which affects the respiratory chain. Effects on processes taking place below the NOAEL should be considered stress which under favorable circumstances can be compared with a reference value. DNA lesions produced by genotoxic compounds have been shown experimentally to be linear down to extremely low doses without threshold. This does not support the threshold concept on the molecular level (Neumann 2009).

## Reference Doses and Concentrations

There are other limit values, which are different from those above. They do not represent a certain state (background stress), but a target or reference value. The so-called chronic reference dose (RfD) is defined as the daily burden of a human, who tolerates this lifelong exposure without appreciable health risk.

These values are derived usually from animal experiments as a NOAEL or LOAEL which is modified by one or more uncertainty factors (10, 100, 1000). The RfD depends on the applied uncertainty factor and is not derived from human data. For inhaled chemicals, a reference concentration exists analogously. The concept is based on the premises that (1) a threshold exists, below which no adverse effects occur; (2) the most sensitive individuals are included in the population distribution; and (3) a critical effect exists, which covers all other adverse effects.

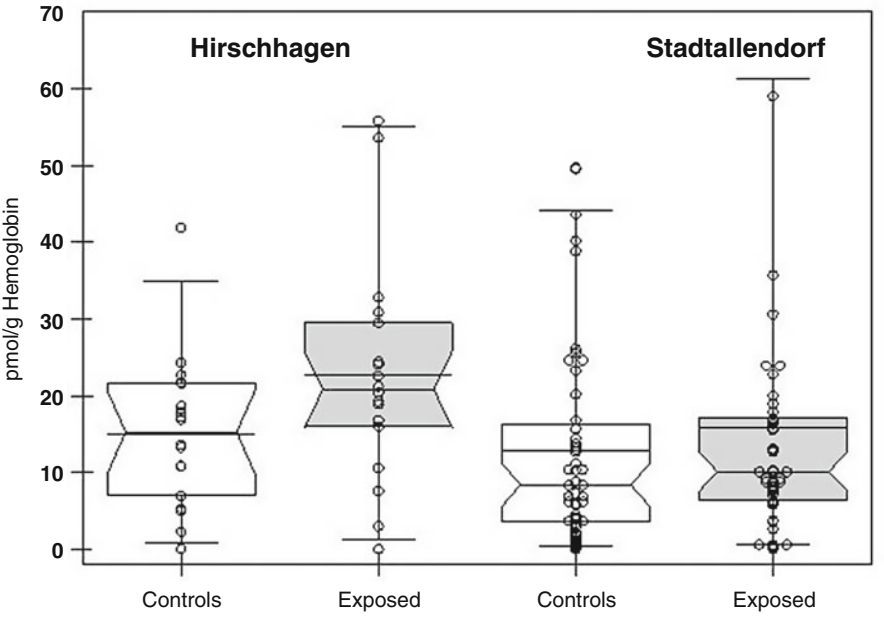



**Fig. 2** Biomonitoring of residents living in an area where soil was contaminated with explosive wastes and not knowingly exposed controls ($n = 18, 18, 34, 34$). Dinitro- and trinitrotoluenes were used to represent explosive wastes in this study. Hemoglobin adducts from 2,4- and 2,6-dinitrotoluene (carcinogenic) were hydrolyzed and typical cleavage products measured. Each point represents one individual. Shown are box plots indicating mean, median (waist), and 95 % percentile of values. The great difference within the groups was expected and may be partly due to different external exposure. However, the presence of adducts in the controls was quite unexpected and points to a widespread background exposure to genotoxic nitroarenes. Even rather high external exposure to genotoxic nitroarenes in soil and groundwater did not increase the internal exposure. It was concluded that residents of the contaminated area do not bear an additional cancer risk (Ewers et al. 2000)

Similarly, the Food and Drug Administration (USA) created the acceptable daily intake, ADI, and the Occupational Safety and Health Administration (OSHA), a permissible exposure concentration PEL. These limits express an estimated exposure, which does not produce any harm with reasonable certainty. In both cases, the external stress of a representative population is assessed without being related to measurable human parameters.

In 2002, a biological guiding value was introduced by the German MAK- and BAT-Value Commission for chemicals without BAT value. This is based on experience from handling these chemicals at the workplace supported by general toxicological knowledge. If such a value is established, it is combined with the mandate to improve the toxicological knowledge to aim at lowering the respective exposures. Arsenic and bromomethane were the first chemicals which were given such a guiding value.

## References

Ewers U, Zwirner-Baier I, Neumann HG, Zelder E, Seuren-Kronenberg K (2000) Hämoglobin-Addukt Konzentrationen sprengstofftypischer nitroaromatischer Verbindungen im Blutr von Bewohnern von Rüstungsaltstandorten, Teil 2 Studie Stadtallendorf (ehemaliges DAG- und WASAG-Gelände) Umweltmed Forsch Praxis 5, pp 277–284

Kommission "Human Biomonitoring" des Umweltbundesamtes (1996) Konzept der Referenz- und Human Biomonitoring (HBM) in der Umweltmedizin. Bundesgesundheitsblatt 6/96

National Research Council (USA) (1974) Science and judgement in risk assessment. National Academy Press, Washington, DC

Neumann HG (2009) Risk assessment of chemical carcinogens and thresholds. Crit Rev Toxicol 50:449–461

Neumann HG, van Dorp C, Zwirner-Baier I (1995) The implication for risk assessment of measuring the relative contribution to exposure, environment and lifestyle: haemoglobin adducts from amino- and nitro-arenes. Toxicol Lett 82(83):771–778

Neumann HG, Thielmann HW, Gelbke HP, Greim H, Kappus H, Norpoth KH, Reuter S, Vamvakas P, Wardenbach HE (1997) Proposed changes in the classification of carcinogenic chemicals in the work area. Regul Toxicol Pharmacol 26:288–295

Senatskommission (2002) Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe der Deutschen Forschungsgemeinschaft MAK- und BAT-Werte-Liste. Wiley-VC, Weinheim

Sugimura T, Nagao M, Wakabayashi K (2000) How we should deal with unavoidable exposure of man to environmental mutagens: cooked food mutagen discovery, facts and lessons for cancer prevention. Mutat Res 447:15–25

# Adverse Effects Versus Non-adverse Effects in Toxicology

Norbert Englert and Robert L. Maynard

## Contents

The Meaning of the Term *Adverse* ..... 450
Definition of Adverse Effects ..... 450
Dimensions of Adversity ..... 450
Severity of an Effect ..... 451
Detectability ..... 451
Subjective Perception ..... 452
Reversibility ..... 452
Probability and Number of Persons Concerned ..... 453
Groups Concerned ..... 454
Causality ..... 454
Final Remarks ..... 454
References ..... 454
Recommended Reading ..... 455

**Abstract**

The term "adverse" is used with the meaning "disadvantageous" or "harmful." An effect should be avoided if it is adverse but could theoretically be tolerated when non-adverse. Generally it is more or less clear what is meant with "adverse," but in a real situation, it may be difficult to position the line separating adverse from non-adverse.

N. Englert (✉)
Formerly: Federal Environmental Agency, Berlin, Germany
e-mail: norbert.englert@web.de

R.L. Maynard
Bideford, Devon, UK
e-mail: robertmaynard3@gmail.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_61, 

## The Meaning of the Term *Adverse*

The term "adverse" is an adjective which is often used to describe negative effects on health. We take the term "adverse" to imply some degree of harm or the likelihood of unfavorable consequences for the individual or population concerned. The term "undesired" seems, to us, less appropriate as a synonym for "adverse": not all effects that might reasonably be described as undesirable need necessarily be adverse.

The term "adverse" is commonly used without reference to a clear definition. Using imprecisely defined terms in assessments of effects, or possible effects on health, is not at all uncommon. For example, Article 3 of the Charter of Fundamental Rights of the European Union says: "Everyone has the right to respect for his or her physical and mental integrity."

## Definition of Adverse Effects

The IPCS/WHO definition of adverse effects, published in 1994 (Environmental Health Criteria 170), is often quoted, but the second and very important sentence of the definition (our underlining below) is frequently omitted:

> Adverse effect: change in morphology, physiology, growth, development or life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences. Decisions on whether or not any effect is adverse require expert judgement.

In 1985 and 1999, the American Thoracic Society (ATS) tried to define adversity in context of air pollution. Supplementing their 1985 statement, in 1999 the ATS referred to health-related quality of life. However, the ATS emphasized that this statement does not offer strict rules or numerical criteria, but rather proposes principles which may be used in weighing the evidence and setting boundaries between adverse and non-adverse health effects, and the placement or positioning of dividing lines should be a societal judgement (ATS 1985, 1999).

In including quality of life and well-being, ATS is supported by the 1948 WHO definition of health (www.who.int/about/definition/en/print.html): "Health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."

## Dimensions of Adversity

The ATS statements and other summarizing reviews indicate several dimensions or characteristics of adversity, in part referring to an individual concerned, in part in addition or exclusively to a population, and also referring to qualitative or general aspects.

Dimensions of adversity:

- Severity of an effect
- Detectability of an effect

- Reversibility of an effect after end of exposure
- Probability of an effect
- Particularly concerned subgroups ("environmental justice") and effect on current or next generation
- Causal relationship between exposure and effect

## Severity of an Effect

According to ATS, death or an increased risk of death and any consequence on life expectancy as well as clinically significant effects should be classified as adverse effects.

For respiratory symptoms and changes in indices of physiological function, the ATS sees transition to adversity if quality of life is impaired. In this context, health-related quality of life refers to factors including capacity to look after oneself, mental health, pain, and generally feeling well. Decrease in health-related quality of life is classified as adverse, e.g., shown by clinically significant findings when diagnostic tools, including questionnaires, are used to assess health status.

Keeping in mind that there is a considerable lack of knowledge of the meaning of changes in biomarkers, ATS does not generally classify changes in biomarkers as being adverse.

## Detectability

Concerning detectability of effects, usually statistical significance is demanded. Tests of statistical significance are used to distinguish, with an acceptable but arbitrary level of confidence, between effects that might be taken to imply some actual relationship between a potential cause and an effect and those which might occur by chance. Thus, unless an association reaches "statistical significance," it is likely to be ignored. The possibility of error should be noted. Large epidemiological studies have great statistical power and can detect very small effects. Thus, in the air pollution field, a change in the daily average concentration of particles defined by $PM_{10}$ has been shown to be associated with a less than 1 % increase in the risk of death over a short period. This is a small effect but the coefficient that specifies the effect is statistically significant and is thus regarded as unlikely to have resulted from chance.

Statistical significance should not be taken as proof of a causal relationship: the possibility of confounding needs to be considered. Death is clearly an adverse effect on health, but is a small increase in the risk of death also, by definition, an adverse effect? It should be noted that when death is discussed, the risk, for all, is absolute: we all die. What is usually meant is an increased likelihood of death in a specified period or a shortening of life expectancy. This opens up the question of what degree of shortening of life expectancy should be regarded as adverse. Many would say that any effect on life expectancy is an adverse effect. This leads to a rather philosophical

point: the effect may be adverse but is it important? There is no scientific way of answering this question: the answer will vary from person to person.

In the 1985 statement, the ATS showed a pyramid or triangle to illustrate the relationship between severity of an effect and number of persons likely to be affected. At the top of the pyramid is death as the most severe effect, followed by disease and finally respiratory symptoms. This pyramid, broadening from top to bottom, is thought to illustrate that with decreasing severity the frequency of an effect increases in a population. The hypothesis that influences on mortality are severe but rare effects, however, does no longer correspond to current knowledge at least with respect to particulate matter. It seems reasonable to think that less exposure to some toxic material is needed to produce a minor effect than a major effect, but work in the air pollution field has shown, especially with regard to particles, that at all ambient concentrations studied, effects on all outcomes (deaths, hospital admissions, symptoms, restriction of daily activity) occur. Thus, it is now felt that the idea of a series of thresholds separating effects on, for example, symptoms from effects on the likelihood of death is incorrect. Whether or not a very small decrease in life expectancy should really be seen among the most severe health effects may be debated. In any case, we can no longer assume that effects on mortality generally only concern a very small part of the population exposed.

The fact that the traditional classification distinguishing between substances without effect threshold (carcinogenic substances) and those with effect threshold is not supported by recent findings makes the classification of toxicologically active materials by their effects more difficult. Without an effect threshold, a purely qualitative statement (effect/no effect) is no longer adequate. There will always be the quantitative aspect to be added (i.e., how large or how frequent an effect may be or should not be).

## Subjective Perception

On an individual level, the more an effect is perceived as imposing a limitation on physical or mental activity and as having negative emotional overtones, the more the effect is likely to be classified as adverse. Aspects like assessing individual possibilities of influencing effects or – in contrast – perceptions of helplessness can act as modifiers.

The subjectively perceived probability of an effect occurring at a certain exposure does not always correspond to "objective" reality, but it contributes to the subjective assessment of adversity.

## Reversibility

Reversibility of effects after the end of exposure is at least very important for symptoms and functional changes. Complete or partial irreversibility would suggest adversity (the occurrence of adverse effects); complete reversibility might support non-adversity, especially in the case of effects of very low severity. With respect to functional physiological parameters, the ATS supposes minor transient deteriorations

of lung function values not to be automatically classified as adverse, but if connected with symptoms, they should. A detectable, permanent deterioration of lung function, however, is always classified as adverse.

## Probability and Number of Persons Concerned

In clinical parameters, at a population level even a minor degree of effects of air pollutants is generally declared not acceptable by the ATS. This may be an example of the fact that changes classified as being not adverse at an individual level may demand different consideration if they occur in a group or a population. Figure 1 schematically shows that a possible classification as adverse or non-adverse depends on the severity of the effect in an individual concerned as well as on the percentage of persons concerned in a population at a certain exposure.

Below the line through y, the severity of effect in an individual concerned is so low that the effect would not be classified as being adverse even if the whole population would be concerned. Left to the line through x, the number of individuals concerned is so low that a relationship to exposure is no more verifiable due to statistical reasons. To the left of and below the dashed curved red line, an effect is not adverse because the severity of the effect is small even considering the number of persons concerned. To the right and above the dashed curved line, an effect is classified as adverse because considering the severity in an individual concerned as well as the number of persons concerned in the population exposed seems to be not tolerable. In this case, the large fraction of persons concerned suggests the classification of an effect as adverse even if it would be classified as non-adverse at an individual level.

However, the difficulties begin when you try to find the adequate position of x and y on defined and scaled axes.

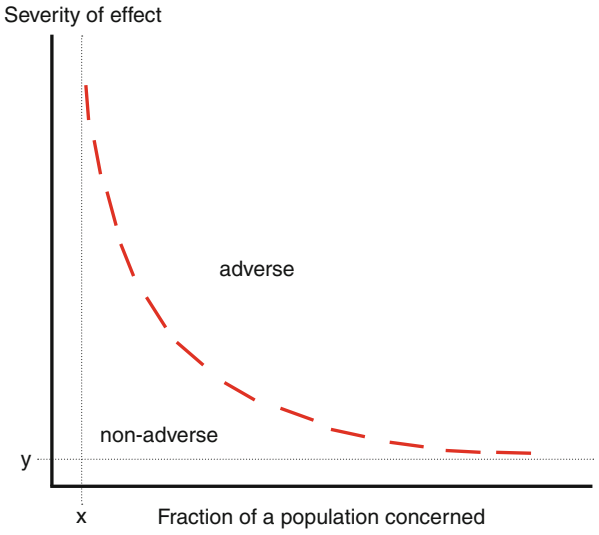



**Fig. 1** Pragmatic classification of an effect as adverse in relation to the number of persons concerned and to the severity of the effect in concerned individuals

## Groups Concerned

If effects are limited to certain subgroups of the population, this will need to be considered when assessing effects at both an individual and population level. Effects focussed on certain subgroups may lead to different assessments on an individual level ("I am not concerned, so it is not so severe") and at the level of effects on public health ("it is a particular injustice because some groups are at a much higher risk").

Effects probably appearing for the first time in the next generation tend to be classified as "adverse," although due to the potential manifestation in the future, the uncertainties are larger than in effects without large temporal latency.

## Causality

The question of causality is a matter of discussion in all epidemiological findings. The Bradford Hill features of causal associations may be helpful (Hill 1965), but whether or not an effect – be it directly measured or "only" calculated – is attributed to its real cause cannot always be clearly decided.

Whether or not a causal chain like *cause – change in a biomarker of exposure – change in a biomarker of effect – functional change – symptom – disease – death* could be demonstrated or only hypothesized and whether that chain describes a possible or an unavoidable course should be considered when assessing the adversity or non-adversity of the initial steps of such a known or hypothesized chain.

## Final Remarks

Generally, the IPCS/WHO definition of 1994 – extended by aspects of (subjectively perceived) quality of life – seems to be a reasonable basis for deciding on whether an effect should be regarded as "adverse." For classifying an effect as adverse or non-adverse, it is not possible to give precise criteria which in any particular case result in an "objective" and transparent assessment. Expert judgement remains a necessity. This should not be seen as an unavoidable deficiency but rather as an opportunity for a discussion exactly adapted to each particular situation. Which persons are the experts authorized to perform such a judgement is a question similarly difficult as that of the threshold between adverse and non-adverse. In a larger context this is subject to societal agreement.

## References

American Thoracic Society (1985) Guidelines as to what constitutes an adverse respiratory health effect, with special reference to epidemiologic studies of air pollution. Am Rev Respir Dis 131:666–669

American Thoracic Society (1999) What constitutes an adverse health effect of air pollution. http://www.thoracic.org/statements/resources/eoh/airpollution1-9.pdf
Hill AB (1965) The environment and disease: association or causation. Proc R Soc Med 58:295–300
IPCS/WHO (1994) Environmental Health Criteria 170. http://www.inchem.org/documents/ehc/ehc/ehc170.htm

## Recommended Reading

Englert N (1996) Ableitung von Grenzwerten für Stoffe in der Luft. In: Transparenz und Akzeptanz von Grenzwerten am Beispiel des Trinkwassers, UBA-Berichte 6/96. Erich Schmidt, Berlin, pp 78–87
Lippmann M (1988) Health significance of pulmonary function responses to airborne irritants. J Air Pollut Control Assoc 38:881–887
Samet JM (1993) Indoor air pollution: a public health perspective. In: Indoor Air ′93. Proceedings of the 6th international conference on indoor air quality and climate, Helsinki, vol 1, pp 3–12
Schimmelpfennig W (1999) Adverse Effekte als humantoxikologisches Problem. Beiträge zur umwelthygienischen Standardsetzung. Schriftenreihe des Vereins für Wasser-, Boden- und Lufthygiene, vol 103. Berlin, pp 108–138

# Health-Based Threshold ADI Versus MOS in Toxicology

Ursula Gundert-Remy

## Contents

Health-Based Threshold Values ..... 458
Dose Without (Adverse) Effect ..... 458
Safety Factors/Uncertainty Factors/Adjustment Factors ..... 460
Margin of Safety (MOS)/Margin of Exposure (MOE) ..... 460
Exposure Assessment ..... 461
Risk Assessment ..... 461
References ..... 461
Resources ..... 461

**Abstract**

The derivation of health-based threshold values in various fields of regulatory toxicology is based on consented rules laid down in regulatory guidance papers (EFSA EFSA J 8:1325, 2010; ECHA, 2010) Guidance on information requirements and chemical safety assessment Chap. R.8: characterisation of dose [concentration]-response for human health ECHA-2010-G-19-EN). The rules were developed according to the field of application and are improved when scientific evidence became available showing that elements of the framework have to be changed. No principle difference exists in the guidances from several European agencies.

The MOS concept applies the same principles; it is however more flexible as it allows to introduce scientific judgement.

U. Gundert-Remy
Institute for Clinical Pharmacology and Toxicology/Institut für Klinische Pharmakologie und Toxikologie, Charité Universitätsmedizin Berlin, Berlin, Germany
e-mail: ursula.gundert-remy@charite.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_62, 

## Health-Based Threshold Values

The basic concept of health-based threshold values, such as ADI values (acceptable daily intake), was introduced by JECFA (Joint Food and Agriculture Organization – World Health Organization Expert Committee on Food Additives) already in the 1950s. Applying the evaluation scheme, food additives are assessed by an international panel of experts. Later, the concept was adopted by the Joint Food and Agriculture Organization – World Health Organization Meetings on Pesticide Residues (JMPR). In the beginning ADI values were developed only by the two scientific panels. Later, when the European Food Agency (EFSA) was established in 2002, ADI values were derived by scientific panels working for EFSA.

Other European regulatory bodies, in particular the European Chemicals Agency (ECHA), also used the concept to derive so-called derived no-effect levels (DNELs) whereby some differences exist between the two European agencies as EFSA derives a single ADI, whereas ECHA derives several DNELs depending on the route of exposure, even for specific end points separately, and separate DNELs for healthy workers and for the general population.

The principle of the concept is to define a dose, which, based on the scientific evidence, can be assumed to be safe, i.e., without adverse health effects in humans even if the dose is taken up on a daily basis for a lifetime. In most of the cases, the point of departure (POD) to derive a safe dose is experimental data from toxicological studies in animals. The dose which did not produce an adverse effect in animals (NOAEL, BMD) serves as the POD. The dose is adjusted to the human situation by using a factor accounting for the interspecies difference and a second factor accounting for the variability in the human population to derive the dose which is assumed to be safe during a daily lifelong exposure by food (ADI), dermal contact, and inhalation (DNEL). Knowing this dose allows regulating exposure at a level which will not result in adverse health effects in the exposed population.

## Dose Without (Adverse) Effect

The dose without an (adverse) effect is the dose for which, in an experimental study, no effect has been observed compared to the control treatment. It should be taken into consideration that the dose critically depends on the experimental conditions such as number of animal tested, dose range and dose interval, and the range of end points tested (e.g., histopathology, clinical chemistry, and functional tests). It has not yet been decided whether the existence of an effect has to be demonstrated by a statistically significant difference compared to control or whether biological plausibility is sufficient.

The limitation of the procedure on how to derive a dose without effect is obvious. We must determine a dose with an adverse effect in order to be able to define the neighboring lower dose as the dose without an effect. Hence, the number

**Table 1** Cumulation factors for substances with long half lives: 28 day study versus 90 day study

| Half life (days) | % of steady state reached after 28 days | % of steady state reached after 90 days | Accumulation factor |
|---|---|---|---|
| 7 | 93.75 | 100 | 1.07 |
| 14 | 75 | 100 | 1.3 |
| 28 | 50 | 90 | 1.8 |

of animals and the spacing of doses are crucial in this respect. If we don't see an effect, this is not proof for absence of an effect. Animal protection requires reducing the number of animals, whereas statistical power considerations would require a high number of animals. We should be aware that OECD guidelines are a compromise between the two principles which does not prevent us in overlooking effects because of the low statistical power of the study.

It has to be discussed whether the point of departure (POD) to derive health-based threshold values is a no-effect level (NEL) irrespective if the effect is adverse or adaptive or compensatory or just a biochemical change. In particular, in the era of genomics, studies at the level of genes do allow to observe changes which, however, cannot be attributed to be adverse. NAEL is used to describe a theoretical no-adverse-effect level, and NOAEL is the not-observed-adverse-effect level. An example for an adaptive effect is the induction of drug metabolizing enzymes in the endoplasmic reticulum in the liver, which will lead to an enhanced metabolic capacity and thus a reduced toxicity if the parent compound is the toxicant. An example for compensatory changes is the increased inhalation rate in metabolic acidosis. In the International Program on Chemical Safety (IPCS/WHO), definition of adverse effect is as follows: change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences. Today, the NOAEL is taken as POD to derive health-based threshold values.

The duration of the study is also influencing the level of the NOAEL: this is partly due to the fact that in subacute and subchronic studies, the number of animals and also the number of parameters investigated are lower than in chronic studies which may lead to higher NOAELs in subacute and subchronic studies as compared to chronic studies. Sometimes, adverse effects may only be developing after long-term exposure. One explanation can be the kinetics. Substances with long half-life will cumulate and reach the maximum level only after prolonged exposure as demonstrated in Table 1.

Toxic effects may develop only after prolonged exposure, e.g., effects on the testes or thyroid as secondary effects with a primary effect on the liver. It is currently becoming clear that the whole database has to be taken into account including the studies for developmental toxicity and fertility. Those studies produce additional information not seen in repeated dose testing.

It is to be noted that using NOAEL as the POD does not use all available information on the dose–response relationship. International agencies (e.g., IPCS–WHO, EFSA) have therefore given advice to use all information by modeling the dose–response relationship and using the curve to derive a dose which corresponds to a low effect level (e.g., 5 % for continuous data or 10 % for categorical data). This is called the Benchmark dose approach. The Benchmark dose (BMD) is a dose level, derived from the estimated dose–response curve, associated with a predefined change in response, the Benchmark response (BMR) which can mean a change in incidence or magnitude.

## Safety Factors/Uncertainty Factors/Adjustment Factors

Point of departure (POD) for the derivation of a health-based threshold value is the NOEAL/the Benchmark dose from a chronic dose in man, often in the rat. Adjustment factors are used to "adjust" the dose in the rat to the respective dose in man. It is assumed that in general the human organism is more susceptible when compared to the rat. To bridge the species difference between rat and man, a factor of 10 is used. The interspecies factor is subdivided into a factor accounting for the differences in toxicokinetics and a factor accounting for differences in toxicodynamics. The toxicodynamic factor is 2.5, a value which is not well supported by data. The toxicokinetic factor is dependent on the species and based on allometric considerations. For the rat, the factor is 4, rendering the total interspecies factor to 10. For mice the toxicokinetic factor is 7, rendering the total interspecies factor to 17.5. For rabbit the toxicokinetic factor is 2, rendering the total interspecies factor to 5. The factors can be modified (so-called chemical-specific adjustment factors) if chemical-specific scientific data are available (WHO 2005).

An additional factor is used to account for the variability within the human population. The intraspecies factor is subdivided into a factor accounting for the toxicokinetic variability and a factor accounting for the toxicodynamic variability. The default value which is used is 10 whereby a factor of 3.14 accounts for toxicokinetics and a factor of 3.14 for toxicodynamics. Data from clinical studies showed when retrospectively analyzed that the factor of 10 is empirically supported. Only with chemicals/drugs metabolized by polymorphically expressed CYPs (such as CYP 2D6), the factor of 10 is not appropriate and a much larger factor is needed. If chemical-specific data are available, it is advised to use the data-derived factors instead of the default value ("chemical-specific factors").

## Margin of Safety (MOS)/Margin of Exposure (MOE)

There are situations where human exposure occurs, but no guideline level (such as ADI or TDI) is available that would help to assess the health risk of the chemical. In such circumstances the value for the NOAEL in the available study is divided by the exposure level. The quotient is called the MOS or MOE (in cases of genotoxic

carcinogens). The margin of safety is also used to assess the health impact in cases in which the exposure is higher than the ADI/TDI. The following aspects are to be taken into consideration when assessing the MOS/MOE: (1) scientific rigor of the database, (2) possible difference in the route of exposure between animal and man, (3) differences in the exposure scenario between animal experiment and human situation (duration, dosing, frequency of dosing: very often the total daily dose is given in one dosing, whereas in humans the dose might be divided in three meals), (4) steepness of dose–response relationship, (5) nature and severity of the effect, (6) differences between species, and (7) variability in the human population including possible sensitive subgroups.

## Exposure Assessment

It should be mentioned here that the assessment of exposure is as important as the hazard identification and dose–response assessment. The first step is the identification of the appropriate scenario, the second the parameterization of the scenario. Whereas for food intake databases have been collated and the content of chemicals in food is well known for most of the food items, the exposure situation is far from being known for other substances and other circumstances such as consumer products. In order to take a cautious approach, "worst case" assumptions are made which bear the potential to grossly overestimate the exposure.

## Risk Assessment

If the exposure is lower than the health-based threshold, it can be concluded that there is no health concern. Normally a factor of 100–300 is sufficient for non-carcinogens and non-genotoxic carcinogens. For genotoxic carcinogens, a MOE greater than 10,000 is interpreted in the way that no urgent measures have to be taken. In cases where the exposure is higher than the health-based threshold value, the MOS approach can be informative to assess the possible health impairment. For example, given the same effect (e.g., hepatotoxicity) a MOS of 10 is of higher urgency for measures as compared with a MOS of 90.

## References

WHO (2005) Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration-response assessment. ISBN 92 4 154678 6

## Resources

http://www.who.int/pcs/jecfa/jecfa.htm

# Precaution Principle Versus Danger Prevention in Toxicology

Ludwig Müller and Neill H. Stacey

## Contents

Principle of Hazard Prevention ..... 464
Suspected Threshold of Danger ..... 465
Principle of Precaution ..... 465
Distinction of the Area of Precaution ..... 466
References ..... 467
Recommended Reading ..... 467

**Abstract**

The pollution of water, soil, air, food, and everyday products with harmful chemicals is accompanied by risks for public health. The active defense or control of these risks can be effected using the principles of hazard prevention or precaution, respectively. Toxicological information is a basic contributor to preventing and controlling hazards together with data from other disciplines.

Protection of the health of consumers and their environment is dependent on scientific information and associated policy with preservation of public health through various measures. More specifically, consumers of food and everyday products are afforded protection by official regulations which concentrate on surveillance of the market and manufacturers, and warnings against goods recognized as presenting actual or potential adverse effects for the public.

L. Müller (✉)
State Health Administration, unit 44 (Pharmaceutics, Toxicology, Genetic Engineering), Bremen, Germany
e-mail: Ludwig.Mueller@gesundheit.bremen.de

N.H. Stacey
Southern Cross Pharma Pty Ltd, Malua Bay, NSW, Australia
e-mail: nstacey@southernxp.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_63, 

Environment-related health protection aims at safeguarding the public from toxic (carcinogenic, genome-altering, and other) effects that may come from contaminated water, soil, and air. Necessary tasks include the recognition and description of environmental influences adverse to health, the prevention or the removal of these influences where applicable, and the development and the transfer/mediation of findings such that harmful inputs from the environment may be avoided.

The administrative regulation of substance-related risks in this field is based on hazard prevention and/or precaution.

## Principle of Hazard Prevention

In general law, the term "danger" is described as a situation, which leads to damage of a protected legal good within a reasonable timeframe and with sufficient probability, if the expected course of events is not stopped. The basis is a safe prediction of the course of events. The requirements for the indication of danger are less demanding if the legal good in question is highly ranked (e.g., human life) and if serious damage is expected (e.g., health damage). The prevention of the development of such a situation is called hazard prevention.

To initiate official measures of hazard prevention concerning chemical substances, usually, a numerically fixed minimum triggering level, a threshold of danger or adverse effect is required. Because of the enormous legal consequences in some cases, pure suggestion of hypothetical damage 1 day is not sufficient to allow (legal) stipulation of the fixing of an absolute limit. Instead, the threshold must be based on scientific or otherwise obvious knowledge of a particular limit above which human health effects may occur.

The toxicologist may essentially contribute to the characterization of a threshold of danger by:

Definition of the relevant route(s) of exposure

Estimation of the extent of exposure

Characterization of risk groups

Determination of mechanism of action of a so-called adverse effect: an important health effect of not just a temporary nature out of a range of effects

Assessment of a dose–response-relationship

Estimation of a NOAEL (No Observed Adverse Effect Level) or a NOAEC (No Observed Adverse Effect Concentration; for instance, in the context of air quality values) or otherwise derived thresholds of effect (for non-genotoxic substances, for instance, using the benchmark procedure)

Determination of a limit dose by using assessment algorithm (e.g., Unit Risks) for genotoxic carcinogens based on "politically" agreed levels of acceptance or tolerance

Such characterization is based on appropriate animal studies (the quality of which are preferably categorized by the so-called Klimisch-criteria, Klimisch et al. 1997), epidemiologic findings, and additional reliable data. In some cases,

single observations in humans after accidents or disasters/catastrophes may also be of use in this endeavor.

## Suspected Threshold of Danger

During toxicological assessment, there are often imponderable aspects that arise due to the lack of useful epidemiological data and the ensuing need to extrapolate animal data (often at high doses) to long-term effects in humans linked to illness (mostly at lower doses relevant in the environment of the affected people). The resulting uncertainties need to be compensated by different (un)certainty and extrapolation factors, such as those proposed by the WHO, the ECHA (ECHA 2010), or the German Committee for dangerous substances (AGS 2010). With respect to legal requirements, in some cases, this is also true for additional so-called danger-linked factors.

The quality of the basis of evaluation, the extent of the broadly agreed factors involved, the experience and the expertise of toxicologists, and finally political considerations influence the determination of the regulatory limit as shown as the area of danger marked in the risk plot, here better described as suspected threshold of danger (Fig. 1). Because of the mainly toxicologically based derivation, the principle of protection by hazard prevention is regarded as a toxicological principle.

## Principle of Precaution

The principle of precaution is based on the assumption of a risk continuum. It follows that the possibility of a health risk below the threshold may not necessarily be totally excluded, especially when investigating worst cases with respect to vulnerable groups of people. This is of particular relevance when considering genotoxic carcinogens. However, with the depletion of the concentration of the pollutant in the environmental milieu, the risk declines and the initiation of health deterioration will become less probable (Fig. 1). The principle of precaution finally aims to reduce this risk to the greatest possible extent.

The application of the precautionary principle is thought to compensate for possible uncertainties (for instance, due to gaps of the toxicological data base) within the evaluation of substances/groups of substances (Mitteilung der Kommission 2000).

One special manifestation of the principle of precaution is the principle of minimization, as found in several legal acts. This means, that – for instance – the concentration of chemical substances polluting the environmental source or adversely influencing its quality (e.g., drinking water) has to be kept as low as ultimately possible according to the state of the art, while considering the circumstances of the particular case with reasonable expense/effort. In brief, it is referred to the ALATA (as low as technically achievable) or the ALARA principle (as low as reasonably achievable).

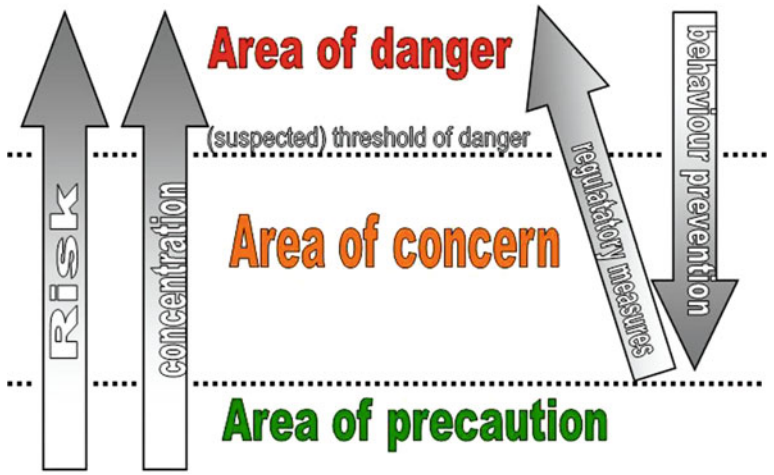



**Fig. 1** Assessment areas for harmful substances in the environment

The principle of precaution also involves the predictive or forward-looking protection of people against adverse exposure by the development, by recommendations, and by the enforcement or implementation of measures on the basis of health quality goals. This is strongly linked to the term of sustainability, the future viability of quality goals, the results of which satisfy the needs of living people. At the same time, these results should not reduce the chances of future generations for a healthy existence.

The burden of exposure of human beings from environmental sources not only should be minimized but rather should be removed or eliminated wherever possible.

Overall, the principle of precaution therefore incorporates the general aspects of environmental health.

## Distinction of the Area of Precaution

In regulatory affairs, it is often necessary to complete such qualitative considerations by quantitative assessments/evaluations to enable administrative/official measures, if needed.

On the scale of a continuum between risk at the (suspected) threshold of danger and (unrealistic) zero-risk, in principle, every value may be accepted for the entry into the area of precaution (precaution value). However, usually, the assumption is followed that a small deviation from the (suspected) threshold of danger is not enough to enter the precaution area. Instead, below the danger area, an area of concern is assumed in which individual hazards are not yet excluded. This area is tied to the area of precaution, in which a health hazard does not exist anymore or would be extremely unlikely (Fig. 1).

However, due to a lack of scientifically reliable data, the threshold to the area of precaution cannot be derived just by toxicological methods. Rather, it is oriented on technical, aesthetic, or general aspects of human well-being. Due to these considerations, the largest possible margin to the (derived) thresholds of effects (suspected threshold of danger) is usually chosen. It is expected that this approach can allow for current imponderables and differences among individuals.

In this context, the toxicologist's expertise is needed with regard to, e.g., information about and weighting of the extent of – for instance – carcinogenic, immunotoxic, neurotoxic, and development blocking effects in the lower risk area, as well as guiding principles for further yet-to-be-fully-resolved exposures, vulnerabilities, and potentials of hazard.

This envisaged level of protection which is beyond the scope of protection against danger is regarded as mainly based on general aspects of environmental health.

This principle manifests itself in the development of (precaution) standards and quality goals with contributions of toxicologists in official working groups, committees, and commissions, for instance, to solve questions with respect to health impact assessment, land-use planning, and so on. As for risk management, the implementation of such working results is usually modified to a high degree by the political weighting of various interests.

Along with administrative/regulatory measures, official recommendations and/or advice are addressed to users and consumers, such as aiming to avoid sources of pollution (behavioral prevention) (Fig. 1).

## References

AGS (2010) Kriterien zur Ableitung von Arbeitsplatzgrenzwerten. Bekanntmachung zu Gefahrstoffen. BekGS 901, Ausgabe April 2010. Ausschuss für Gefahrstoffe. GMBL 32:691–696

ECHA (2010) Guidance on the information requirements and chemical safety assessment. Chapter R8: Characterization of dose (concentration)-response for human health. Version December 2nd 2010. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r8_en.pdf?vers=16_12_10

Klimisch HJ, Andrae M, Tillmann U (1997) A systematic approach for evaluation the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5

Mitteilung der Kommission der Europäischen Gemeinschaften über die Anwendbarkeit des Vorsorgeprinzips. Kom (2000) end; Ratsdok. 6055/00, BR-Drucksache 141/00. http://ec.europa.eu/dgs/health_consumer/library/pub/pub07_en.pdf

## Recommended Reading

Müller L (1998) Gesundheitsbezogene Prüfwerte für Schadstoffe im Boden. Gesundheitswesen 60:439–444

# Hygienic Versus Toxicological Approaches in Regulation

Roland Suchenwirth

## Contents

Introduction ..... 469
Toxicology ..... 470
Hygiene ..... 471
Example: Pesticide Regulation in the Drinking Water ..... 472
The German Philosophy ..... 472
The Philosophy of the WHO: ..... 473
Regulation of "Unknown" Substances ..... 474
Recommended Reading ..... 474

**Abstract**

The hygienic approach aims at keeping the environment free of avoidable loads, which means to minimize environmental contamination and thus at the same time prevent chemically induced illnesses. The toxicological approach assumes that an exposure height can be defined, that does not lead to specific health risk and derives tolerable exposures from this starting point. Both approaches often complement each other in regulatory considerations.

## Introduction

While toxicology derives tolerable doses on the basis of present knowledge, hygiene aims at keeping the environmental media free of pollution/contaminants, which has the effect to reduce and avoid exposures (Fig. 1). The two concepts have a right to exist in parallel in regulations. They use different approaches

R. Suchenwirth
Head of Department of Environmental Medicine, Governmental Institute of Public Health of Lower Saxony, Hannover, Germany
e-mail: Roland.Suchenwirth@nlga.niedersachsen.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_64, 

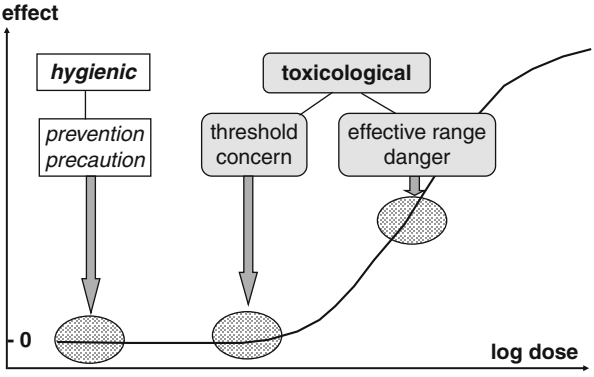



**Fig. 1** Hygenic and toxicological view

but have a common purpose: protection of the population and the living nature. The differences are less in the nature or content of applied methods, but rather in the way of thinking already at the beginning of a regulatory process.

## Toxicology

The assessment in toxicology is primarily based on the knowledge of effects caused by an individual substance and is evaluated under the motto of "dosis sola facit venenum."

Absolute thresholds without detrimental effects are derived mostly from experiences with (high) toxic doses for a single substance or group of substances under strictly defined conditions (e.g., certain occupation-types or animal species). For example, most of the Occupational Exposure Limit values (OEL) or also Acceptable Daily Intake values (ADI) are generated in this way (see chapter "► Assessment of Limit Values in Regulatory Toxicology" in this book).

Environmental toxicology has the objective to assess health risks associated with substances of geogenic or anthropogenic nature and their distribution in the environment. The human being as well as the animated nature is in its focus. Bioassays and studies with in vitro systems are used to determine or model the physical factors and the toxicity of substances that occur in the environment as intended active ingredients, residues, or resulting contaminants.

Limit values for pollutants in environmental media consider – in comparison with workplace regulations – the potentially longer duration of stay (24 h per day, each day of a year) and the higher diversity of influencing conditions, such as the potentially higher sensitivity of special population subgroups. It can be stated, however, that in most areas of regulation (work, environment etc.), the idea of prevention and the hygienic approach to protection becomes increasingly important.

## Hygiene

Hygiene is more far reaching, but at the same time, it is also more indistinct and less definable: Its scientific and educational approach aims at the prevention and control of illness as well as health preservation in particular through health protection and health promotion. Hygiene investigates all illness-causing factors in the natural, technical, and social environment. Based on this, the discipline develops counterstrategies and countermeasures.

Inherent to the vision of hygiene is the "precautionary principle" to protect health against detrimental risks from a contaminated environment with natural and anthropogenic pollutants. This is a guard or protective shield also against possible not yet sufficiently understood environmental-toxicological interactions, for the benefit of the population today and protection of the basis of life of future generations.

For regulatory purposes and risk management, hygiene also uses toxicological tools and methods in "risk assessment," but many more imponderabilities have to be taken into account in the development of limit values. Environmental hygiene deals with water-, soil-, air-, and food-mediated, potentially harmful influences on the living nature and man. The environmental-hygienic evaluation has to pay attention to a much larger variation width of influencing factors than, for example, the traditional occupational toxicology. Broad variances of the life circumstances and considerable differences in the length of the exposure, the number of substances, and further factors (see Table 1) exist. Also the prevention claim of the hygiene discipline goes on a lot further and has recently been integrated in many regulation philosophies, including occupational medicine.

The setting of toxicologically reasonable limit values, for example, OEL values, is always dependent on the state of scientific knowledge at the moment of implementation. Thus, it is also hardly disputable that in some cases in the past, limit values were subject to be corrected and judged more strictly a few years later, and some substances subsequently had to be classified as carcinogen. This may be considered as just barely tolerable for occupational (40 h/week) exposures and under consideration of the employers' liability insurance for economic compensation in case of damage. But the possible and nearly unavoidable impact of scientific uncertainty appears not acceptable when the population is exposed during the whole lifetime and when considering the cumulative effects for future generations (e.g., ground pollution, refuse dumps).

With this in mind, any complex weighted environmental-hygienic limit value usually provides a wider scope of protection compared to a similar value that is toxicologically derived. These additional weighing processes will be clarified further using the historical example of setting drinking water limit values for pesticides (see also chapter "► Assessment of Limit Values in Regulatory Toxicology").

**Table 1** Constraints and assumptions

| | Toxicology/Occupational Health | Hygiene and Environmental Medicine |
|---|---|---|
| **Exposure Substances** | Some few | Many |
| **Exposure type** | Mono-media (e.g., air) | Polymedial (water, soil, air, food, toys, etc.) |
| **Duration** | 8 h/day | 24 h/day |
| | Working life (40–45 a) | Lifelong (70–90 a) |
| **Substances (nature, number of)** | Definable, known | Inconsistent known, unknown |
| ***Use, handling*** | As intended | Unpredictable handling |
| **Health Status** | Healthy; under occupational medical control | Healthy and sick, old and young; no targeted control possible |
| ***Protection*** | Protective clothing, ventilation, air extraction | Not possible; substitution |
| ***Monitoring Surveillance*** | Targeted occupational surveillance & investigation, targeted measurement (e.g., human biomonitoring) | Incidental findings (e.g., population-based human biomonitoring) |
| **Substance combinations (combined effects)** | Few | Many |

## Example: Pesticide Regulation in the Drinking Water

The different approaches can be exemplified by comparing the earlier regulations of pesticides in drinking water in Germany (largely hygienic-environmental) with that of the World-Health Organization (largely toxicological). Although both are aimed at apparently nearly the same objective of protection and conservation, the numerical values show that different approaches were applied: use of the chemical detection limit in the German regulation but toxicologically derived values in the WHO guidelines.

## The German Philosophy

> The environmental impact of pesticides e.g. due to (useful) agricultural activity would reach an absolute upper limit, if such activities would inevitably lead to a (harmful) contamination of groundwater or drinking water. Its absolute lower limit, however, is defined by the amount of active ingredients, which must reach the (pest) target organism in order to be effective, however, without contaminating the non-target-compartments soil or groundwater.
> The mixture of harmfulness, usefulness and avoidability of pesticides leads therefore not to a „zero" value, but to 0.1 µg/l per single active substance in ground- or drinking-water. This tolerance threshold corresponds to the state of the art and makes agricultural activity equally acceptable for positive and negatively concerned persons (Dieter 1995).

For most pesticides, this drinking water limit value corresponds to hardly 1 % of the lifetime innocuous dose. So it is guaranteed that damages due to combination effects, barely investigated metabolites or reaction products during drinking water treatment, can be excluded with practical certainty. This means, however, at the same time that a temporary limiting value violation would result rarely in an immediate health risk. Thus, countermeasures are possible that should start soon, considering the sometimes decades long "contamination memory" of soil and groundwater.

## The Philosophy of the WHO:

WHO has the task to generate and distribute scientifically derived health standards. The procedure is fundamentally different from that in the German regulation: departing from an ADI or TDI value as a convention 10 % of the tolerable intake is allocated to the drinking water path. For lipophilic pesticides such as aldrin, DDT, lindane, and some others, for which it is assumed that the main transfer in humans occurs, e.g., via the food path, the allocation is only 1 %. For substances which are probably carcinogenic for man, concentration values were derived departing from a reference risk of $10^{-5}$ using the usual exposure parameters for drinking water.

It is however not expected by any means that the toxicologically derived limit values will be adopted into laws of the various countries. In the introduction to the "Guidelines for drinking-water quality," WHO states that for the derivation of national standards, it is necessary to consider the context of the local and national environment as well as the social, economic, and cultural conditions. In spite of references to necessary cost-benefit weighing, there is also a hint, that "*every effort should be done, to achieve a drinking water quality that is as high as possible. The best protection of the drinking water consists in avoiding the pollution of the raw water*". And at another place*: "Although the guideline values describe a drinking water that meets the claim of lifelong health protection, their derivation must not be understood in a way, as if this approved a replenishment of a given drinking water quality up to the mentioned values. On the contrary, the protection of a drinking water quality that is as good as possible, demands and earns continuous effort"* (Dieter 1993).

WHO as supranational organization could hardly be more clear than quoted comments in expressing its support for a minimization principle which exceeds the mere compliance with strictly toxicologically derived guideline values. This all the more, because WHO must always keep in mind the specific conditions, in particular the economic situation of the less developed countries.

For the national adoption of the guidance values, WHO advises, to take the specific ecological, geological, socioeconomic and technical prerequesites in to account. Therefore, the comparison of the WHO guideline values with the German legal limit values shows that the environmental-hygienic demands seems to be

absolutely appropriate. In particular, they fulfill the claims for a sustainable protection of the environmental resources.

## Regulation of "Unknown" Substances

There are situations, such as incidents of raw-water contamination, where drinking water regulation can unexpectedly have to deal with formerly unregulated substances and a lack of toxicology data (e.g., TOSU or PFT Contamination in Germany in 2002). In such a situation, the public health authority must check whether, despite the presence of the new contaminant in drinking water, there is no hygienic concern and whether the contamination is still compliant with the requirements of the national drinking water regulation. Such a specific approach was developed, e.g., by the German National Drinking Water Commission and published as a Recommendation of the German Federal Environmental Agency (FEA/UBA 2003).

## Recommended Reading

Dieter HH (1993) Die neuen Trinkwasserleitwerte der WHO für chemische Stoffe und ihre praktische Bedeutung. Bundesgesundhbl 36:176–180

Dieter HH (1995) Grenzwertkultur. Bundesgesundhbl (38):169

Dieter HH (2010) The relevance of "non-relevant metabolites" from plant protection products (PPPs) for drinking water: the German view. Regul Toxicol Pharmacol 56(2):121–125

FEA/UBA (2003) Evaluation from the point of view of health of the presence in drinking water of substances that are not (yet) possible or only partially possible to evaluate. http://www.umweltdaten.de/wasser-e/empfnichtbewertbstoffe-english.pdf last accessed 29 July 2013

Recommendation of the Federal Environmental Agency after consultation with the National Drinking Water Commission. Bundesgesundheitsbl - Gesundheitsforsch -Gesundheitsschutz 2003 (46):249–251. (Link: http://www.umweltdaten.de/wasser-e/empfnichtbewertbstoffe-english.pdf) last accessed 10 May 2013

World Health Organization (1993) Guidelines for drinking-water quality. Vol.1, Recommendations, 2nd edn. WHO, Geneva

World Health Organization (2011) Guidelines for drinking–water quality, 4th edn. WHO, Geneva http://www.who.int/water_sanitation_health/publications/2011/dwq_chapters/en/ last accessed 29 July 2013

# Protected Property and Protection Level in Regulatory Toxicology

Bernhard Liebl and Ines Liebl

## Contents

Subjects of Protection ..... 475
Protection Goals and Levels of Protection ..... 478
Complete Protection – Partial Protection ..... 478
Hazard Control – Prevention ..... 478
Substantiation of Protection Goals: Deduction of Standards ..... 479
Recommended Reading ..... 480
Resources (Last Date of Access: April 17, 2013) ..... 480

**Abstract**

The modern determination of standards (benchmarks, threshold values, etc.) is achieved in a multistep process, beginning with the definition of the subjects of protection as well as protection goals and levels of protection, respectively (Fig. 1). The process is not strictly divided from step to step. The assessment of data from one step often requires a feedback to the primary subjects of protection and protection goals.

## Subjects of Protection

Human being itself or the animated or inanimated environment can represent subjects of protection. In this context, two objects of legal protection are of significant importance:

B. Liebl (✉)
Bavarian Health and Food Safety Agency, Oberschleissheim, Germany
e-mail: bernhard.liebl@lgl.bayern.de

I. Liebl
Bavarian State Ministry for the Environment and Public Health, Munich, Germany
e-mail: ines.liebl@stmug.bayern.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_65, 

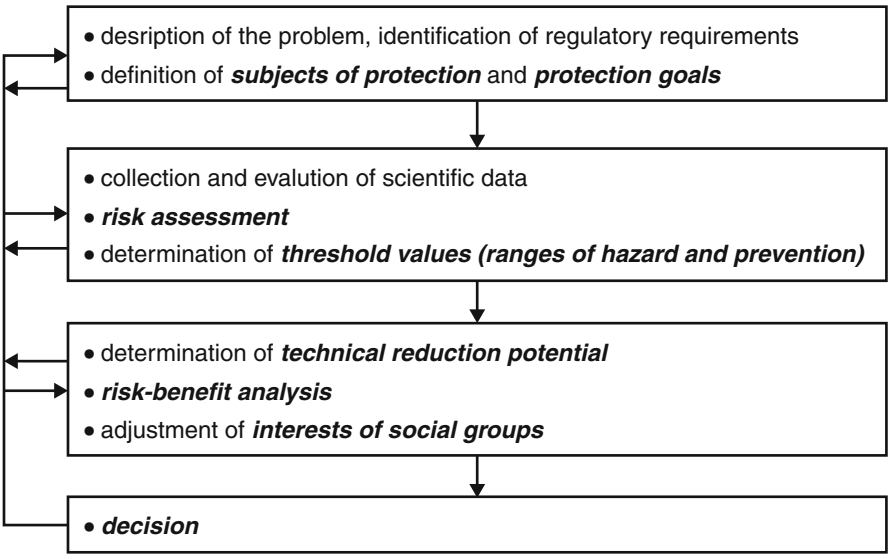



**Fig. 1** Determination of environmental standards

- "Physical integrity" (physical health)
- "Conservation of natural resources" (environment: ground, water, air, fauna, and flora)

In many countries, these objects of legal protection are firmly established in the constitution. Therefore, they have to be respected even if they are not explicitly addressed in a relevant law. Additional constitutionally protected objects, which have to be considered in this context, are "**professional freedom**" and the "**common freedom of action**." These basic rights are very relevant in the economic sector. They ensure the freedom to perform the profession of one's own choice, the use of manpower against payment, the possibility for businessmen to compete, and the entrepreneurial freedom of action.

Against this background, for example, in Germany, the ad hoc commission "reorganization of proceedings and structures for risk assessment and standardization in environmental health protection" (risk commission) defined three subjects of protection:

- Human life
- Diversity of species and types
- Economic power

These three subjects of protection depend on each other. They are fundamental in context of the global action program for the twenty-first century "Agenda 21" and the resulting strategy of "Sustainable Development," compiled in 1992 in Rio de Janeiro by the "Conference of the United Nations on Environment and Development."

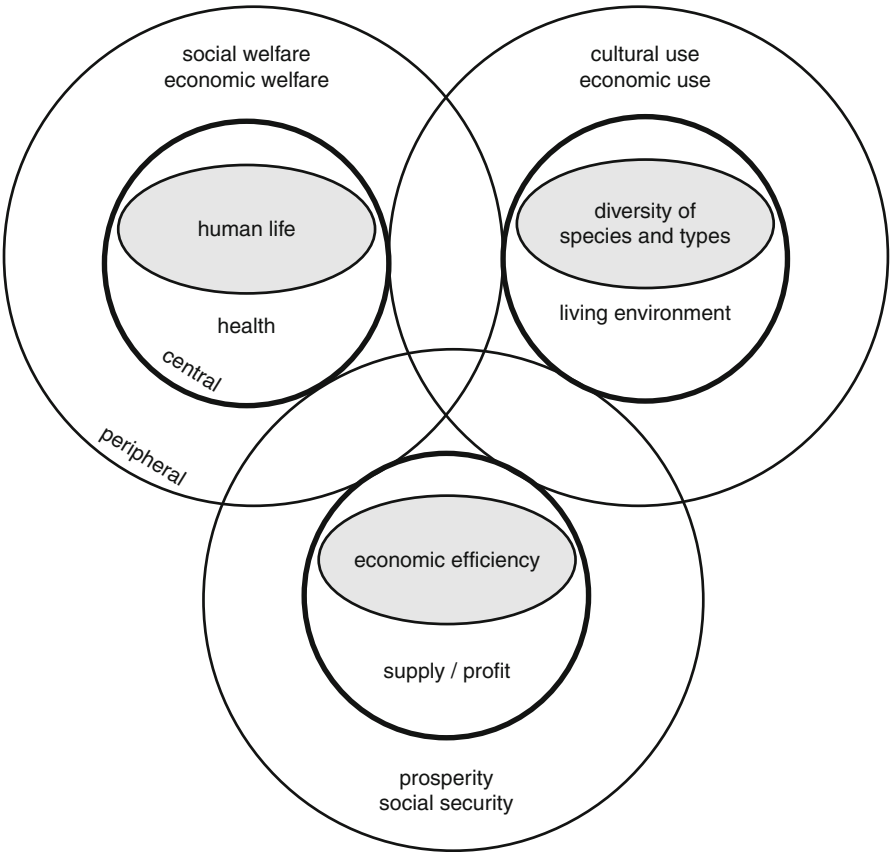



**Fig. 2** Areas and subjects of protection

When concrete measures are planned or evaluated, these three subjects of protection can come into conflict with each other. In such cases, it is recommended to distinguish between **central and peripheral areas within the subjects of protection** (Fig. 2). For human beings, the protection of health and, for nature, the protection of the natural living environment represent the central area (anthropocentric versus ecocentric protection of the environment or nature). The peripheral areas cover especially socially, culturally, and economically associated subjects which influence and determine the central areas. These subordinated, peripheral areas overlap and often cannot be precisely assigned to a distinct subject of protection. If it comes to a conflict between the central areas of the different subjects of protection, one should seek a measure which shifts the conflict into the peripheral areas, in order to protect the central areas as much as possible. In the peripheral areas, activities that carry risks become comparable and calculable. Additionally, in a concrete situation, it has to be

considered that upper-level objects of legal protection – normally, life and health of human beings – are favored compared to, e.g., economic objects. Compensatory measures should be considered for more affected subjects of protection. Moreover, risks depending on external influences should receive more weight than self-dependent risks.

## Protection Goals and Levels of Protection

Besides the definition of subjects of protection, it is also important to define how far the protection should go.

**Protection goals** describe the degree of intended protection and thereby the **level of protection** aimed at. Their definition has significant impact on the quantification of standards and the following implications. Protection goals can be classified in two ways:

- Complete protection – partial protection
- Hazard control – prevention

### Complete Protection – Partial Protection

In this context, depending on the risks that are to be regulated and the subjects of protection, the following questions arise:

- Is complete protection of subjects of protection intended or are certain risks tolerable, because their complete exclusion is not possible, too expensive, or socially not accepted?
- Are entire systems (i.e., populations, ecosystems) to be protected or additionally each therein contained individual component, possibly including particularly sensitive components?

In the discussion of these questions, also constitutional criteria have to be considered, for example, suitability, requirement, and adequacy of a planned measure.

### Hazard Control – Prevention

In many countries, law differs between damage, danger, prevention, and remaining (residual) risk. **Damage** means that the probability of a negative event (adverse effect) amounts to one, i.e., a negative event occurs with certainty or has occurred already. **Danger** means that damage is expected with a (inacceptable) high probability. In context of law, dangers have to be defended. The borderline separating danger from the range of prevention is determined by the level of non-tolerable risk. The borderline separating the range of prevention from a remaining (residual) risk is defined as **tolerable risk** (**traffic light principle**, Fig. 3).

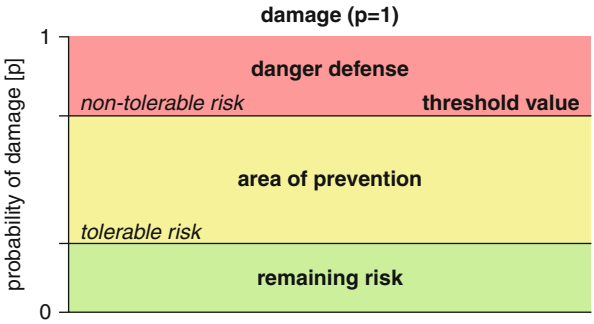



**Fig. 3** Traffic light principle

## Substantiation of Protection Goals: Deduction of Standards

If a protection goal is defined, this can – as far as possible and necessary – be substantiated for both protection levels, i.e., danger defense and area of prevention, respectively, by **quantitative risk assessment (QRA)**.

Generally, **danger defense** is implemented by definition of a normative **threshold value**. Threshold values generally separate the area of danger from the area of prevention. Exposures lower than the threshold values usually imply that affected objectives have no risk of damage. On the other hand, this does not imply that an exposure exceeding the threshold value automatically leads to damage.

An important source for the deduction of threshold values is toxicological data resulting from dose-effect or dose-probability estimations, respectively. In this context, it is important to differ between agents with dose-effect curves revealing a level beneath which no effect is observable or expected from agents for which such a level is not apparent. The last applies particularly for genotoxic agents, e.g., benzene or benzo(a)pyrene.

For **agents with a threshold of effect**, regulatory values are generally defined using the ADI concept of the WHO. Point of origin in this context is the "no observed [adverse] effect level" (NO[A]EL) or alternatively the "lowest observed [adverse] effect level" (LO[A]EL). The threshold for human beings, at which lifelong no harm for health can be expected (convention, not toxicologically evidenced), is calculated by division by a safety (respectively uncertainty) factor (normally 100).

A method used for **agents without a no observed effect level** (e.g., genotoxic agents) is, for example, the **unit risk method** of the Environmental Protection Agency (EPA). The unit risk of an agent describes the estimated additional lifelong cancer risk posed on a person exposed for 70 years with 1 μg of the agent per $m^3$ air. An additional lifelong cancer risk between 1:10,000 and 1:1,000,000 is discussed as acceptable. The dose corresponding to a risk of 1:1,000,000 is called "virtually safe dose."

The protection philosophy of threshold values based on quantitative risk assessment can be found e.g., in the WHO "Air Quality Guidelines" and the "Guidelines for Drinking-Water Quality" for Europe or the "Maximum Residue Limits" of the WHO.

The **precautionary principle** implies that (environmental) exposure should be prevented or reduced far before the risk of danger occurs. This principle is particularly applied in case of a suspected risk of agents for which scientific data for (quantitative) assessment are not yet sufficient to define threshold values. This is, for instance, the case when causal correlation between an exposure and damage is likely but not (yet) proven. In these cases, the principle of exposure reduction as far as economically and socially justifiable (ALARA, "as low as reasonably achievable") or as far as technically possible (ALATA, "as low as technically achievable") can be applied. In these cases, the precautionary principle is often not related to measurable effects and refers to the principles of "sustainable development" and protection of environment for further generations.

The **protection level** aimed at the individual case (i.e., how safe is safe enough? definition of "tolerable" or "negligible" risks, respectively) and the subsequent options of action are generally defined in the course of a normative (political) process of decision making. At best, science can contribute by describing scenarios using objective scientific data. Modern, socially accepted regulatory processes additionally require adequate information and participation of the public and transparent reproducible decision-making policies.

## Recommended Reading

European Commission: first report on the harmonisation of risk assessment procedures, Part 1: the report of the Scientific Steering Committee's Working Group on harmonisation of risk assessment procedures in the scientific committees advising the European Commission in the area of human and environmental health, 26–27 Oct 2000

Fehr R, Mekel O, Lacombe M, Wolf U (2003) Towards health impact assessment of drinking-water privatization–the example of waterborne carcinogens in North Rhine-Westphalia (Germany). Bull World Health Organ 81:408–414

Füllgraff G (2000) Bedeutung von Grenzwerten (Umweltstandards). In Wichmann, Schlipköter, Fülgraff (Hrsg.) Handbuch Umweltmedizin

Kappos AD (2003) Standardization of procedures and structures for risk evaluation in germany–the work of the risk commission. Int J Hyg Environ Health 206:231–235

Renn O, Klinke A (2004) Systemic risks: a new challenge for risk management. EMBO Rep 5(Suppl 1):S41–S46

The Federal Office for Radiation Protection (Bundesamt für Strahlenschutz BfS) (2002) ad-hoc Kommission „Neuordnung der Verfahren und Strukturen zur Risikobewertung und Standardsetzung im gesundheitlichen Umweltschutz der Bundesrepublik Deutschland (Risikokommission) Abschlussbericht über die Arbeit der Risikokommission. Limbach Druck- und Verlag GmbH, http://www.bfs.de

Wiedemann PM, Schütz H (2005) The precautionary principle and risk perception: experimental studies in the EMF area. Environ Health Perspect 113:402–405

## Resources (Last Date of Access: April 17, 2013)

Academy of technology assessment in Baden-Württemberg. http://elib.uni-stuttgart.de/opus/portal/ta/
Action program environment and health. www.apug.de
Council of experts for environmental issues. www.umweltrat.de

# Ethical Issues in Science. Focus on Regulatory Toxicology

Beate Henrikus and Wolfgang Eisenmenger

## Contents

Historical Development ..... 482
Ethical Principles ..... 484
Respect and Protection ..... 484
Informed Consent and Transparency ..... 484
Favorable Risk-Benefit Ratio ..... 484
Fair Participant Selection ..... 485
Scientific Validity ..... 485
Ethical Review Criteria ..... 485
Types of Experiments ..... 487
Animal Welfare ..... 487
Recommended Reading ..... 489
Resources ..... 490

**Abstract**

Toxicological, pharmacological, and biomedical trials in humans or animals imply ethical issues. Due to legal requirements, these studies are subject to an ethical assessment in most countries. Although the ethical principles, the review criteria, and the legal basis have been well established and harmonized for many years, the formal ethical assessment procedure differs on several factors.

Investigators have multiple and comprehensible interests: the desire to conduct high-quality research, to complete the research quickly, to protect research participants, to obtain funding, and to advance their careers. The very nature of many ethical issues in research means that they cannot easily be defined as clearly right or wrong. The resolution of these issues relies upon the person's values and beliefs, thus requiring an independent opinion. Independent review (frequently in the form

B. Henrikus (✉) • W. Eisenmenger
Ethics Committee Ludwig-Maximilians-University Munich, Munich, Germany
e-mail: Beate.Henrikus@med.uni-muenchen.de; Wolfgang.Eisenmenger@med.uni-muenchen.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_90, 

of an ethics committee) provides public accountability and minimizes potential conflicts of interest.

Much has been written about the bureaucratic downside of formal ethics review systems. On the one hand, ethics review uses up precious time and can be seen as delaying the research. On the other hand, through the continuous dialogue between researcher and the review committee, a positive reflective process is embedded throughout the experiment's life span.

In order to gain a deeper appreciation of ethical principles, it is helpful to consider the historical debate.

## Historical Development

In 1833, William Beaumont, a US Army surgeon, advocated for the right to perform human experiments. In 1865 Claude Bernard, a French physiologist, argued for animal experimentation as part of the standard scientific method. Both scientists only focused on the researchers' rights. About the same time the scientific community in medicine became aware of ambivalence in medical studies. The discrepancy between an experimental therapy, aiming at scientific interests, and an individual treatment experiment, serving patient's welfare, was published by Charles Nicolle in his views on the moral responsibility of scientists. At the end of the nineteenth century, as the experimentation on human beings and animals increased, criticism and controversy began. The public began to demand that the welfare of the patient is respected as well as the interests of researchers.

In Prussia, research regulations were introduced in 1900, following the increased governmental awareness of the lack of standards in medical research. These regulations were among the earliest and clearest pronouncements on the importance of informed consent in medical research. The reason for these research regulations was based on vaccine trials, conducted on prostitutes and abandoned children without consent.

In 1931, a directive from the German Ministry of Interior demanded that innovative or experimental therapy could only be conducted on human subjects if the person concerned (or his legal representative) had unambiguously consented after being informed in advance about the nature of the procedures and their risks.

In 1947, the "Nuremberg Code" was published. This code was based on ethical principles developed by the Nuremberg Military Tribunals during the prosecution of physician researchers, accused of conducting horrible medical experiments on prisoners of war during the Second World War. Being the first international standard for the conduct of medical research, the code was designed to protect the rights and the well-being of human subjects in medical experiments and to establish voluntary consent in research.

In 1964 the World Medical Association issued new recommendations on "Ethical Principles for Medical Research involving Human Subjects," based on the principles of the Nuremberg Code. They are known as the "Declaration of Helsinki." The Declaration has been adapted and amended several times, most

recently by the 59th World Medical Association in Seoul in 2008. The declaration modified the Nuremberg Code's first principle that the voluntary consent of the human subject is essential, with the recognition of the legitimacy of proxy consent for research involving children and persons with cognitive impairment. The Declaration of Helsinki states: "The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration."

In 1979 the Belmont Report was published. It summarizes the basic ethical principles developed by the United States Department of Health, Education, and Welfare, due to the problems arising from the Tuskegee Syphilis Study, an experiment in poor, rural black men. In this study the researchers knowingly failed to treat patients appropriately in order to study the natural progression of untreated syphilis.

In 1997 the International Conference on Harmonisation (ICH) published international ethical guidelines on Good Clinical Practice (GCP). These guidelines seek to harmonize clinical studies worldwide and to ensure that the data generated from studies are valid.

In 2004 the European Union implemented the principles of Good Clinical Practice. They have been laid down in the EU Directive 2001/20/EC. This Directive is law in all EU Member States. In the United States they appear in FDA Federal Regulations Title 21, Subchapter A, Part 56.

In 2006, the disastrous results of the first application of the monoclonal CD 28 human antibody TGN1412 in healthy volunteers, a so-called first-in-man study (FIM), raised many serious medical and ethical issues. The applied humanized monoclonal antibody acted in different fashion in humans as compared to the toxicological tests in laboratory animals. Nothing in the preclinical and toxicological tests predicted the overwhelming systemic reaction to the antibody; no previous animal tests demonstrated the toxicological response seen in humans. The severe adverse reactions occurred due to adverse immune-mediated drug reactions (such as cytokine storm, autoimmunity, immunosuppression). As a consequence a guidance for first-in-man studies was enacted by the European Medicines Agency (EMA) in 2007. Special care has to be paid to the novel mechanism of action (extent, amplification, duration, reversibility of the effect), the nature of the target, the relevance of animal species and models (questionable relevance implies an additional risk), the estimation of the first dose in human (when the methods of calculation (e.g., NOAEL, MABEL) give different estimations of the first dose in man, the lowest value should be used), the sequence and the interval between dosing of subjects within the same cohort, the dose escalation increments, the transition to next dosing cohort, the stopping rules, responsibilities for making decisions, monitoring, and communication of adverse events/reactions.

The differences in target affinity, mechanism of action, and immunogenicity between established toxicological models (i.e., NHP) and the human immune system, the functional potency of humanized monoclonal antibodies to modulate the target, and the new toxicology of these complex protein products (i.e., high target specificity, lack of metabolite toxicity) require the development and validation of new toxicological models.

Financial aspects must also be considered: the compensation of volunteers for the assumption of risk and the fair compensation of trial participants in case of injury.

## Ethical Principles

EU Directive, FDA Regulations, as well as the Declaration of Helsinki require an ethical review on a legal basis. Furthermore, they introduce legal obligations and specifications for the scope of the ethical assessment. Ethics committees have to guarantee that investigators act in compliance with fundamental ethical principles. These principles are:

### Respect and Protection

A fundamental principle is respect and protection of the individual. This includes the well-being of research participants, their right for self-determination, protecting privacy by assuring confidentiality of personal information, and respecting anonymity. The privacy of research participants and the confidentiality of their personal information have to be protected to minimize the impact of the study on their social integrity.

### Informed Consent and Transparency

A further principle is the right of a participant to make informed decisions, regarding participation in medical studies, both initially and during the course of the medical study. Voluntary consent must be guaranteed. No competent individual may be enrolled in a clinical trial unless he or she freely agrees. A research participant also has the right to withdraw consent at any time for any reason, without affecting their subsequent care. The participant must also be informed of all potential trial risks and burdens and of any newly discovered risks or benefits during the course of a clinical trial. Participants shall be informed of the results of the medical study.

### Favorable Risk-Benefit Ratio

It is based on the principles of “non-maleficence” and “beneficence.” Every medical trial has some degree of potential risk and benefit; therefore, investigators have to insure that risks to study participants are minimized. Consequently,

a careful assessment of the possible risks and benefits must be carried out for the trial participants. Medical studies involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the study subjects. There has to be a reasonable likelihood of benefit to the population studied. Experimental studies should always be compared to the best methods, but under certain circumstances a placebo or no-treatment group may be utilized. Special attention has to be paid to patients involved in placebo arms. The subsequent treatment of the research subjects after the end of the study is part of the ethical assessment. This includes the assurance that they will have access to the best-proven medical procedures. Investigations that are contrary to morals and conventions are ethically not acceptable. An example for such an experiment is a trial in humans for detecting the threshold of injuring effects of pesticides or herbicides. Such studies provoke only harms but no benefit for the individual.

### Fair Participant Selection

This principle is based on the principle of justice. To be ethical, the selection of participants must be fair. Investigators need to ensure that:

On the one hand, stigmatized and vulnerable individuals are not targeted for risky medical experiments. Medical studies involving a vulnerable population are only justified if there is a reasonable likelihood that the population benefits from the result of the research. When a study participant is incompetent, physically or mentally incapable of giving consent, or is a minor, the investigator needs the consent of a legal representative or proxy acting in the subject's best interest. On the other hand, not only rich and socially powerful individuals should be favored for potentially beneficial research. This demand is especially relevant for nations form the so-called Third World or nations without public health insurance coverage.

### Scientific Validity

To be ethical, clinical research must be conducted in a methodologically rigorous manner and must be of scientific value. Bad science is bad ethics because it does not emerge better medical knowledge but may provoke additional risks and harms for study participants. Furthermore, bad science may generate incorrect and nonvalid data which may entail harmful, risky, or ineffective treatments. The allocation of safe and effective drugs is a mandatory ethical and legal requirement.

## Ethical Review Criteria

The primary task of an ethical assessment by ethics committees is the review of research proposals and their supporting documents. Therefore, special attention is paid to the informed consent process, documentation, and the suitability and

feasibility of the protocol. Ethical reviews need to take into account previous scientific reviews and the requirements of applicable laws and regulations. The ethical review is focused on – but not exclusively limited to – the following issues:

- Are the risks acceptable?
- Are there any precautionary measures to minimize the risks?
- What are the potential hazards and how they are handled? An example is the use of magnetic resonance tomography (MRT) as a diagnostic tool instead of a CT in order to eliminate radiation burden.
- What is the scientific validity of the proposal – will it achieve its stated objectives?
- Is the methodology appropriate to the study?
- Is the drug and its dose adequate? Is the dose used to examine the efficacy the same as used for the safety research?
- Is the sample size adequate?
- Is the use of placebo in the control arm justified? If there is a best care regime available as control that is well recognized and commonly applied, a placebo arm is ethically not justified.
- What are the criteria for withdrawing a research participant prematurely from the research or for suspending or terminating the research as a whole? Has the welfare of the participants been protected? This includes physical and emotional welfare, discomfort, and distress. The impact of the study on the participants must be anticipated.
- What are the characteristics of the population from which the research participants will be drawn? This includes gender, age, literacy, culture, economic status, and ethnicity.
- Are adequate provisions made for monitoring and auditing the conduct of the research, including the constitution of a data safety monitoring board (DSMB)?
- In which manner will the results of the research be reported and published?
- Are the conditions of insurance (insurance coverage) adequate?
- Are provisions for data protection according the corresponding law?
- Are the study sites suitable and the staffs adequately trained?
- Have human rights been respected? Was the consent obtained voluntarily? Any coercion invalidates the consent made. Is the informed consent form understandable to the potential participants, in particular, if vulnerable groups, such as children or partly incompetent patients, are involved? Is the research participant adequately informed about the nature, significance, risks, and implications of the medical study, as well as about his or her right to withdraw from the experiment at any time without affecting his or her subsequent care? A generally comprehensible information sheet is to be handed out to him. Furthermore, the person concerned is to be given the opportunity to have a counselling session with an investigator about the other conditions surrounding the conduct of the medical study. Is the right of privacy respected? The consent must refer particularly to the collection and processing of health-related data. The participant should know which data will be collected and who will have access to them.

## Types of Experiments

The spectrum of experiments with ethical implications is divided into different types. One of the main types is the clinical study on drugs or medical devices in humans. This type of study is well regulated and harmonized at the EU level and the United States, respectively. ICH Topic E 6 Guideline for Good Clinical Practice Note for Guidance (www.eudra.org/ema) presents detailed written instructions to achieve uniformity of the performance of specific drugs. The methodology must be clearly described and copies of the patient's information and consent will be required.

Another main type is the epidemiological study. It seeks to detect the incidence or the prevalence of diseases (i.e., epidemiological studies led to the discoveries of the relationship between smoking and cancer and to the identification of heart disease risk factors). Population studies demonstrated the mechanism of the transmission of AIDS and other infectious diseases and also showed how these diseases can be prevented. It also includes studies of a new medical procedure in the context of diagnosis or radiotherapy, surgery, transplantation, psychotherapy, or studies in complementary or alternative medicine as well as research experiments performed to determine how health care is delivered or might be improved or to examine personal or social behavior, opinions, or attitudes. For this type of study a set of recommendations is available, the so-called good epidemiologic praxis (GEP). These recommendations seek to standardize epidemiological studies and include ethical aspects, research questions, study protocol, biological sample banks, quality assurance, data management and documentation, analysis, data protection, contractual conditions/frameworks, interpretation, communication, and public health. In contrast to the GCP, the GEP are only recommendations without legal basis.

Another form of studies is the category "biomedical studies" which includes many subtypes. It includes studies on human materials (i.e., blood, tissue, urine) or on human data such as questionnaire-based projects. This form of study has not yet been standardized or been regulated at the EU level. A further main type is the animal study.

## Animal Welfare

In the middle of the nineteenth century, animal experiments were established as part of the standard scientific method. They include pure research such as genetics, developmental biology, behavioral studies, as well as applied research such as biomedical research, xenotransplantation, drug testing, and toxicology tests, including cosmetics testing.

One of the first opposition to the use of animals in medical research arose in the United States and resulted in the passing of the Animal Welfare Act (AWA) in 1966 that regulates the treatment of animals in research. Other laws, policies, and guidelines may include additional species coverage or specifications for animal care and use, but all refer to the AWA as the minimally acceptable standard for animal treatment and care.

Early objections to animal testing came from the belief that animals were so different to humans that results from animals could not be reliably applied to humans (i.e., contergan). There were also objections on an ethical basis, contending that the benefit to humans did not justify the harm to animals.

In 1985 a set of ethical principles known as the 3Rs, Replacement, Reduction, and Refinement were outlined into 11 principles by the Council of International Organizations for Medical Sciences (CIOMS) and have become the international standard governing animal experimentation. In the European Union the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes was adopted in 1986. In November 2010, "Directive 2010/63/EU on the protection of animals used for scientific purposes," which updates and replaces the 1986 Directive 86/609/EEC, was finalized and came into force. Full implementation of the new EU directive starts on January 1, 2013.

In the last decades there was an enormous success in the replacement of animals. Meanwhile nonanimal test methods become more and more numerous and have been formally validated and accepted by most countries as replacements for an existing animal test. Examples include models for tumor biology with a multifunctional microfluidic-based approach as well as sophisticated in vitro, genomic, and computer-modelling techniques or cell and tissue culture, healthy or cancerous or otherwise morbid human tissue (in vitro) investigating prevalent human diseases like diabetes, cancer, heart failure, or rare diseases like cystic fibrosis and muscular dystrophy. In vitro genetic research has isolated specific markers, genes, and proteins associated with Alzheimer's disease, Parkinson's disease, muscular dystrophy, schizophrenia, and other inherited diseases. A three-dimensional model of breast cancer has recently been developed that will allow investigators to study the earliest stages of breast cancer and test potential treatments. Rather than studying cancer in rodents, this model, which uses both healthy and cancerous human tissue, effectively allows the study of cancer as it develops in humans. An embryonic stem cell test, using mouse-derived cells to assess potential toxicity to developing embryos, has been validated as a partial replacement for birth-defect testing in rats and rabbits. The 3T3 Neutral Red Uptake Phototoxicity Test uses cells grown in culture to assess the potential for sunlight-induced ("photo") irritation to the skin. Human skin model tests are now in use, including the validated EpiDerm™ test, which has been accepted almost universally as a total replacement for skin corrosion studies in rabbits. The use of human skin leftover from surgical procedures or donated cadavers can be used to measure the rate at which a chemical is able to penetrate the skin.

Another example for a well-regulated animal welfare is the system in UK (Animals (Scientific Procedures) Act). It requires three levels of regulation:

– A project approval for the scientific substance of the project, which details the numbers and types of animals to be used, the experiments to be performed, and the purpose of them. The experiment can be performed on an animal if it can be successfully argued that it is scientifically justified and there are good reasons to cause an animal harm.

– An approval of the institution (it ensures that the institution has adequate facilities and staff).
– A personal approval for each scientist or technician who conducts any procedure. The clarification on responsibilities needs to be addressed for staff members who carry out research on animals as well as implementation of good animal welfare practices to ensure compatibility with scientific needs. In deciding whether to grant an approval, the regulatory agency has to refer to "the likely adverse effects on the animals concerned against the benefit likely to accrue as a result of the program to be specified in the license." An approval should not be granted if there exists a "reasonably practicable method not entailing the use of protected animals." The experiments must use "the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress, or lasting harm and [be the] most likely to produce satisfactory results." All three licenses must be obtained before starting the animal experiment. Animal experiments can be performed based on a review and approval of the institutional official.

All regulations contain provisions to ensure that animals used in research receive a certain standard of care and treatment. Animal care and use in research are largely controlled by Institutional Animal Care and Use Committees. Most governments aim to control the number of times individual animals may be used, the overall numbers used, and the degree of pain that may be inflicted. Furthermore, there exist numerous standard operating procedures (SOPs) for animal care. Animal care duties include – but are not exclusively limited to – housing (i.e., well-controlled airflow, room temperature), daily health checks (observation for sign of illness or injury, pathogen control, general medical surveillance procedures), body weight measurements, feed, and transport (a minimum acclimation period of 72 h, physical separation of animals accomplished by housing different species in separate rooms, the extent of the quarantine period).

Although the regulations that apply to animals in experiments vary across species (i.e., stronger rules for vertebrates) and around the world, the spirit of the regulations is always the same: animal welfare!

## Recommended Reading

Animals (Scientific Procedures) Act 1986. www.legislation.gov.uk/ukpga/1986/14/enacted. Accessed 7 Nov 2012

Animal welfare Act (AWA) Public Law 89–544 Act of August 24, 1966; Edition 2009. www.gpo.gov/fdsys/pkg/CFR-2009-title9-vol1/xml/CFR-2009-title9-vol1-chapI-subchapA.xml. Accessed 9 Oct 2012

DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:121:0034:0044:en:PDF. Accessed 17 Sept 2012

Good Epidemiological Practice (GEP: Proper Conduct in Epidemiologic Research). http://www.dundee.ac.uk/iea/goodpract.htm. Accessed 7 Oct 2012

Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. http://www.archive.official-documents.co.uk/document/hoc/321/321.htm. Accessed 9 Oct 2012
ICH-GCP Topic E6. www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002874.pdf. Accessed 17 Sept 2012

## Resources

COMMISSION DIRECTIVE 2005/28/EC of 8 April 2005. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:091:0013:0019:en:PDF. Accessed 12 Sept 2012
GUIDELINE ON REQUIREMENTS FOR FIRST-IN-MAN CLINICAL TRIALS FOR POTENTIAL HIGH-RISK MEDICINAL PRODUCTS. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002989.pdf. Accessed 5 Nov 2012

# Part IV

# Risk Assessment and Evaluation

Risk considerations play a central role in social and economic life. In many fields, such as the insurance industry or health care, risk analysis is a prerequisite for risk minimization steps. Likewise, recurrent toxicological risk assessment and evaluation is a core activity of regulatory toxicology and a motor for risk minimization through improved regulation. Risk assessment relies on information from toxicological tests. It should be clearly differentiated from risk evaluation. Risk evaluation takes scientific/toxicologic properties and sets them within a context that also includes psychological, sociological, and political arguments. Risk regulation must be carried out in two different situations. First, in the context of regulation of intentional or incidental exposures, normal exposure conditions are defined and a cautious approach applied. Second, in the context of chemical incidents, which are typically associated with temporarily increased exposures, the regulation aims at defining a situation-bound tolerable risk. While the second part of this book deals with the experimental methods for detecting toxicological hazards and risks, the following section shows how results from toxicological testing are incorporated in risk assessment and evaluation.

**The Risk Concept**

When humans make a decision, this usually involves the question of whether the decision is seen as an opportunity or a risk and depends on the individuals' attitude to and awareness of risk. This may become a source of conflict within society. Individuals tend to reject relatively low levels of imposed risks (often even when there is a benefit to society as a whole), but accept or even love much higher levels of voluntarily taken risks. Risk reduction cycles are important in technical development. These drive continuing risk reduction in many areas of life, notably in environmental protection. Each cycle is intended to minimize an existing risk, preferably without a corresponding minimization of benefit.

**Process of Risk Assessment**

Toxicological risk assessment uses the experimental findings about substance properties from toxicological tests, the dose-response relationship, and exposure levels to characterize a hazard and estimate a risk. Each of these steps requires acquisition of information. The toxicologist must be able to critically interpret

results and draw appropriate conclusions from them. Although identification of the hazard of a substance is at the core of a toxicological risk assessment, the risk can be strongly influenced by the level of exposure at the site of action and, hence, be affected by both the toxicokinetics and the toxicodynamics of the interaction between substance and organism.

**Process of Risk Evaluation**

The risk evaluation process has the aim to find out which kind of risk is acceptable to those affected. It takes into consideration psychological, sociological, and political attitudes. Risk evaluation uses the data of risk assessment and tries to resolve the question as to whether a particular situation or exposure is acceptable at the societal level. This may lead to the second question, "what is the maximum exposure that is considered to be 'safe' (i.e., pose an acceptable risk)." In risk evaluation, specific safety factors may be applied, and they may differ depending on the circumstances of exposure (e.g., for normal people and for sensitive population groups). Risk-benefit considerations can be taken into account in the risk evaluation. Risk comparisons may be included, however, not to the extent that the current level of risk of ill-health from some widely accepted risks (e.g., smoking) should be considered as a standard for other regulations. Nonscientific aspects, such as conflicts of interests, economic aspects, tradition, or fear, may be obstacles for acceptance by those individuals who are affected.

# Current Role of the Risk Concept in Regulatory Toxicology

Rolf Hertel, Michael Schwenk, and H. Paul A. Illing

## Contents

Risk and Harm ..... 494
History of the Term ..... 494
Dimensions of the Concept of Risk ..... 495
Risk and Potential Danger ..... 496
Quantification of Probability ..... 497
Risk Comparison ..... 497
Phantom Risk ..... 498
Dealing with the Concept of Risk ..... 499
Recommended Reading ..... 499
Internet ..... 499

**Abstract**

Since people live together, they must try to answer the question whether what they do causes a risk to others. When people live in proximity to one another, society sets goals. These include avoidance of creating unnecessary risks to others, minimization of unavoidable risks, and seeking to make the residual risks as predictable as possible. However, what one person perceives as a necessary

R. Hertel (✉)
Formerly Federal Institute for Risk Assessment, Berlin, Germany
e-mail: rolf.hertel1@freenet.de

M. Schwenk
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

H.P.A. Illing
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK
e-mail: paul@sherwood37.demon.co.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_66, 

risk or an evil that should be tolerated may be considered by others as a threat. In Regulatory Toxicology, this phenomenon must be considered when determining courses of action.

## Risk and Harm

Many risks are associated with human activity. When the consequences of an activity are uncertain, this activity may be beneficial or it may be harmful (cause detriments). The concepts of risk and benefit characterize the consequences of any action. Risk involves a probability statement, the likelihood or frequency of the event occurring or the effect being observed, as well as a quantitative statement, identifying the extent and type of harm (detriment).

Damage (harm, detriment) has occurred when a physical or functional impairment is recognized to be the result of an activity. Damage can be determined only on a relative scale; for the toxicologist, this is the ill-health effects (including death), either on humans or on other species. In addition to acute damage and possible harmful effects visible only after prolonged or repeated dosing or in successive generations (e.g., cancer or detrimental effects on reproduction and development of offspring), reversibility of the damage must be included in an overall assessment. An internationally accepted standard methodology for quantifying damage is not yet available, although quantification is being attempted using methods based on direct monetary values and on values associated with particular effects in terms of quality of life for those harmed.

## History of the Term

The concept of risk has its origins in Italy and symbolizes semantically the process of venturesome circumnavigating a cliff. If you want to capture the historical dimension of the concept of risk, you will find the first hints of a deliberative decision-making in the ancient skeptics. In Pharisaic Judaism from 500 AD on, the text of the Bible and tradition were interpreted according to the requirements of the situation. Probability was considered, but without the ability to undertake calculations of probability. In Europe until the late Middle Ages, the Christian personality was so dominated by religious forces that he or she was not able to make a free decision between alternatives that would be based on his own power and conviction.

The first systematic solution to a probability problem, that of playing dice, was recorded in the correspondence between Fermat and Pascal in 1654. At the beginning of the Enlightenment, Thomasius gave a hint that decision-making requires "courage and boldness." In overcoming medieval thinking (or lack of thinking) concerning both the continuing craft skills and the explicability of natural phenomena, there was a need to see the development of technology as purposeful. Groundbreaking is the invention of the lightning rod in 1752, which put the people in a position to protect their property during a thunderstorm. The realization that the lightning strike is no longer solely an act of God but a manageable phenomenon led

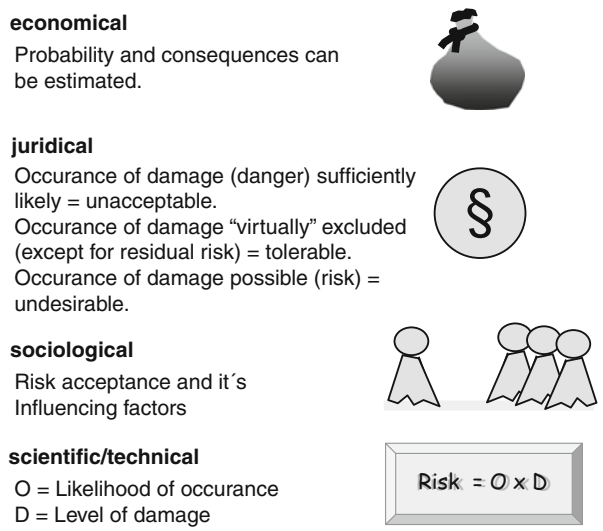



**Fig. 1** Dimensions of risk

to the analysis of other events which were previously considered as uncontrollable. It certainly was of importance that Jesuitical casuistry was taken up as part of the general philosophy of science. Once this had happened, it was possible to develop the modern concept of risk, based on mathematical descriptions of phenomena and probability theory. Today, the term "risk" is used with varying meanings in economic, legal, sociological, and scientific/technical fields (Fig. 1).

## Dimensions of the Concept of Risk

**Economically** speaking, decisions can be made with uncertainty concerning the risk be taken: We call it uncertainty when all the possible consequences of an action are known, but the actual outcome is uncertain. If in addition, the likelihood of occurrence is known, we call it "risk."

The economic benefits of an activity can therefore, be optimized when one of several options for action is preferred as it is the one in which the desired sequence of actions occurs with the highest probability. The risk of being confronted with undesirable consequences of action is low in this case, but still exists. This approach can be demonstrated using examples based on different and varyingly successful investment strategies.

**Legal perspectives** on the concept of risk will depend on the jurisdiction. From a German legal perspective, the concept of risk can be made clear, as it is distinct from the terms danger and residual risk. The term "danger" comes from the police law. There, the consequences of an action or activity must result with reasonable certainty in unacceptable damage. In our society, the state is obliged to avert such danger. This is the basis of laws that society has imposed on itself; the concept of danger is therefore defined by society. It therefore also has important significance in

safety- and environmental legislation. When one speaks of a residual risk, absolute certainty is not given and the non-excludable damage is accepted.

When there is a risk, a detrimental event cannot be excluded. Such a consequence is undesirable, but still possible. If the damage is likely to be delayed, severe and/or irrecoverable, the risk should be minimized by minimizing exposure (this is a statement of one form of the precautionary principle).

**Sociologists and psychologists** analyze how society and individuals deal with risk and with the insecurity and uncertainty of the consequence of an action. They are essentially concerned with the ways in which different groups within society perceive risks. The risk discussion is mainly concerned with risk acceptability. Often scientists are thought to deal in objective (or numerical) risk and the general public to consider risk in a subjective or judgmental manner. Depending on the perspective, the consequences of an action are considered by some sociological groups as manageable risk and by others as a threatening danger (others may feel unaffected by the risk); affected people articulate their concerns. Often, the residual risk is considered unacceptable by some or many of these groups of individuals, and they may then participate in public discussion of an issue in order to influence wider opinion.

The **scientific/technological** risk concept defines risk as the product of the extent of damage (disease/danger) and probability or frequency of the event occurring. From this simplified mathematical formula, a continuous description of all possible risk scenarios can be derived due to the variability of the factors. Here, risk is quantifiable. The Division of Toxicology of the IUPAC (International Union of Pure and Applied Chemistry), in its Glossary (IUPAC 2007, 2nd ed) gives two definitions of risk: (1) the probability of adverse effects and (2) the expected frequency of occurrence of a harmful event.

## Risk and Potential Danger

In regulatory toxicology, the distinction between hazard (potential danger) and risk is of great practical importance.

The qualitative description of the harmful effects, imposed by a substance (inherent toxicity, hazard), is used to characterize and classify this material. As part of the IOMC (Inter-Organization Program for the Sound Management of Chemicals), the "United Nations Globally Harmonized System of the Classification and Labeling of Chemicals" (GHS) was adopted in 2002 after long discussion. GHS has the purpose to contribute to the worldwide harmonization of national communications systems on hazards posed by chemicals and thus protect people and the environment worldwide. This was based on the UN Recommendations on the Transport of Dangerous Goods and the earlier classification and labeling systems in the EU, the USA, and Canada. Harmonized were criteria for the classification and definitions of risk potentials of substances and formulations and the elements of labeling. Most of the GHS has now been formally enacted in the European Union through EU Regulation 1272/2008 (the Classification and Labeling Regulation).

To define the toxicological risk posed by the substance, its toxicity expressed as a dose–response assessment is compared with statements regarding the likelihood of exposure. If an exposure is expected that would lead to an adverse effect on basis of the dose–response relationship, the risk can be quantified (see section "Risk Evaluation in Regulatory Toxicology").

## Quantification of Probability

The risk is associated with the likelihood or frequency of exposure(s) of at least a certain duration and magnitude taking place. Such a concept of probability is difficult to describe objectively, although quantification is conducted, for example, using empirical statistics. This differentiates the term "probability" from the term "suspicion." When trying to quantify, both the variability that is the actual scattering of quantifiable parameters and the uncertainty that is the uncertainty of the examiner must be considered.

**Variability** is the actual heterogeneity of the studied parameters; thus, it affects the accuracy of a statement. **Uncertainty**, however, can lead to false statements because it includes not only statements concerning the reliability and adequacy of a validating study at identifying and quantifying known effects but also includes allowance for possible inadequately quantified and nonidentified detrimental effects.

It is important for the further action of the toxicologists that different consequences result from variability and uncertainty. Often, uncertainties can be reduced by undertaking additional tests or involvement of additional expertise (Consilium), although there is always the possibility of nonidentified effects being unknown effects appearing for the first time. Variability cannot be eliminated, however, and therefore prompts the regulatory toxicologists to adjust his protective measures, when a certain level of safety is to be maintained.

## Risk Comparison

Often it is not enough for regulatory toxicologists to describe the risk posed by a substance, but he has also to compare the risk with that of potential "substitutes." For this, comparison of the **toxicological potency** of different substances (see chapter "► Assessment of Limit Values in Regulatory Toxicology") is required.

In the derivation of parameters, one has to distinguish between those substances for which a threshold can be specified on the dose–response relationship and those substances for which such a threshold is considered inappropriate. One threshold measure is the acceptable daily intake (ADI). Originally, the ADI was for food additives and then pesticides. It is the maximum level, according to present knowledge, considered to exclude a risk even when the consumer is exposed daily during his lifetime. The corresponding parameter for industrial chemicals

is the "derived no effect level." These are obtained by applying standardized factors to the "no observed adverse effect level." For substances for which no threshold dose can be determined, there are several approaches. One, the unit risk and potency factor were originally introduced by the US EPA (1986). In the European Union the dose descriptor T 25 can be applied to create a potency factor. T 25 is defined as the dose (in mg/kg body weight/day), which causes a tumor incidence of 25 % in experimental animals after lifetime exposure. This value can be converted to the corresponding human HT 25 by being divided by an appropriate "scaling factor." This metabolically and physiologically legitimated factor is derived from the comparison of metabolic rates. The third approach is to derive a "derived minimal effect level" using, as the starting point for extrapolation, a dose at which no excess of tumors was experimentally detectable and applying factors.

Just as the concept of risk can be understood in different ways, so the perception of risk is possibly different and subjective. The broad approach, which the sociological risk discourse opens, allows for the conclusion that a comparative risk assessment and evaluation is only effective if all the discourse participants have similar basic characteristics (e.g., social background, education, interests, similar life experiences, lifestyles, and desired goals in life).

A comparative examination of the risk posed by, for example, a defined household chemical with the risk posed by a substitute can be used meaningfully if effects and exposure scenarios are directly comparable. Whether different less serious and reversible toxic effects are equivalent may need discussing. It therefore remains to note that each risk requires an individual decision and that due to methodological problems (e.g., death from smoking cigarettes vs. prize in the lottery), there are limitations to the comparison of different risks (but see chapters "▶ Risk Comparison in Toxicology" and "▶ Risk-Benefit Considerations in Toxicology").

## Phantom Risk

The different perceptions of risk can lead to a phenomenon that is described by the term **phantom risk**. In general, this means that different assumptions about risky cause-effect relationships are made, which may remain unprovable. Such an approach results in an assessment that unduly increases a potential risk (exaggerated fears). New information that is made available usually lead to a perception, in which the risk appears greater than it actually turns out to be later. To what extent this behavior is phylogenetically useful to sustain human life during evolution, and thus is largely unalterable, remains to be established. However, the regulatory toxicologist has to take into account this phenomenon since it directly influences the general political decision on the classification of risks, be it unacceptable or undesirable, although it cannot be proven on a rational basis.

## Dealing with the Concept of Risk

Both the definition of "risk" and the methodological processes that must be applied when dealing with questions of risk management are dependent on expert judgements. Thus, although the GHS system can provide a basis for common judgements concerning the hazard, one cannot assume that these judgements will be internationally applicable when applied to risk or that the same legal base for managing the risks will pertain internationally. In the event that no binding requirements/laws exist, all aspects of the decision finding must be presented as far as possible in a transparent way. Both generally, and specifically, within European Union countries, risk management appears to be based on increasing levels of risk aversion and precaution.

## Recommended Reading

Begemann C (1987) Furcht und Angst im Prozess der Aufklärung zu Literatur und Bewusstseinsgeschichte d. 18. Jh. Athenäum, Frankfurt am Main

CEC Commission of the European Communities (2000) Communication from the Commission on the precautionary Principle. COM (2000) l, Brussels

CWG (Commission Working Group on the Technical Meetings for Risk Assessment for Existing Substances) (1999) Guidelines for quantitative risk characterization of non-threshold carcinogens in the framework of existing chemicals following Council Regulation (EEC) NO_NL 793/93, ECB/ISPRA/01/99_Rev.l

IUPAC International Union of Pure and Applied Chemistry. Subdivision on Toxicology and Risk Assessment (2007) Glossary of terms used in toxicology, 2nd edition (IUPAC Recommendations 2007). Pure Appl Chem 79(7):1153–1344

Rabinovitch NL (1973) Probability and statistical inference in ancient and medieval Jewish literature. University of Toronto Press, Toronto

SRU (The Advisory Council on the Environment) (1999) Environment and health special report. Metzler Poeschel, Stuttgart

U.S. Environmental Protection Agency (U.S. EPA) (1986) Guidelines for carcinogen risk assessment. Fed Regist 51:33992–34003

## Internet

Information about GHS. http://www.ilo.org./public/english/protection/safework/ghs/
http://www.oecd.org.7ehs/
http://www.unece.org/trans/danger/danger.htm

# Risk Cycles in Toxicology

H. Paul A. Illing and Michael Schwenk

## Contents

Perceptions of Risk ..... 502
What Are Risk Cycles? ..... 502
A Framework for Risk Evaluation ..... 503
Risk-Reduction Cycles ..... 504
Product Development Risk ..... 505
Life Cycle Cost Risk ..... 505
Health Risks ..... 505
The "Risk Cycle" ..... 506
Future of Risk-Reduction Cycles ..... 507
References ..... 507
Recommended Reading ..... 508

**Abstract**

Risk cycles can lead to promotion of an existing risk or its reduction. Risk-reduction cycles are an effective means to minimize exposures of man and the environment to pollutants. Risk perception is an essential precondition for risk-reduction efforts.

H.P.A. Illing (✉)
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK
e-mail: paul@sherwood37.demon.co.uk

M. Schwenk (✉)
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_67, 

## Perceptions of Risk

**Perceived risk** was defined in the UK Royal Society's (1983) report as the combined evaluation that is made by an individual of the likelihood of an adverse event occurring in the future and its likely consequences. The chapter on risk perception in the 1992 report from the UK Royal Society Study Group states that:

> Risk perception involves peoples beliefs, attitudes, judgements and feelings, as well as the wider social or cultural values and dispositions that people adopt, towards hazards and their benefits. . .. Furthermore the perception of risk is multidimensional, with a particular hazard meaning different things to different people (depending, for example, upon their underlying value systems) and different things in different contexts. In some circumstances, important aspects of risk perception and acceptability involve judgements not just of the physical characteristics and consequences of an activity but also social and organizational trustworthiness of risk management and regulatory institutions.

An important point that flows from this is that there is often a serious disjunction between how the technical expert (the risk assessor) sees risk (so-called objective risk) and how the public perceives risk. In addition, chemical products, in particular, may be rejected not because they are "unsafe" in any conventional sense, but because the public is insufficiently persuaded that they serve a legitimate social need.

## What Are Risk Cycles?

The risk cycle involves the interplay of activities belonging to the three basic components of risk assessment, risk management, and risk communication. These are defined elsewhere (IPCS 2004).

Risk cycles can occur in two contrasting forms. "Risk **enhancement cycles**" show themselves in the form of enhanced pesticide performance (in terms of removing the pest) and enhanced levels of risk associated, e.g., with stock market speculation, willingness to partake in dangerous sports, and preparedness to escalate armed conflicts. When human behavior is involved, Adams (1995) has called this group "*Homo aleatorius*" – dice man, gambling man, or risk-taking man. They are willing to take risks, and they may be driven by mass psychological phenomena, such as a "spiral of violence."

"Risk-**reduction cycles**," however, are, in Adams' terms, undertaken by "*Homo prudens*" – those who strive to avoid "accidents." The aim is to reduce risk, based on a factual analysis and willingness to find technical solutions. This approach is rarely spectacular but is usually considered to be essential for sustainable and positive development. It occurs, for example, in the form of security measures, dispute settlement, and prevention measures. It also plays an important role in project management of companies and chemical safety.

A third variant involves no real change in risk. It is **fatalism**. Fatalists believe they have minimal control over their lives. They accept risks, are resigned to their

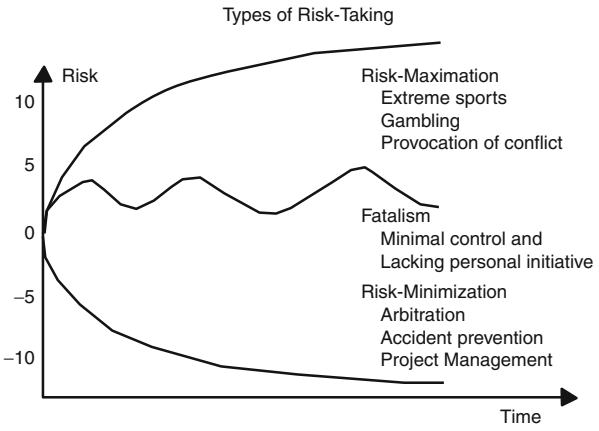



**Fig. 1** Contrasting forms of risk modulation

fate and see no point in trying to change it. Thus, they are unlikely to modify the risks to which they are exposed to (Fig. 1).

Generally, risk taking is seen as "good" ("nothing ventured – nothing gained" or "no risk – no reward") and a necessary part of "progress." Nevertheless, the propensity to take risks, when combined with human fallibility, is often asserted to be the root causes of dangerous exposures, i.e., human error due to miscalculation, lapse of concentration, or ignorance concerning the dangers leads to inappropriate exposure. When society imposes risk management measures that reduce risk, the individual may seek to restore the balance of risk by behavior that accepts higher risks (including so-called "macho" behavior). Human nature often leads individuals towards *Homo aleatorius* when society as a whole wants to encourage the behavior associated with *Homo prudens*. Understanding the sociological and psychological background concerning how risks are perceived is essential if risks are to be reduced.

## A Framework for Risk Evaluation

The Royal Society study group put forward a risk evaluation framework in 1983. Essentially this is concerned with "objective risk" – the probability that a particular adverse event occurs during a stated period of time or results from a particular challenge. It was originally developed to handle engineering risk, but it is equally applicable to health risks from chemicals. Illing and Marrs (2009) have discussed the application of this framework to the evaluation of health risks arising from exposure to chemicals.

Criteria for reaching decisions can be classified according to three "pure" criteria (UK Health and Safety Executive 2001). These are:

- An **equity-based** criterion, which starts from the premise that all individuals have unconditional rights to certain levels of protection. This leads to standards,

applicable to all, held to be usually acceptable in normal life. In practice this leads to fixing a limit to represent the maximum level of risk above which no individual can be exposed. If the risk characterization indicates that the risk is above this limit, the risk is held to be unacceptable – whatever the benefits.

- A **utility-based** criterion, which applies to the comparison between incremental benefits of measures to prevent the risk of injury or detriment [for health effects, ill-health] and the cost of the measures. The utility-based criterion compares the relevant benefits (e.g., statistical lives saved, life-years extended, reduced ill-health, and better quality of life) obtained by adoption of a particular risk prevention measure with the net cost of introducing it and requires that a balance be struck between the two. This balance can be deliberately skewed towards risk reduction by ensuring gross disproportion between costs and benefits.
- A **technology-based** criterion, which essentially reflects the idea that a satisfactory level of risk prevention is attained when "state of the art" control measures (technological, managerial, organizational) are employed to control risks, whatever the circumstances.

These criteria underlie the regulatory process first outlined by the Royal Society (1983). The scheme is based on:

- An upper limit of risk which should not be exceeded for any individual ("unacceptable")
- Further control, so far as is reasonably practicable, making allowances if possible for aversions to the higher levels of risk or detriment ("tolerable")
- A cutoff in the deployment of resources below some level of exposure or detriment judged to be trivial ("broadly acceptable")

This approach to risk evaluation can be applied to health effects, both to the target species and to incidentally affected species. For many health effects, the risk evaluation is concerned only with determining what constitutes a "broadly acceptable" risk and hence with the equity criterion. This is the case if any equity criterion for "safe" (the "broadly acceptable" level of risk), such as a residue level in a foodstuff, is exceeded, thus resulting in its immediate withdrawal from the market. It is also applied to the indirect risks to the environment and to humans mediated via the environment.

## Risk-Reduction Cycles

The aim of risk reduction is to reduce risk levels to those regarded as "broadly acceptable" or, if this is not possible and the benefits to society are required, at least to keep risks within "tolerable" levels while seeking improvements aimed at eventually achieving the "broadly acceptable" level of risk. Measures to mitigate risks are found in many areas of human activity. Such risks can be detected early through effective project management, which includes periodical simulation of risk-reduction cycles. Here are some examples:

## Product Development Risk

Different types of risks may arise during the development and use of new materials and products such as new chemicals, pharmaceuticals, and medical devices. Appropriate management can be critical for the economic success of a company:

1. The technical risk, that the desired product (or the process for its manufacture) has problems and cannot be produced economically.
2. The regulatory risk, that the product does not receive approval.
3. The market risk, that the intended sales success does not happen.
4. The risks arising from litigation when a product is perceived by the individual as having caused damaged or ill-health.

Reduction of product-development risks is often a consequence of product optimization.

## Life Cycle Cost Risk

The life cycle cost risk is the risk of a customer or his insurer in connection with a bought product. Especially for long-lived and fragile products, the long-term costs can be much higher than the purchase price. That can be considered before purchase, making a life cycle cost risk estimation. It includes not only the purchase price but also the maintenance and repair costs, disposal costs, and the possible cost for unforeseeable events "force majeure risks". Such a life cycle cost assessment can help to make decisions between alternative products.

## Health Risks

Risk-reduction cycles have made possible the safe use of new techniques in many areas of modern life. For example, in the field of car accident prevention, the periodic improvement of occupant safety of modern cars includes introduction of crumple zones, seat belts, headrests, airbags, and antilock brakes. Comparable cycles existed in the protection of the chemical worker from harmful exposures in the workplace (occupational safety), the reduction of pollutants in consumer goods (consumer protection), and the attempts towards clean environmental media (environmental protection). Thus, in the twentieth century, the consequent reduction of uncontrolled emissions into the ambient air led to a drastic decrease of air pollution. Initially this was through the reduction of dust emissions. Later, the emissions of sulfur dioxide, nitrogen oxides, CFC propellants, lead, chlorinated hydrocarbons, dioxin, and benzene were systematically reduced. The effort is not over yet. Future tasks include the reduction of carbon dioxide, diesel particles, domestic heating emissions, and the management of new sources of emissions.

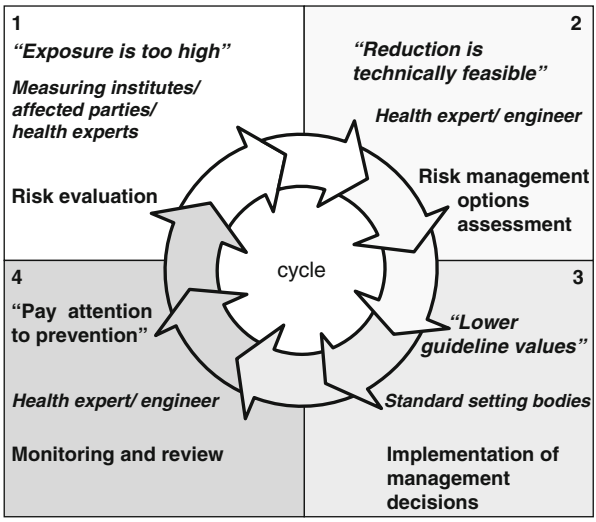



**Fig. 2** The four steps of risk cycles

## The "Risk Cycle"

Risk-reduction cycles in the field of workers protection, consumer protection, and environmental protection typically can be divided into steps. An EU expert scientific committee has divided the cycle into four stages (EU 2000): risk evaluation, risk management options assessment, implementation of management decision, and monitoring and review (Fig. 2).

IPCS (2004) identified that risk management consists of risk or risk-benefit evaluation, emission and exposure control, and risk monitoring, with the risk options assessment being implicit in the process and risk options implementation being the decisions taken concerning emission and exposure.

In the EU scheme, phase 1, measurement organizations detect elevated levels of pollutants in the air, soil, water, or housing and suggest that this may represent a health hazard. Residents are shocked and proclaim that such a high risk is unacceptable. Health experts get involved and conclude that the risk should be avoided or reduced. In EU phase 2, a range of control options are identified and an appropriate option is selected. For EU phase 2, engineers and other experts offer technical solution, leading to an emission/exposure reduction or sanitation improvement. In EU phase 3, the chosen solutions are implemented. For EU phase 4, the new level of exposure is measured and the effects were evaluated. A new, and almost always tougher criterion for the exposure (normally called a standard) may be developed and the lowered guideline levels monitored. This new criterion is considered as acceptable until, some years later, new findings and insights lead again to concern, initiating a second risk-reduction cycle. Many existing limit values and guideline values were developed in the context of such cycles. When multiple cycles occur in a row, one can talk of a risk-reduction spiral.

In the environmental context, risk-reduction cycles often start in an unplanned and chaotic "scandal." They are often promoted by "concerned" people or lobbying groups, including citizens' groups and environmental organizations. Because these usually demand zero risk quite aggressively (possibly combined with attempts to denigrate the experts), they initially are considered by experts (including toxicologists) with skepticism. Whenever possible, this difference between perceived risk and objective risk should be resolved as, usually, cooperation is required between all involved parties to achieve an acceptable solution.

Sometimes cycles end with an unsatisfactory result. This may be the case when the claim is too high or the solution too expensive or technically not feasible. It is also the case when obvious opportunities for improvement are ignored.

## Future of Risk-Reduction Cycles

Risk reduction has contributed a great deal to the steadily decreasing pollution in many parts of the world in the past decades. It has reduced the exposures of workers, consumers, and the living environment. As a beneficial side effect, the development of environmental protection technology has become an important economic benefit. The potential for further improvements is almost endless, especially when considering the global dimensions.

Sustainable development (development that meets the needs of the present without compromising the ability of future generations to meet their own needs) can and must be the way forward. But this is only possible when society considers the maintenance of a clean and healthy environment as an important goal. Experts will be required who support this idea and its implementation, both technically as well as at the regulatory level. Thus, it is likely that risk-reduction cycles will continue to play an important role in the future.

## References

Adams J (1995) Risk. UCL Press, London

EU (2000) First report on the harmonisation of risk assessment procedures. Part 1. The report of the Scientific Steering Committee's Working Group on harmonisation of risk assessment procedures in the scientific committees advising the European Commission in the area of human and environmental health 26–27 Oct 2000. European Commission. http://europa.eu.int/comm/food/fs/sc/ssc/out83_en.pdf

Illing HPA, Marrs TC (2009) Regulatory toxicology. In: Ballantyne B, Marrs TC, Syverssen T (eds) General and applied toxicology, 3rd edn. Wiley, Chichester, pp 2527–2553

IPCS (2004) IPCS risk assessment terminology Part 1 IPCS/OECD key generic terms used in chemical hazard/risk assessment. Part 2 IPCS glossary of key exposure assessment terminology. Harmonisation document no 1. Inter-Organisation Program for the Sound Management of Chemicals. International Program on Chemical Safety. World Health Organisation, Geneva

Royal Society Study Group (1983) Risk assessment. A study group report. Royal Society, London

Royal Society Study Group (1992) Risk: analysis, perception and management. Royal Society, London

UK Health and Safety Executive (2001) Reducing risks, protecting people. HSE's decision-making process. HSE Books, Sudbury

## Recommended Reading

Illing HPA (2009) The role of veterinary pharmacovigilance in risk analysis and the influence of risk perception. In: Woodward KN (ed) Veterinary pharmacovigilance – adverse reactions to veterinary medicinal products. Wiley-Blackwell, Chichester, pp 691–708

# Risk Minimization in Drug Development. Regulatory Aspects

Elke Roehrdanz and Klaus Olejniczak

## Contents

Risk and Risk Minimization ..... 510
Risk Level ..... 510
Unknown Risks ..... 511
Strategies for Risk Minimization in Nonclinical Development ..... 511
Adaption of Trial Protocols to the Stages of Drug Development ..... 511
Chronological Order ..... 512
Potential Areas of Risk for Humans ..... 512
Extrapolation to Humans ..... 513
Feedback of the Results from Early Clinical Trials ..... 513
Role of Clinical Development ..... 513
Prerequisites for Use in Humans ..... 514
Harmonization of Drug Assessment ..... 514
Carcinogenicity Studies (ICH S1) ..... 514
Reproduction Toxicity Studies (ICH S5) ..... 515
Criteria for Inclusion of Volunteers/Patients in Clinical Trials (ICH M3 (R2)) ..... 515
Men ..... 515
Women Not of Childbearing Potential ..... 516
Women of Childbearing Potential ..... 516
Pregnant Women ..... 517
Clinical Trials in Pediatric Populations ..... 517
Tolerable/Non-tolerable Risks Using the Example of Safety Pharmacology ..... 518
Example QT-Interval Prolongation ..... 518
Recommended Reading ..... 519
Resources ..... 519

E. Roehrdanz (✉)
Federal Institute for Drugs and Medical Devices, Bonn, Germany
e-mail: elke.roehrdanz@bfarm.de

K. Olejniczak
Non-clinical Regulatory Consultant, Berlin, Germany
e-mail: klausolejniczak@yahoo.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_68, 

**Abstract**

Risk minimization plays a central role in different areas of regulatory toxicology. Extremely complex and time-consuming methods are applied for risk minimization in drug development with the aim to exclude potential health risks for humans as far as possible. Therefore, the nonclinical and clinical drug development comprises a program whose results shall ensure a maximum amount of safety for each phase of clinical development (risk-benefit assessment).

## Risk and Risk Minimization

"Risk" means that something undesirable may occur. "Probable" means that the occurrence of a risk cannot be evaluated with absolute certainty, but that it will remain relative, i.e., it can be classified anything from "low" to "high," but can never be fixed at "zero" or a "100 %." Therefore, nothing can ever be excluded or anticipated with certainty.

Risk minimization in the framework of drug development implies that the probability of occurrence and the extent of a possible damage caused to the health of a volunteer or patient should be kept as low as possible. Opposed to that may possibly be the entrepreneurial risk to stop the further development of a compound out of safety reasons although the compound might be generally safe and efficacious (low risk).

### Risk Level

Experience and knowledge are indispensable for risk identification. If the assessment of a graded risk shows that the probability of the risk occurring is too high to be considered irrelevant (i.e., the suspicion having arisen requires clarification), then a suitable nonclinical experiment must precede studies in humans. It is understandable that during medicinal product development, there will be a continuous increase in the amount of those data available, which suggest that a certain degree is (not) sufficient to raise a suspicion, which can only be clarified by an experiment.

The question whether there is a risk leading to such a degree of suspicion that would require scientific clarification must be answered on the basis of all available information. The assessment of the degree of risk, which ranges from "low" to "high," can only lead to one of the following decisions:

- No, there is no suspicion for a risk requiring (experimental) clarification.
- Yes, there is suspicion for a risk requiring (experimental) clarification.

The impact of this assessment on the requirement for particular nonclinical studies as well as on the decision about the use of the developmental compound in humans will become evident and can be categorized with the help of special flowcharts covering all typical areas of possible damage to humans.

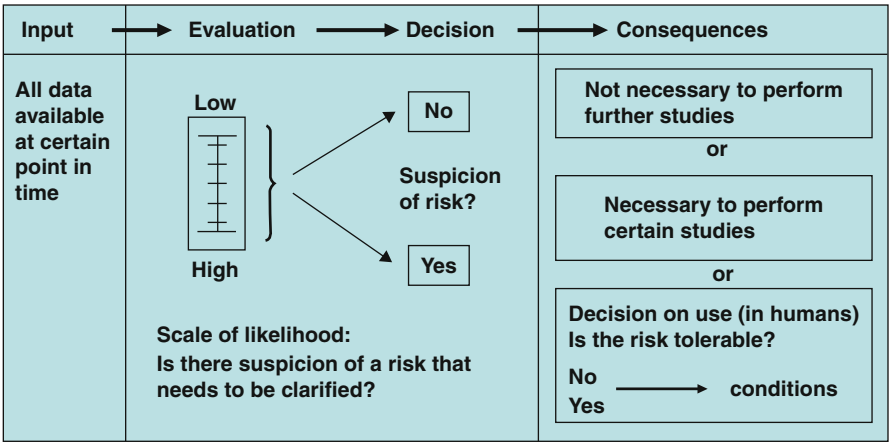



**Fig. 1** Nonclinical testing strategy for risk identification

The different experimental areas are usually of interest during various phases of drug development, i.e., they will be dealt with earlier or later during drug development (Fig. 1).

### Unknown Risks

Unknown risks cannot be investigated scientifically. The effects of chloro-fluoro-carbons (CFCs) on the stratospheric ozone layer are a well-known example. The risk from the ozone-destroying activity was discovered not until 1974, resulting in a stepwise reduction in CFC consumption up to the complete abandonment. The same applies to medicinal uses.

## Strategies for Risk Minimization in Nonclinical Development

### Adaption of Trial Protocols to the Stages of Drug Development

The nonclinical development of drugs covers studies on toxicology and safety pharmacology in animals and in vitro. These studies are performed to minimize the risk for the use of a new drug substance in humans from the first orienting administration to volunteers/patients up to the broad therapeutic use in practice.

Due to average drug development times of 5–15 years, the nonclinical development program inevitably ranges over a period of several years. This program does not represent an isolated sequence of different studies but is embedded in the whole process of drug development. During drug development, a constant adaptation of

both individual study plans and the whole study program to the constantly changing progress in knowledge is needed. The same applies for its execution, which happens stepwise and is coordinated with the clinical studies.

## Chronological Order

Chronologically, certain nonclinical studies usually precede certain clinical trials. The required contents of nonclinical studies can be deduced from the scientific questions arising from the planned clinical trials. Generally, the characterization of the pharmacodynamic effects is followed by an investigation into toxicodynamic effects before the substance can be used in humans at all. Findings from the use in humans usually have an effect on the kind and content of further nonclinical studies and their logical and chronological position in the development process. Thus, a cycle of accumulating knowledge and its influence on the design of the remaining nonclinical program is completed. This sequence gives only an extremely rough guidance for the course of the development process for a new drug substance, regarding its contents and chronological order. However, it clearly indicates that the cycle in question can be – and usually is – repeated as development progresses.

## Potential Areas of Risk for Humans

In an attempt to cover all relevant areas of potential risks, it is necessary to consider an exhaustive list of adverse reactions that are generally expected to occur and to compare them with the risks observed in the clinical trial situation. Consequently, identified and potentially meaningful risks have to be as far as possible investigated experimentally.

Typical areas of possible damage to humans are:

- Acute toxicity
- Repeated-dose toxicity
- Adverse effects on male or female fertility
- Genotoxicity
- Tumorigenicity
- Sensitization/immune suppression and stimulation
- Local or other particular adverse events

Drug development is a stepwise process where information about safety from animal as well as human studies is assessed. The aims of nonclinical studies comprise: characterization of toxic effects on target organs, dose-effect relationship, relation to exposure and potential reversibility. Such information is important regarding the evaluation of the safe starting dose in humans and to determine which parameters have to be monitored to detect possible adverse effects during the clinical trial.

## Extrapolation to Humans

Before a potential medicinal product is used in humans for the first time, there are, with regard to risk assessment, only results available from nonclinical investigations and possibly some hints on potential effects in humans derived from experience with related compounds. Based on this knowledge and considering particular results from nonclinical or clinical areas under investigation, it can be stated which relation exists, e.g., between substance related effects and amounts of bioavailable substance. Perhaps it can be predicted if effects were provoked by the applied substance itself or biotransformation products. However, at this point of time, no reliable statement can be made about the degree of similarity of the experimental models to the situation in humans.

As a result, the investigator is forced to use the potential medicinal product in a variety of testing models (various models of animal species, application forms and experimentation, and different duration of studies) in order to increase the chances of having included relevant models for the situation in humans.

## Feedback of the Results from Early Clinical Trials

If this was the case can only be stated after the first studies in humans have been performed. With an increase in scientific knowledge resulting from various steps of the clinical development, certain findings obtained in the nonclinical stage will invariably lose their meaning for risk assessment.

On the other hand, through the flow of information from clinical studies, those nonclinical models can be identified that are particularly appropriate for risk assessment on the basis of their similarity to the human situation. Results from these studies together with those from early clinical trials form the basis of risk assessment, which must always be carried out before the next phase of clinical trials can be entered.

## Role of Clinical Development

Clinical trials are performed in humans with the objective to show the safety and efficacy of the respective medicinal product. The first phase starts with a relatively low exposure in a low number of volunteers to investigate compatibility. In the following clinical trials, exposure will generally be increased in relation to dose, duration, and/or size of the exposed patient population. Clinical trials will be extended if appropriate safety was proven in prior clinical trials plus additional safety information from nonclinical studies, which will be obtained during clinical development.

## Prerequisites for Use in Humans

Prior to use in humans, it is necessary to build up a risk assessment using the most sensitive nonclinical testing model following medicinal-ethical and also legal aspects. This rule remains valid until it can be convincingly shown that the models have no or only limited biological impact on risk assessment.

The depicted approach, i.e., drawing conclusions from nonclinical test results with a view to potential results and risks for humans, and the feedback from relevant information obtained from clinical trials, turns the development and application of nonclinical testing strategies into a complex and dynamic process beyond fixed plans or checklists.

A reasonable approach implies the possibility for a critical analysis of planning, performance, interpretation, and assessment of nonclinical and clinical studies. It is acknowledged that any individual kind of investigation may be of limited relevance. One should be aware that results obtained can influence the type and extent of subsequent nonclinical and clinical studies. The design of testing strategies must, therefore, be accompanied by a high sense of responsibility reconciling the volunteers', patients', and doctors' requests for new safe medicines and the need to protect laboratory animals. Adhering to this principle will reveal, after thorough evaluation, which practical steps should be taken for each step of drug development.

## Harmonization of Drug Assessment

The European Union's (EU) strive for harmonization with a view to a common market for medicinal products and the trilateral negotiations between Japan, the United States, and the EU led in October 1989, to the initiation of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). ICH is hosted by the International Federation of Pharmaceutical Manufacturers Associations (IFPMA). The ongoing program has, among others, the following objectives:

- To identify and eliminate the differing technical requirements in the three states/regions
- To avoid repetition of all kind of tests
- To accelerate drug development, thus giving patients quicker access to new medicinal products without negatively affecting quality, safety, and efficacy

In the field of nonclinical testing of drugs, 14 guidelines have been adopted since the initiation of the ICH process (Table 1).

## Carcinogenicity Studies (ICH S1)

Carcinogenicity studies are generally not required to be completed prior to the conduct of clinical trials except there is a cause of concern. For pharmaceuticals,

**Table 1** Nonclinical ICH guidelines: check list

| ICH-Code | Topic |
|---|---|
| S1A | Need for Carcinogenicity Studies of Pharmaceuticals |
| S1B | Testing for Carcinogenicity of Pharmaceuticals |
| S1 (R2) | Dose Selection for Carcinogenicity Studies of Pharmaceuticals |
| S2 (R1) | Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use |
| S3A | Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies |
| S3B | Pharmacokinetics: Guidance for Repeated-Dose Tissue Distribution Studies |
| S4 | Duration of Chronic Toxicity Testing in Animals |
| S5 (R2) | Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility |
| S6 (R1) | Preclinical Evaluation of Biotechnology-Derived Pharmaceuticals |
| S7A | Safety Pharmacology Studies for Human Pharmaceuticals |
| S7B | The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT-Interval Prolongation) by Human Pharmaceuticals |
| S8 | Immunotoxicity Studies for Human Pharmaceuticals |
| S9 | Nonclinical Evaluation for Anticancer Pharmaceuticals |
| S10 (draft only) | Phototoxicity |
| M3 (R2) | Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals |

which are developed for certain life-threatening diseases, carcinogenicity studies, if applicable, may be completed after market approval.

### Reproduction Toxicity Studies (ICH S5)

Reproduction toxicity studies should be conducted as is appropriate for the population that is to be exposed.

## Criteria for Inclusion of Volunteers/Patients in Clinical Trials (ICH M3 (R2))

### Men

Men (volunteers/patients) can be included in phase I trials and phase II clinical trials before the conduct of the male fertility study in rodents since an evaluation of the male reproductive organs is performed in the repeat-dose toxicity studies. Repeated-dose toxicity studies of at least 2 weeks are considered to be adequate for evaluation of male reproductive organs.

## Women Not of Childbearing Potential

Women of not childbearing potential (i.e., permanently sterilized, postmenopausal) can be included in clinical trials without reproductive toxicity studies if the relevant repeated-dose toxicity studies (which include an evaluation of the female reproductive organs) have been conducted, again repeated-dose toxicity studies of at least 2 weeks are considered appropriate.

## Women of Childbearing Potential

For women of childbearing potential, there is a high level of concern for the unintentional exposure of an embryo or fetus before information is available concerning the potential benefits versus potential risks. The recommendations on timing of reproduction toxicity studies to support the inclusion of women of childbearing potential in clinical trials are similar in the EU, United States, and Japan.

It is important to characterize and minimize the risk of unintentional exposure of the embryo or fetus when including women of childbearing potential in clinical trials. One approach to achieve this objective is to conduct reproduction toxicity studies to characterize the inherent risk of a drug. A second approach is to limit the risk by taking precautions during exposure of women of childbearing potential in clinical trials. Testing for pregnancy during the trial and subject education should be sufficient to ensure compliance with measures designed to prevent pregnancy during the period of drug exposure. To support these approaches, informed consent should be based on any known pertinent information related to reproduction toxicity. If no relevant reproductive information is available, the potential for unidentified risks to the embryo or fetus should be communicated.

In all three regions, women of childbearing potential can be included in early clinical trials without nonclinical development toxicity studies (e.g., embryo-fetal studies) in certain circumstances. One circumstance could be intensive control of pregnancy risk over a short duration (e.g., 2 weeks) clinical trials. Precautions to prevent pregnancy include pregnancy testing, use of highly effective methods of birth control, and study entry only after a confirmed menstrual period.

Generally, where appropriate preliminary reproduction toxicity data are available from two species, and where precautions to prevent pregnancy in clinical trials (see above) are used, inclusion of women of childbearing potential (up to 150) receiving investigational treatment for a relatively short duration (up to 3 months) can occur before conduct of definitive reproduction toxicity testing. This is based on the very low rate of pregnancy in controlled clinical trials of this size and duration.

In the United States, assessment of embryo-fetal development can be deferred until before phase III for women of childbearing potential using precautions to prevent pregnancy in clinical trials (see above). In the EU and Japan, other than the situations described above, definitive nonclinical developmental toxicity studies should be completed before exposure of women of childbearing potential.

In all three regions, women of childbearing potential can be included in repeated-dose phase I and II trials before conduct of the female fertility study since an evaluation of the female reproductive organs is performed in the repeated-dose studies. Nonclinical studies addressing female fertility should be completed to support inclusion of women of childbearing potential in large-scale or long duration clinical trials (e.g., phase III trials).

In all three regions, the pre-postnatal development study should be submitted for marketing approval. All female reproductive toxicity studies and the standard battery of genotoxicity tests should be completed before inclusion, in any trial, of women of childbearing potential not using highly effective birth control or whose pregnancy status is unknown. Further details on the inclusion of women of childbearing potential in clinical trials are given in ICH M3 (R2).

## Pregnant Women

Before the inclusion of pregnant women in clinical trials, all female reproductive toxicity studies and the standard battery of genotoxicity tests should be conducted. In addition, safety data from previous human exposure should be evaluated.

## Clinical Trials in Pediatric Populations

When pediatric patients are included in clinical trials, safety data from previous adult human experience would usually represent the most relevant information and should generally be available before initiation of pediatric clinical trials. The appropriateness and extent of adult human data should be determined on a case-by-case basis. Extensive adult experience might not be available before pediatric exposures (e.g., for pediatric-specific indications).

Results from repeated-dose toxicity studies of appropriate duration in adult animals, the core safety pharmacology package, and the standard battery of genotoxicity tests should be available before initiation of trials in pediatric populations. Reproduction toxicity studies relevant to the age and gender of the pediatric patient populations under study can also be important to provide information on direct toxic or developmental risks.

The conduct of any juvenile animal toxicity studies should be considered only when previous animal data and human safety data, including effects from other drugs of the pharmacological class, are judged to be insufficient to support pediatric studies.

The appropriateness of carcinogenicity testing should be addressed before long-term exposure in pediatric clinical trials. However, unless there is a significant cause for concern, carcinogenicity studies are not recommended to support the conduct of pediatric clinical trials. Further recommendations for clinical trials in the pediatric population are depicted in ICH M3 (R2).

## Tolerable/Non-tolerable Risks Using the Example of Safety Pharmacology

Additionally to the characterization of the desirable pharmacodynamic effects of a drug, studies investigating secondary pharmacodynamic effects are requested. Pharmacodynamic effects relevant for safety fall into the category of "safety pharmacology." Safety pharmacology studies concerning effects of the medicinal product on vital functions like the cardiovascular, central nervous, and respiratory system should be performed prior to first administration in humans. If not covered by results from previous toxicology studies, supplemental safety pharmacology studies may be necessary for the renal/urinary system, the autonomic nerve system, the gastrointestinal system, etc. with respect to further drug development.

## Example QT-Interval Prolongation

A relatively recent finding is that drugs intended for a non antiarrhythmic indication may lead to an abnormal QT-interval prolongation displayed in the electrocardiogram (ECG). In this context, potential life-threatening cardiac arrhythmias belonging to the type of torsade de pointes may occur. At international level, this potentially serious adverse reaction raised a question: How can the data material collected for assessment of the arrhythmogenic potential of a drug in nonclinical studies be improved and how can a more precise risk assessment be guaranteed?

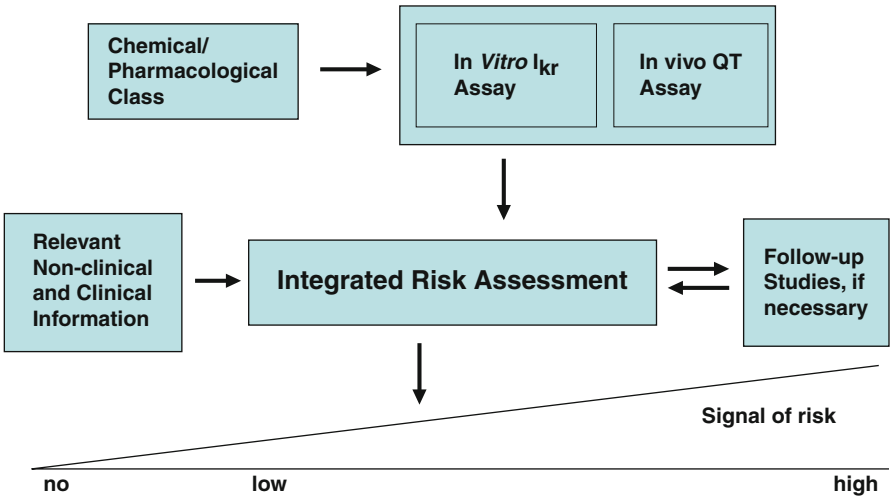



**Fig. 2** Nonclinical testing strategy for assessing risk for QT-interval prolongation (Refer to ICH 7B)

The QT-interval of the ECG is a measure of the duration of ventricular depolarization and repolarization. Repolarization of the heart ventricle is mainly influenced by the activation of the delayed rectifier $K^+$ current ($I_k$) which is composed of a rapidly ($I_{Kr}$) and a slowly ($I_{Ks}$) activating component. The rapidly activating component ($I_{Kr}$) is encoded by HERG (human ether-a-go-related gene). Substances which block the $I_{Kr}$ prolong the action potential of the heart. Whether the medicinal product under investigation belongs to a chemical/pharmacological class with the potential to prolong the QT-interval should be assessed prior to first administration in humans.

Nonclinical methodologies address investigations using HERG encoded $K^+$ channels, action potential parameters as well as ECG parameters taken from non-rodent species. All data available are included in an "integrated risk assessment" to detect a potential risk for a potential to prolong the QT-interval (refer to Fig. 2). The result "no risk," "low risk," or "high risk" can be crucial for further drug development. A new medicinal product with QT-interval prolonging properties has to be clearly defined concerning its therapeutic significance especially in comparison to drugs with similar or comparable indications.

## Recommended Reading

Olejniczak H, Bode G (2002) ICH topic: note for guidance on safety pharmacology studies for human pharmaceuticals. Fundam Clin Pharmacol 2:79–81

Olejniczak K, Günzel P, Bass R (2001) Preclinical testing strategies. Drug Inf J 2:321–336

Special issue (2010) Themed section: QT-safety. Br J Pharmacol 159:1–251

Zünkler BJ (2006) Human ether-a-go-go-related (HERG) gene and ATP-sensitive potassium channels as targets for adverse drug effects. Pharmacol Ther 112:12–37

## Resources

International conferences on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH). http://www.ich.org

# Importance of Physical-Chemical Properties for Toxicological Risk Assessment

Hans-Uwe Wolf and Michael Schwenk

## Contents

Solubility ..... 521
Molecular Size ..... 523
Dissociation ..... 523
Boiling Point ..... 523
Other Physical and Chemical Parameters ..... 524
Recommended Reading ..... 524

**Abstract**

The toxicokinetic as well as the pharmacokinetic behavior of a foreign substance is determined largely by **solubility**, **molecular size**, **dissociation** behavior, and **vapor pressure**. All of these parameters have a significant effect on the absorption, distribution, and excretion. Moreover, physicochemical properties ultimately have a strong influence on the environmental behavior and on the toxic activity of a foreign substance. Taken all together, the physicochemical properties are important parameters for risk assessment.

## Solubility

The solubility of a foreign substance is determined essentially by its water solubility and fat solubility. Only few foreign substances such as methanol, ethanol, and dimethyl sulfoxide are soluble in both hydrophilic media such as water and

H.-U. Wolf
Department of Pharmacology and Toxicology, University of Ulm, Ulm, Germany

M. Schwenk (✉)
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_113, 

hydrophobic (= lipophilic) solvents. For the large majority of foreign substances, the water solubility decreases approximately in parallel with the increase of fat solubility.

To characterize the solubility of a foreign substance, one can use its fat solubility, which significantly influences the ability to migrate into lipophilic compartments of the organism and to remain there for a long time. The extent of fat solubility is quantitatively determined by measuring the partition (P) ratio, which is the ratio of solubilities in *n*-octanol (hydrophobic phase) and in water ("P o/w"). Predominantly water-soluble foreign substances usually exhibit values below 1, while fat-soluble substances show values above 1. Since lipophilic compounds can have distribution ratios of up to 10,000,000, it is common to use the logarithm of the values ("log P o/w"). Predominantly water-soluble substances then have values below 0 (i.e., negative values), and predominantly fat-soluble substances have values from 0 to 7. Highly fat-soluble substances such as polychlorinated biphenyls or polychlorinated dibenzodioxins and furans reach values of 6–7.

The *lipid solubility* of foreign substances is crucial for their absorption. Moderately fat-soluble foreign substances are generally readily absorbed by the surface cells of the gastrointestinal tract, the lungs, and the skin of an organism. Thus, solubility influences to a decisive extent the bioavailability of a foreign substance, as is well known for lipophilic drugs in suitable formulations, which are readily absorbed via the skin or mucous membranes. However, this can have limits. If, for example, a bolus of highly lipophilic paraffin is swallowed, only a very small portion will be absorbed in the intestine due to the limited access of paraffin molecules to the hydrophilic barrier on top of the absorbing epithelial cell layer. On the other side, foreign substances with good water solubility, such as some water-soluble heavy metal compounds, will be poorly absorbed, since they cannot transverse the lipophilic cell membrane.

The *insolubility* of certain substances in water, hydrophobic media and diluted hydrochloric acid, usually results in a lack of absorbability, which is generally favorable from a toxicological point of view. Pharmacologically, this insolubility in the gastrointestinal tract allows application of certain therapeutic agents for which an absorption is undesirable. These include, for example, activated charcoal to bind lipophilic toxic compounds in the gastrointestinal tract, iron (III) hexacyanoferrate (II) ("Prussian blue") to interrupt the enterohepatic circulation during thallium decontamination, or barium sulfate as gastrointestinal contrast medium. In connection with toxicological risk management, these aspects of solubility are very important, since they allow to estimate, if and under which conditions a foreign compound will be absorbed (gastrointestinal tract, lung, skin), and thus affect the emergency recommendations to treat people who have been exposed.

Many foreign compounds are amphiphilic, which means they have functional groups that are water soluble and others that are lipid soluble. Once in the circulation, amphiphilic compounds can be bound to one of the unspecific binding sites of serum albumin, so that their free concentration in the blood decreases.

## Molecular Size

The molecular size is another physicochemical parameter, affecting the absorption and distribution of foreign substances in an organism. Both the absorption at all three relevant entry sites (gastrointestinal tract, skin, lungs) and the diffusibility of foreign substances within the organism, which means the passage from one compartment to another, depend critically on the molecular size. Both absorption rate and diffusibility generally decrease sharply with increasing molecular size. Apparent exceptions to this rule are found even in high-molecular-weight substances. An example is botulinum toxin with a molecular mass of about 150,000 Da, which, contrary to expectations, can penetrate the gastrointestinal epithelial barrier in toxicologically significant amounts.

There are membrane carriers in the liver, kidney, and other cell types, which catalyze the membrane transport of compounds with amphiphilic features. Their substrates have a molecular size of about 400–600 Da depending on species.

## Dissociation

The dissociation of weak acids and weak bases is another influencing factor which co-determines membrane penetration of foreign substances. The kinetic behavior of such substances with regard to absorption, distribution, and excretion is largely dependent on the state of dissociation: The charged forms of the substances (acid anions, base cations) penetrate biological membranes much less, if at all, than the corresponding uncharged forms. This effect generally results in a reduced absorption of weak bases from an acidic medium (stomach) and a reduced absorption of weak acids from an alkaline medium (upper small intestine). This is also relevant for renal excretion of foreign compounds. Here pharmacological acidification of the urine favors the excretion of weak bases, while alkalinization of the urine augments the excretion of weak acids.

## Boiling Point

The boiling point of a foreign substance may also affect the kinetic behavior. At a given temperature, the resultant vapor pressure of a liquid and the vapor concentration in the ambient air is inversely related to the height of the boiling point. The extent of absorption into the body, especially the extent of pulmonary absorption, is directly related to the vapor concentration of the contaminant in the ambient air, so that with decreasing boiling point of the substance, the pulmonary absorption increases. Foreign substances with a low boiling point, e.g., solvents, are generally referred to as “volatile” substances.

While the distribution of a foreign substance in the body is practically independent of its boiling point, the rate of exhalation via the lungs is again strongly

influenced by the boiling point. The gas phase concentration of a foreign substance in the pulmonary alveoli will be inversely related to the value of the boiling point. This relationship gains practical importance for such volatile foreign substances, for which exhalation is a relevant path of excretion from the organism.

## Other Physical and Chemical Parameters

Other physicochemical parameters such as **melting point** and **light absorption** are usually of comparatively low importance, except in single cases, where, for example, light absorption of a foreign substance in the skin may lead to photochemical reactions and phototoxicity. For the chemist, **odor** is a phenomenon that helps characterize a substance. In many cases, the airborne concentrations of odorous compounds do not necessarily cause toxic effects (low levels of mercaptans), while conversely, some very toxic chemicals virtually lack an odor (phosgene). In any case, odors must be considered as a warning sign. Even if a bad odor does not cause a toxic effect, it must be considered as harmful in itself. Thus, bad odors due to emissions from factories, animal farms, or the neighborhood may have considerable relevance for the quality of life and may induce sick feeling and vomiting in susceptible individuals. Odors must therefore be regulated.

## Recommended Reading

Blaauboer BJ (2003) The integration of data on physico-chemical properties, in vitro-derived toxicity data and physiologically based kinetic and dynamic as modelling a tool in hazard and risk assessment. A commentary. Toxicol Lett 138:161–171

OECD Guidelines for the Testing of Chemicals, Section 1; Physical-chemical properties. http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-1-physical-chemical-properties_20745753, Accessed May 13 2013

# Importance of Intrinsic Toxic Properties for Risk Assessment

Hermann Kappus and Michael Schwenk

## Contents

Chemical Characteristics ..... 526
Acute Toxicity ..... 526
Subacute, Subchronic, and Chronic Toxicity ..... 527
Reproductive Toxicity ..... 528
Genotoxicity ..... 529
Carcinogenicity ..... 529
Irritation ..... 530
Allergic Reactions ..... 530
Recommended Reading ..... 531

**Abstract**

Identification of intrinsic toxic properties is the first step in toxicological risk assessment. Toxic properties are not constants of nature but depend on the study program and the observed organism. Reasonably reproducible results are usually gained in standardized animal testings. Available findings in humans and results from in vitro and in silico tests are also taken into account. It is often impossible to prove certain properties, such as carcinogenicity, because other toxic properties, such as very strong irritant effects, dominate. Such "interferences" must be considered in risk assessment.

H. Kappus
Formerly MAK-Commission, Freising-Weihenstephan, Germany

M. Schwenk (✉)
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_115, 

## Chemical Characteristics

Chemical characteristics of a substance, such as physicochemical properties, environmental behavior, and reactivity, have a strong influence on exposure levels and on the disposition of a substance in the living organism. Therefore, these properties can be considered as components, affecting the intrinsic toxic properties of a chemical (Fig. 1). However, aspects of physicochemical properties will not be considered here, since they are dealt with in other sections of this volume (see chapters "► Characterization of Physicochemical Parameters in Toxicology" and "► Importance of Physical-Chemical Properties for Toxicological Risk Assessment").

## Acute Toxicity

The LD50 or LC50 or related parameters from animal experiments are usually used as a measure of acute toxicity. Such values are useful to compare the acute toxicity of different substances but have the disadvantage that there are significant species differences. The transferability to humans is very limited. Moreover, these values report simply a toxicity parameter (mortality) but do not provide information about the underlying mechanism. A standardized follow-up after a large single dose of a systemically active compound often results in false interpretation, since the distribution in the body and possible accumulation in certain tissues remain unconsidered.

When examination of acute toxicity includes the monitoring of disease parameters, such as behavior and organ functions, this can provide information about the underlying mechanism of toxicity. More important would be the detection of specific effects on target organs. Such investigations would of course have to include the symptom-free animals. A longer follow-up period of surviving animals would also allow to say something about the reversibility of the effect.

In the case of substances for which experiences in humans exist, these often provide valid evidence for symptoms and severity of acute toxic effects. Such data can be obtained in connection with accidents where high human exposures occurred or from observations at the working place, where, years ago, exposures were much higher than they are today. Thus, exposure-specific clinical findings at the workplace have often been documented in occupational medicine and can be used to derive a provisional limit value for acute toxicity in humans.

In recent years, numerous in vitro models have been introduced for the testing of acute toxic effects on specific biochemical or cellular parameters. They often reveal information on underlying mechanisms. However, such models lack the interactions between organs and ignore possible counter-regulations against the toxic effects. They can therefore serve as additional toxicity markers but not entirely replace in vivo tests.

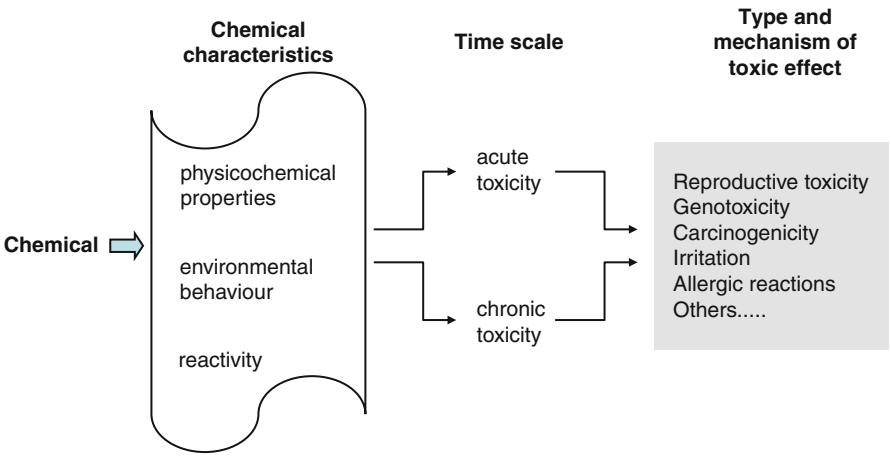



**Fig. 1** Parameters of intrinsic toxic properties

## Subacute, Subchronic, and Chronic Toxicity

These three types of toxicity tests have in common that the substance is applied repeatedly or continuously for a certain period of time. The resulting toxic effects usually differ only gradually. When untypical toxicokinetic behavior is involved, this may lead to wrong conclusions about the mode of action. The three terms are often summarized. For many substances, a 90-day animal study (subchronic) is sufficient to detect all types of toxic effects that are known, except carcinogenicity. The 90-day trial is very important for risk assessment. Observation of the general behavior of the animals, their organ functions, and possible mortality allows to make statements on the toxicity and, if measured, on the kinetics of a substance during the test period. Examination of the organs during and after the trial period includes clinical chemistry and histopathology and provides important information regarding the organotrophy of a toxic effect. Chronic toxicity studies in animals often provide information on the type of organ damage but no clear information about the underlying mechanism. Additional knowledge on these mechanisms often comes from short-term trials or appropriate in vitro studies.

However, even without knowledge of the mechanism of action, the dose-response relationship of such animal studies often allows to derive a threshold value, below which no toxicity occurs. These "no-observed-adverse-effect levels" are the basis for regulatory levels, derived for different types of exposure, such as the workplace, the environment, and food. The focus can either be on "no-observed-adverse-effect level" (NOAEL) or on "lowest-observed-adverse-effect level" (LOAEL). From the LOAEL, a NOAEL can be derived. These two values, however, are not material constants but depend on experimental conditions, and they differ between different animal species. Therefore, it is often difficult to

establish a limit value for humans that is solely based on the observed threshold in animal experiments. Within an identical experimental setup, these values are well suited to compare different substances, especially if the same target organ is affected. In order to improve transferability to humans, there is an increasing demand to get information on the mechanism of action of subchronic and chronic toxicity. If the mechanism of action differs between animal species and man, then the animal trial provides at most partial information for regulatory purposes. Moreover, the risk assessment from subacute, subchronic, and chronic toxicity studies is complicated by the fact that standardized time intervals for the dosage of the individual substances are necessary to receive reproducible results, while in the reality of human exposure, varying concentrations/doses of different durations are more probable.

Epidemiological studies can sometimes reveal subacute, subchronic, and chronic toxicity findings (hazard) of investigated substances, but quite often it is not possible to derive a threshold value in humans. In cases of strongly accumulating substances, where the total dose adds up over a certain time period, there is a better chance, that threshold values may be determined.

There are increasingly more in vitro models that allow to study subacute, subchronic, and chronic toxicities and the underlying mechanisms. It can be expected that with the advances of cell culture and organ culture models, this may further improve in the future.

## Reproductive Toxicity

Toxic effects on fertility and the development of the embryo and fetus are actually part of the acute, subacute, subchronic, and chronic toxicity testing. However, they constitute a very specific hazard and are therefore seen separately in the process of risk assessment. Reproductive toxicity is generally investigated by appropriate animal studies. Epidemiological observations in humans only rarely provide information about toxic effects on fertility or on the development of embryo and fetus. Exceptions are accidents. One of the sad examples is the damage to embryos due to methylmercury poisoning in Iraq in 1971. Wheat seed, treated with the fungicide methylmercury, was eaten by the starving population. Brain damage was induced in many people notably in the developing brain of unborn children. For fetotoxic compounds, there is usually a brief time window, sometimes less than a day, where the tissue is responsive to the toxic developmental effect due to the transient expression of specific developmental structures. Earlier or later application of the teratogenic substance may be without effect. Thalidomide is an example.

When the effects of heavily accumulating substances on the fertility of female animals are studied, one should be aware that toxic effects on the developing embryo and fetus may occur. In general, however, one tries to investigate these toxicity endpoints separately. When toxic effects on fertility are detectable, it is important to try to elucidate the underlying mechanism. Today this is possible often without too much effort.

The situation is different in developmental toxicity. Here the toxic effect is often described, but the underlying mechanisms remain largely unknown. In addition, dose-response studies are often lacking because such animal studies are time consuming and expensive and in conflict with the aims of animal welfare. When teratogenic effects are induced by a substance in animal studies, one often abstains from further investigations that would allow to determine the dose that is without effect. There is virtually no in vitro model available that allows to make a quantitative risk assessment of reprotoxic effects. However, in vitro models are important for the detection of the underlying mechanisms.

## Genotoxicity

Genotoxicity is usually assessed in the context of carcinogenicity. But it is actually a toxic endpoint that has its own purpose, because genotoxic effects of substances often lead to cell toxicity and to cell death. Moreover, genotoxicity is often accompanied with mutagenicity in germ cells so that toxic damage will be inherited by the next generation. Genotoxicity is typically assessed in vitro either in bacteria or in mammalian cell systems. More meaningful, however, are suitable in vivo experiments. It has been shown in recent years that in vitro genotoxicity tests deliver very good information about underlying mechanisms but that in vivo studies are more suitable to get information for risk assessment. Even if in vitro systems are reinforced by addition of drug-metabolizing systems, they never contain all the factors that are present in vivo. This may have consequences not only with regard to the activation by the drug-metabolizing system to a reactive and genotoxic metabolite but also for the inactivation to less genotoxic products. When an in vitro system delivers a negative result with regard to genotoxicity, this is not a proof that the substance does not exhibit genotoxic properties in vivo. Likewise, a positive genotoxicity result in an in vitro test is only an indication for possible genotoxicity in the living organism and no proof that this is detectable in vivo.

## Carcinogenicity

If genotoxicity is detected, there is always the suspicion of a carcinogenicity of a substance. The proof can be provided only in long-term animal studies. In vitro models that study cell transformation and related cellular biological systems also provide relevant information. Epidemiological findings sometimes provide good correlations between exposure levels and tumor incidence. However, due to confounding factors and mixed exposures, epidemiological results are often not unambiguous. Chronic animal studies rarely observe mechanisms, and almost never the kinetics of the substance to be considered. Moreover very high doses are usually administered that may lead to other toxic effects. Therefore, it is now viewed as essential that in addition to positive tumor findings, it is also necessary to provide information on the kinetics and mechanism of the substance-specific tumorigenesis.

Moreover, most animal experiments do not reveal clear dose-response relationships, and the toxicologist has to rely on extrapolations from the high-dose concentration range to lower doses. A number of short-term animal studies have emerged in recent years.

Knowledge of the mechanisms of carcinogenic effects has led to the insight that there are substances for which one may define a "threshold concentration" or "threshold doses." This is true especially for substances that show no genotoxic effects but nevertheless give positive tumor findings in animals. For these so-called epigenetic carcinogens, it appears to be possible to derive medically safe limit values. Moreover, there are numerous research approaches that allow to analyze the mechanisms of carcinogenic effects in more detail, so that there are cases where, at least in the lower concentration and dosage range, carcinogenic risks can be excluded (see chapter "► Do Carcinogens Have a Threshold Dose? Pro and Contra").

## Irritation

Irritant effects are usually observed on the skin, eye, and mucous membranes. These acute effects are usually strictly concentration- and dose-dependent. The underlying mechanisms are not always well understood. They are often the result of overt tissue damage including cell death and its consequences, such as edema, inflammatory responses, and tissue repair. An activation of nerve endings can be involved, causing neurogenic inflammation and pain. Due to their local expansion, irritations are usually evaluated as endpoints per se. Irritation after oral administration of corrosive chemicals occurs mainly in the upper gastrointestinal tract. But irritation can also occur in susceptible inner organs. Chronic irritation may have far-reaching consequences, such as tumor formation. It is usually possible to define a threshold concentration or threshold dose for irritating substances.

## Allergic Reactions

Allergic sensitization by substances often affects the skin, the respiratory tract, and the gastrointestinal tract. Allergic reactions are not toxic reactions but reactions of the immune system that tries to neutralize intruding foreign macromolecules. Allergic reactions do not show any strict dose-response relationship. Most relevant information about allergenic compounds comes from observations in humans. Tests are available which allow to study sensitization (readiness of the immune system to interact with the foreign compound) and the type of allergic response in affected people. Animal tests can also give a hint on a possible allergenic potential. When dealing with allergic substances, one should be aware that there are various types of allergic mechanism (type 1 to type 4), leading to different types of disease. Allergy to specific substances is not rarely associated with the genetically predetermined MHC patterns on the surface of cells of an individuum. Typically, allergenicity is a feature of large molecules such as (glyco)proteins, which are foreign to the

organism and recognized as such by the immune system. But some small molecules can also be allergenic, notably when they bind to proteins in the body. These allergens are then called haptens. Formation of a reactive intermediate that binds to body proteins is, one way, how a hapten can become allergenic.

Risk assessment of allergenic effects differs from risk assessment of toxic effects. Due to their potential hazard at very low concentrations, allergens must be considered with great care. An example for a reasonable European regulation is the obligation for manufacturers to declare allergenic food ingredients in prepackaged food. At the same time, the allergen-sensitive person has a co-responsibility to avoid substances, to which he/she is allergic.

## Recommended Reading

Lam CW, Aguirre MP, Schischke K, Nissen NF, Ogunseitan OA, Schoenung JM (2012) International harmonization of models for selecting less toxic chemical alternatives: effect of regulatory disparities in the United States and Europe. Integr Environ Assess Manag 8(4):723–30

Winder C (2012) Chemical hazards in the organisation. Experientia 101:1–19

# Importance of Metabolism. Mechanistic Considerations Relevant for Toxicological Regulation

Franz Oesch and Jan G. Hengstler

## Contents

Fundamentals ..... 534
Examples of Metabolism-Associated Toxicity ..... 537
MeIQx (2-Amino-3,8-Dimethylimadazo[4,5-f]Quinoxaline) ..... 537
Aflatoxin B1 ..... 538
Vinyl Acetate: The Relevance of Practical Thresholds ..... 541
References ..... 545

**Abstract**

Most xenobiotics do not remain unchanged in the human organism (and in other organisms), but rather are metabolized. The change in chemical characteristics of the thereby produced metabolites as compared with the chemical characteristics of the corresponding parent compounds usually leads to changes in both the desired properties, e.g., therapeutic efficacy, and also in undesired properties, i.e., xenobiotic metabolism usually is toxicologically not neutral, but rather leads in most cases to toxication or detoxication of the respective compound (overview see Oesch-Bartlomowicz and Oesch 2007). Thereby xenobiotic metabolism becomes one of the most important factors controlling the toxicity of the respective compound. This, in turn, makes xenobiotic-metabolizing enzymes to control factors for xenobiotic toxicity.

F. Oesch (✉)
Oesch-Tox Toxicological Consulting and Expert Opinions GmbH&Co.KG, Wackernheim, Germany
e-mail: oesch@uni-mainz.de

J.G. Hengstler
Leibniz Research Centre for Working Environment and Human Factors at the Technical University of Dortmund (IfADo), Dortmund, Germany
e-mail: hengstler@ifado.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_71, 

## Fundamentals

These xenobiotic-metabolizing enzymes drastically differ quantitatively and in many instances even qualitatively between animal species, organs, cell types, developmental stages, and physiological states such as health and individual diseases and most often even between strains and genders (for a succinct review, see Hengstler and Oesch 1999). For an extrapolation of toxicity findings in experimental systems to humans, cognizance of differences in xenobiotic-metabolizing enzymes between the systems used and humans is therefore critical.

Quantity and also chemical identity of the formed metabolites depend on many factors. This complexity leads to difficulties to predict from experimental systems which metabolites and how much of them will be generated in humans. Difference in xenobiotic metabolism between species is one of the factors which most profoundly limit the extrapolation of toxicological results obtained in experimental systems to humans (for a comprehensive review, see Hengstler et al. 1999). The later considerations in this chapter will therefore especially take this interspecies parameter into account. However, in order to be comprehensive, it must also be stated that in addition to these crucial species differences, further important differences exist also within a given species. This includes genetic differences in xenobiotic metabolism between strains and between individuals. In humans genetic polymorphisms in xenobiotic-metabolizing enzymes known to date account for up to 40 % of cytochrome P450 (CYP)-dependent xenobiotic metabolism (Modak 2010). The generally used pharmaco/toxicokinetic default uncertainty factor of 3.2 to account for human interindividual differences in the extrapolation of toxicity data to human may need to be enlarged if it does not encompass human polymorphisms from poor to extensive metabolizers of the xenobiotic compound in question (Schroeder et al. 2011). Moreover, differences caused by different gene expressions during development or disease states may drastically influence xenobiotic metabolism, most profoundly if the liver, the organ responsible for the largest portion of the mammalian xenobiotic metabolism, is involved. In addition, environmental factors, nutrition, and drug treatment can profoundly modulate xenobiotic metabolism by enzyme induction, repression, activation, or inhibition (for an overview see Oesch and Arand 1999; updated version in German: Arand and Oesch 2004). These numerous factors may interact with each other, generating a high complexity of xenobiotic metabolism control and consequent toxicities. For instance, very early on, it had already been shown that differences in nutritional status profoundly influenced drug-metabolizing enzyme induction (e.g., by DDT) and the consequent toxicity of a third compound (e.g., carbon tetrachloride) (McLean and McLean 1966).

In order to improve the water solubility and excretability of xenobiotics, the organism most often uses conjugation with endogenous water-soluble building blocks such as glutathione, glucuronic acid, or sulfate. Such conjugations need the preexistence of suitable substituents in the xenobiotic compound in question which, if not preexisting in the parent compound, first have to be introduced or

liberated. This step in xenobiotic metabolism is called phase I, and the subsequent conjugation is called phase II (the then frequently following active excretion of the generated water-soluble metabolite from the cell of origin is often called phase III).

The phase I metabolites possess at the site to be conjugated electrophilic (such as epoxides, α,β-unsaturated carbonyls) or nucleophilic (such as hydroxyl, sulfhydryl, amino, carboxyl) structural components. Depending on their relative chemical reactivities, electrophilic moieties can have high toxicological potential by reacting with nucleophilic moieties of endogenous compounds, toxicologically most significant if they thereby modify the structures of macromolecules such as proteins, RNA, and – especially important – DNA, the latter potentially leading to significant genotoxicity. In contrast to this, nucleophilic metabolites usually do not covalently react with endogenous molecules and therefore usually are toxicologically less problematic. However, they can, in some cases, have affinity to receptors and thereby lead to desired therapeutic or undesired toxic interactions.

The conjugating phase II reactions in most cases lead to a large increase in the water solubility of the compound in question, to its efficient excretion, and to termination of its biological activity, be it beneficial (therapeutic) or undesired (toxic). However, some exceptions exist, for instance, some glucuronides (e.g., of morphine) possess high biological activities and some conjugates (e.g., of vicinal halogenated alkanes) with glutathione possess higher genotoxic potential than the parent compound (for an overview see Oesch-Bartlomowicz and Oesch 2007).

The enzymes catalyzing phase I reactions include oxidoreductases and hydrolases. Oxidoreductases relevant for xenobiotic metabolism include cytochromes P450 (CYP), flavin-containing monooxygenases (FMO), monoamine oxidases (MAO), and cyclooxygenases (COX). In most cases these oxidoreductases introduce oxygen into xenobiotic molecules or abstract electrons. CYPs are quantitatively especially often involved in xenobiotic metabolism. Thus, two-thirds of the top 200 drugs prescribed in the United States (year of survey: 2002) are cleared through metabolism that involves CYPs (Williams et al. 2004). Further important xenobiotic-metabolizing oxidoreductases include dehydrogenases and reductases such as alcohol dehydrogenases (ADH), aldehyde dehydrogenases (ALDH), and carbonyl reductases. They abstract or add hydrogen atoms. Diverse xenobiotic-metabolizing hydrolases catalyze the hydrolysis of esters, amides, glucuronides, sulfates, or epoxides.

In the phases II reactions, electrophilic substrates are conjugated by glutathione *S*-transferases (GST), nucleophilic substrates by UDP-glucuronosyltransferases (UGT), sulfotransferases (SULT), *N*-acetyltransferases (NAT), acyl-CoA-amino acid-*N*-acyltransferases, and methyltransferases (for an overview see Oesch and Arand 1999; updated version in German: Arand and Oesch 2004).

A correct prediction of toxicity is especially important in cases of long latencies such as cancer, since a wrong prediction leads to accumulation of numerous

**Table 1** Reactive metabolites: some important prototypes

| Parent compounds | Reactive metabolites | Enzymes involved in the control |
|---|---|---|
| Aromatic/olefinic hydrocarbons | Epoxides | Cytochromes P450 |
| | | Glutathione S-transferases |
| | | Epoxide hydrolases |
| Aromatic/heterocyclic amines | Reactive esters | Cytochromes P450 |
| | | Sulfotransferases |
| | | Acetyltransferases |
| | | Glutathione S-transferases |
| | | UDP-glucuronosyltransferases |
| Dialkylnitrosamines | Carbonium ions<br>Electron deficient alkyl groups | Cytochromes P450 |
| Vicinal dihaloalkanes | Episulfonium ions | Glutathione S-transferases |

irreversible damages before the error becomes manifest. For such toxicities electrophilically reactive metabolites are especially important which frequently have a short life span and are formed in low quantities. For such cases cognizance of reactive metabolites and xenobiotic-metabolizing enzymes responsible for their control (formation, detoxication, sequestration into alternative pathways) is especially important. Examples of some important electrophilically reactive metabolites and xenobiotic-metabolizing enzymes involved in their control are given in Table 1. An important consequence of the fact that quantitatively minor metabolites may be responsible for toxic (especially for genotoxic) effects is that species-specific divergent pathways leading to such minor but toxicologically important metabolites may become crucial. When a human-only metabolite is not formed in the experimental species chosen for toxicity testing, an incomplete xenobiotic safety assessment may result leading to an underestimation of toxicological risk. The FDA/CDER guidance on safety testing of drug metabolites (U.S. Department of Health and Human Services 2008) therefore states that a unique human metabolite must itself be tested for toxicity when the metabolite level reaches >10 % parent systemic exposure at steady state.

Some overall approximations in relatively high similarities of some xenobiotic-metabolizing enzymes or their response to exogenous stimuli between certain experimental animal species and humans may be attempted. Although the different animal models have many differences in the ligand-binding domain of the respective nuclear receptors involved in the control of xenobiotic-metabolizing enzymes induction compared with humans (Mohutsky et al. 2010), induction responses compared with humans appear to be most similar in rats and mice for CYP1A; in rats, mice, and pigs for CYP3A; in monkeys for CYP2C; and in dogs for CYP2D (Martignoni et al. 2006; Zuber et al. 2002; Bogaards et al. 2000). However, some exceptions of outstanding practical importance highlight the fact that a priori acceptance of these overall relatively high similarities may be dramatically misleading for an

individual xenobiotic compound under consideration. Thus, rifampicin does not induce CYP3A in rats or mice, but does so in humans (leading to unwanted pregnancies in combined use of contraceptives and rifampicin) and in rabbits (Kocarek et al. 1995; Back et al. 1988). Inversely, pregnenolone-16α-carbonitrile (PCN), which strongly induces CYP3A in rats and mice, causes no induction in humans or rabbits, and CYP3A induction by 5α-pregnane-3,20-dione is seen only in humans and mice, but not in rats or rabbits (Mohutsky et al. 2010). For improved predictions animal models have been genetically modified in which some nuclear receptors controlling induction of a xenobiotic-metabolizing enzyme of that species have been knocked out and replaced by the corresponding human gene (Scheer et al. 2008; for a similar approach see Ma et al. 2007).

## Examples of Metabolism-Associated Toxicity

Having discussed the basic aspects of drug metabolism, the following chapter will focus on examples of compounds where drug metabolism plays an important role for risk assessment. Usually risk assessment is based on animal experiments. For identification of acceptable human exposures, NOAELs (see chapter "► Examination of Acute and Chronic Toxicity") from laboratory animals are used and multiplied with safety factors (see chapter "► Extrapolation Factors and Safety Factors in Toxicology"). Usually this procedure identifies exposure levels that are safe for humans. However, working with safety factors, e.g., a fixed safety factor of ten to consider possible interspecies differences of metabolism, may under certain circumstances lead to mistakes. This is the case when interspecies differences in metabolism between humans and the relevant animal species are huge. To illustrate this problem, some examples of well-characterized compounds will be discussed in the following paragraphs (from: Hengstler et al. 1999 and Hengstler et al. 2003) and references cited herein. It should be considered that they represent extreme and rare cases. Nevertheless, they are important to illustrate how mistakes in risk assessment can be avoided.

### MeIQx (2-Amino-3,8-Dimethylimadazo[4,5-f]Quinoxaline)

MeIQx represents an intensively studied heterocyclic amine found in fried as well as cooked meat. It is formed by a heat-dependent reaction between muscle creatinine and amino acids. MeIQx is a strong colon carcinogen in rats and mice. However, it does not cause colon cancer in cynomolgus monkeys. Therefore, a critical question is whether human risk assessment should be based on the rodent or monkey data. Because of the small evolutionary distance, one might be tempted to favor the monkey for this purpose. However, a relatively simple experiment demonstrates that risk assessment must be based on the more susceptible rodents. An Ames mutagenicity test using microsomes from livers of human, rat, and

cynomolgus monkeys as a metabolizing system reveals major interspecies differences. Human and rat microsomes strongly activate MeIQx to a mutagen, whereas microsomes from cynomolgus monkeys are almost inactive (Fig. 1). This corresponds to the mechanism of metabolic activation of MeIQx to a carcinogenic nitrenium ion (Fig. 2). Human and rat cytochrome P4501A2 form a hydroxylamine that is further metabolized to a reactive *N*-acetoxyester. In contrast, cynomolgus monkeys lack an activity corresponding to human or rat cytochrome P450 1A2. However, it should be considered the cynomolgus monkey represents an exception with respect to MeIQx metabolism. Even other monkey species, such as marmosets, form the hydroxylamine from MeIQx and are therefore susceptible to its carcinogenic effect. In conclusion, humans are similarly susceptible to MeIQx-induced carcinogenicity as rats and do not represent a resistant species, such as cynomolgus monkeys. Therefore, risk assessment must be based on the more resistant species.

## Aflatoxin B1

Aflatoxin B1 is one of the most potent liver carcinogens for humans and rats. However, the TD50 (the dose that induces tumors in at least 50 % of the animals) shows large interspecies differences, ranging between 1 and 6 μg/kg/day for different rat strains, whereas even doses of 2,000 μg AFB1/kg/day did not yet cause liver tumors in 50 % of the C57/BL6 mice. Therefore, the interspecies differences between rats and mice are larger than a factor of 1,000, a difficult scenario for human risk assessment.

To study whether humans are as susceptible to AFB1 as rats or rather as resistant as mice, genotoxicity assays were performed using liver microsomes of all three species as a metabolizing system (Fig. 3). Sister chromatid exchanges (SCE) in human lymphocytes were analyzed as a genotoxic end point. Incubation of AFB1 (10 μM) with liver microsomes of all three species caused a clear increase in SCE when NADPH was added to the incubation mixture, whereby NADPH acts as a cofactor of the cytochrome P450-mediated metabolic activation of AFB1. However, metabolic activation by mouse liver microsomes was stronger compared to human and rat. It should be considered that lower AFB1 concentrations (only 1 μM for mice compared to 10 μM for human and rat) were used. This seems to be in contrast to the aforementioned carcinogenicity studies where mice appeared to be more resistant than rats. However, this discrepancy could be explained by an additional in vitro experiment (Fig. 3). In microsomal preparations the cofactors of phase II metabolism, such as glutathione (GSH), are too diluted to allow an in vivo like phase II metabolism. Therefore, GSH and cytosol of the corresponding species (containing, e.g., glutathione *S*-transferases) were added to the microsomal incubations. These experiments showed a strongly reduced SCE induction when mouse cytosol was added (Fig. 3). In contrast, addition of cytosol and GSH did not reduce genotoxicity of human and rat microsomal incubations. Therefore, mouse liver microsomes have a higher capacity to activate AFB1 to a genotoxic species

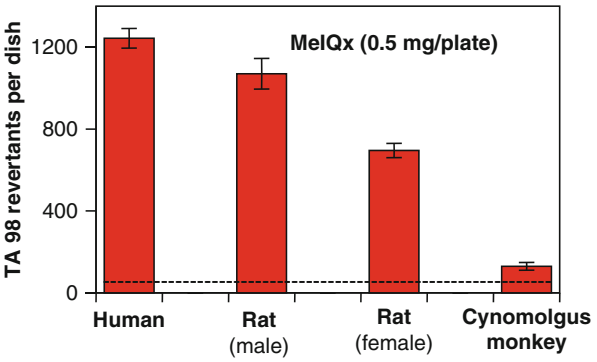



**Fig. 1** Activation of the heterocyclic amine MeIQx (2-amino-3,8-dimethylimidazo(4,5-f) quinoxaline) to a mutagenic metabolite by liver microsomes of humans, rats, and cynomolgus monkeys. The *dashed line* shows the number of spontaneous revertants in the Ames test (Davis et al. 1993; review: Hengstler et al. 1999)

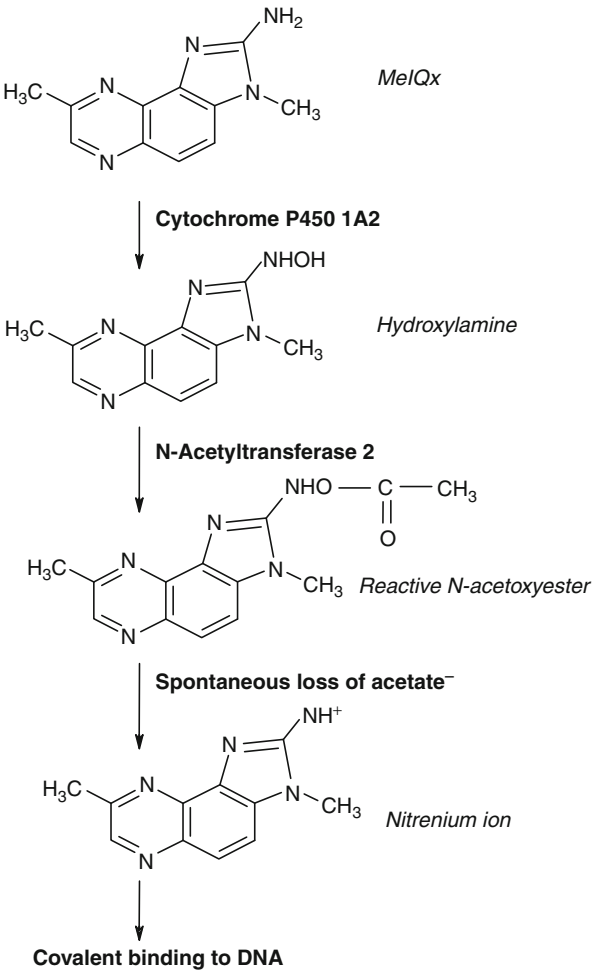



**Fig. 2** Mechanism of metabolic activation of MeIQx to a DNA-binding nitrenium ion by cytochrome P450 1A2 and *N*-acetyltransferase 2. Humans and rats efficiently form the hydroxylamine. However, cynomolgus monkeys lack a corresponding activity

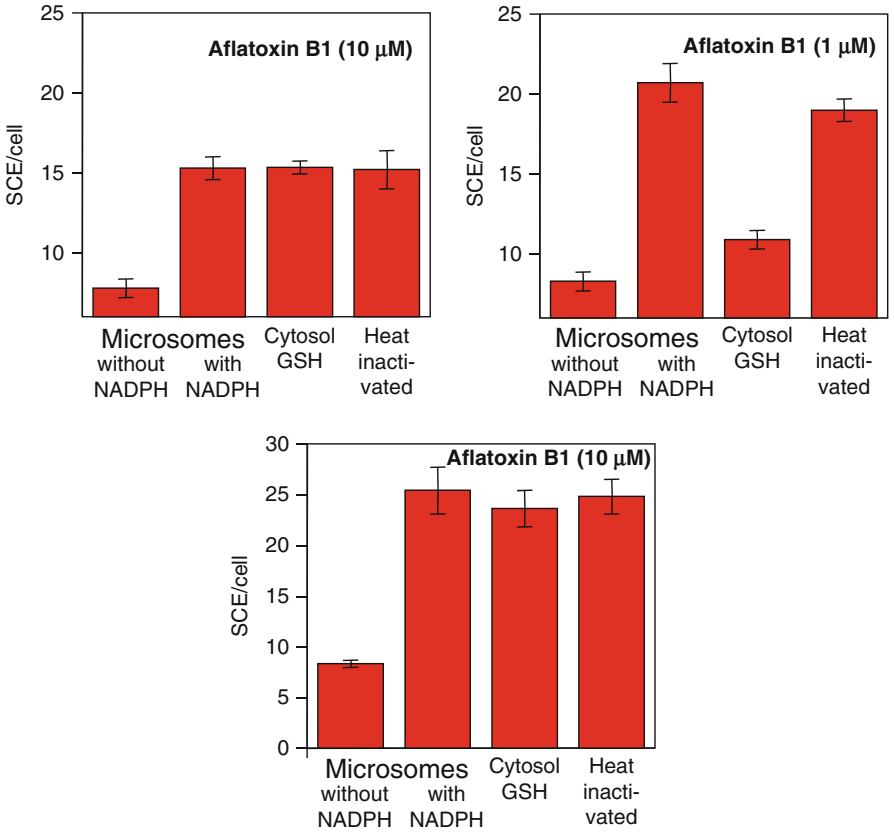



**Fig. 3** Influence of phase I and phase II metabolism on the genotoxicity of aflatoxin $B_1$ (AFB1), as evidenced by the sister chromatid assay in human lymphocytes. Venous blood of a volunteer was incubated with $AFB_1$ in the presence of human, mouse, and rat liver microsomes. Concentrations of 10 μM $AFB_1$ were used for incubations with human and rat microsomes. For incubations with mouse microsomes, only 1 μM $AFB_1$ was used since higher concentrations were no longer in the linear concentration-effect range. *First column*: negative controls with microsomes but without the cofactor NADPH. *Second column*: influence of phase I metabolism in the presence of microsomes and NADPH. *Third column*: combined influence of phase I metabolism and glutathione *S*-transferases in presence of microsomes, NADPH, cytosol, and 2.5 mM glutathione. *Fourth column*: negative control, with the same conditions as for the third column but using heat-inactivated cytosol (Wilson et al. 1997; review: Hengstler et al. 1999)

compared to humans and rats. On the other hand, the cytosolic compartment of mice also shows a higher capacity to detoxify AFB1.

Today, the mechanisms underlying these observations are known. Activation of AFB1 to a genotoxic carcinogen, namely, AFB1-exo-8,9-epoxide, is catalyzed mainly by cytochrome P450 1A2 and 3A4 (human). The extremely efficient inactivation of AFB1-exo-8,9-epoxide in mouse liver cytosol is catalyzed by the glutathione S-transferase isoenzyme mGSTA3-3 (synonym, mGST-Yc).

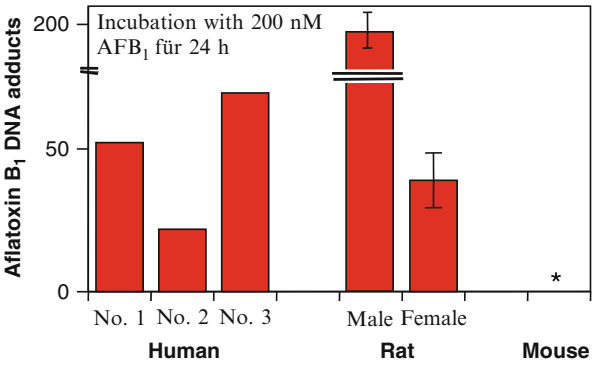



**Fig. 4** DNA binding of $^{3}$H-aflatoxin $B_1$ in cultivated hepatocytes of humans (three donors), rats (Sprague Dawley, male and female), and mice (CD-1, male) after incubation for 24 h (Cole et al. 1988; review: Hengstler et al. 1999)

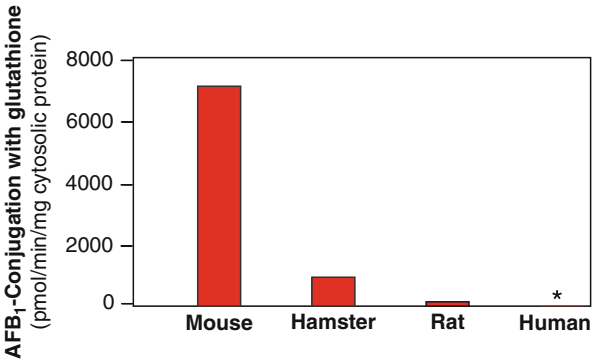



**Fig. 5** Conjugation of aflatoxin $B_1$-exo-8,9-epoxide (generated by incubation with microsomes) with glutathione by cytosol of livers from mice, hamsters, rats, and humans (Slone et al. 1995). *Below detection limit

In contrast, humans and rats do not express phase II enzymes with a similarly high capacity to detoxify AFB1-exo-8,9-epoxide.

An adequate technique for identification of the interspecies difference in AFB1 susceptibility is the analysis of DNA adducts in primary hepatocytes (Fig. 4). While an approximately similar extent of DNA adducts was formed in human and rat hepatocytes, the corresponding data of mice were below the detection limit. Finally, the glutathione conjugation capacity can directly be analyzed, illustrating the low capacity of human liver cytosol to detoxify AFB1-exo-8,9-epoxide compared to mice (Fig. 5). In conclusion, humans are more susceptible to $AFB_1$-mediated carcinogenesis than mice. Therefore, human risk assessment should be based on rat data.

## Vinyl Acetate: The Relevance of Practical Thresholds

The examples of MeIQx and $AFB_1$ have illustrated the importance of basing risk assessment on toxicity data of speZcies that resemble the human situation. This is particularly relevant in case of huge interspecies differences of metabolic activation

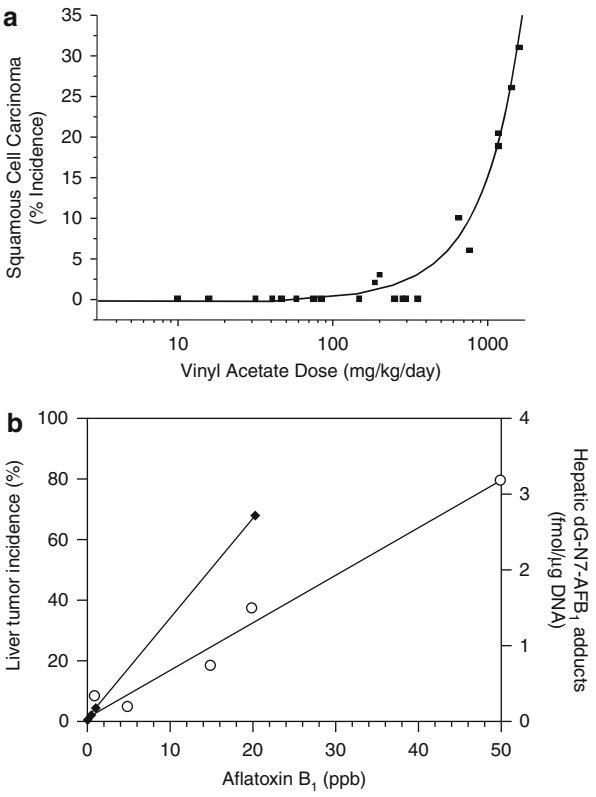



**Fig. 6** (**a**) Dose–response relationship between exposure to vinyl acetate in the drinking water and induction of carcinomas in rats and mice. Since rats and mice respond similarly to vinyl acetate on a mg/kg/day basis, data for rats and mice were summarized in one figure (details in Hengstler et al. 2003). Carcinomas induced by vinyl acetate under these conditions are tumors of the oral cavity, esophagus, and forestomach. The data show an increase of tumor incidence only at doses higher than 100 mg/kg/day. (**b**) Induction of liver tumors (○) and DNA adducts (◆) by aflatoxin $B_1$. In contrast to vinyl acetate a linear dose–response relationship was obtained by aflatoxin $B_1$ (review: Hengstler et al. 2003)

or detoxification. A further important aspect for risk assessment is the dose–response relationship at low in vivo relevant doses. In this subchapter we discuss the example of vinyl acetate to illustrate the relevance of threshold mechanisms. Similar principles can be applied for acrylonitrile and 1,3-butadiene which also are produced in large amounts. Vinyl acetate is carcinogenic in rats and mice. After oral administration only tumors of the oral cavity, esophagus, and forestomach have been observed. Inhalation studies with rats led to tumors of the olfactory epithelium. Therefore, vinyl acetate represents a typical "site of contact carcinogen." Vinyl acetate is known to induce DNA-protein adducts, chromosomal aberrations, and sister chromatid exchanges. Therefore, it represents a genotoxic carcinogen. Nevertheless, metabolism and mechanism of action of vinyl acetate show some relevant differences compared to MeIQx and $AFB_1$ that should be considered for risk assessment. Importantly, vinyl acetate is rapidly metabolized to acetaldehyde and acetic acid. This reaction is catalyzed by carboxylesterase and aldehyde dehydrogenase. Acetaldehyde can cause DNA-protein cross-links and finally chromosomal aberrations at high concentrations. Acetaldehyde represents the only genotoxic metabolite of vinyl acetate. The parental compound is not genotoxic. Also the second metabolite, acetic acid, may contribute to vinyl acetate

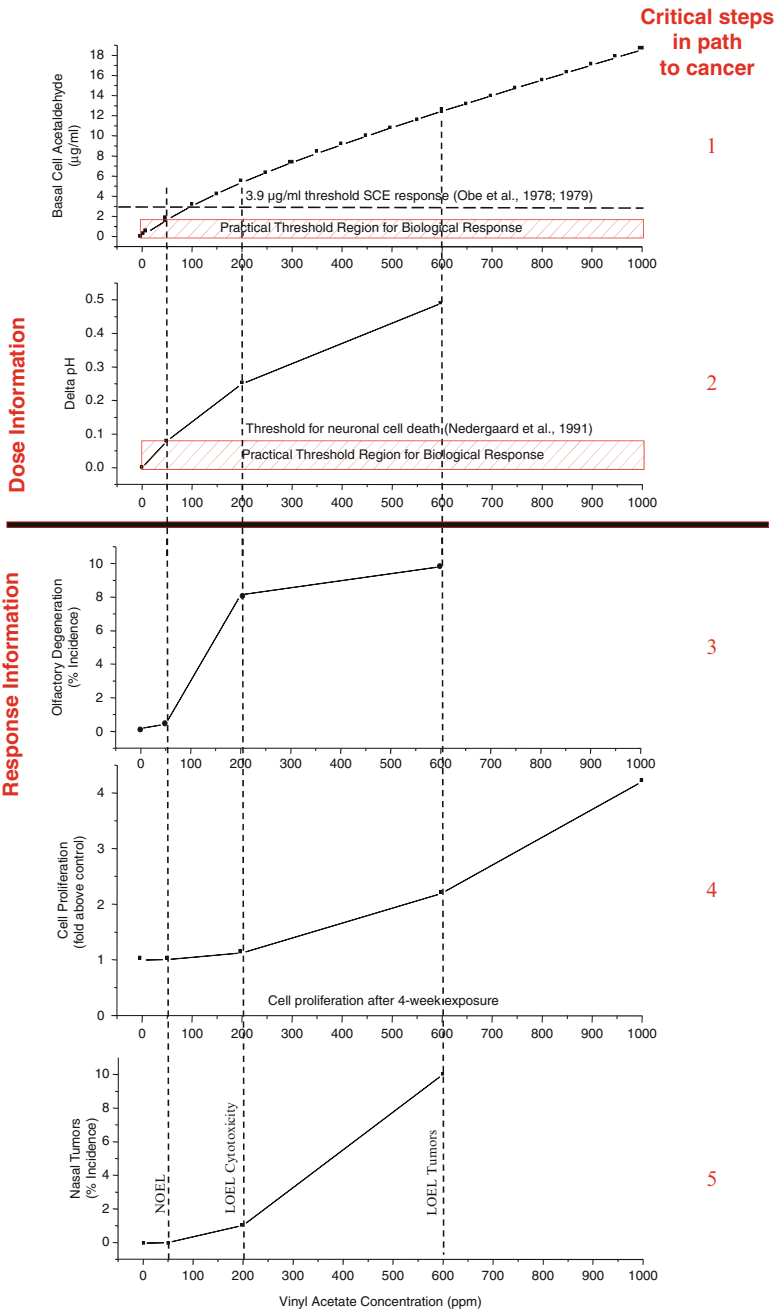



**Fig. 7** Five critical steps on path to cancer induced by vinyl acetate in the olfactory epithelium (From Hengstler et al. 2003). *Panel 1*: concentrations of acetaldehyde in the basal cells of the olfactory epithelium, the cells of origin of nasal tumors. *Panel 2*: reduction of pH in relation to

cytotoxicity by decreasing the pH value. Decreases of the pH value of less than 0.15 units usually remain without toxic consequences. However, a further decrease in pH may cause cytotoxicity and replacement proliferation. This will promote carcinogenesis resulting from acetaldehyde-induced DNA lesions.

For risk assessment it is important to consider that both metabolites of vinyl acetate, acetaldehyde, and acetic acid also are endogenously formed in the organism. Acetaldehyde is formed in threonine metabolism. Endogenously, acetaldehyde is formed in concentrations of approximately 0.3 μg/ml blood. Exposure to vinyl acetate at levels that increase acetaldehyde and acetic acid within this endogenously occurring range does not induce tissue damage or carcinogenesis, which will be shown below. Therefore, it can be concluded that the organism has established protective mechanisms that avoid tissue damage at physiological levels of both vinyl acetate metabolites. Exposure to vinyl acetate should be acceptable if the resulting increase in acetaldehyde and acetic acid at the highest exposed cells of the organism is lower than endogenously formed concentrations.

Although the aforementioned theoretical considerations may seem plausible, the assumption of a "practical threshold" is only acceptable when proven by experimental data. Dose–response experiments for vinyl acetate-induced carcinogenicity show a wide dose range without increased tumor incidence (Fig. 6a). However, a clear increase is observed at doses higher than 100 mg vinyl acetate/kg body weight/day. The shape of the dose–response curve of vinyl acetate clearly differs from that of the no-threshold carcinogen $AFB_1$, for which both tumor incidence and DNA adducts do not show any evidence for a threshold (Fig. 6b).

The threshold of the dose–response relationship for vinyl acetate is due to the fact that critical concentrations of acetaldehyde and acetic have to accumulate up to certain concentrations where mechanisms relevant for carcinogenesis are activated. For the olfactory epithelium, five steps of vinyl acetate-mediated carcinogenesis seem to be critical (Fig. 7). According to our PBPK model exposure of 50 ppm, vinyl acetate leads to acetaldehyde concentrations of 1.7 μg/ml (step 1 in Fig. 7). Moreover, the resulting acetic acid causes a pH reduction of 0.08 units in basal cells of the olfactory epithelium, the cells of origin of carcinogenesis (step 2). This pH decrease is less than the critical value of ΔpH 0.15 which may cause cytotoxicity. Therefore, degeneration of olfactory cells (step 3) and replacement proliferation (step 4), steps critical on path to cancer, do not yet occur at 50 ppm vinyl acetate. However, increasing vinyl acetate exposure to 200 or even 600 ppm will activate these mechanisms. Vinyl acetate exposure of 600 ppm will lead to a concentration of 12.4 μg/ml acetaldehyde in basal cells (step 1). The pH value will decrease by 0.49 units (step 2) which will cause degeneration of the olfactory epithelium (step 3) and will lead to replacement proliferation (step 4) of basal cells. Therefore, all steps critical for carcinogenesis are active at 600 ppm vinyl acetate. This leads to

**Fig. 7** (continued) vinyl acetate exposure. Reduced pH is responsible for cytotoxicity in the olfactory epithelium (*Panel 3*), which causes replacement proliferation (*Panel 4*) and finally promotes induction of nasal tumors (*Panel 5*)

a clear increase of tumor incidence (Fig. 7). The model demonstrates that the mechanisms critical for carcinogenesis (steps 1-4) become active only when threshold concentrations of acetaldehyde and acetic acid are exceeded. These threshold concentrations will only be exceeded when vinyl acetate exposure occurs above certain levels (Fig. 7). In conclusion, two metabolites, acetaldehyde and acetic acid, are responsible for the toxic and carcinogenic effects of vinyl acetate. Both metabolites also occur endogenously. Only above certain threshold concentrations carcinogenicity can be expected. Therefore, risk assessment of vinyl acetate has to take into account quite different principles as for, e.g., aflatoxin $B_1$ or heterocyclic amines where similar threshold mechanisms are not known.

## References

Arand M, Oesch F (2004) Fremdstoffmetabolismus. In: Marquardt H, Schäfer S (eds) Lehrbuch der Toxikologie. Wissenschaftliche Verlagsanstalt Stuttgart, Germany, pp 89–116

Back DJ, Grimmer SF, Orme ML, Proudlove C, Mann RD, Breckenridge AM (1988) Evaluation of committee on safety of medicines yellow card reports on oral contraceptive-drug interactions with anticonvulsants and antibiotics. Br J Clin Pharmacol 25:527–532

Bogaards JJ, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ, Walther B (2000) Determining the best animal model for human-cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. Xenobiotica 30:1131–1152

Cole KE, Jones TW, Lipsky MM, Trump BF, Hsu IC (1988) In vitro binding of aflatoxin $B_1$ and 2-acetylaminofluorene to rat, mouse and human hepatocyte DNA: the relationship of DNA binding to carcinogenicity. Carcinogenesis 9:711–716

Davis CD, Adamson RH, Snyderwine EG (1993) Studies on the mutagenic activation of heterocyclic amines by cynomolgus monkey, rat and human microsomes show that cynomolgus monkeys have a low capacity to N-oxidize the quinoxaline-type heterocyclic amines. Cancer Lett 73:95–104

Hengstler JG, Oesch F (1999) Interspecies differences in xenobiotic metabolizing enzymes and their importance for interspecies extrapolation of toxicity. In: Ballantyne B, Marrs TC, Syversen T (eds) General and applied toxicology. Macmillan, London, pp 271–290

Hengstler JG, van der Burg B, Steinberg P, Oesch F (1999) Interspecies differences in cancer susceptibility and toxicology. Drug Metab Rev 31:917–970

Hengstler JG, Bogdanffy MS, Bolt HM, Oesch F (2003) Challenging dogma: thresholds for genotoxic carcinogens? The case of vinyl acetate. Annu Rev Pharmacol Toxicol 43:485–520

Kocarek TA, Schuetz EG, Strom SC, Fisher RA, Guzelian PS (1995) Comparative analysis of cytochrome P4503A induction in primary cultures of rat, rabbit, and human hepatocytes. Drug Metab Dispos 23:415–421

Ma X, Shah Y, Cheung C, Guo GL, Feigenbaum L, Krausz KW, Idle JR, Gonzalez FJ (2007) The pregnane X receptor gene humanized mouse: a model for investigating drug–drug interactions mediated by cytochromes P450 3A. Drug Metab Dispos 35:194–200

Martignoni M, Groothuis GM, de Kanter R (2006) Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2:875–894

McLean AE, McLean EK (1966) The effect of diet and 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane (DDT) on microsomal hydroxylating enzymes and on sensitivity of rats to carbon tetrachloride poisoning. Biochem J 100:564–571

Modak AS (2010) Single time point diagnostic breath tests: a review. J Breath Res 4:1–6

Mohutsky MA, Romeike A, Meador V, Lee WM, Fowler J, Francke-Carroll S (2010) Hepatic drug-metabolizing enzyme induction and implications for preclinical and clinical risk assessment. Toxicol Pathol 38:799–809

Oesch F, Arand M (1999) Xenobiotic metabolism. In: Marquardt H, Schäfer S, McLellan D, Welsch C (eds) Toxicology. Academic, San Diego, pp 84–110

Oesch-Bartlomowicz B, Oesch F (2007) Mechanisms of toxication and detoxication which challenge drug candidates and drugs. In: Triggle D, Taylor J (eds) Comprehensive medicinal medicine. Elsevier, Philadelphia, pp 193–214

Scheer N, Ross J, Rode A, Zevnik B, Niehaves S, Faust N, Wolf CR (2008) A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. J Clin Invest 118:3228–3239

Schroeder K, Bremm KD, Alépée N, Bessems JG, Blaauboer B, Boehn SN, Burek C, Coecke S, Gombau L, Hewitt NJ, Heylings J, Huwyler J, Jaeger M, Jagelavicius M, Jarrett N, Ketelslegers H, Kocina I, Koester J, Kreysa J, Note R, Poth A, Radtke M, Rogiers V, Scheel J, Schulz T, Steinkellner H, Toeroek M, Whelan M, Winkler P, Diembeck W (2011) Report from the EPAA workshop: in vitro ADME in safety testing used by EPAA industry sectors. Toxicol In Vitro 25:589–604

Slone DH, Gallagher EP, Ramsdell HS, Rettie AE, Stapleton PL, Berlad LG, Eaton DL (1995) Human variability in hepatic glutathione S-transferase-mediated conjugation of aflatoxin $B_1$-epoxide and other chemicals. Pharmacogenetics 5:224–233

U.S. Department of Health and Human Services, FDA, Center for Drug Evaluation and Research (CDER) (2008) Safety testing of drug metabolites. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079266.pdf. Accessed November 2009

Williams JA, Hyland R, Jones BC, Smith DA, Hurst S, Goosen TC, Peterkin V, Koup JR, Ball SE (2004) Drug–drug interactions for UDP-glucuronosyltransferase substrates: a pharmacokinetic explanation for typically observed low exposure (AUCi/AUC) ratios. Drug Metab Dispos 32:1201–1208

Wilson AS, Williams DP, Davis CD, Tingle MD, Park BK (1997) Bioactivation and inactivation of aflatoxin $B_1$ by human, mouse and rat liver preparations: effect on SCE in human mononuclear leucocytes. Mutat Res 373:257–264

Zuber R, Anzenbacherova E, Anzenbacher P (2002) Cytochromes P450 and experimental models of drug metabolism. J Cell Mol Med 6:189–198

# Dose–Response Relationship and Extrapolation in Toxicology. Mechanistic and Statistical Considerations

Werner K. Lutz, Roman W. Lutz, David W. Gaylor, and Rory B. Conolly

## Contents

Dose–Response Curve in Textbook ..... 549
The Logarithm "Catch" ..... 549
Continuous Response Variables ..... 552
Linearity as Default Extrapolation for Rates of First-Line Interactions ..... 552
Deviation from Linearity Due to Saturation of Processes that Modulate Biomarker Levels ..... 554
Nonmonotonic Shape if Background Is Reduced at Low Dose ..... 555
Confidence Limits on Low-Dose Effect and Comparison with Background Variation ..... 556
Mechanistic Background of Nonmonotonic Dose Response ..... 558
"Incidence" as a Different Type of Response Variable ..... 560
A Dose–Incidence Relationship Reflects Differences in Susceptibility ..... 560
Chemical Carcinogenesis and Cancer Incidence ..... 562
Multiplicative Combination of Susceptibility Factors Results in a Lognormal Distribution ..... 563
Extrapolation of a Dose–Cancer Incidence Relationship ..... 563
It Is Time for a Revival of the Lognormal Distribution for Cancer Risk Extrapolation ..... 563
Tolerance Distribution Versus Stochastic Modeling ..... 566

W.K. Lutz (✉)
Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg, Germany
e-mail: lutz@toxi.uni-wuerzburg.de

R.W. Lutz
Formerly: Seminar for Statistics, Swiss Federal Institute of Technology, Zürich, Switzerland

D.W. Gaylor
Formerly: National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA

R.B. Conolly
Integrated Systems Toxicology Division, NHEERL/ORD, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_72, 

How to Incorporate a Nonlinearity of an Experimental Biomarker in a Dose–Incidence Relationship? ..... 566
Monte Carlo Simulations to Differentiate the Dose Response for a Population Average Versus Individuals ..... 567
References ..... 568

**Abstract**

Controversy on toxicological dose–response relationships and extrapolation of an incidence to low dose can be the consequence of misleading data presentation, diverging mechanistic understanding, or lack of differentiation between a continuous response variable, such as any concentration of a biomarker, and an incidence derived from a binary response (yes or no?) in individuals (dichotomous variable). In this chapter, we address respective issues and illustrate them with examples for genotoxicity, mutagenicity, and cancer incidence. The rate of any interaction of a toxicant with a biological target molecule at low dose is proportional to its concentration. Linear extrapolation is therefore a reasonable default for rates of first-line interaction in the low-dose range. In toxicity testing however, (i) we do not measure rates of interactions but concentrations of biomarkers, and (ii) we deal with a dose range that usually expands to overt toxicity. Deviation from linearity is observed with increasing dose whenever saturation, inhibition, or induction of a process involved comes into play. A nonmonotonic shape of the dose–response curve may be observed as a special case of nonlinearity, if a background measure in untreated controls is decreased at low dose but increased at high dose. A dose response can appear as a threshold if two processes that affect the background level in opposite directions cancel each other out. A mathematical threshold, where there is no effect at all up to a defined breakpoint of the dose–response "curve," cannot be advocated for any continuous response measure. We use computational modeling to characterize how competing influences that are dominant over different dose ranges combine to generate different shapes. The situation is different for an incidence of a defined effect, e.g., a diagnosis of cancer. On an individual level, the response is given by a binary "yes or no." For dose response, each individual has its own "threshold dose" to switch from "no cancer" to "cancer"; the dose–incidence "curve" represents a staircase of individual threshold doses and reflects the tolerance distribution in the examined population. Extrapolation to low dose therefore follows differences in individual susceptibility and cannot be predicted by the mode of interaction between toxicant and biological target. For complex endpoints of toxicity such as cancer, individual susceptibility is determined by numerous genetic and in-life factors, such as enzymatic activation and detoxification of endogenous and exogenous carcinogens, DNA repair, or cell cycle control. Multiplicative combination of the individual activity of these factors and application of the central limit theorem of statistics suggests that the tolerance distribution – and with this the dose–incidence relationship – is approximated by a cumulative normal curve against log (dose). Using this model

for a dose–incidence extrapolation, the cancer risk drops faster than by linear extrapolation, the more we approach dose zero. In the last section, we combine a mechanistically supported nonmonotonic dose response with individual differences for the rate of the underlying counteracting processes. Monte Carlo simulations indicate that a nonmonotonic shape of a dose response for a biomarker, determined as an average of a dose group, does not exclude a monotonic shape for some individuals. An observation of a nonmonotonicity in animals cannot be carried over by default to a dose–incidence response in a human population.

***EPA Disclaimer***
*This chapter has been reviewed by the United States Environmental Protection Agency and approved for publication but it may not reflect the views of the Agency and no official endorsement should be inferred.*

## Dose–Response Curve in Textbook

The usual representation of a dose–response relationship is the cumulative normal distribution against the logarithm of the dose (Fig. 1). It is based on the finding that a lognormal curve often provides a good fit to data of different types of response variables. This holds for continuous response variables such as any rate of a process or concentration of a biomarker, as well as for an incidence of a defined effect, which is based on a binary (yes-or-no) response in individuals.

For an extrapolation to background (dose zero), the logarithmic dose scaling may be misleading because the sublinear appearance at the low-dose end may be interpreted as indicating a threshold. For an appropriate discussion of dose–response curves, it is crucial to understand (i) the consequences of logarithmic scaling of the dose axis and (ii) to clearly define the term "threshold."

Figure 2 shows that "threshold" could mean different things (Lutz and Lutz 2009). One curve starts with a positive but statistically insignificant slope and bends up at the "threshold dose"; the second is a mathematical threshold that is defined by an initial slope zero, followed by slope $>0$ at some breakpoint; and the third has an initially negative slope, which results in a nonmonotonic shape over the whole dose range. We will later discuss mechanism and conditions that may lead to the different types of "threshold."

## The Logarithm "Catch"

First of all, we must caution against the use of a logarithmic scale for the dose axis in connection with a discussion of dose–response extrapolation (Lutz et al. 2005). Problem #1: Since log (0) is indefinite ("- $\infty$"), the response measure of the control group at dose zero cannot be plotted in the same graph together with the treated groups, and visual inspection of the dose range of extrapolation to background is not possible. Problem #2: Logarithmic scaling of the dose axis distorts a straight

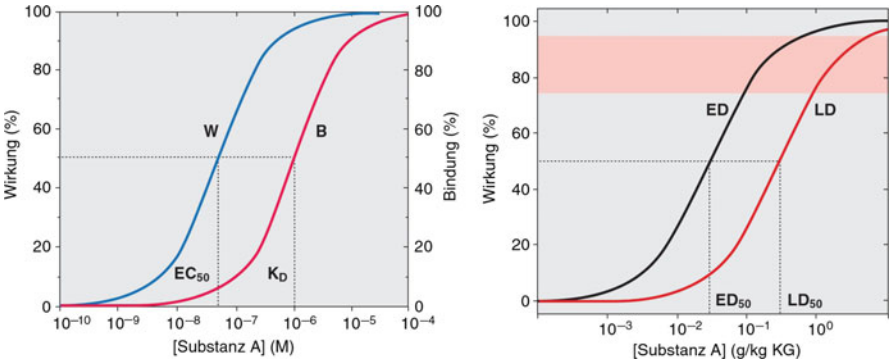



**Fig. 1** Typical sigmoid shape of dose–response curves in textbooks of pharmacology and toxicology for different types of response variables, using a logarithmic scaling of the dose axis. *Left* (continuous response variables): ligand–receptor binding (*B*) and receptor-mediated response (*W*, "Wirkung") as a function of the logarithm of the ligand concentration (*substance A*). *Right* (dichotomous variable): incidence of a therapeutic effect (*E*) or of death (*L*) shown as a function of log (*dose*) (Reprinted with permission from Aktories/Förstermann/Hofmann/Starke, Pharmakologie und Toxikologie, 9. Auflage 2005, Abb. 1–4 and 1–7, Urban & Fischer, München)

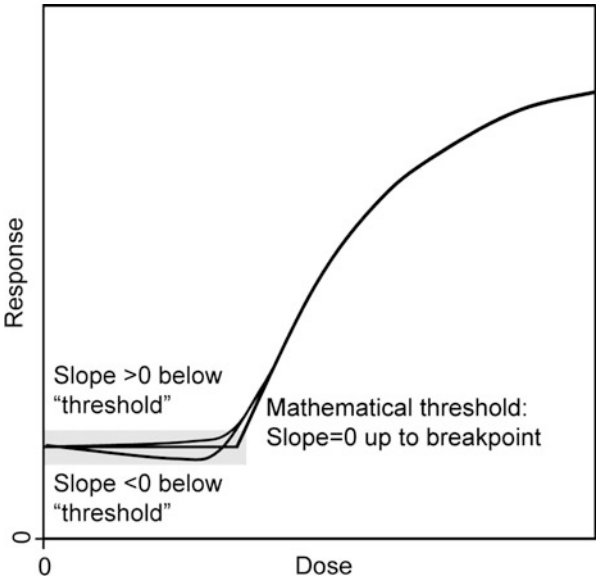



**Fig. 2** Three shapes of dose–response curves that could be interpreted as indicating some type of "*threshold*": linear–sublinear (a nonzero slope at low dose, bending up at an undefined threshold), mathematical threshold (*slope* = 0 up to a defined breakpoint dose), and nonmonotonic dose response (*slope* < 0 *below* the "threshold") (Reprinted with permission (Lutz and Lutz 2009))

line ("linearity") into a threshold-like curve. Figure 3 shows different representations of the same linear function y = 5 + 10 * x. An arithmetic scale is used for the left panel. The center panel uses a logarithmic scale and spans doses between 0.4 and 4. A sublinear shape is seen. The right panel spans six orders of magnitude down to dose $10^{-6}$. The dose groups that show an increase above control are

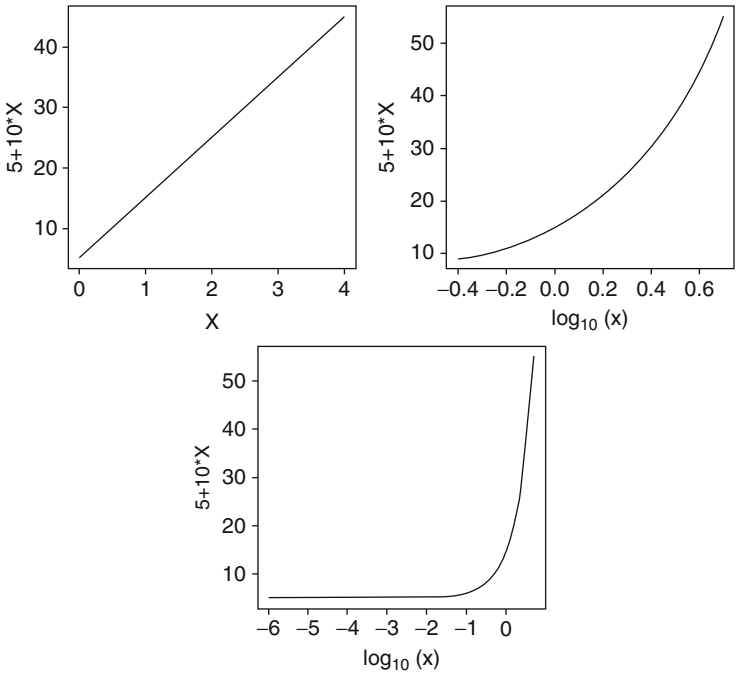



**Fig. 3** Logarithmic representation of the dose axis distorts the straight line of a linear dose response into a threshold appearance. All three representations show the same linear dose–response relationship y = 5 + 10*x. *Left*: arithmetic dose scale; *center*: log scale spanning one factor of ten for dose; *right*: log scale spanning seven factors of ten down to dose $10^{-6}$

compressed into one factor of ten and appear with a steep slope. Such a delusive appearance of a threshold still shows up in publications. It is easily generated by the use of doses that are many orders of magnitude below the no-observed-effect level.

The low-dose part of the sigmoid shape shown for receptor–ligand binding (R + L → RL) in Fig. 1b is another example of the result of logarithmic dose scaling. The underlying function [RL]/[Rtot] = [L]/([L] + Kd) is not sigmoid but a hyperbola, and linearity is a good approximation at low concentration of ligand [L]. Figure 4 shows this function on a logarithmic and an arithmetic scale (left and right panel, respectively). Note that Michaelis–Menten kinetics of enzyme reactions follows the same function. This also means that the rate of enzymatic product formation is approximately proportional to the substrate concentration at concentrations below the Michaelis constant.

**Conclusions**

*The fact that logarithmic scaling of the dose axis provides good data fit by a cumulative normal distribution, data both for continuous variables and for incidences (dichotomous variable), is misleading in two ways. On the one*

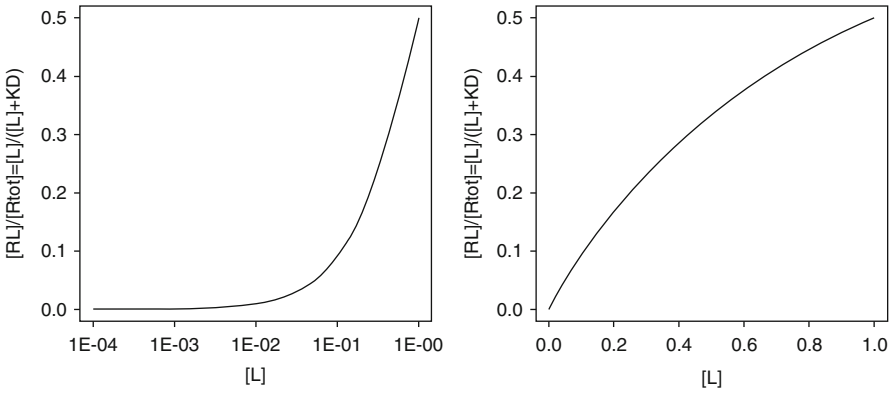



**Fig. 4** Low-dose part of receptor–ligand (RL) complex formation as a function of ligand concentration $[RL]/[R_{tot}] = [L]/([L] + K_D)$ for the dissociation constant $K_D = 1$, shown on a logarithmic scale (*left*; as used in Fig. 1) and on an arithmetic scale (*right*)

*hand, the sublinear appearance of the low-dose part can mimic a threshold even for a linear dose response; on the other hand, it is suggestive of the misconception that the sigmoid shape of a dose–incidence relationship adheres to the same principles as a dose response for a continuous variable.*

## Continuous Response Variables

### Linearity as Default Extrapolation for Rates of First-Line Interactions

Many biomarkers measured in toxicity testing are concentrations, e.g., products of physical or chemical interaction of a toxicant with a biological target (binding to a receptor or an enzyme; reaction with protein or DNA). According to the law of mass action, the rate of interaction is approximately proportional to the concentration of the reaction partners at low dose. Linear extrapolation is therefore an appropriate default for the low-dose end. This includes situations of complex metabolic activation where the toxic reaction product is the result of multiple steps and includes competing reactions. In Fig. 5, the mycotoxin aflatoxin $B_1$ ($AFB_1$), a potent hepatocarcinogen, is shown to react with the seven-position in guanine to form the respective promutagenic DNA adduct. Metabolic activation to the chemically reactive, electrophilic epoxide (in brackets) is a necessary intermediate step. The up-and-down arrows indicate that a number of concurrent reactions (other pathways of elimination; reaction with other nucleophiles, e.g., water or glutathione) take place. All reactions – toxification as well as detoxification – are approximately proportional to the concentration of the reactant as long as capacity-limited processes are not approaching saturation.

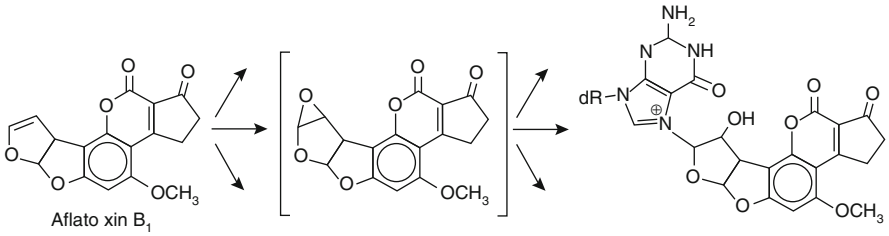



**Fig. 5** Two-step formation of a guanyl-7-adduct in DNA by the carcinogenic mycotoxin aflatoxin $B_1$ via metabolic activation to the chemically reactive epoxide. *Up*-and-*down arrows* indicate competing processes of detoxification

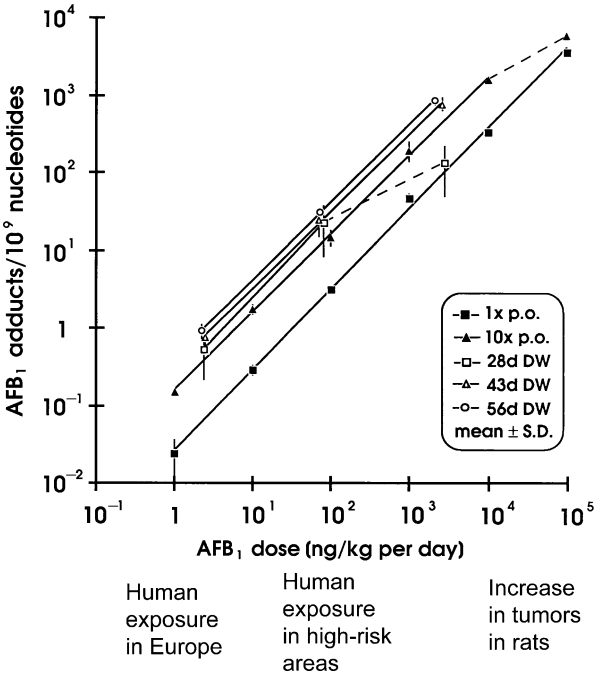



**Fig. 6** Linear dose–response relationships at the low-dose end for [$^3$H]aflatoxin $B_1$-DNA adducts in rat liver. Single and multiple daily oral dosing, as well as application in drinking water (*DW*) for up to 56 days. Note the *double-log plot*: slope = 1 for all *full lines* are indicating proportionality between dose and response, i.e., linear dose response

Considering extrapolation of a biomarker to low dose, it means that reducing the dose by a given factor is expected to result in a reduction of the biomarker by the same factor. For DNA-adduct levels, for instance, this also means that the rate of formation cannot drop to zero at any low dose. A mathematical threshold for a dose response as shown in Fig. 2 is therefore not possible. As an example of linearity down to the ng/kg dose range, DNA adducts in the liver of rats treated with [$^3$H]$AFB_1$ of high specific radioactivity decreased in a dose-proportional manner for all treatment scenarios at dose levels below 100 ng/kg per day (Fig. 6).

## Deviation from Linearity Due to Saturation of Processes that Modulate Biomarker Levels

Toxicity studies usually include dose levels beyond the range of proportionality for the reaction rates that determine the response measure. Deviation from linearity is therefore the rule rather than the exception. For biomarkers of genotoxicity, one important mechanism that results in sublinear deviation of the dose response for mutation is saturation of DNA repair. Figure 7 shows the formation of a GC → AT base-pair substitution mutation resulting from methylation of guanine at the $O^6$-position (G*). The full process of mutagenesis requires two rounds of DNA replication. It starts with mispairing of G* with thymine (T) at the first round, followed by the correct pairing of T with adenine (A) in the second round. Repair is possible at all stages of the process. At low dose, i.e., at slow rate of DNA-adduct formation, repair may be proportional to the damage, so that mutation rates stay low. The resulting slope of the dose-mutant frequency relationship is positive, but may not be significant. With further increase in dose, repair will become saturated, which results in a steep increase in slope for mutant formation.

Superposition of the rates of formation and repair of DNA are shown schematically in the left panel of Fig. 8. It shows a linear dose response for

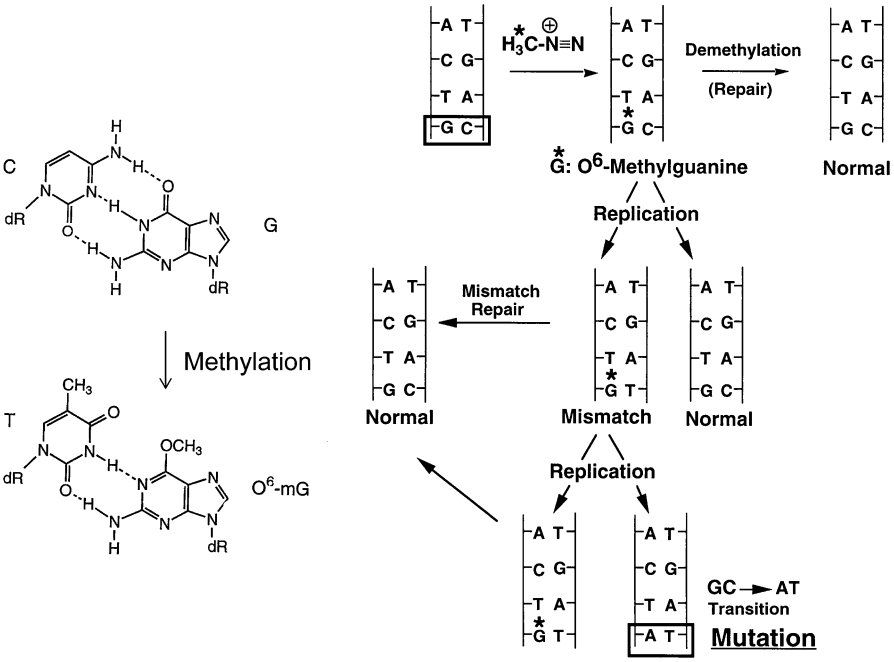



**Fig. 7** *Left*: methylation of guanine (*G*) at the $O^6$-position and mispairing of $O^6$-mG (*G**) with thymine (*T*) instead of cytosine (*C*). *Right*: from a GC base pair to AT, i.e., a transition mutation requiring mispairing and two rounds of DNA replication

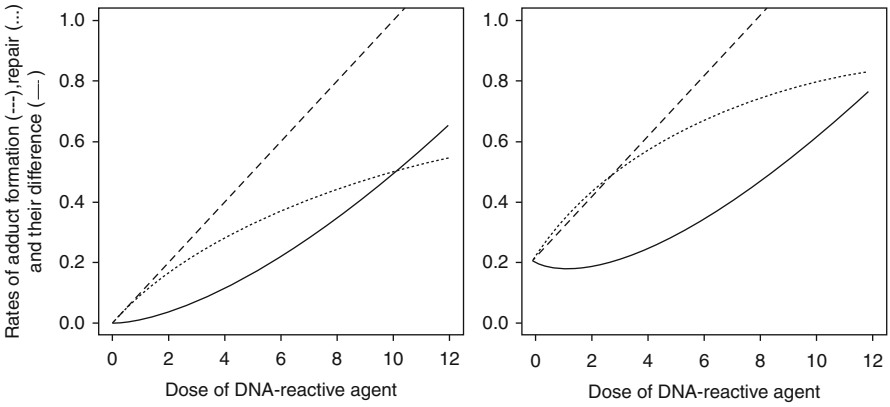



**Fig. 8** *Left* panel: schematic representation of the superposition of a dose linear increase for adduct formation (*dashed line*) by a saturable rate of DNA repair (*dotted line*). The result is a sublinear curve for mutant frequency as a function of dose (*full line*). *Right* panel (includes background DNA damage at dose 0): The slope for exogenous adduct formation is the same as in the *left* panel. The repair activity is assumed to be induced (steeper slope at low dose) and active also on background adducts. Superposition results in a nonmonotonic dose response for mutant frequency

the rate of DNA-adduct formation (dashed line) and a saturation curve for DNA repair (dotted line). The difference between the two curves (adduct formation minus repair; full line) assumes a sublinear shape for the dose response for mutagenicity.

This situation is considered the mechanistic basis of a threshold-like dose response for the mutagenicity of ethyl methanesulfonate (EMS) in mice. The respective data had been collected in the follow-up of a drug contamination by this ethylating agent (Lutz 2009). Figure 9 shows the induction of lacZ mutants in MutaMouse® treated daily for 28 days with EMS. At low dose, DNA ethylation was probably repaired almost as rapidly as it had been formed. When the enzymatic DNA repair came into saturation with further increase in DNA ethylation, the dose–response curve for mutant induction bent upward. A hockey stick threshold model provided much better fit to the data than a linear dose response and showed a lower limit of a 90 % confidence interval for a hypothetical breakpoint at 23 mg/kg per day (dashed line) (Lutz and Lutz 2009).

## Nonmonotonic Shape if Background Is Reduced at Low Dose

In view of the general understanding that both adduct formation and repair are approximately proportional to low dose and the fact that repair always lags somewhat behind, one would expect minute, though positive slope for mutant induction also below the "threshold dose." In our example, however, linear regression of the mutant

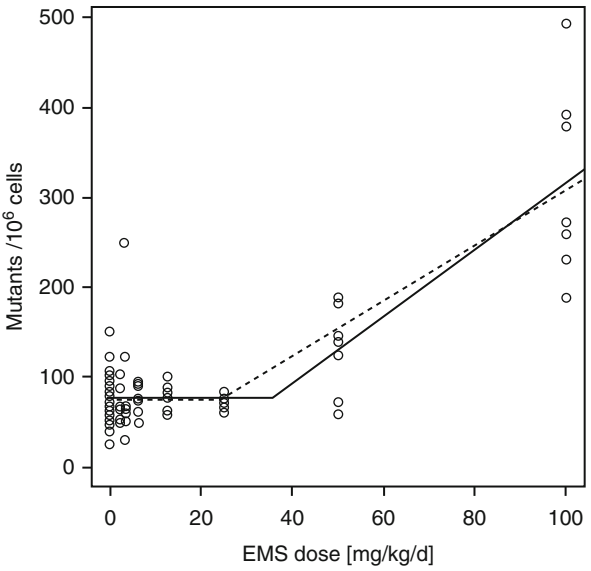



**Fig. 9** Dose response for lacZ mutant frequency in bone marrow cells of MutatMouse™ treated daily for 28 days with ethyl methanesulfonate (EMS; $CH_3–SO_2–O–CH_2–CH_3$). *Circles* represent individual mice. The *full line* indicates the best fit by a hockey stick model; the *dashed line* represents the lower limit of a two-sided 90 % confidence interval for the respective breakpoint (23 mg/kg per day) (Reprinted with permission (Lutz and Lutz 2009))

frequency data shown in Fig. 9 below the "threshold" shows a slightly negative slope (Fig. 10). It appears as if treatment of the mice with ethyl methanesulfonate below the putative threshold dose had resulted in a minute reduction of the background mutant frequency. If true, how could this be explained mechanistically?

DNA methylation of guanine by S-adenosyl methionine forms an important part of promutagenic background DNA damage. In view of the high mispairing potency of $O^6$-methylguanine, inducible repair has evolved to limit this dangerous type of DNA damage. The negative slope could therefore be explained by the hypothesis that $O^6$-ethylguanine, a DNA adduct similar in structure to $O^6$-methylguanine, induced DNA repair even at lowest doses of EMS. If the induced repair was not only active on DNA ethylation but also on background DNA methylation, one could explain the negative slope for mutant frequency. Over a wide dose range then, a nonmonotonic shape appears because of saturation of the induction of repair.

This hypothesis is illustrated schematically in the right panel of Fig. 8. It differs from the schema in the left panel in that it includes a background DNA damage and exhibits a steeper initial slope of repair due to additional induction of repair by DNA ethylation. Superposition of the linear dose response for the formation of adducts by the saturable rate of repair now results in a nonmonotonic shape for total DNA damage.

## Confidence Limits on Low-Dose Effect and Comparison with Background Variation

While linear regression shows a negative, while not statistically significant, slope as best fit to the data, the true slope could also be positive or more negative. This is

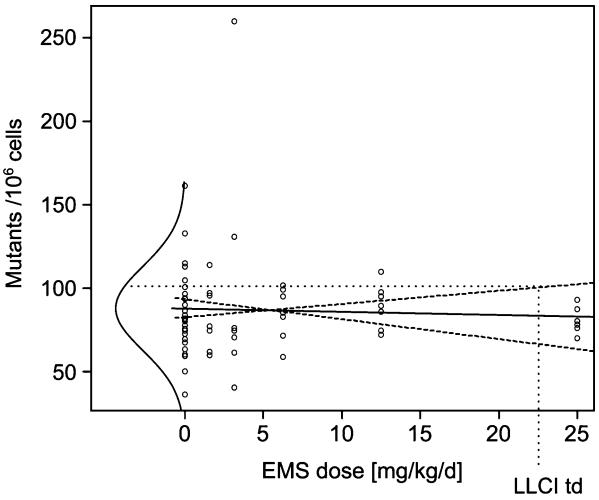



**Fig. 10** Linear regression of the dose–response data below the threshold dose for lacZ mutant induction by ethyl methanesulfonate shown in Fig. 9 (see respective legend for experimental details). The best estimate of the slope is imperceptibly negative (*full line*). *Dashed lines* indicate the limits of a 90 % confidence interval for the slope. The *horizontal dotted line* connects the upper bound of the regression at the threshold dose to the variance of response in control animals shown as a normal distribution (Reprinted with permission (Lutz and Lutz 2009))

indicated in Fig. 10 by the dashed lines that show upper and lower limits of a 90 % confidence interval for the slope of the linear regression. The question now is whether induced mutant frequency at the threshold dose of 23 mg/kg per day would be of concern if the upper limit of the confidence interval for the slope were true. This can be discussed on the basis of a comparison of the hypothetical increase in mutant frequency with the variation in background observed in the 27 control mice. The horizontal dotted line in Fig. 10 intersects at percentile 72 of a normal distribution fit to the data points, which allows the conclusion that even a statistically unlikely positive slope for mutant frequency would vanish within less than one standard deviation of the background variation.

**Conclusions**

*The dose–response curve for continuous response variables of early biomarkers of toxicity is the result of a superposition of a number of processes that contribute to the response measure. Each single process shows a monotonic dose response that is approximately linear at low dose and usually saturates with increasing dose. Superposition of the contributing dose responses results in sublinear or supralinear deviation from linearity. If one of the processes reduces the background response level, a nonmonotonic shape may also be observed. A mathematical threshold, where slope zero changes at a defined breakpoint of the curve to slope >0, can hardly be*

*explained by a biologically based mechanism. For practical purposes, however, i.e., to provide an estimate and its confidence limits for the point of transition, a simple statistical threshold model such as the hockey stick model might be useful.*

## Mechanistic Background of Nonmonotonic Dose Response

Several scenarios that can give rise to nonmonotonic dose responses had been addressed before publication of the EMS data (Conolly and Lutz 2004):

1. Formation of cyclic AMP as a function of the binding of phenylisopropyladenosine to adenosine receptors: Data showing a nonmonotonic shape are explained by the antagonistic action of the adenosine receptors A1 and A2, given the differences in ligand affinity and efficacy of signal transduction. A1: antagonistic, high affinity, low efficacy; A2: agonistic, low affinity, high efficacy.
2. Androgen-mediated gene expression: Combined exposure to native androgen and a synthetic analog interacts competitively at the androgen receptor to form a series of homo- and heterodimers with differing abilities for promotion of gene expression.
3. DNA adducts and mutation: Induction of repair also repairs DNA damage due to a background process. This theoretical example in fact predicted the observations for ethyl methanesulfonate and lacZ mutant induction in transgenic mice discussed above.
4. Cell cycle checkpoints: DNA damage activates checkpoints in the cell cycle. Long-duration checkpoints provide additional time for DNA repair before DNA replication can fix the damage as a mutation.

The four examples, though diverse, are all characterized by the presence of more than one influence on the shape of the dose–response curve, with each influence being dominant over a different range of doses (Table 1).

It is possible within these four examples to distinguish two classes of mechanisms that give rise to nonmonotonic dose response. The latter two examples involve adaptive responses of the exposed tissue – induction of DNA repair and activation of cell cycle checkpoints. The first two examples – modulation of adenylyl cyclase activity and androgen-mediated gene expression – do not involve adaptation. Rather, they reflect constitutive biology. Adaptation requires some amount of time, hence any appearance of a nonmonotonic dose response has a temporal aspect – nonmonotonicity will not be seen if the interval between exposure and measurement of the relevant endpoint is too brief. For risk assessment, we are usually concerned with longer-term exposures, so that nonmonotonic responses on all kinds of adaptation must be considered relevant.

Computational modeling can be used to characterize how these influences combine to generate different dose responses, including nonmonotonicity

**Table 1** Examples of nonmonotonic dose response for processes that are dominated differently over different dose ranges. Response is below background at low dose and returns to background (for androgen-mediated gene expression) or is above background (other three examples) with increasing dose (Conolly and Lutz 2004)

| Endpoint | Toxicant or ligand | Dominant influence at low dose | Dominant influence at high dose |
|---|---|---|---|
| Activity of adenylyl cyclase: formation of cAMP | Phenylisopropyladenosine | Adenosine A1 receptor | Adenosine A2 receptor |
| Androgen-mediated gene expression | Hydroxyflutamide (in the presence of dihydrotestosterone) | Homodimers (dominant at low and high dose) | Heterodimers (dominant at mid-dose) |
| Total DNA damage (endogenous plus exogenous) | DNA adduct-forming agent | Induction of DNA repair | Exogenous DNA adducts |
| Mutation | DNA adduct-forming agent | Cell cycle checkpoint | Exogenous DNA adducts |

(Conolly and Lutz 2004). This involves "parameter sweeps" where the value of the key parameter is varied across a range to produce a corresponding set of dose–response curves. As an example, for nonmonotonicity due to induction of DNA repair, a sweep on the parameter "induction of DNA repair" was conducted (Fig. 11). When the efficacy of induction is low (panel B; level 1), the dose–response curve for total adducts is monotonic (panel D, showing the sum of exogenous and background adducts). High levels of induction (levels 4–7) generate nonmonotonic curves of increasing degree. Interestingly, an intermediate efficacy (level 3) leads to a dose–response curve where, at low dose, the increase in the adduct burden due to the xenobiotic is closely balanced by the induction of repair capacity, resulting in a threshold-like curve.

It is tempting to speculate that this result explains the data for ethyl methanesulfonate and lacZ mutation (see Figs. 9 and 10). The data are consistent with an intermediate efficacy for induction of a repair process that acts on both the background burden of promutagenic DNA damage and the damage due to ethyl methanesulfonate. Differentiation between (i) a monotonic curve with a shallow slope $>0$, (ii) a seeming threshold, and (iii) a weakly nonmonotonic curve must be based on plausible mechanistic considerations. Data fitting by different models may find the statistically best fit, but this is no proof of the true shape of the dose response.

Similar results are obtained for the other three cases listed above (Conolly and Lutz 2004). Sweeping on a key parameter leads from a monotonic dose response, through an intermediate, threshold-like regime to a clearly nonmonotonic response. These results suggest that the conditions, under which nonmonotonicity arises, may be only subtly different from those generating monotonic responses. It possibly involves no more than a quantitative difference in one of the background components of the effect under study.

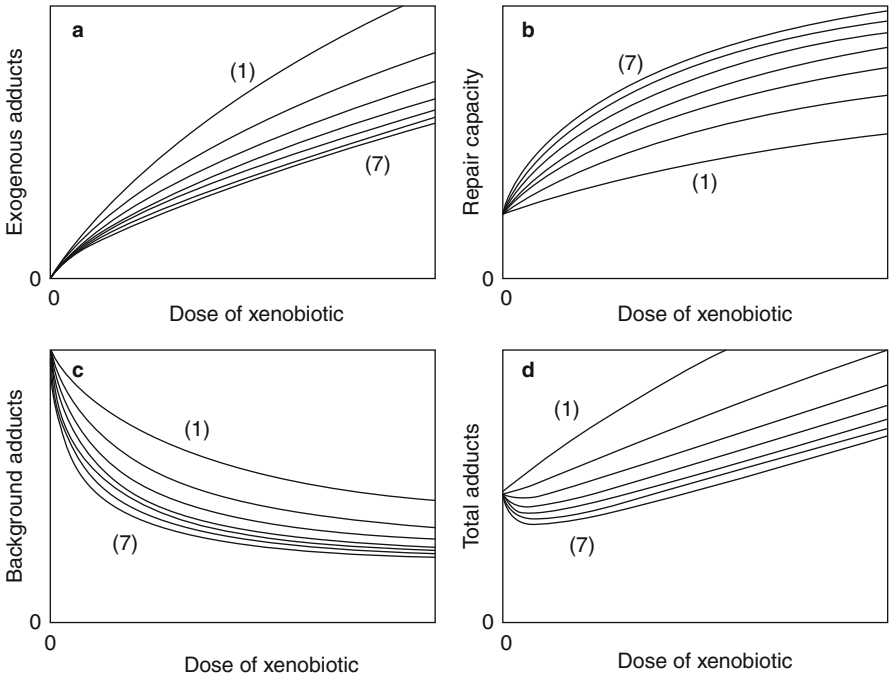



**Fig. 11** Modeling dose–response relationships for DNA adducts as a function of dose of an exogenous mutagen (**a**), modulated by different levels (*1–7*) of repair induction (**b**). (**c**) Decrease of background DNA adducts due to the induced repair. (**d**) Total adducts (background plus exogenous) obtained by superimposition of (**a**) and (**c**) (Reprinted with permission (Conolly and Lutz 2004))

## "Incidence" as a Different Type of Response Variable

### A Dose–Incidence Relationship Reflects Differences in Susceptibility

The risk of an exposure-related increase in a defined disease is measured as an incidence in a group of animals or humans. Each individual can either manifest this effect ("yes"; response value 1) or not show the effect ("no"; value 0). The incidence is given by the fraction or percent of responders in the group and increases with dose.

Figure 12 shows a hypothetical example of the dose–incidence relationship for the effect of alcohol on a group of ten humans. The yes-or-no criterion of toxicity is defined as the loss of balance to keep walking straight on. The group is given increasing volumes of wine at weekly intervals, and the test is made after 15 min. The graph shows that one individual manifested the adverse effect already when the dose increased from 100 to 150 mL. At the other end of the dose response, it took more than 400 mL to knock out the most tolerant individual. In other

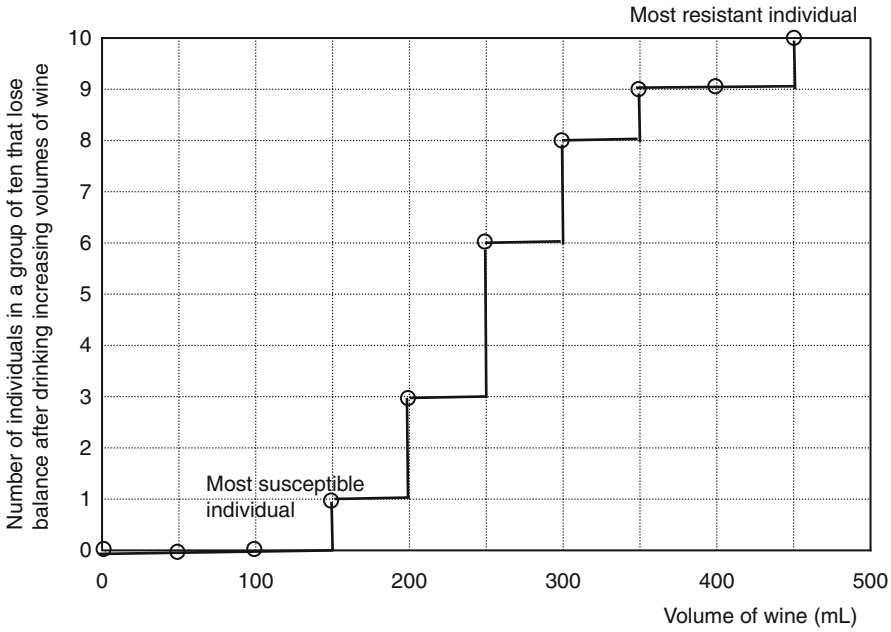



**Fig. 12** Representation of a dose–incidence relationship for the effect of alcohol on a group of ten individuals. Dose steps: 50 ml of wine. Each individual has its own threshold dose range to lose balance

words, the threshold dose for the most susceptible individual was somewhere between 100 and 150 mL; the threshold dose for the most tolerant individual was between 400 and 450 mL. The resulting "curve" therefore represents the distribution of susceptibility (or tolerance) of the individuals in the group exposed.

The problem of risk extrapolation to low dose therefore boils down to the question about whether individuals in a large population show a lower threshold dose than observed in a small group of ten. In order to answer this question, we must investigate the criteria that are responsible for differences in susceptibility. For our example of tolerance of the acute effect of alcohol on the equilibrium, the most important criterion is the volume of distribution for ethanol. Since this is largely determined by the body weight, the most susceptible individual was probably a slim female, the most tolerant a heavy male. Other factors that you may mention are stomach content at the time of drinking (rate of absorption) and habituation to alcohol. Knowledge of the type of interaction of alcohol with its biological target (s), on the other hand, does not help predict the shape of the curve in the dose range of extrapolation. Information on the molecular mode of action is of interest only in the search of factors that may modulate the susceptibility. This limitation of the usefulness of mechanistic information for dose–incidence relationships is not commonly recognized.

**Conclusions**

*As opposed to the situation of continuous response variables of biomarkers, a dose–incidence relationship is not a smooth curve but a flight of steps that represents the sequence of individual threshold doses to switch from "no" to "yes." The flight of stairs reflects the tolerance distribution in the respective group of individuals. Mode of action does not account for the shape of the dose–incidence relationship, but its knowledge may help define susceptibility factors, characterize and model their distribution in the population, and identify susceptible groups and individuals.*

## Chemical Carcinogenesis and Cancer Incidence

Tumor induction is a complex process with numerous modulatory factors that determine the individual's probability to manifest the disease after carcinogen exposure. Figure 13 shows a number of factors in rectangular boxes that express important interindividual differences: metabolic activation of a carcinogen, metabolic detoxification, rates of DNA repair and replication, inheritance of activated cancer genes or inactive tumor-suppressor genes, and immune surveillance, to name a few. How should these factors and activities be combined to result in

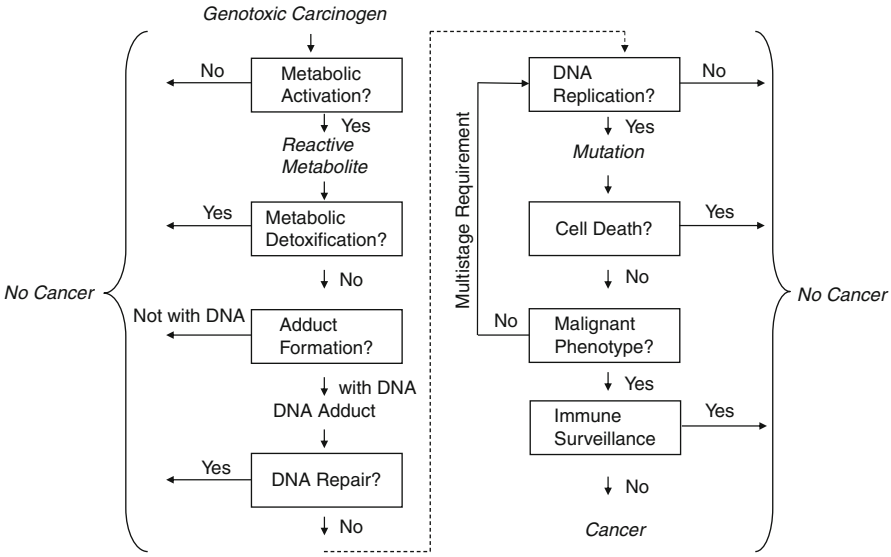



**Fig. 13** Schematic representation of the process of chemical carcinogenesis by a genotoxic carcinogen. The *boxes* show factors for which individuals express different activity, which modulates the rates of the steps towards the manifestation of cancer

a susceptibility expressed as an individual threshold dose? An example with two factors could illustrate the approach. Assume two individuals who differ by a factor of two for both the rates of detoxification of the aflatoxin epoxide and of repair of the respective DNA adduct. As a consequence, the rate of mutation will be four times as high in the individual with the lower activity for detoxification and DNA repair. In order to generate the same rate of mutation for both individuals, the aflatoxin dose has to be reduced by a factor of four for the more susceptible individual. As a conclusion, for quantitative combination of susceptibility factors, individual rates have to be multiplied.

### Multiplicative Combination of Susceptibility Factors Results in a Lognormal Distribution

The central limit theorem of statistics states that *sums* of a large number of independent random variables are approximately normally distributed. The Galton board (1889) shown in the top left panel of Fig. 14 illustrates the principle, for the simplest situation of the sum of ten binary variables, where balls that can fall either to the left or to the right (Limpert et al. 2001). *Multiplicative* combination of the ten variables calls for different shapes of the triangles, as illustrated in the top right panel of Fig. 14; the distribution now has a positive (right) skew. The bottom panels demonstrate that logarithmic transformation of the x-axis reverts the right skew to the symmetry of the normal distribution defined by its mean and a *multiplicative* standard deviation.

To implement these finding for a discussion of a dose–cancer incidence relationship means that the x-axis represents the dose axis; balls represent human individuals with different threshold doses of carcinogen to get cancer. The binary factors chosen for the Galton board obviously do represent the world of biology. The factors that modulate the rate of chemical carcinogenesis as shown in Fig. 13 can assume different types of distribution and variance. It will be a future task to collect the respective information in the human population. The larger the number of factors and the larger their variances, the larger will the multiplicative standard deviation of the lognormal distribution become.

## Extrapolation of a Dose–Cancer Incidence Relationship

### It Is Time for a Revival of the Lognormal Distribution for Cancer Risk Extrapolation

The first model employed by the US regulatory agencies to estimate a cancer risk at low dose utilized the Mantel–Bryan procedure (Mantel and Bryan 1961). Starting from animal data and considering a wider variability in the human population, the susceptibility was suggested to follow a normal distribution against $\log_{10}$ (dose) with a conservative multiplicative standard deviation of 10 (called "slope 1" [$\log_{10}$ of 10]).

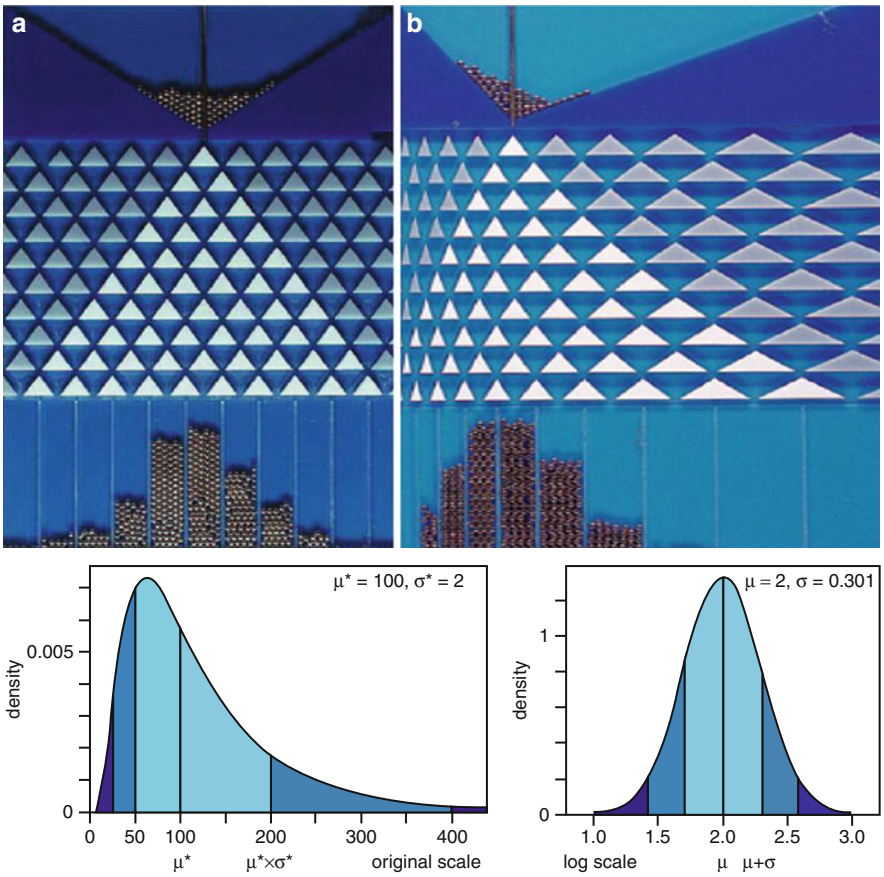



**Fig. 14** *Top*: the Galton board (1889). Physical models illustrating the central limit theorem of statistics. *Left*: additive superposition of ten "good or bad" random susceptibility factors. *Right*: multiplicative superposition of factors, which generates a right-skewed distribution. *Bottom*: using a log scale, the *right* skew (*left panel*) converts back to the normal distribution (*right panel*) (Reprinted with permission (Limpert et al. 2001))

In other words, reduction of the dose by a factor of ten was assumed to result in a decrease of the incidence by one standard deviation. The approach was abandoned because of the uncertainty associated with the assumption on the slope and because of arguments of the stochastic aspects of carcinogenesis.

Dose–response data for tumor incidence in humans are very limited. For lung cancer incidence as a function of cigarette smoking, the data available for British physicians were analyzed using different models, including a lognormal distribution. Best fit was achieved with a multiplicative standard deviation of 5.75 (Whittemore and Altshuler 1976). The drop in risk for drop in dose for this particular example is therefore steeper than when using the default assumption of Mantel and Bryan.

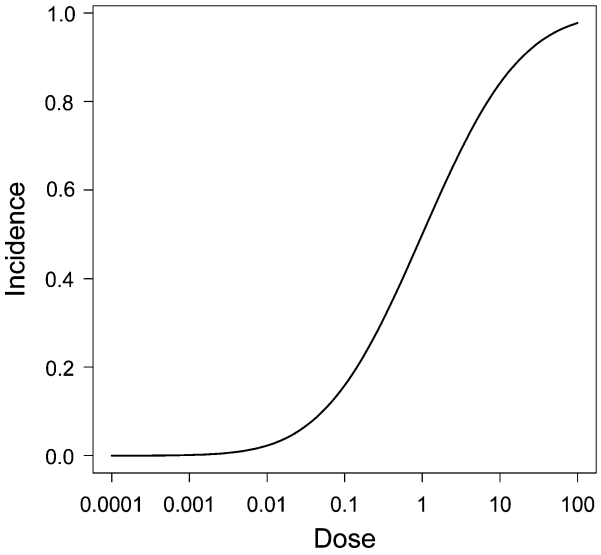



**Fig. 15** Dose–cancer incidence relationship following on a lognormal susceptibility distribution. Carcinogenic potency $TD_{50} = 1$ (incidence 0.5 [50 %] at dose 1). Multiplicative standard deviation $\log_{10}(10) = 1$, i.e., a dose-reduction factor of 10 results in an incidence reduction by 1 standard deviation (Mantel and Bryan 1961). The extrapolated cancer incidence for dose 0.1, 0.01, 0.001, and 0.0001 is 0.16, 0.023, 0.0014, and 0.00003, respectively (see also Table 2)

**Table 2** Low-dose extrapolation of cancer incidence in a population of 100,000. Comparison between a linear and two lognormal dose–response relationships with different standard deviations. Assumptions: tumorigenic potency: $TD_{50} = 1$ dose unit; dose-reduction factor: 10

| | Linear extrapolation | | Lognormal extrapolation multiplicative SD = 10 ($\log_{10}$ probit slope 1) | | Lognormal extrapolation multiplicative SD = 5.75 ($\log_{10}$ probit slope 0.76) | |
|---|---|---|---|---|---|---|
| Dose | Incidence in 100,000 | Risk reduction factor | Incidence in 100,000 | Risk reduction factor | Incidence in 100,000 | Risk reduction factor |
| 1 ($TD_{50}$) | 50,000 | | 50,000 | | 50,000 | |
| 0.1 | 5,000 | 10 | 15,865 | 3 | 9,412 | 5.3 |
| 0.01 | 500 | 10 | 2,275 | 7 | 425 | 22 |
| 0.001 | 50 | 10 | 135 | 17 | 4 | 107 |
| 0.0001 | 5 | 10 | 3 | 45 | 0.007 | 558 |
| 0.00001 | 0.5 | 10 | 0.03 | 110 | 0.000002 | 2987 |

The dose–cancer incidence curve that follows a lognormal susceptibility distribution is shown in Fig. 15. As starting point it assumes a dose of 1 for a 50 % tumor incidence; the drop in risk with drop in dose follows the conservative assumption of Mantel and Bryan. The graph shows the respective drop in risk from 0.5 to 0.16 (16 %) with the first step in dose reduction by a factor of 10 and a reduction from 16 % to 2.3 % by another factor of ten. The graph does not allow visual assessment of the cancer risk with further decrease in dose. Table 2 provides this information down to $10^{-5}$ times the $TD_{50}$, both for the

Mantel–Bryan assumption and the human lung tumor data for smokers. Comparison with a linear extrapolation allows the following conclusions: At relatively high-dose levels in the range of 0.1 times the $TD_{50}$, linear extrapolation drops faster than the lognormal extrapolations. The ranking reverses with every further dose step towards zero. At dose 0.0001 times the $TD_{50}$, for instance, linear extrapolation predicts a much higher incidence than following the lognormal curve postulated for lung cancer in smokers.

### Tolerance Distribution Versus Stochastic Modeling

Knowledge on susceptibility factors for the rate of carcinogenesis as shown in Fig. 13 has increased dramatically in the last few decades. Major advances can be noted for the inheritance of mutant "cancer genes" of individual differences for DNA repair and metabolic activation and detoxification. The use of tolerance distribution models for cancer risk extrapolation therefore deserves a revival. Yet, a number of aspects of the process of chemical carcinogenesis may keep a stochastic element. For instance, the question of whether a DNA adduct is formed in a critical gene (oncogene or tumor-suppressor gene) or in an innocuous gene will not be fully predictable on an individual level.

**Conclusions**

*A dose–cancer incidence relationship for a given population is predictable to the extent of our knowledge of the distribution of individual risk factors. Confidence limits will have to be widened to account for remaining stochastic aspects. These limitations do not invalidate the general statement that linear extrapolation of a treatment-related excess cancer risk to background incidence is inappropriate.*

## How to Incorporate a Nonlinearity of an Experimental Biomarker in a Dose–Incidence Relationship?

The threshold-type dose response shown above for mutant induction in mice treated with ethyl methanesulfonate (Figs. 9 and 10) leads us to the question how this knowledge can be used for a dose–incidence curve for humans exposed to this genotoxic agent. Since a dose–incidence curve is not directly dependent on mode of action but follows the distribution of tolerance within the population, the question must be addressed whether the factors that result in the deviation from linearity in the transgenic mice also operate in the human population. How is the activity of the protective factor(s) distributed among individuals? In the particular case of repair of DNA ethylation, it will be important to investigate whether there are individuals that may not benefit from this type of DNA repair and its induction.

Since DNA methylation is one of the most critical types of background DNA damage, an individual with little or no respective repair would probably accumulate lethal mutations already during fetal development. It could therefore be assumed that all newborn are able to repair DNA methylation to an extent required for survival and show a nonlinear dose response for exogenous DNA ethylation. Whether there is even a nonmonotonic shape as shown as an average response in mice will depend on the distribution in the human population of the inducibility of repair activity.

## Monte Carlo Simulations to Differentiate the Dose Response for a Population Average Versus Individuals

Our model for induction of DNA repair is based on a normal distribution for the parameter that determines the efficacy with which DNA damage due to the xenobiotic induces DNA repair. Monte Carlo sampling allows prediction of dose–response curves for individual members of a population (Fig. 16). The four panels show how individuals in a population can differ in their response to genotoxicant exposure, given interindividual variation in ability to induce DNA repair.

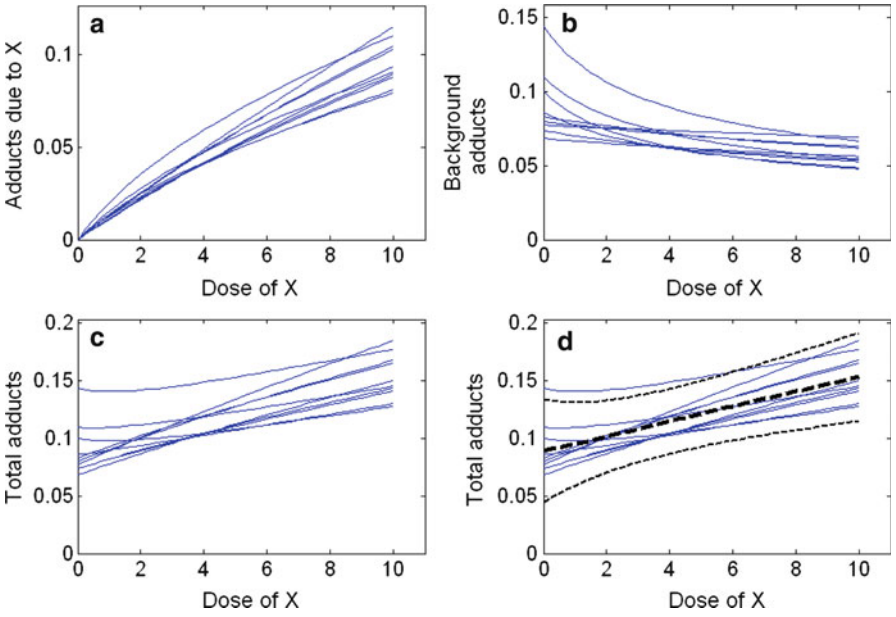



**Fig. 16** Monte Carlo simulation with ten runs to generate dose–response curves for ten individuals who vary in their ability to induce DNA repair capacity. (**a**) formation of DNA adduct from xenobiotic as a function of dose. (**b**) background (endogenous) DNA adducts as a function of the dose of X. (**c**) total DNA adducts (endogenous plus exogenous). (**d**) same as (**c**) plus *dashed lines* to show the *upper* and *lower* 95 % confidence limits and the mean for the population (Reprinted with permission (Conolly et al. 2005))

All types of dose–response curves are seen: monotonic, seeming thresholds, and nonmonotonic. Note that the mean and the lower confidence limit are monotonic, while the 95 % upper confidence limit on the mean response is nonmonotonic. Similar results are obtained for the Monte Carlo version of the model predicting how activation of cell cycle checkpoints affects the rate of mutation (Conolly et al. 2005).

While computational studies and simulations are theoretical and while we know of no dataset that actually shows different behaviors of dose response in human individuals, we do think that the results suggest mechanisms by which individuals within a population may have quite different susceptibilities to xenobiotic stressors.

**Conclusions**

*A nonmonotonic shape of a dose response shown for a population average does not exclude a monotonic shape for subpopulations or individuals. This limitation holds both for a dose response of a continuous biomarker and for a dose–incidence relationship. Nonmonotonicity cannot be considered a default for a population unless there are convincing arguments that all individuals meet the same quantitative criteria for the underlying modulatory factors.*

## References

Conolly RB, Lutz WK (2004) Nonmonotonic dose–response relationships: mechanistic basis, kinetic modeling, and implications for risk assessment. Toxicol Sci 77:151–157

Conolly RB, Gaylor DW, Lutz WK (2005) Population variability in biological adaptive responses to DNA damage and the shapes of carcinogen dose–response curves. Toxicol Appl Pharmacol 207:S570–S575

Galton F (1889) Natural inheritance. Macmillan, London

Limpert E, Stahel WA, Abbt M (2001) Log-normal distributions across the sciences: keys and clues. Bioscience 51:341–352

Lutz WK (2009) The Viracept (nelfinavir) – ethyl methanesulfonate case: a threshold risk assessment for human exposure to a genotoxic drug contamination? Toxicol Lett 190:239–242

Lutz WK, Lutz RW (2009) Statistical model to estimate a threshold dose and its confidence limits for the analysis of sublinear dose–response relationship, exemplified for mutagenicity data. Mutat Res 678:118–122

Lutz WK, Gaylor DW, Conolly RB, Lutz RW (2005) Nonlinearity and thresholds in dose–response relationships for carcinogenicity due to sampling variation, logarithmic dose scaling, or small differences in individual susceptibility. Toxicol Appl Pharmacol 207(2 Suppl):565–569

Mantel N, Bryan WR (1961) "Safety" testing of carcinogenic agents. J Natl Cancer Inst 27:455–470

Whittemore A, Altshuler B (1976) Lung cancer incidence in cigarette smokers: further analysis of Doll and Hill's data for British physicians. Biometrics 32:805–816

# Importance of Exposure Level for Risk Toxicological Assessment

Hans Drexler and Anuradha Shukla

## Contents

Local Toxicity ..... 570
Irritation ..... 570
Local Carcinogenicity in the Airways ..... 572
Systemic Toxicity ..... 572
Biomarkers ..... 573
References ..... 574

**Abstract**

A health risk for an individual due to an exposure to a hazardous substance depends upon the properties of the substance, the amount of the substance, and the susceptibility of the individual. If an individual is susceptible to a particular hazardous substance, only the amount of the substance determines the risk resulting from the exposure to this substance. In the case of local effects, the concentration of the hazardous substance in the environment and the duration of the contact determine the risk, with the exception of allergic reactions where the susceptibility is more significant. In the case of systemic effects, only the internal exposure or dose is relevant for the risk. Therefore, it is important for the marker of exposure to be a good surrogate for the dose in the target organ. If appropriate methods are available, a biological monitoring is more significant for risk assessment as compared to ambient monitoring. If biomarkers of effect are available, not only the general but also the individual risk can be assessed.

Risk assessment is the quantification of the likelihood that a quantitatively defined exposure of an individual (or a group of individuals) to a given chemical might

H. Drexler (✉) • A. Shukla
Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, FAU, Erlangen, Germany
e-mail: hans.drexler@ipasum.uni-erlangen.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_73, 

result in some adverse health effects. The probability depends on three elements: the risk factor itself (hazard or hazardous substance), the level and duration of exposure, and the individual susceptibility.

This can be described with the simple equation:

$$\text{Risk} = \text{Hazard} \times \text{Exposure} \times \text{Susceptibility}$$

The equation states that for an existing level of risk to be present, each of the three components must be different from zero (Manno et al. 2010). A risk assessment needs information about the hazard and the susceptibility and must be based on a valid exposure assessment.

Exposure assessment requires a monitoring of the concentration of the hazardous substances in the air or in materials (ambient monitoring) or the concentration of the substances or their metabolites in the body fluids of exposed persons (biomonitoring). For this purpose, it is necessary to use analytical methods which have been tested for reliability and practicability. An appropriate internal as well as an external quality assessment of the applied methods is essential to assure the accuracy and the comparability of results. For example, the international program of the German External Quality Assessment Scheme (G-EQUAS) provides proficiency testing for most of the human biomonitoring parameters, which are commonly used for the assessment of the human exposure to chemicals (Göen et al. 2012).

If scientifically based threshold limits in drinking water, food, or air are available, the exposure assessment is often used in terms of a risk assessment for human health. This means that in cases where the threshold limits are exceeded, it would result in a concrete risk to the individual. This is best possible for local effects caused by the hazardous substance. For a systemic effect, however, this would be justified only when the measured value in water, food or air is a good surrogate for the effective dose in the human body.

Hazardous substances from the environment come into contact with the human body via the mucous membranes, skin, lungs, and the gastrointestinal tract. The critical toxicity of a hazardous substance can be its local toxicity or its systemic toxicity (Fig. 1).

## Local Toxicity

### Irritation

Exposure of persons to hazardous substances can cause irritation or erosion of the skin or the mucous membranes. These effects depend on the characteristics of the hazards and their concentration in the environment. At workplaces, the concentration of a hazardous substance in air is of importance. Many occupational exposure limits for hazardous substances in air are based on irritative effects seen in man or in animals. For these hazards, a time-weighted average threshold limit value (8 h) would not be protective. Therefore, either short exposure threshold limit value

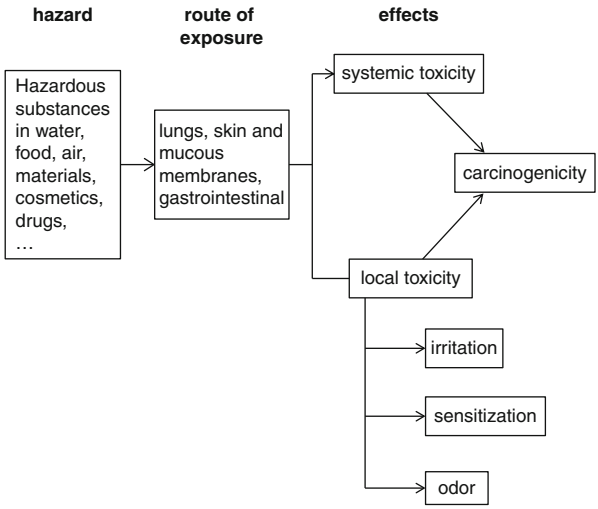



**Fig. 1** Hazards and effects

for a 15-min period or, in the case of a substance with a very high irritative potential, even ceiling threshold limits are evaluated.

## Sensitization

Allergies caused by chemical substances affect mostly the skin (contact eczema, contact urticaria), the respiratory passages (rhinitis, asthma, alveolitis), and the conjunctiva (blepharoconjunctivitis). The kind of allergy is mainly determined by the chemical properties of the substance. The development of a contact allergy of the delayed type is determined by several factors like the sensitization potential resulting from the chemical properties of the substance, the exposure concentration, the size of the exposed skin area, the duration and manner of exposure, the genetic disposition of the person, and, last but not least, the state of the tissue with which the substance makes contact (DFG 2012). Therefore, apart from the concentration, the susceptibility is also significant for skin sensitization. The size of the skin area correlates with the number of dendritic cells in the skin which come into contact with the allergen and, thus, also influences the risk of sensitization. A quantitative dermal exposure assessment that is valid is very difficult to do (Ness 1994) and not practicable for a routine exposure assessment. As the sensitization depends on the concentration of the substance, there are ceiling concentrations for many allergens (e.g., formaldehyde, nickel, fragrance) in consumer products. This should prevent sensitization; however, for already sensitized individuals, the risk of an allergic reaction remains.

The allergic reactions of the airways and conjunctiva which take the form of bronchial asthma or rhinoconjunctivitis mostly involve reaction of the allergen with specific IgE antibodies and belong to the manifestations of the immediate type. Most respiratory allergens are macromolecules, mainly peptides or proteins. But low molecular weight substances can also produce specific immunological reactions in the airways. Allergic reactions of the immediate type can also cause systemic reactions and even anaphylactic shock. The development of allergies of

the respiratory passages, like that of contact allergies, is dependent on a number of factors. In addition to the substance-specific potential for causing sensitization, the exposure period and the genetically determined disposition of the exposed person play a decisive role. Particular attention should be drawn to atopic diathesis which is characterized by an increased susceptibility to atopic eczema, allergic rhinitis, and allergic bronchial asthma with increased IgE synthesis (Schnuch et al. 2002). But the concentration of the allergen in air is very important for sensitization (Drexler et al. 2000) as well as for the provocations of symptoms (Drexler et al. 1999). So far it has been possible to evaluate health-based threshold limit values only for a few allergens (isocyanates, flower).

For individuals who are already sensitized, the individual susceptibility is very significant for the risk assessment. At least for the high molecular type-1 allergens, persons with a so-called atopic diathesis have a considerably higher risk for sensitization than nonatopic individuals. After sensitization, the hazards cause the allergic symptoms only in sensitized individuals. For non-sensitized individuals, the susceptibility is zero, and according to the equation mentioned in the beginning of the text, the risk is also zero independent of the exposure.

### Local Carcinogenicity in the Airways

Airborne carcinogenic substances can cause the risk of a systemic or a local cancer (e.g., asbestos, cadmium for lung cancer) in the airways. In the case of a local cancer risk, only the concentration in air is relevant for the extent of the hazard. In the case of dust, it is very important to differentiate between total dust and the inhalable dust fraction which can enter the alveoli. Only the inhalable fraction of the dust is the fraction which is relevant to health. The aerodynamic diameter of the particles determines the fraction which enters the thorax (thoracic fraction). Smaller solid particles and droplets are deposited in the tracheobronchial region or in the alveolar region. For this reason, it is not enough to measure only the mass ($mg/m^3$ or ppm). The number of particles and their geometry are also very important for the resulting health risk (DFG 2012).

## Systemic Toxicity

For hazardous substances which cause a systemic toxicity, the quantification of biomarkers of exposure is a better surrogate than the quantification in food, water, materials or air because only the amount of the hazardous substance which is incorporated into the body is relevant for the internal effective dose. The amount of the hazardous substance incorporated is very difficult to assess based only on the values in food, water, and other materials because the question as to how much of the hazardous substance is released and how much is absorbed can never be answered correctly. This is even true for hazardous substances in air because the amount of ventilation, the distribution of the hazardous substance during the time

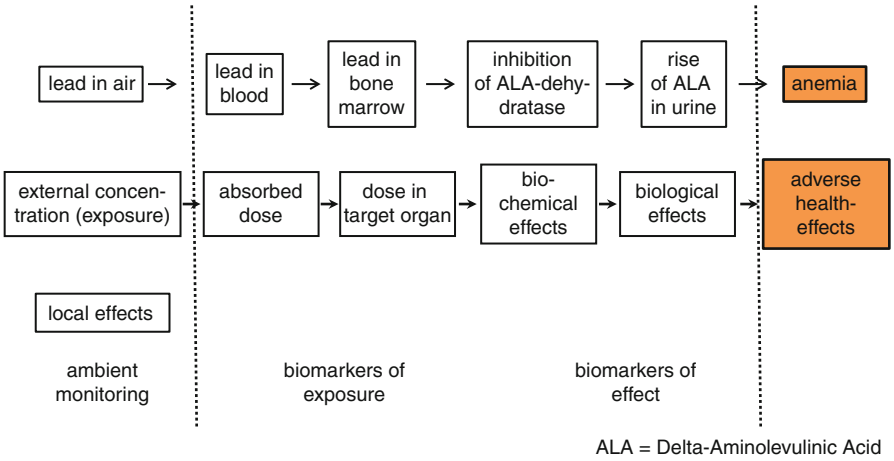



**Fig. 2** Monitoring hazards and effects

period, and the local distribution have a relevant influence on the dose. Also at workplaces, it is often the additional skin contact which can be quantified only by means of a biological monitoring that is relevant.

In Fig. 2 the course of an externally caused health effect, e.g., a lead-induced anemia, is shown. A part of the hazardous substance is absorbed from the environment and can be quantified as the internal exposure, like lead levels in blood in the above example. The amount which gets into the target organ correlates in most cases with the internal dose like lead in blood and lead in bone marrow. Early biochemical effects like the inhibition of the delta aminolevulinic acid (ALA) can be compensated without a biological effect. Chronic or intensive exposures result in initial biological effects (rise of ALA in urine) before the adverse health effect (anemia) is seen.

## Biomarkers

Biomarkers of exposure are the concentration of either a substance or its metabolites. There are various methods for analysis of hazardous substances in biological materials that have been published (DFG 2010). Important for the correct interpretation of the results is among others the knowledge of the half-life of the chemical which could be in the range of a few minutes (e.g., some solvents in blood) to many years (e.g., PCB, dioxins).

Detectable effect parameters like protein and DNA adducts are also biomarkers of exposure as long as they have no role in the pathogenesis. The most commonly used protein adducts are hemoglobin adducts. The number of adducts with the amino acids in hemoglobin is so low that the adducts do not influence the function of hemoglobin. The advantage of the use of hemoglobin adducts is their half-life. Taking into account the life span of erythrocytes, the hemoglobin adducts are used

to assess the exposure during the last 3 months before blood sampling. DNA adducts are biological target dose markers which reflect the exposure of the last 10 days before sampling (Henderson et al. 1989).

Another advantage of the adduct biomarker is that one can estimate the proportion of the toxic metabolites. As a rule, it is the metabolic intermediate that is produced in phase 1 metabolism and not the hazardous substance itself that is responsible for the cancerogenic effect and for the formation of the hemoglobin adducts. Persons with a high activation (phase 1 metabolism) and a low deactivation (phase 2 metabolism) rate are more susceptible, resulting in a higher cancer risk. For example, aromatic amines are activated by hydroxylation and deactivated by acetylation. The hydroxylated metabolite is excreted with the urine and forms the cancerogenic agent in the bladder. Under the same exposure conditions, persons with a higher rate of acetylation have lower hemoglobin adducts and a lower risk of developing bladder cancer as compared to those with a low rate of acetylation.

Biomarkers of exposure quantify the dose, whereas biomarkers of effect indicate early biochemical or functional alterations including a wide array of biological responses, ranging from physiological adaptation to disease. They represent a heterogeneous group of indicators and have different applications depending on the toxicological significance (Manno et al. 2010). The quantification of CO-hemoglobin as biomarker of an exposure to carbon monoxide and the activity of acetylcholine esterase as a biomarker of an exposure to inhibitors of this enzyme are well-known examples. Other endpoints, such as proteins in urine of subjects exposed to nephrotoxic solvents or metals, have been largely used as early indicators of biological effect. This application requires, of course, that the target organ and preferably also the mechanism of chemical toxicity be known. Effect biomarkers used as early predictors of clinical disease can improve health risk assessment and contribute to implement new effective disease prevention in occupational and environmental settings, but they must be first validated. Validation also involves the clarification of the biomarker's toxicological significance, which means its relation with the chemical's mechanism of action and its ability to detect or predict a specific toxic effect (Manno et al. 2010).

Biomonitoring is used successfully in many occupational and environmental exposure studies as well as during routine diagnostic by physicians. In the field of occupational medicine, biomonitoring is the most important tool to assess the individual exposure to specific chemicals, to characterize exposure pathways, and to assess potential individual risk factors.

## References

DFG: List of MAK and BAT values (2012) Commission for the investigation of health hazards of chemical compounds in the work area. Wiley, Weinheim

DFG: The MAK-collection for occupational health and safety (2010) Part IV: biomonitoring methods, vol 10–12. Wiley, Weinheim

Drexler H, Schaller K-H, Nielsen J, Weber A, Weihrauch M, Welinder H, Skerfving S (1999) Efficacy of hygienic measures in workers sensitised to acid anhydrides and the influence of selection bias on the results. Occup Environ Med 56:145–151

Drexler H, Jönsson BAG, Göen T, Nielsen J, Lakemeyer M, Welinder H (2000) Exposure assessment and sensitisation in workers exposed to organic acid anhydrides. Int Arch Occup Environ Health 73:228–234

Göen T, Schaller KH, Drexler H (2012) External quality assessment of human biomonitoring in the range of environmental exposure levels. Int J Hyg Environ Health 215:229–232

Henderson RF, Henderson WE, Bechtold JA, Bond JD (1989) Sun: the use of biological markers in toxicology. Crit Rev Toxicol 20:65–82

Manno M, Viau C; in collaboration with, Cocker J, Colosio C, Lowry L, Mutti A, Nordberg M, Wang S (2010) Biomonitoring for occupational health risk assessment (BOHRA). Toxicol Lett 192(1):3–16. doi: 10.1016/j.toxlet.2009.05.001. Epub 13 May 2009

Ness SA (1994) Surface and dermal monitoring for toxic exposures. Van Nostrand Reinhold, New York

Schnuch A, Lessmann H, Schulz K-H, Becker D, Diepgen TL, Drexler H, Erdmann S, Partasch M, Greim H, Greim H, Kricke-Helling P, Merget R, Merck H, Nowak D, Rothe A, Stropp G, Uter W, Wallenstein G (2002) When should a substance be designated as sensitizing for the skin ('Sh') or for the airways ('Sa')? Hum Exp Toxicol 21:439–444

# Risk Characterization in Regulatory Toxicology

Maged Younes

## Contents

Definitions and Goals ..... 577
Elements of Risk Characterization ..... 578
Hazard Characterization ..... 578
Exposure Characterization ..... 579
Uncertainty and Variability ..... 580
Weight of Evidence ..... 581
Transparency of the Process ..... 582
Recommended Reading ..... 582

**Abstract**

Risk characterization is the final step in the risk assessment process. All scientific data are summarized, reviewed, and evaluated in an integrated manner. Risk characterization should provide a clear description of the potential risk and outline the strengths and weaknesses of the whole risk assessment process. This includes a description of all assumptions and uncertainties of applied procedures, as well as a delineation of how the decision-making process.

## Definitions and Goals

The goal of risk characterization is to provide decision makers with all the information necessary to take risk management actions in a logical and clear manner. The main question to be answered here is "Which effects, in the sense of a possible occurrence of a harmful effect, or in the sense of an increased risk, respectively, are linked with a

M. Younes
Former Director, Food Safety and Zoonoses, World Health Organization (WHO), Mies, Switzerland
e-mail: magedyounes@gmail.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_74, 

certain given or expected exposure?" Typical risk management questions that require answers in the process of risk characterization are outlined in the text box below. The risk manager should have an understanding of, and a feel for, how exact risk predictions are, as well as to where the data described lie in the continuum from actual human data to data extrapolated from animal studies or in vitro experiments.

**Some Risk Management Questions to Risk Characterization**

1. What is the bottom line/final conclusion of the risk assessment?
2. Does the risk assessment provide sufficient information to justify regulatory action?
3. What is the range of uncertainty that characterizes the calculated exposure and the extrapolated number of potentially exposed individuals? Do we know if the calculated exposure corresponds to the actual one? Does the actual (or expected) exposure constitute a health (or environment) problem?
4. Which lacking data could give rise to criticism of the risk values or the risk management options?
5. Are there ongoing studies that could, eventually, provide lacking critical data in reasonable time?
6. Did the risk assessment undergo a peer-review process? If yes, by whom? What was the outcome?
7. Is there a possibility of "zero risk"? Was this really excluded?
8. Which key parameters drove the outcome of the risk assessment?
9. If studies were excluded from the risk assessment, which consequences did this have on its outcome? Why were these studies not considered?

It is also very important to identify vulnerable population groups or subgroups that are at particular risk under certain circumstances. An increased vulnerability could be the consequence of a higher exposure or an increased susceptibility. An example of an elevated exposure is that of population groups that consume very high amounts of fatty fish. Such groups may ingest higher amounts of, for instance, dioxins, polychlorinated biphenyls, methyl mercury, and other persistent compounds that accumulate in fat tissue. Children, in particular small children, constitute a group with higher vulnerability towards a number of risk factors, since their organ systems and their physiological defense mechanism against toxic compounds are still not fully developed.

## Elements of Risk Characterization

### Hazard Characterization

Hazard characterization, the description of the potential to harm, requires an interpretation of all data on the toxicity and the dose–response relation. At the onset, information concerning the completeness and the quality of the database is assessed.

Studies are evaluated with respect whether or not they have been conducted according to accepted scientific and ethical principles and if they have been adequately assessed and documented. With respect to ecological risk assessment, this applies to laboratory and field studies, and with respect to health risk assessment, they apply to human data, both epidemiological and volunteer studies, animal experiments, and in vitro tests. In the case of epidemiological studies, for example, it must be clarified if the exposed and control groups have been appropriately selected, if the length of the observation was adequate, if latent effects and confounding factors have been fully considered, if a causal relation between exposure and effect seems logical, and if the level of exposure/dose was adequately captured.

With experimental animal data, the main issue is about the integrity of the studies conducted. Here, a number of factors play a crucial role, among others if the studies have been conducted according to GLP principles (and if not, if an adequate and reproducible operative approach has been applied and described), how the choice of the test species and strain was made, the number of animals per dose group, the choice of dose or exposure levels, and the intervals between repetitive exposures, as well as the duration of the experiment. Often there are no data on certain endpoints. In such cases it is important to evaluate, based on the existing information, to what extent the missing studies might change the outcome of the risk assessment.

An important step in hazard characterization is the identification of the critical effect (or critical effects). In some instances, several toxic endpoints are observed. The decision as to which of these effects can be considered as critical (and there may be more than one) depends on the severity of the respective toxicological endpoint and the exposure/dose level at which it first occurs. Eventually, more than one such endpoint need to be considered, especially when particular toxic outcomes affect particular population groups as is the case with developmental toxicity.

When conducting hazard assessment, a distinction is often made between toxic effects that have a threshold of toxicity and those that show an effect at any observable exposure level regardless of how low it is (non-threshold effects). In the first case, it is assumed that the exposure must exceed a certain level before a toxic effect is manifested. Consequently, a "safe" maximal exposure can be calculated, below which damage is not likely to occur. In the second case, it is assumed that an effect would occur at every exposure, be it so low. In such cases, the probability of a damage (e.g., 1 in 1,000,000) is often calculated for extremely low doses using a variety of mathematical models. Such an approach is mainly applied in the case of genotoxic carcinogens. Modern approaches to risk assessment suggest that such a differentiation is artificial and that all data should be treated in the same manner.

Hazard identification should, to the extent possible, include a description of the mode of action or, if data are available, the exact mechanism of action.

## Exposure Characterization

In characterizing exposure it is important to start by describing the applied exposure assessment methods, as well as their strengths and weaknesses.

If different approaches to exposure assessment were employed in parallel, a comparative assessment of all of them should be presented.

Exposure characterization should include a description of all exposure sources and the contribution by all relevant environmental media (air, water, food, soil) to the total exposure. While the total exposure must be determined, it may be important under certain conditions to consider exposures related to different routes (e.g., inhalation and ingestion) separately. This is particularly important in cases where different toxicological effects are observed following exposure via different routes. Furthermore, it is important to consider all environmental compartments that lead to an exposure (such as the workplace or the general environment) separately when determining and describing the overall exposure.

Finally, it is crucial to determine the exposure of particularly vulnerable groups of the population and to identify all such groups that are subject to higher exposure levels.

## Uncertainty and Variability

In the process of risk assessment, uncertainties may stem from the lack of data or because extrapolations are necessary. These must be identified in the risk characterization. In addition, variability aspects must be fully considered, be they between individuals or between particular groups of the population. A good risk characterization will address and describe both aspects.

Questions related solely to uncertainty are those that address a lack of knowledge or information. Examples include the eventual need (due to lack of data) for extrapolation from short- to longer-term exposures (e.g., from subchronic to chronic exposure), the extrapolation from lowest dose that results in an adverse effect (lowest observed [adverse] effect level, LO[A]EL) to the highest dose that shows no adverse effect (no observed [adverse] effect level, NO[A]EL). This category of pure uncertainty aspects also includes all deficiencies of the database, for example, the lack of certain studies. Such studies could be ones linked to certain exposure duration or ones that assess certain toxic endpoints (e.g., studies on neurotoxic or reproductive effects). In such instances it is critical to evaluate to what extent the missing information could change the overall assessment and to provide a clear and logical description of such an evaluation. This requires appropriate experience and often a knowledge of the mechanism of action, besides information on exposure and toxicokinetics. Generally, with increasing information the certainty of the risk assessment increases as well (Fig. 1).

Questions related to both, uncertainty and variability, are encountered in cases where interspecies extrapolation is necessary. This is the case, for example, when the health risk to humans is evaluated based on data from animal experiments. The variability between individuals in a given population group (intraspecies variability) also plays an important role. In both cases, the variability encompasses aspects of toxicokinetics and toxicodynamics, including, inter alia, the contact rate,

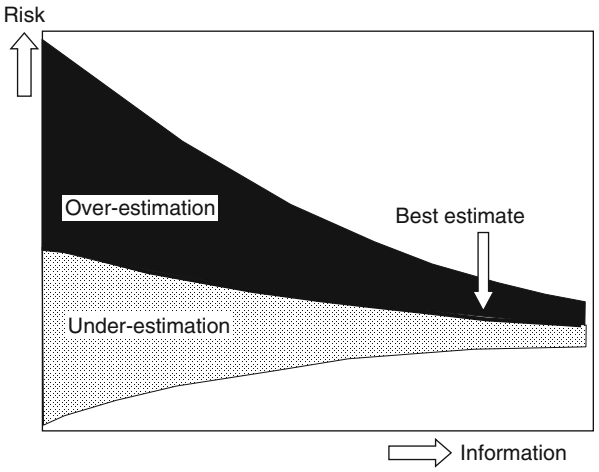



**Fig. 1** Precision of a risk estimate as a function of available information. The more data are available, the lower the uncertainty around the risk estimate

uptake or absorption, general systemic availability, systemic elimination, active site concentration, physiological parameter changes at site of effect, and the functional reserve capacity. Aspects of variability must all be fully considered in the risk characterization process.

In certain cases, special issues may play a role in risk characterization. In the case of persistent compounds that bioaccumulate, such as PCBs, dioxins, and persistent chlorinated pesticides, risk characterization should rather be based on the total body levels over exposure time (body burden) rather than on the external exposure or a dose over a limited period of time. With these compounds it is also important to consider that an exposure in utero is not only related to an eventual exposure during pregnancy, but rather on the body burden of the mother at that time. Another example is that of substances that show cumulative effects, such as cholinesterase inhibitors, which exert their toxic effects through a common point of action.

## Weight of Evidence

The analysis of all data in the process of risk characterization provides a possibility of reviewing all information available, together with available knowledge on uncertainty and variability aspects. Depending on the amount and quality of information, a decision is made as to the risk associated with certain exposures. The term "weight of evidence" has been applied to describe the fact that it is the amount of scientific evidence that guides the final conclusion. The term has not been generally used in a uniform manner, though, but is mostly related to a qualitative evaluation of the data rather than following a clear methodological approach.

## Transparency of the Process

In risk characterization, the whole process of risk assessment must be clearly outlined, and all elements necessary for decision-making on managing potential risks sufficiently described. In this respect information on the scope of the risk assessment (why was it conducted?), on the extent and quality of the database, as well as on the date of the last literature search were conducted. Furthermore, it must be stated if, and if yes why, adjustment or uncertainty factors were used. Finally, all elements of the decision process must be described, including the mechanism of peer review.

## Recommended Reading

Dybing E, Doe J, Groten I, Kleine J, O'Brien J, Schlatter J, Steinberg P, Tritscher A, Walker R, Younes M (2002) Hazard characterization of chemicals in food and diet: dose–response, mechanisms and extarpolation issues. Food Chem Toxicol 40:237–282

Vermeire T, Stevenson H, Pieters MN, Rennen M, Slob W, Hakkert BC (1999) Assessment factors for human risk assessment: a discussion paper. Crit Rev Toxicol 29:439–490

Weed DL (2005) Weight of evidence: a review of concept and methods. Risk Anal 25:1545–1557

World Health Organization (1999) Principles of the assessment of risks to human health from exposure to chemicals, vol 210, Environmental health criteria. WHO, Geneva

Younes M, Meek ME, Hertel RF, Gibb H, Schaum J (1999) Risk assessment and management. In: Herzstein JA, Bunn WB, Fleming LE, Harrington JM, Jeyaratnam J, Gardner IR (eds) International occupational and environmental medicine. Mosby, St Louis, p 62

# Risk Evaluation in Regulatory Toxicology

Maged Younes

## Contents

Introduction and Definitions ..... 583
Elements of Risk Evaluation ..... 584
Context of the Risk Problem ..... 585
Risk Acceptance ..... 585
Economic Factors ..... 586
Sociopolitical Factors ..... 586
Uncertainty and Variability: Scientific and Economic Aspects ..... 587
Outlook ..... 587
Recommended Reading ..... 587

**Abstract**

Risk evaluation is the step within the risk analysis process that links risk assessment (the final step of which is risk characterization) with risk management. This intermediary step is mostly not explicitly mentioned, or it is seen as a preliminary step in risk management. The goal of risk evaluation is to link exposure levels with corresponding risks and to identify sources of uncertainty in the scientific data used.

## Introduction and Definitions

Based on the outcome(s) of the risk assessment, options for risk management need to be developed and evaluated for the decision-making process. Risk management covers all actions and decisions as to whether or not, and, if yes, how certain risks

M. Younes
Former Director, Food Safety and Zoonoses, World Health Organization (WHO), Mies, Switzerland
e-mail: magedyounes@gmail.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_75, 

should be eliminated or reduced. Options and strategies developed for their possible implementation can be of regulatory, economic, informational, or technological nature. They need not to be mutually exclusive. In order to reach adequate and rational decisions, a risk must be seen in the context of other risks and evaluated considering various different factors.

Good risk management decisions should follow certain principles. Thus, the problem must be discussed within its health or ecological context and should consider the views of all those who would be affected by possible decisions. Such decisions must be based on a balanced scientific assessment and build on a full analysis of different regulatory and nonregulatory options for action. They must lead to a reduction or an elimination of the risk under consideration and be implementable in a rapid and efficient manner and with the support of all relevant stakeholders. Actions must, indeed, be proven to affect the risk to be minimized or eliminated. They should offer the possibility for being revised or changed if new information becomes available that would justify it.

## Elements of Risk Evaluation

To allow for sound risk management decisions to be reached, risk evaluation should offer ways to eliminate or reduce the risk(s) under consideration that fulfill certain criteria. Risk evaluation should be based on scientific, technical, and economic data of the highest possible quality. It should take account of the mostly existing context of multiple risks. Recommended actions must be technically, politically, and economically feasible and should offer clear advantages with regard to cost. They should give preference to prevention and place innovation in the center of the decision-making process. Finally, they should take account of sociopolitical aspects. Some elements of risk evaluation are discussed in more detail in other chapters of this book. In this chapter, the main aspects of risk evaluation will be briefly discussed and relevant terms will be explained (Fig. 1).

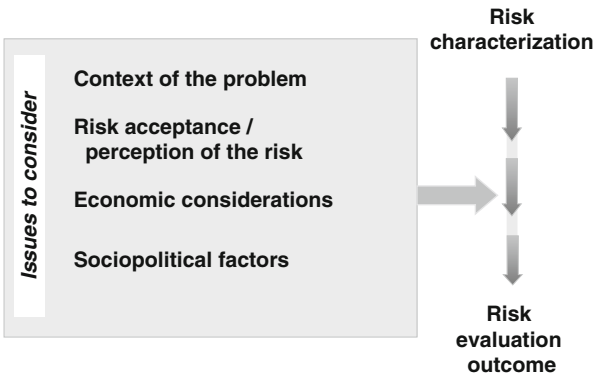



**Fig. 1** Issues to consider within the framework of the risk evaluation process

## Context of the Risk Problem

A risk cannot be evaluated in an isolated manner. Often, a risk factor, for example, a chemical substance, has several sources that contribute to the overall exposure. In such cases the risk should be evaluated within the "multiple source context." The risk evaluation must consider the contribution by each of these sources to the overall exposure in order to permit the development of effective risk management options that will, indeed, reduce the risk. The question here is mostly about the point of intervention that would achieve the most effective protection.

An exposure to the same risk factor could occur through different environmental media (e.g., air, water, drinking water). One example is lead. Human exposure is via air (inhalation; the main sources here are car exhaust emissions if lead-containing petrol is used, lead-containing paint, and various industrial processes), via drinking water (ingestion, e.g., in the case of lead water pipes), via food (ingestion, mainly through the use of lead-containing food cans), as well as via direct exposure through the use of lead-containing cosmetics (e.g., dermal exposure). A risk evaluation must therefore also consider exposure through all relevant environmental media ("multimedia context").

The source of one risk factor might also release other substances that may pose an additional risk. Dioxins, for example, are encountered as food contaminants (e.g., in fish), often in combination with, inter alia, polychlorinated biphenyls and methyl mercury. The three compounds have all neurotoxic effects among other toxic actions. The combined effects must be considered jointly. In such cases, individual compounds should not be evaluated independently from accompanying exposures ("multifactor context").

Finally, a risk must be evaluated in comparison to other risks in the same group of population. This is important in order to set priorities for action and to initiate actions which are most urgently needed first ("multiple risk context").

## Risk Acceptance

The decision as to whether or not a risk is acceptable requires a judgment in the context of social, political, and economic aspects. Of special importance to the risk evaluation is the way society judges the particular risk under consideration and to what extent certain exposures would be tolerated. Risk acceptance depends to a large extent on the perception of risk (see chapter "► Risk Communication"). Risks are not always seen by the public in scientific terms but often also based on qualitative perception. Thus, risks are accepted if they are common and known, if they are easy to control, if their mode of action is known, if the exposure is voluntary, if the effects are immediately seen and do not affect future generations, and if potential effects are reversible and/or are not of catastrophic nature. Trust in responsible institutions, lack of media interest, and clearly visible economic benefits also increase risk acceptance. In general, risks are more accepted if they are easy to see and their control appears to be easily accessible. Thus, the risk of a nuclear reactor accident is judged to be higher

than that of a motorcycle accident or that of smoking. Aspects of risk acceptance must therefore be clearly delineated and fully considered in risk evaluation. Risk comparisons may help in providing an objective view in this context.

## Economic Factors

An economic valuation provides important information for risk management. Economic considerations should therefore form an integral part of risk evaluation, including potential benefits which would be brought about through an improved health and environmental protection. In particular, two aspects should be looked at. The cost-benefit analysis considers economic and/or social gains emanating from a risk-producing process in comparison to its costs, which should also include those costs related to eventual damages to human health and environmental integrity. The cost-effectiveness analysis, in contrast, evaluates rather the "efficiency of a certain intervention" (e.g., a regulatory measure or a technological evaluation) in controlling a certain risk. Here, the expected economic and/or social benefit due to a certain proposed measure is quantified and compared to the cost caused by such a measure. In both processes, the quantification of positive and negative effects on human health and the environment in economic/monetary terms is a major problem.

## Sociopolitical Factors

Risk management decisions are political in nature. Therefore, options developed in risk evaluation need to reflect social and political considerations. Among the questions to be addressed, the issue of other risks that occur concomitantly and need also to be managed figures prominently. In this context, different risks are evaluated in a comparative manner, and a rational weighting is performed. Often, it is necessary to assess which risks should be given priority in reaching risk management decisions and which can be addressed at a later stage, since it is difficult, if not impossible, to address all risk factors at the same time. This process of comparing risks and weighing risk management options is defined as "risk balancing."

Sometimes, an action taken to control a given risk factor may lead to the appearance of new risks to health and/or the environment. Replacement of a chemical in a technological process with another, for example, can produce new risks. A practical example would be to abandon the disinfection of drinking water to avoid the risk due to chlorination by-products: the expected reduction of the health risks due to chlorinated organic compounds in drinking water would be linked to a significant increase in the risk of waterborne infectious diseases. Considerations of this kind fall under the term "risk-risk tradeoffs." The main question to be addressed here is: "which risks do we take if we control another through certain measures?"

Another aspect of sociopolitical and ethical nature is that of "environmental equity." In this context, considerations are made as to whether or not the population

group(s) that benefits from a certain activity is the same as those who carry the risk. The aim is to avoid situations where a group carries all (or the larger part) of risk, without profiting from the risk-producing process, and that all benefits come to another group that carries no or a substantively lower part of the risk.

### Uncertainty and Variability: Scientific and Economic Aspects

Since risk evaluation is an intermediary step linking risk characterization with risk management, uncertainty and variability issues that emerged and were discussed in risk characterization must also be fully considered when developing options to minimize or eliminate risks. Problems that could be of relevance at this step could target scientific or economic aspects. Examples of scientific issues include the relevance of toxicological studies under real-life conditions (risk prediction), the possibility to detect and consider differences in susceptibility among exposed populations, as well as the identification of highly exposed groups. In addition, questions concerning realistic exposure scenarios and on interactions between different risk factors may be of relevance. Economic problems include the difficulty of quantifying health and environmental aspects from an economic point of view. Other issues target the inconsistency of economic analyses and the uncertainties connected with it, as well as the inadequacy of methods to validate the advantages of potential risk management actions for human health and the environment.

## Outlook

Aspects described in this chapter are within the context of evaluating a given risk in the context of other risks and with consideration given to risk acceptance, as well as political and economic factors. Such considerations constitute a judgment of the characterized risk in connection with the development of control options as a prerequisite for managing the risk. Risk evaluation, thus, has a bridging function between the pure science and the political decision-making process. Even though this step is not explicitly included as a separate process in the usual risk assessment and risk management paradigms, risk evaluation is an important basis for decisions. Modern approaches to risk analysis, such as the one proposed by the US National Research Council in 2009, promote a more integrated approach to risk assessment and management, during which questions related to risk evaluation are addressed from start (problem formulation) to end (risk management), ensuring stakeholder involvement and risk communication throughout.

## Recommended Reading

Abt E, Rodericks JV, Levy JI, Zeise L, Burke TA (2010) Science and decisions: advancing risk assessment. Risk Analysis 30:1028–1036

National Research Council (2009) Science and decisions: advancing risk assessment. National Academy Press, Washington

National Research Council (NRC) (1994) Science and judgment in risk assessment. National Academy Press, Washington, DC

National Research Council (NRC) (1996) Informing decisions in a democratic society. National Academy Press, Washington, DC

Presidential/Congressional Commission on Risk Assessment and Risk Management (1997a) Framework for Environmental Health Risk Management. First Report – vol 1. Presidential/Congressional Commission on Risk Assessment and Risk Management, Washington, DC

Presidential/Congressional Commission on Risk Assessment and Risk Management (1997b). Risk Assessment and Risk Management in Regulatory Decision-Making. First Report – vol 2. Presidential/Congressional Commission on Risk Assessment and Risk Management, Washington, DC

# Risk Comparison in Toxicology

Franz-Xaver Reichl

## Contents

Introduction ..... 590
Definitions ..... 590
Risk Structures ..... 591
Future Risks ..... 593
Mortality Risks in the Population ..... 596
Acceptance of Risks ..... 598
References ..... 598
Recommended Reading ..... 598
Resources ..... 599

**Abstract**

Statisticians have calculated probabilities for most of the circumstances of everyday life, including the chances that an individual will become ill, have an accident, or die. The danger profile for a single individual is divided into a multitude of individual risks, which are unequally distributed, sometimes starting from birth. For example, 2 % of all diseases are genetically determined. Even people who arrive in the world healthy, however, have disadvantages, but factors such as success in an occupation and high income are protective against early death. Statistically high risks are associated with smoking and poor nutrition, whereas the risk of death from viruses, radiation, or chemicals is low.

The calculation of risks is difficult and dangers often arise. Experts as well as laypeople may fall victim to "cognitive dissonance," where knowledge that

F.-X. Reichl
LMU Munich, Munich, Germany
e-mail: reichl@lmu.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_76, © Springer-Verlag Berlin Heidelberg 2014

disturbs established beliefs and habits is not perceived. Risk assessment thus becomes difficult, as seen by the following phenomena:

- The occurrence of rare hazards (e.g., snake bite) is overestimated, whereas that of frequent hazards (e.g., death caused by cardiac infarct resulting from obesity) is underestimated.
- People are subject to an imperfect assessment of probabilities; for example, driving in a car is more dangerous than flying in a plane, but the fear of flying is common compared with the fear of driving.
- People tend to overestimate their own abilities (e.g., they believe they are immune from disease).
- Fears are minimized for things people believe they can control (e.g., smoking).
- Fears are maximized for things people believe they cannot control (e.g., a toxic waste repository).

Psychological studies show that events with a high "horror factor" (e.g., being eaten by a shark) are particularly feared, even if they occur extremely rarely. Human behavior is less determined by numbers and facts than by faith, desires, and fears.

## Introduction

Insurance companies and security specialists define risk as the product of probability of occurrence and level of damage, and calculate premiums accordingly. So the risk remains the same, regardless of whether minor damage occurs frequently or major damage occasionally. According to this principle the risk for 1,000 road accidents, each with one adult killed, is exactly the same as that of a school fire in which 1,000 children die. In general, however, the word "risk" encompasses both danger and chance. It describes both the objective threat which cannot be avoided and also the subjective gamble which is assumed voluntarily. A danger survived can therefore also become a chance for a better and safer life.

The idea that each person holds his fate in his or her own hand did not emerge until after the Middle Ages. While prior to this many people believed in evil or good-natured gods, who at least partly determined fate, in modern times each individual rose to become important producers of dangers and chances. And because at that time there was no term for this concept, a new word had to be coined. The work "risk" derived etymologically in the sixteenth century from the Italian word *risco* (gamble, hazard), which was in turn probably derived from the Greek *rhiza* (root; secondary meaning: cliff) or from the Arabic *rizq* (livelihood which depends on God and fate).

## Definitions

*Absolute risk* in an equally affected group of persons is the ratio of the number of illnesses to the total number of persons.

*Relative risk* is the ratio of the absolute risk of the affected group to the unaffected group.

*Risk appraisal* (risk description) is the quantitative determination of possible health risks due to chemicals or radiation depending on efficacy, length of exposure, and level of exposure or the dose absorbed.

*Risk assessment* is the evaluation of a risk with regard to its tolerability under social and health political aspects.

## Risk Structures

Statisticians have calculated the probabilities for all possible circumstances of everyday life that an individual will, e.g., contract an illness, have an accident, or die. Their calculations of the risk of illness ("morbidity") and the risk of dying ("mortality") are however applicable only to an imaginary being: the statistical average person.

His existence follows a risk profile which changes dramatically with age. Already on the first day after birth, one in every 600 newborns dies in Germany, as the result of, for instance, having too low a birth weight or pregnancy complications. In the first year of life, the rate increases to one in 125 babies. After the first birthday, survival odds then rise steeply. Ten years after birth and survival of childhood diseases, the safest phase of life is reached. The annual risk of death reduces to the lowest level of 1 in 6,000, before increasing again between the ages of 15 and 20 for the average male teenager to 1 in 1,000 due to the propensity to take risks (e.g., driving). In addition, the willingness to take their own lives increases: Around one in five men who die around the age of 30 commit suicide. Diseases (e.g., cardiac and circulatory diseases, cancer), which are the cause of death for 95 % of people, only dominate from the age of 40, and in the decade before retirement bring the mortality rate back up to the level of infants.

This basic risk structure has not changed for thousands of years. Already in the Paleolithic Age, death claimed mainly infants and old people and granted security in late childhood. Equally, young people took risks, although in those times not on the roads but perhaps in hunting.

But any attempt to derive one's personal destiny from these figures is senseless. The **risk profile** for the individual is made up of millions of individual risks which are often unequally distributed right from birth. Two percent of all diseases are, for instance, genetically determined. And a child born healthy but male already has a disadvantage. In their very first year of life, around one third more boys die than girls. Women are less likely to commit suicide and do not have as many accidents on the road or at work. They also drink and smoke less than men – an advantage which is however diminishing in the name of emancipation.

Professional success, high income, and a good education are on the other hand factors which protect men in particular against an early death. According to a US study rich men, for instance in Canada live four-and-a-half years longer than the poorest ones there. Background, poverty, poor living conditions, and unemployment reduce **life expectancy** (Reichl and Ritter 2011). This becomes particularly clear in the New York district of Harlem which is populated almost entirely by non-whites, almost 50 % of whom live

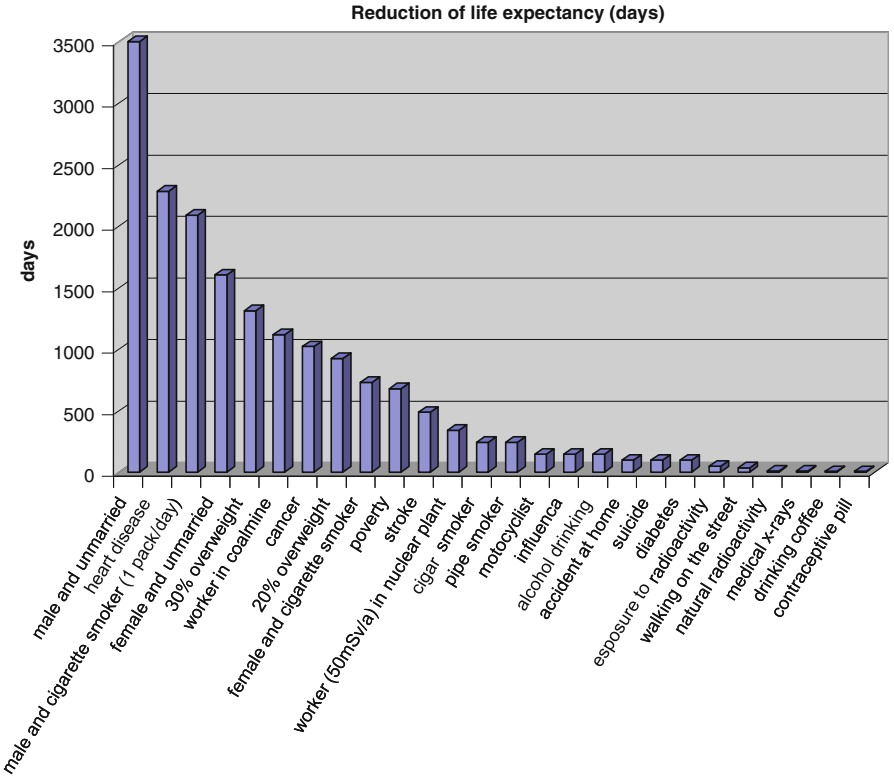



**Fig. 1** Reduction of life expectancy through various risks in the population of Europe (as at 2007, updated according to Reichl 2011)

below the poverty line. In this area, mortality rates in women between 25 and 34 and in men between 35 and 44 are six times higher than the US average. The likelihood of reaching the age of 65 in Harlem is less than it is in Bangladesh.

Other various risks also contribute to reducing life expectancy, e.g., the life expectancy of men who smoke cigarettes reduces on average by almost 7 years (Fig. 1). One way to reduce personal risk slightly (on a purely statistical basis) is a trip to the registry office. But whether marriage really offers more security is not certain. Perhaps single people between the ages of 35 and 45 have a riskier way of life. Divorce and even more so the death of a partner drive many to an early grave – although women cope with the loss considerably better.

Today, statistically speaking, Germans are getting around three times as old as they were 300 years ago at 82 years (women) and 76 years (men) (Fig. 2). The main reason for this is the decline of infant mortality. For the year 2040, in Germany a life expectancy of 92 years (women) and 87 years (men) is forecast. The life expectancy of persons in Afghanistan has up to now not got beyond 45. Almost all over the world, women live longer than men, with the exception of countries such as Afghanistan or Bangladesh, in which they are severely disadvantaged (Fig. 3).

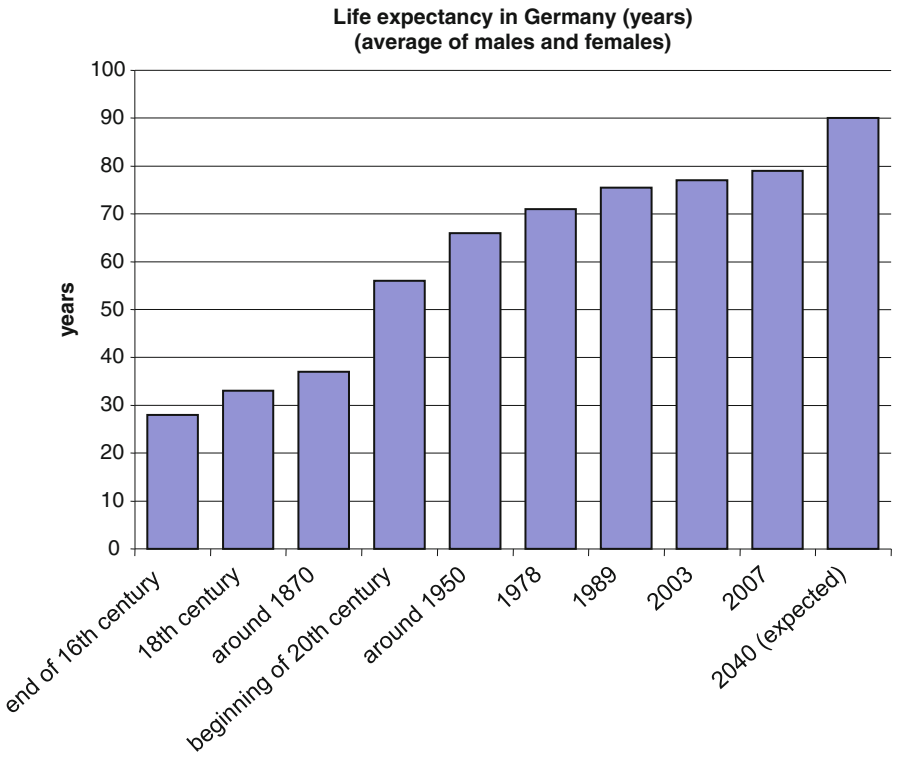



**Fig. 2** Development of life expectancy in Germany (years; average of men and women) (as at 2007, updated according to Reichl 2011)

## Future Risks

Compared to smoking and nutrition, today **viruses**, **radiation**, and **chemicals** (still) represent relatively minor risks. In the future however, three risks from the environment could be a particular threat:

- Damaging ultraviolet (UV) radiation: according to US calculations by 2100, skin cancer will claim the lives of an additional 10,000 people as a result of increased UV radiation on the Earth due to the reduced (protective) ozone layer.
- In the USA, passive smoking today already causes up to 8,000 deaths a year.
- The radioactive gas radon, which comes up through the ground and penetrates natural building materials, today also already claims the lives of around 20,000 Americans.

The risk of contracting cancer from the 60,000 chemicals is relatively low. According to the most recent studies, this contributes a total of only around 1–2 % of the overall cancer risk to humans. However, 50,000 materials have still not even been tested for their carcinogenic potential. It is still disputed just how dangerous these over 600 substances are which have proved carcinogenic in

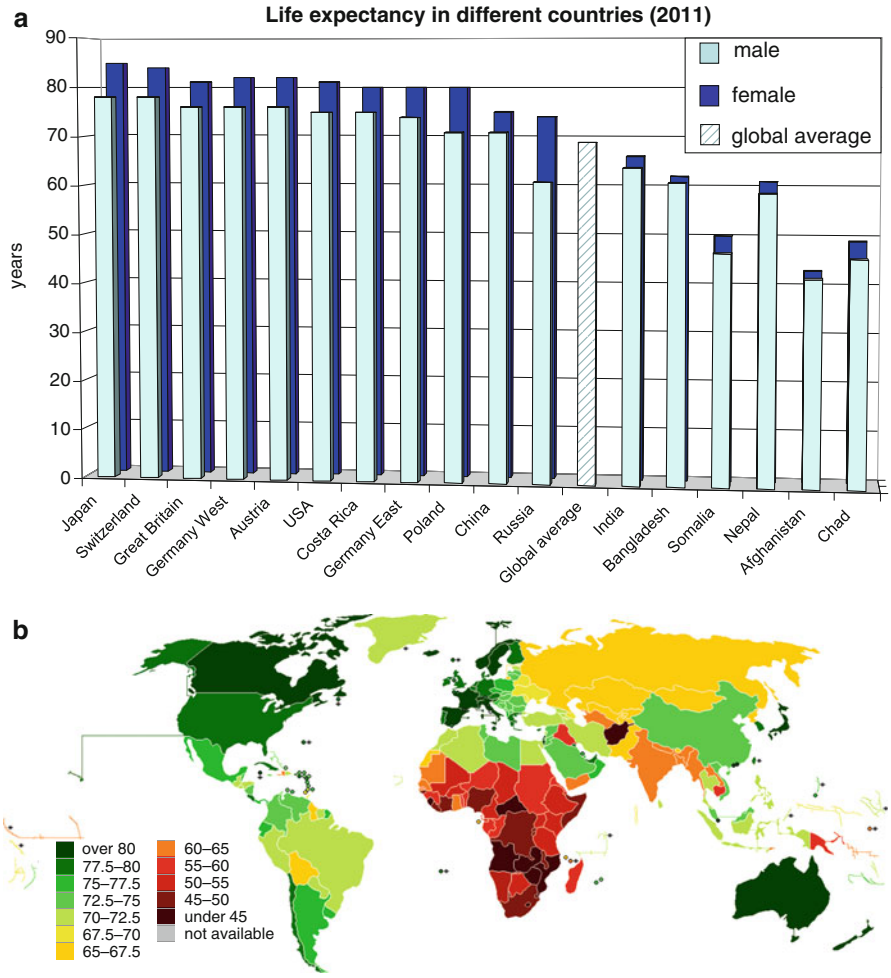



**Fig. 3** (**a**) Life expectancy in various countries (as at 2007, updated according to Reichl 2011). (**b**) World map indicating Life expectancy at birth in UN member states as of 2007

animal experiments. What induces cancer in a rat may have no effect on a mouse and vice versa.

Another problem still unresolved is the effect of carcinogens in combination. The effect of radon contaminated inside air, asbestos, or alcohol must not simply be added to the risk factor of smoking. In combination, these substances may even exponentiate the risk of lung cancer.

Patients are also increasingly complaining of the onset of symptoms after a tooth restoration, because they believe they are being slowly poisoned by the substances released from the inserted dental materials in combination with other

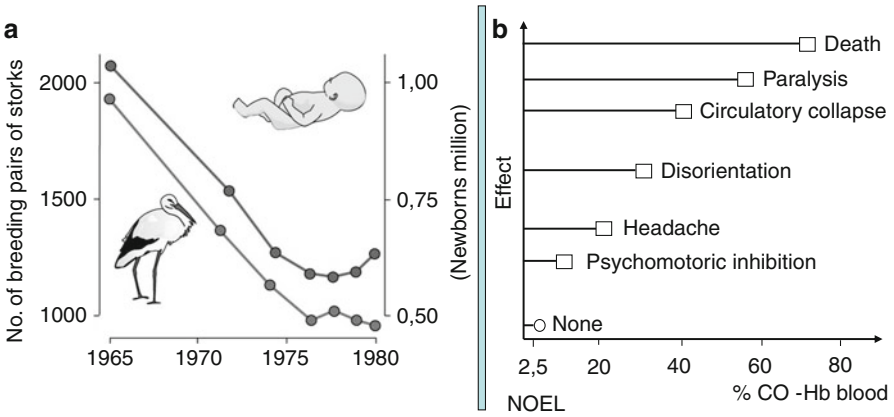



**Fig. 4** Useful and senseless correlations. (**a**) Statistically significant correlation, but no causal link: decline of breeding pairs of storks and decline of birth rate in the Federal Republic of Germany from 1965 to 1980. (**b**) Statistically significant correlation, causal link: effect of an increasing carbon monoxide content in the blood

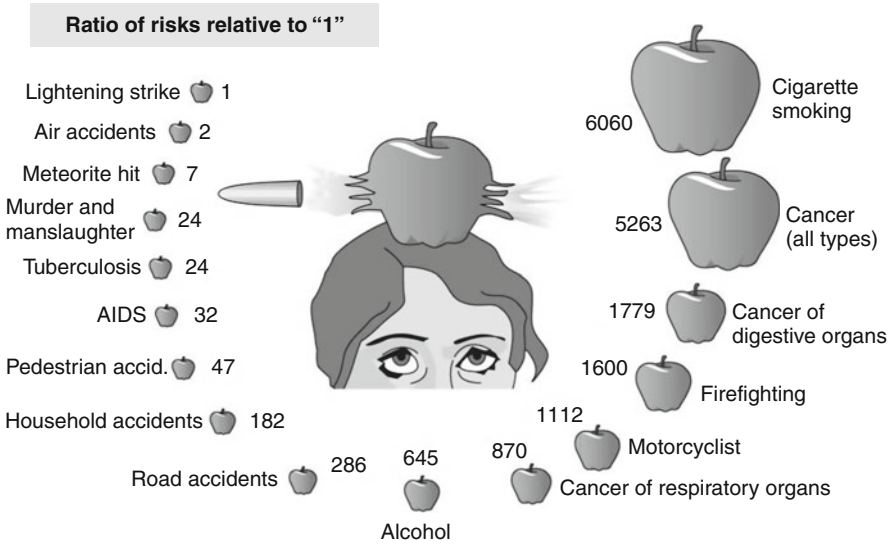



**Fig. 5** Mortality risks in the population of Europe (as at 2007, updated according to Reichl 2011)

(environmental) pollutants. What risk actually exists for these patients can be established by a recognized international toxicological dental advice center (e.g., at the Ludwig-Maximilian University of Munich: *reichl@lmu.de*).

Because the analysis of health risks is fraught with many uncertainties, statisticians tend to resort to averages calculated from many individual findings. This does

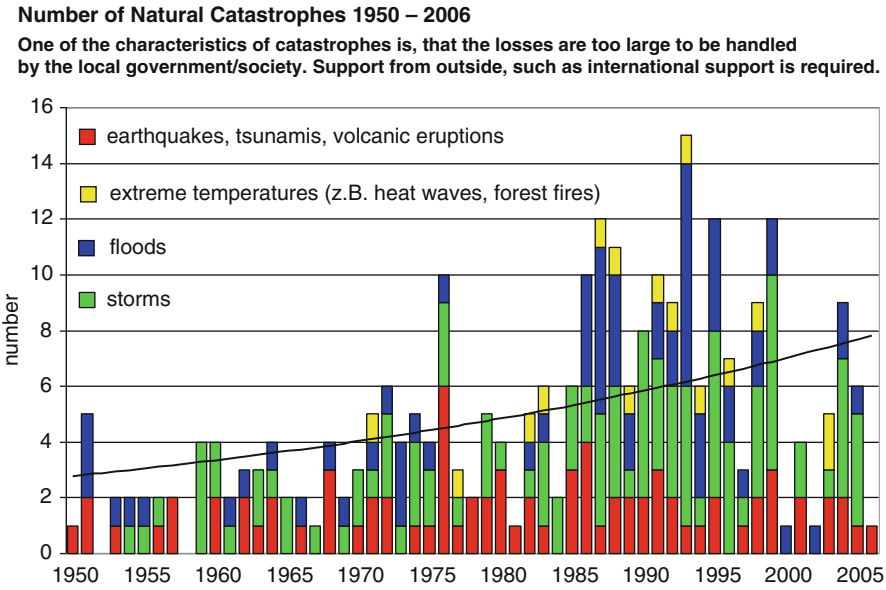



**Fig. 6** Major natural disasters from 1950 to 2006 (worldwide; source: Münchener Rückversicherungs-Gesellschaft/GeoRisikoForschung, NatCatSERVICE). Major natural disasters are, according to the UN definition, disasters which claim more than 1,000 lives and make more than 100,000 homeless. These are disasters which are beyond the capacity of self-help in the regions affected and require international aid

however have its pitfalls: Risks which are meaningless for the general public can be a major threat to those in certain risk groups, e.g., children who sit in classrooms contaminated with asbestos. Although in the average population of the USA 100 times more schoolchildren die at football games than from asbestos poisoning, anyone who is exposed to the deadly dust bears a high individual risk.

Comparisons such as "asbestos versus football" are intended to put risks into perspective. But one risk is not like any other, and not every relation is useful. This becomes particularly clear by considering, for instance, the correlation between the reduction of breeding pairs of storks in Germany to the reduction of the birth rate in Germany in the years 1965–1980 (Fig. 4): An accurate correlation, but not a useful one!

## Mortality Risks in the Population

The calculation of risks is very difficult and often involves dangers. Experts are namely just as susceptible as lay people to what is known as **cognitive dissonance** (Reichl and Ritter 2011), the phenomenon by which knowledge which disrupts long-established habits is simply not perceived. Thus, for instance, in

**Table 1** Comparisons across decades of the number of major natural disasters occurring, the damage to the national economy, and the insured damage in billions of US dollars (worldwide; values from 2006; source: Münchener Rückversicherungs-Gesellschaft/GeoRisikoForschung, NatCatSERVICE)

**Major natural catastrophes 1950 -2006**

| | Dekade 1950-1959 | Dekade 1960-1969 | Dekade 1970-1979 | Dekade 1980-1989 | Dekade 1990-1999 | last 10 1997-2006 |
|---|---|---|---|---|---|---|
| Number | 21 | 27 | 47 | 63 | 91 | 51 |
| Overall damage | 51,4 | 89,5 | 155,2 | 252,0 | 742,9 | 550,7 |
| Insured damage | 1,6 | 7,3 | 14,9 | 30,5 | 137,7 | 180,2 |

Comparison of the last 10 years to the 1960s shows the dramatic increase

| Factor last 10:60s |
|---|
| 1,9 |
| 6,2 |
| 24,7 |

• Damage in billions of US$ – values from 2006

the USA in 1985 NASA published a risk assessment on the Space Shuttle, according to which the probability of crash for the shuttle was 1: 100 000. Studies by other committees however assumed a risk ranging from 1: 270 to 1: 57. In fact, the shuttle "Challenger" exploded on its 25th flight and the shuttle "Columbia" broke up on its 28th flight.

According to more recent studies, being killed by lightening is around 650 times more unlikely than dying as a consequence of alcohol – but dying as a result of cigarette smoking is almost 10 times more likely (Fig. 5). The risk of being killed by a meteorite crash is in fact greater than that of dying in a plane crash. Although the likelihood of being hit by a celestial body is astronomically small, if it did happen millions of people could die – so the result is an increased risk.

The probability for the average citizen of dying in a terrorist attack or a natural disaster is infinitesimal. It remains infinitesimal even though the number of, e.g., major natural disasters has been increasing globally since 1950 (Fig. 6). It is this increase which is often the reason for escalating fear in the population, although in fact this is not justified. But escalating fears would be justified if you consider the indirect consequences of terror attacks or natural disasters, which affect every one of us, i.e., even the unaffected average citizen, and could thus precipitate his downfall. Because the national economic damage caused by disasters has in fact increased dramatically over the last 50 years (Table 1), bringing some countries to the brink of ruin and even causing some reinsurance companies to topple (just the terrorist attack on the World Trade Center on 11 September 2001 caused national economic damage of almost half a billion Euros). The loss of immense sums of money means that there is then no longer enough funding available for necessary projects. Whole population groups can thus be thrust suddenly into economic poverty, which is in turn a factor for a shorter life.

## Acceptance of Risks

The risk acceptance in the population is a complex and unpredictable phenomenon.

1. Rare risks (e.g., a snake bite) are overestimated, common threats (e.g., fatal heart attack due to excess weight and lack of exercise) are underestimated.
2. People are liable to falsely evaluate probabilities (e.g., driving a car is more dangerous than flying, but we always talk about a fear of flying and never a fear of driving). People do not worry because the possibility of dying in each individual car journey is one in four million and thus lower than the chance of having a fatal accident when mowing the lawn. But if you consider that in the course of your life you undertake several thousand car journeys, the actual risk increases. One in one hundred Germans dies as the result of a road traffic accident.
3. People overestimate their own abilities (e.g., they believe they are immune to disease or will have a long life).
4. Things which we believe are under our control minimize fear (e.g., smoking, drinking alcohol, driving, or climbing).
5. Things which we obviously cannot control ourselves increase fear (e.g., toxic waste facilities, invisible toxins in foodstuffs, atomic power plants).

Psychological studies show that events with a high "gruesomeness factor" (e.g., plane crashes, death by lightening, or being eaten by a shark) are especially feared, even though they are extremely rare. This is made particularly clear by the following study: When asked to choose between two forms of treatment, patients, and even doctors, preferred the treatment with a 90 % chance of survival over that with a 10 % mortality rate, although both figures express exactly the same thing: one in ten dies.

Thus, human behavior is steered less by facts and figures than by beliefs, desires, and anxieties. That is why in the future too, acceptance of risks will remain a fascinating and unpredictable social psychological phenomenon for us all.

## References

Facts and figures on the subject of health protection, environmental protection, consumer protection, risk evaluation and risk assessment:

Reichl F.-X, Ritter L (2011) Illustrated handbook of toxicology. [A pocket guide to toxicology]. Thieme, New York

Pollutants or toxins and environmental factors:

Reichl F.-X (ed) (2011) Moderne Umweltmedizin. [A pocket guide to environmental medicine]. Lehmanns Media, Berlin

## Recommended Reading

UV radiation, ozone layer and cancer risks:

Norval M, Cullen AP, de Gruijl FR et al (2007) The effects on human health from stratospheric ozone depletion and its interactions with climate change. Photochem Photobiol Sci 6(3):232–235; updated in: www.climate.org/topics/health/index.shtml

Passive smoking and cancer risks:
CDC, Centers for Disease Control and Prevention (2006) State-specific prevalence of current cigarette smoking among adults and second-hand smoke rules and policies in homes and workplaces – United States. MMWR Morb Mortal Wkly Rep 55(42):1148–1151; updated in: www.repace.com/fact_cardio.html www.who.int/entity/tobacco/en/atlas10.pdf
Radon gas and cancer risks:
Krewski D, Lubin JH, Zielinski JM et al (2006) A combined analysis of North American case–control studies of residential radon and lung cancer. J Toxicol Environ Health A 69(7):533–597; updated in: www.radon.com/radon/radon_facts.html
Risk assessment of chemicals:
An Introduction CJ van Leeuwen, TG Vermeire Springer Verlag, 2007

## Resources

Bibliographic collections (some with summaries) of articles from numerous specialist journals on the subject of risk in the environment:
European databases: www.ecdin.etomep.net, UNO databases: www.irptc.unep.ch
OECD (2002) Technical guidance document on the use of socio-economic analysis in chemical risk management decision making. updated in: series on risk management no. 14; IOMC, Paris. www.oecd.org./ehs, German databases: www.dimdi.de
Mortality risks, insurance and natural disasters:
www.munichre.com
Constantly updated information on the subject of risk assessments of (environmental) toxins:
www.toxikologie.de

# Risk-Benefit Considerations in Toxicology

Rolf Hertel, Michael Schwenk, and H. Paul A. Illing

## Contents

Risk-Benefit Assessment ..... 602
Cost-Benefit Analysis ..... 603
Utility Analysis ..... 605
Recommended Reading ..... 605

**Abstract**

If an action involves risk, the outcome is not completely predictable in advance. This raises the question as to whether there are decision-making tools that could help to identify the consequences as much as possible and then to evaluate them and weigh them. Both positive consequences (benefits) and negative consequences (risks) are considered together. The evaluation depends on being able to quantify the risks and benefits using the same units, such as monetary value or length and quality of life. When governments and agencies decide to take a risk, they should do so after considering formal risk-benefit and cost-benefit analyses and a utility analysis.

There are known knowns (those well-characterized and quantified risks and benefits), known unknowns (effects that can be identified but not quantified), and

R. Hertel (✉)
Formerly Federal Institute of Risk Assessment, Berlin, Germany
e-mail: rolf.hertel1@freenet.de

M. Schwenk
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

H.P.A. Illing
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK
e-mail: paul@sherwood37.demon.co.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_77, © Springer-Verlag Berlin Heidelberg 2014

unknown unknowns (risks or benefits not known at the time of the evaluation). Risk-benefit analysis is an attempt to quantify known knowns and known unknowns, with, for the known unknowns, an attempt to quantify the uncertainties involved. It cannot address unknown unknowns, which may be risks or benefits. It is not unknown for a drug or other chemical developed for one purpose to have a much more important use discovered later – and therefore not taken into account in the original risk-benefit analysis. Also, sometimes a new toxicity is described after a drug or other chemical has been marketed that changes the risk-benefit equation. In the case of a drug, this often results in its withdrawal from the market. Thus, there is always a residuum of risk that cannot be included in a risk-benefit assessment.

## Risk-Benefit Assessment

The **risk-benefit analysis** compares the identified risks with the identified benefits. A variety of methods can be used (Fig. 1). From an economic perspective, the benefit/utility of an object is based on a subjective judgment of the value of the benefit gained or the risk foregone. Both the object itself and the estimation of its value are considered. As no useful universally valid measure exists for the determination of a benefit, a benefit can only be measured indirectly. Care must be taken to identify whether the property that should result in a benefit is intrinsic or whether it depends on further conditions which are not dependent on the risk decision or whether it appears just accidentally to be advantageous.

Simultaneously, both the benefits and the consequential risks must be allowed for, although these consequential risks will need to be tolerable. Household disinfectants are a good example. The aim of using the material to be evaluated is to disinfect. Disinfectants have the purpose to reduce germs. However, they can cause irritation of the skin, eye, and respiratory tract of the user if not handled appropriately. Besides the desired benefits, that is, "reducing the germs," other features, i.e., the corrosive/irritant properties of the substance, must also be taken into account. In the present example, the benefits are evident, and warning labels identifying the hazardous properties and some basic precautions inform users so that they can minimize their exposure. However, if the hazardous properties of the active ingredient are identified as an unavoidable concomitant to achieving the objective of disinfection, and serious, such a product may either be banned or some limitation as to user (e.g., "for professional use only") imposed.

From an economic perspective, there are fundamental concerns with such considerations, since the evaluation of the benefits is determined by the market and includes the question as to whether there is a demand for such a material.

**Risk comparison** is one method by which substitute products can transparently be evaluated. As stated in chapter "▶ Current Role of the Risk Concept in Regulatory Toxicology," a comparison of risks is only possible if the assessable parameters of the various risks are realistically comparable, i.e., sufficiently similar in terms of their combination, chronologic order, and the distribution of advantages

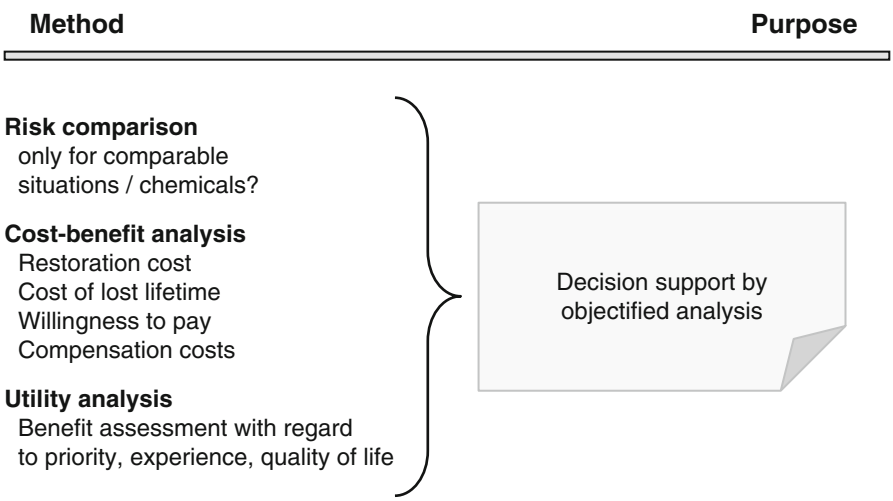



**Fig. 1** Ways of carrying out a risk benefit analysis

and disadvantages. A risk comparison can lead to a transparent identification of the critical parameters. When interpreted competently, the comparison of risks is an adequate evaluation framework in which the individual decision to take a specific risk can be made. It is important for the regulatory toxicologists that all information, including information concerning the different ways in which risks are perceived, is considered in the risk comparison. The risk comparison will only be convincing, when the underlying preconditions are clearly presented and possible shortcomings of the comparison are explained (see chapter "▶ Risk Comparison in Toxicology").

Risk comparison may allow decision takers to communicate the decision more effectively. If the comparison shows that the risk posed by a substance or an action is less than a similar risk that has previously been accepted by society, then it is likely that the decision will be generally acceptable.

Risk comparisons are appropriate when they are based on a solid safety assessment. If however the uncertainty is high and/or variable, the risk comparison will not be convincing.

## Cost-Benefit Analysis

While the risk-to-benefit analysis generally requires a more qualitative approach, a cost-benefit analysis contributes a quantitative component to the overall assessment. Here, the risks and the benefits of an action or of a substance/use are set out quantitatively, usually in monetary terms, i.e., the risks and benefits are monetized. Consider the costs and benefits to the state of a premature death caused by a chemical exposure. The direct costs are reduced productivity and cost of treatment

of any illness/infirmity. An earlier death is likely to reduce the costs of treatment of diseases of the elderly and cost of pensions. Funeral expenses will arise in both cases. Generally, possible psychological/psychiatric costs for those affected and their family are not monetized. The valuation shows here an extreme case of how profits and losses for society can be estimated. It should be noted that moral and ethical considerations may result in considerable disproportionation occurring, i.e., that the benefits gained by society may have to grossly exceed the risks taken by the individual before that risk is deemed tolerable. In some cases moral/ethical considerations may completely override any cost-benefit analysis. A detailed guide on how to use such decision aids can be found in a document of the OECD (2002).

**Value of a Human Life**: Four different methods have been used to calculate the value of a human "life." If the costs that arise to eliminate effects of a substance or an action from which a person suffers are determined, we talk about **"regeneration cost."** This approach can be applied in connection with such accident damages that result from a risky decision. The costs can be estimated on the basis of statistical data. The monetary benefits that might result from a professional development of the person (e.g., a promotion) can however only partially – if at all – be considered.

A system which is based on years of life lost assesses the productive contribution of the individual to society. This method calculates the costs (residual working lifetime, employment rate, and national income) and the benefits which can no longer be taken advantage of (consumption, services, medical expenses). A cost calculation is based on this so-called **human capital approach**.

A fundamentally different method for rated human life uses neither statistical data about recovery costs nor the contribution of the individual to the productivity of the society, but personal judgment. A **willingness to pay** analysis is conducted where a court awarded compensation or the amount of a life insurance serves as the base. The costs identified using this technique depend heavily on the tradition and the ethical values of the society in which the individual lives. Examples are the different levels of compensation in the USA and Europe.

Furthermore, appropriate questioning/survey techniques allow us to estimate those costs which the individual would be willing to bear in order to compensate for the consequences of an action or the application of a substance. The acceptance of such "**compensation cost**" depends not only on the social environment of the respondents but also on the individual's concern at the loss and the availability of alternatives, as demonstrated by the example of the marketing of so-called organically grown food.

Each method can give very different results, and the actual results obtained are country specific. In the Federal Republic of Germany, the Federal Highway Research Institute calculated the annual economic cost of road accidents. Therefore, apply in 2009 following personal accident costs: for a slightly injured person 4,416 €, for a seriously injured person 110,571 €, and for a fatal outcome 996,412 €. Although the range of values is extremely broad, UK and US Governmental organizations appear be similar. The mean values for the UK were given as 2,281 million US dollars and the USA is predictably higher, at 3,472 million US dollars (Miller 2000).

## Utility Analysis

Monetization is often rejected not only for ethical reasons but also because of methodological shortcomings. The utility analysis provides a quantification of values independent of money. It does not balance out cost of alternatives, but this analysis captures the benefits/utility as dimensionless value, derived from priorities, ideas, and experience of the decision maker. The focus is on possible consequences of the decision and the probability of their arrival. If the benefits can be subdivided, the various cost-benefit values are weighted and combined to a total benefit.

It is also possible within the frame of a given plausible and/or politically legitimate objective and precondition to summarize individual cost-benefit values, determined by various decisions and include them in the total analysis. Risk managers may choose between different courses of action, and they then have to seek widespread acceptance for the measures taken. This is best sought through a description of the decision parameters. Advantages and disadvantages should be distributed as evenly as possible among the affected individuals or interest groups. Since no pecuniary settlement is involved, even nonmonetary parameters (e.g., improved quality of life) can adequately be used for decision making.

In utility analysis, the initial objectives and requirements in the decision-making process can continuously be questioned. If considered desirable, these objectives can be modified following appeals from stakeholders, and this should result in the greatest possible consensus (even though that may still be a very limited consensus in the case of some projects, such as new airport capacity around London!).

## Recommended Reading

BASt (2011) Socio-economic costs due to road traffic accidents in Germany 2009. http://www.bast.de/cln_033/nn_virgin/EN/e-Home/e-homepage__node.html?__nnn=true. Accessed 9 Apr 2013

Miller TR (2000) Variation between countries in values of statistical life. J Transp Econ Policy 34:169–188

OECD (2002) Technical guidance document on the use of socio-economic analysis in chemical risk management decision making. Series on risk management No. 14. IOMC, Paris. http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono(2002)10&doclanguage=en. Accessed 9 Apr 2013

SRU (Der Rat von Sachverständigen für Umweltfragen) (1999) Umwelt und Gesundheit – Sondergutachten – Metzler-Poeschel, Stuttgart

# Toxicological Risk Assessment in Different Jurisdictions

Dietrich Henschler and Wolfgang Dekant

## Contents

Acceptable Risk, Comparison of Risks, and Establishing Acceptable Levels of Risk ..... 608
Pharmaceuticals ..... 609
Occupational Health ..... 610
Indoor and Ambient Air ..... 612
Food ..... 612
Cosmetics and Consumer Products ..... 613
General Chemical Safety ..... 613
Radiation Protection ..... 614
References ..... 614

**Abstract**

The control of potential health hazards to humans due to the production, use, and disposal of chemicals is a major issue of concern. The concern arises from the increasing numbers of chemicals in production and use and the increasing numbers of chemicals demonstrated to exert toxic effects in sensitive toxicity testing systems. This situation has afforded growing legislative control of the production and application of chemicals. Control measures may limit the presence of hazardous chemicals in the environment or regulate the use of hazardous chemicals.

The assessment of potential human *health risks* resulting from the exposure to chemicals provides the basis for appropriate regulatory and control measures (Table 1). The *health risk assessment* determines whether a chemical may cause adverse health effects, at what level, duration and frequency of exposure, and

D. Henschler (✉) • W. Dekant
Institute for Toxicology, University of Würzburg, Würzburg, Germany
e-mail: popa-henning@toxi.uni-wuerzburg.de; schraut@toxi.uni-wuerzburg.de; dekant@toxi.uni-wuerzburg.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_78, 

**Table 1** Possible measures to reduce *human exposure to hazardous chemicals*

| Application of or exposure to chemical in question | Measures to reduce exposure |
|---|---|
| Industrial chemicals | Reduction or cessation of application, protective measures in the workplace, alternative chemicals with lower hazard |
| Pharmaceuticals | Definition of optimal dosing and dose limits, cost–benefit analysis |
| Alcohol, smoking, pharmaceuticals of abuse | Education |
| Environmental chemicals | Quantitation of exposure, strategies for avoidance, or reduction of environmental pollution |

the probability that adverse health effects will occur. Risk assessment considers the available data on the toxicology of a specific chemical when judging which agents potentially pose a significant risk to the human population. Tolerable exposure levels for humans are derived from the results of animal studies by using margins of safety or defining "acceptable" incidences of adverse health effects in exposed humans.

Health risks due to contact with potentially toxic chemicals are dependent on the conditions of exposure, since not only the intrinsic toxicity of a chemical determines the magnitude of the adverse effect but also the dose. In toxicological terms, risk therefore is the product of the intrinsic toxicity of a chemical and the exposure characteristics.

## Acceptable Risk, Comparison of Risks, and Establishing Acceptable Levels of Risk

In earlier phases of risk assessment, the basic belief was that few chemicals are toxic and all of these toxic chemicals are derived from synthetic processes. To achieve a zero risk, chemical exposure must be reduced below a threshold level under which it does not cause risk. However, where such a threshold cannot be demonstrated, one must assume that a finite risk may occur at any exposure level. These considerations resulted in the *zero-risk concept*. However, the more widespread testing of chemicals for toxicity, the increased sensitivity of analytical instruments to determine chemicals, and the developments in the science of toxicology put the basic assumption of the zero-risk concept into question.

These developments led to the recognition that zero risk was unachievable and, perhaps, unnecessary for the regulation of chemicals. The was based mainly on a few facts: (1) all chemicals, both of synthetic and natural origin, are toxic under *specific exposure conditions*; (2) most of the hazardous chemicals routinely encountered by humans are of natural rather than synthetic origin; (3) most of the exposure to hazardous synthetic chemicals cannot be avoided entirely or be eliminated from the environment without profoundly changing the way of life in many countries; and (4) in the case of cancer risk assessment, DNA damage and

mutations, assumed to be of major significance in the process of carcinogenesis, occur spontaneously, albeit at a low rate.

Given these facts, the *acceptable risk concept* was developed as an alternative. The acceptable risk concept realizes that it is not possible to eliminate all potential health risks associated with chemical exposure due to the lifestyle. According to the concept of acceptable risk, safety – the reciprocal of risk – is no longer an absolute term but is redefined as a condition of certain but very low and thus acceptable risk. This conceptual change improves the ability to deal with potentially very low risks identified by the increased sensitivity of analytical instrumentation and with increasingly sensitive scientific methods to detect *potential adverse effects*. The concept of acceptable risk also permits the definition of limits for the exposure to toxic chemicals that can be considered to have a negligible impact on the incidence of adverse effects in an exposed population. Risk assessment therefore is unavoidable.

In different regulatory frameworks, while the general approaches to risk assessment are used in an identical approach – *hazard assessment*, *exposure assessment*, and *dose–response evaluation* – a number of specific factors and circumstances are influencing *risk characterization*. These are outlined in the following for some major application of chemicals. For most of the areas of applications of chemicals, the responsible authorities or scientific bodies have developed specific guidance documents which in detail define the approaches to be used and are frequently updated to include scientific progress and societal demands.

## Pharmaceuticals

The marketing and application of pharmaceuticals is most highly regulated as compared to other application areas of chemicals. *Unwanted effects* play a major role in risk assessment. In contrast to other regulations concerning chemicals, which often attempt to avoid any negative health effects due to exposure, *risk–benefit considerations* are specifically integrated in the *evaluation*. Risk–benefit considerations are important since any therapeutically active chemical may have unwanted effects even at optimal *therapeutic dosage*. While unwanted effects may be mitigated by specific molecular design and optimized therapeutic schemes, they usually cannot be completely avoided, specifically when treating life-threatening diseases. Risk assessment aims to reduce the incidence of unwanted effects to a tolerable extent. The necessary evaluation therefore includes *risk–benefit analysis*. Benefit is the *beneficial therapeutic effect* for a patient; *risk in this context is the type*, *frequency*, *and intensity of unwanted effects*. The relation between risk and benefit will then be translated in a scientifically supported relation; performing risk–benefit analysis is implied in legal regulations. However, due to the complexity of the disease processes and the potentially different responses of a disease to treatment options (from complete curability to mitigation of severe symptoms and improvement of life quality), the criteria for risk–benefit analysis in different areas of pharmaceutical treatment differ widely. For normative

purposes and harmonization, science-based consensus by highly experienced expert groups and scientific societies has been developed both on a national and an international scale. *Risk assessment of pharmaceuticals* is performed at two levels, at the *level of authorization* and at the *level of supervision* of the incidence of unwanted effects when the pharmaceutical is on the market and applied to a large number of patients.

*Authorization of Pharmaceuticals*. Authorization of pharmaceuticals is regulated by national, European, Japanese, and US laws. Authorization by the *US Food and Drug Administration* (*US-FDA*) is often also used as the basis for authorization of pharmaceuticals by national authorities of other countries. Authorization is based on three pillars: pharmaceutical quality, measurable clinical effects, and aspects of "safety." Definition of "safety" of pharmaceuticals implies risk–benefit assessment.

The applicant, usually a pharmaceutical producer, has to submit a detailed dossier in a defined format, the *Common Technical Document* (*CTD*), to apply for authorization. All information has to be collected according to predefined protocols and to be reported in harmonized format. This common format is requested in Europe, the USA, and Japan. The CTD dossier has to contain all relevant information regarding production, research, and development of the pharmaceutical. In addition, a major focus are the results of the non-clinical, pharmacologic, and toxicological studies and all data from clinical studies investigating efficacy and frequency and intensity of unwanted effects.

Regarding toxicology, harmonization and standardization of the required toxicological testing has been developed by international harmonization between the US-FDA, the *European Medicines Agency* (*EMA*), and Japanese authorities in the *International Committee on Harmonization* (*ICH*). Most of the requested study designs developed by ICH are considered mandatory by these regulatory agencies.

Authorization within the European Union may be performed by a centralized application at the EMA or in a decentralized procedure by application to a regulatory authority of a member state. The regulatory authority evaluates the submitted dossier and may request additional information in case of uncertainties or specific issues. After authorization, effects of pharmaceuticals are further monitored to detect potential risks in larger populations. Low incidence effects with severe health impact or unwanted effects under certain conditions will only be evident after use of a pharmaceutical in large populations.

## Occupational Health

Protection of worker health when handling chemicals is one of the oldest areas of regulation where a science-based risk assessment served as a basis for regulatory decisions. The major protective measures are reduction of exposure due to technical improvements of the work situation and *maximum tolerated concentrations of chemicals* in workplace air. Regarding reversible effects, *threshold limit values* (*TLVs*) are developed; for chemicals with irreversible modes of action such as carcinogens, *quantitative risk assessments* are performed. TLVs for reversible

modes of action are based on thresholded dose–responses for adverse effects; concentrations below the thresholds should not result in health effects in the exposed workers. TLVs are derived from observations in occupationally exposed humans or based on animal toxicity testing using appropriate routes of exposure (usually inhalation). *Safety factors* (*SFs*) as used in risk assessment for food additives and food contaminants are not mandatory in deriving TLVs; if used, they are much smaller than those used for regulation of food additives (SFs of 5–10 as compared to 100). *Surveillance* of TLVs is performed by analytical determination of the air concentration of the respective chemical at the workplace; periodic health surveillance of exposed workers will assure that the aim of health protection is reached. TLVs are usually derived for an exposure of 8 h per day, 5 days a week, and 40-year work life, but a variety of values for shorter duration exposures or specific situations have been developed in the different regulatory frameworks. Deduction of TLVs may consider specific *individual susceptibilities* if procedures to detect such predisposing factors are available. *Sensitizing properties of a chemical* are not generally considered, but such properties will require specific labeling. Issues of costs of compliance and technical measures for compliance are not considered.

TLVs presently are also derived for chemicals with irreversible modes of action, which usually are genotoxic carcinogens. Based on the *basic concept of carcinogen risk assessment*, even very low exposures to genotoxic carcinogens may result in an increased incidence of tumors (although often in extremely low incidences); thus, the aim of complete health protection cannot be reached when exposure to a genotoxic carcinogen at the workplace may occur. Therefore, the basis for the TLVs for carcinogens is a comparison of the calculated tumor risk of an exposure (over the whole work life) with that of other occupational health risks not related to chemical exposures (such as accidents). Such a comparison requires a quantitation of the tumor risk, which is usually done by *extrapolation* of the dose–response curve from animal experimentation to render concentrations expected to result in an acceptable risk. Previously, TLVs for carcinogens were not derived since the risk assessment process has not been considered sufficiently precise. However, in Germany, since 2005, the former "*Technische Richtkonzentrationen* (*TRK*)" for genotoxic carcinogens has been formally replaced by TLVs, which need to be developed over time. Driving force for the decision was the intent to base a value for a TLV for a carcinogen on a scientific evaluation instead of relying on technical capacities, analytical surveillance, and socioeconomic issues, which were drivers for TRKs.

Risk assessments for TLVs are performed by independent scientific committees such as the "Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe (MAK-Kommission)" or the EU Scientific Committee on Occupational Limits (SCOL). In analogy, TLVs in the USA are also derived by the TLV Committee. Members of the committees are recruited from independent toxicologists, occupational physicians, and analytical chemists. A public consultation period regarding the derived TLV is often integrated in the process, and all TLVs are justified in detailed written reports.

## Indoor and Ambient Air

In contrast to occupational exposures which usually only occur for 8 h per day, exposure to indoor and ambient air contaminants occurs for 24 h per day and the whole population is affected. Risk assessment for ambient and indoor air contaminants often is also based on inhalation toxicity studies, but often needs to integrate higher *extrapolation factors* to cover potentially sensitive groups such as infants, the aged population, and predisposed individuals. However, besides a risk assessment-based approach using animal test data, many regulatory tolerance values regarding air contaminants are derived based on observations in large-scale epidemiology studies in humans. In addition, some values are using a precautionary approach or may be oriented regarding socioeconomic considerations and natural background of the contaminant.

## Food

*Food additives* and *food contact materials* have become important over the past 50 years to preserve food or improve appearance and taste and shelf life. More and more contaminants present in low concentrations in food are detected by the increased food surveillance and the significantly improved analytical capabilities. Risk assessment for these types of compounds is performed on national and international levels. In the European Union, the *European Food Safety Authority* (*EFSA*) is responsible for setting tolerable concentrations of contaminants and food contact materials in different food items and for evaluation of maximum content of food additives. EFSA uses the classical risk assessment process integrating safety factors for contaminants with reversible mode of action and quantitative approaches for carcinogens, decisions on the limits are made by advisory panels of independent scientists, and detailed justification documents are published. Tolerable limits for food additives, which require authorization, are also assessed using safety factor methodology. Chemicals with genotoxic properties will not be authorized for these purposes.

Human exposures to food additives are estimated by using maximum addition levels of the additive to food items and surveillance data regarding consumption of food items containing the additive. A similar approach using concentration data for contaminants in different food items and European consumption data are derived to define tolerable intakes for contaminants. Regarding genotoxic contaminants, the *margin-of-exposure* (*MoE*) *concept* is applied. The MoE represents the difference between the estimated human exposure to a genotoxic contaminant and a dose descriptor (usually a benchmark dose, BMD05) from animal testing. A MoE of $>10{,}000$ is considered to be of "low concern" for regulatory action or mitigation. Food contact materials are usually regulated based on *migration testing* by standardized procedures and intended uses of the food contact material with certain food items and its consumption pattern. Similar approaches as used by EFSA are used

by the US-FDA and national authorities worldwide and by international organizations such as the *Joint FAO/WHO Expert Committee on Food Additives* (*JECFA*).

## Cosmetics and Consumer Products

Due to the increased awareness regarding potential risks of synthetic chemicals, risk assessment for chemical ingredients in cosmetics and consumer products is also performed. On one hand, intentionally added ingredients are systematically assessed regarding potential health risks based on information requests by scientific advisory bodies; in addition, assessment of contaminants may be requested by regulatory authorities. Exposure assessments are performed based on the concentration of the chemical to be added to the consumer product and anticipated frequencies of use and use levels. When oral exposure is involved, migration testing determining the release of the agent from the product under predefined conditions (e.g., plasticizers from toys) is used as a major basis for exposure assessment. Regarding cosmetics, where the major exposure to ingredients is likely dermal uptake and inhalation, oral toxicity data are used as a basis for hazard assessment integrating consideration of toxicokinetics. It should be noticed that animal experiments regarding hazard assessment of cosmetic ingredients are banned in Europe after 2013 and new ingredients in cosmetics subjected to animal testing will not be permitted. Non-animal methods for hazard assessment, however, do at present not have the capacity to predict potential toxicities. A solution to this dilemma is not expected in the near future.

## General Chemical Safety

The new European legislation regarding *Registration*, *Evaluation*, *Authorization of Chemicals* (*REACH*) attempts to establish "safe uses" for all chemicals available on the market and used in products. Within REACH, the producer or importer of a chemical has to file a registration dossier containing all available hazard data, anticipated exposures, and a risk assessment for the intended and foreseeable uses. The general approach therefore shifts responsibility for risk assessment from a *regulatory agency* to industry. Due to the large number of chemicals to be registered, it is expected that only a limited number of the submissions will be checked for correctness and scientific soundness. In addition, chemicals of "very high concern" may need to be authorized for specific applications. Specific assessments of priority chemicals are made by a scientific committee (*Committee for Risk Assessment*, *RAC*) consisting of a limited number of independent scientists and mostly of representatives of national regulatory agencies. In addition, socioeconomic consequences of restrictions or bans of certain chemicals will be evaluated by a specific group. Detailed guidance regarding approaches to be used in REACH has been developed over the past decade.

## Radiation Protection

*Radiation protection* aims at limiting the number of people exposed and the probability of exposure to be "*as low as reasonably achievable*" (*ALARA*); if exposed, exposure should also be as low as reasonably achievable. In the process to set exposure limits, social and economic factors are taken into consideration and risk comparisons are performed. Risk assessment for ionizing radiation for the general population is based on a comparison of the natural background radiation and its variation with that of the radiation source to be assessed. Since radiation protection assumes low-dose linearity for risk assessment with the main focus on cancer prevention, risk comparison is performed. The radiation dose expressed in Sv represents the amount of radiation energy deposited in tissue. As Sv is a large unit of measurement, the millisievert (mSv) is frequently used. The average human dose from background radiation is about 2 mSv per year. In many cases of radiation exposures, including background radiation, the radiation dose is evenly distributed throughout the body. Exposure may also be directed to a limited area of the body (radiation therapy) or single organs (e.g., radioactive iodine in the thyroid). As some organs are more sensitive to radiation, tissue weighing factors ($W_T$) are used to determine the equivalent risk of locally limited exposure. When the tissue weighing factor has been applied, the term "effective dose" is used. *The International Commission on Radiological Protection* (*ICRP*) recommends tissue weighing factors. The effective dose puts all ionizing radiation on an equal basis in terms of their potential to cause damage. In the EU, the upper limit for the ionizing radiation is 20 mSv/year in occupational scenarios and 1 mSv for the general population regarding technical sources.

## References

European Food Safety Authority (EFSA) Scientific Committee (2012) Scientific Opinion on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. The EFSA Journal 10:2578.

German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK-Kommission) The MAK collection for occupational health and safety available online at http://onlinelibrary.wiley.com/book/10.1002/3527600418

The Scientific Committee on Occupational Exposure Limits (SCOEL) (2009) Methodology for the derivation of occupational exposure limits, version 6

Williams ES, Panko J, Paustenbach DJ (2009) The European Union's REACH regulation: a review of its history and requirements. Crit Rev Toxicol 39:553–575

# Part V

# Risk Management and Risk Communication

There are two types of risk management. Routine management is in connection with legislation associated with anticipatable exposure, including that requiring registration or labeling of products. Most of them proceed according to clear rules and schedules. In contrast, crisis management of accidental exposures is highly dynamic and an initial lack of information is typical. The regulatory toxicologist is needed in both situations. In the routine work, he or she must deal with the interpretation of complex research results and communicate his or her judgment with colleagues of his or her own and other institutions. In crisis management, the requirement is for rapid comprehension of the situation, quick decisions, and good risk communication. In all of these situations, the proposals are aimed at protection of humans and the environment. The decisions should be pragmatic, acceptable, and affordable.

**Risk Management**

Risk management is the process that includes risk regulation. The goal is to provide adequate protection to humans and the environment. Toxicological risk management includes the setting of limit values, registration, classification, labeling, and monitoring. It also includes the management of chemical incidents. These activities complement each other. A risk management decision may be prepared by a single person, but usually it requires the approval of different institutions, sometimes in very long procedures with tough battles among the different stakeholders. A successful management relies on technical competence, credibility, and flexibility. In the management process, the toxicologist should indicate whether there may be potential conflicts of interest (e.g., through membership of organizations or funding of work) that have to be taken into account when considering his or her opinions.

**Risk Communication and Participation of Affected People**

Risk communication has intended as a means of seeking agreement about the extent of a risk and what to do about the risk. Risk communication should take place before different opinions about an acceptable risk collide. The aim is a consensus concerning a solution to a particular problem. It should be a moderated process. It may only involve decision makers, but it should also include the public.

The toxicologist may have the task of explaining complex scientific interrelations in a clear and, ideally, comprehensive manner and to propose reasonable technical solution alternatives. It may happen that during risk communication a respected toxicologist gets discriminated against by a rhetorically skilled amateur. It may also happen that a patient who feels poisoned opposes the interpretations of the experienced toxicologist. Toxicologists should be capable of communicating effectively.

# Purpose of Risk Management in Regulatory Toxicology

Rolf Hertel, Michael Schwenk, and H. Paul A. Illing

## Contents

Instruments of Risk Management ..... 618
Chemical Risk Assessment ..... 618
Risk-Reduction Strategy ..... 620
Voluntary Agreements and Regulatory Actions ..... 621
Thalidomide and Drugs Legislation ..... 621
Smoke Control and Air Quality Legislation ..... 622
DDT, Malaria Control and Wildlife ..... 623
Preventive Measures ..... 623
Recommended Reading ..... 624
Internet ..... 625

**Abstract**

If the risk resulting from an event, including an event involving exposure to a defined chemical substance, is known and characterized, measures preventing the event or mitigating the damage (including ill-health) can be set up. These may include land use and emergency planning and restrictions on use, including the ultimate restriction, which is prohibition. Such measures can have major socioeconomic impacts. In a democratically organized society, these measures

R. Hertel (✉)
Formerly Federal Institute of Risk Assessment, Berlin, Germany
e-mail: rolf.hertel1@freenet.de

M. Schwenk
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

H.P.A. Illing
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK
e-mail: paul@sherwood37.demon.co.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_79, © Springer-Verlag Berlin Heidelberg 2014

must be acceptable to the public as a whole; thus, effective multidirectional communication between stakeholders (interested parties) is essential.

## Instruments of Risk Management

The term risk management includes those measures which must be taken by individuals or society to cope with an identified risk. The regulatory toxicologist may be asked to suggest measures that are used to control, reduce, or regulate risk. The general objective of risk management is to select appropriate tools to reduce the likelihood or size of risks and thus to prevent or minimize damage. Toxicological risk management includes standard setting, i.e., defining and setting limit values for exposure that represent the maximum acceptable or tolerable risk. Exposure standards exist within a process of risk management, which, when followed, should ensure the safe handling of the relevant substance (see chapter "► Importance of Exposure Level for Risk Toxicological Assessment"). C*lassification and labeling* draws attention to the inherent properties without defining a risk (see chapter "► Health Hazards Classification and Labeling"). For a risk to exist, there must also be sufficient exposure to the substance. Exposure monitoring can then serve as management tool (see chapter "► Human Biomonitoring. Its Importance in Toxicological Regulation").

Important measures to limit the impact of unavoidable damage are appropriate land use planning, proper design of manufacturing plants, restriction on use (e.g., for certain applications or in specified processes, or to specified users – often combined with the use of protective equipment) or sales outlets, and, in extreme cases, prohibitions on the marketing of certain substances (see chapter "► Restrictions and Prohibitions as Tools in Regulatory Toxicology"). The range of these instruments shows that these measures must be decided politically. Usually there is some actual or potential benefit foregone when manufacture or use of a substance is restricted. Since the acceptability of the risk depends on attitudes to risk and in the majority of cases there is no clear, generally accepted solution, management decisions often require detailed discussion. Here, the preferences for *risk-friendly*, *risk neutral* or, *risk averse* options must be weighed against each other. Bans may ultimately lead society into a foregoing of opportunities. Therefore, such a drastic measure must be carefully justified. The choice of policy instruments for risk control is not arbitrary. The regulatory toxicologist may have to explain the basis of the acceptability of a risk in a way that is both intelligible to the public and well founded. The widest acceptance can be achieved if all stakeholders are affected similarly by the management decision. Ideally, when an individual's choice creates extra risk burdens, those burdens should be to that individual rather than to others in society or society in general.

## Chemical Risk Assessment

There are two main streams of chemicals risk assessment. The first is in terms of substances and their uses, and the second is in terms of risk assessment for pollution

prevention and control. The latter includes "major accident hazards", such as those seen at industrial manufacturing plants, and land use planning. Associated with the last named is the "cleanup" (remediation) for preexisting pollution.

Within the EU substances are now dealt with through the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation (Regulation EU No 1907/2006, as amended) and a variety of use-specific schemes (for medicines, veterinary products, food additives and contaminants, plant protection products, biocides, personal care products ["cosmetics"], etc.). Closely associated with this regulation is that for classification, labeling, and packaging of substances (CLP Regulation, Regulation 1272/2008). REACH and CLP replace older regulatory schemes, notably those associated with "new" and "existing" substances. REACH also subsumes the control requirements set out in former "Marketing and Use" Directives. The CLP regulation introduces the UN Globally Harmonized classifications into EU law. This classification system is intended to be utilized worldwide.

REACH requires that a chemical safety assessment is conducted for chemicals registered in quantities of 10 tons or more chemicals identified as causing certain types of toxicity. The guidance to the REACH regulation identifies that the chemical safety report (produced as a result of the assessment) should include an assessment of any hazards that the substance may present (to human health, to physical chemical hazards [fire and explosion, etc.], and to the environment). When the substance meets classification criteria or is persistent, bioaccumulative and toxic, or very persistent and very bioaccumulative, the assessment is likely to be conducted by the regulatory authority. In all cases it includes an attempt to identify the conditions under which the risks can be controlled and therefore requires an examination of exposures and risk management procedures.

Pollution prevention and control involves both prevention and control of incidental and accidental releases of chemicals. Therefore, both land use planning and emergency planning are important. At the base of such prevention is the identification of chemicals and processes that cause harm to human health and the environment, modeling their dispersion and monitoring emitted levels of the substances to ensure that the exposures do not exceed acceptable levels. Incidental exposures are dealt with through processes such as "integrated pollution prevention and control."

In the EU, **major accident hazards** are subject to the "Seveso Directives" – Seveso 1 is Directive 82/501/EEC, Seveso 2 is Directive 96/82/EC, and Seveso 3 is Directive 2012/18/EU. This major accident hazards legislation was the result of chemical exposures following accidents at Seveso (Italy) and Flixborough (UK). The current Directive is Seveso 2; Seveso 3 comes into effect in 2015. The legislation is concerned with both planning for the prevention of major incidents and emergency planning should a major accident occur. It should be noted that many of these major accidents are the result of fire or explosion, but others (e.g., dioxins at Seveso or methyl isocyanate at Bhopal) are due to direct health hazards of chemicals released to the atmosphere. Again, underpinning the assessment for releases of chemicals capable of causing damage to human health and to the environment is identification and characterization of the hazards (i.e., the toxicology and ecotoxicology) as well as the exposure pathways.

In certain parts of the world "**contaminated land**" is an issue – i.e., land that contains substances in or under the land that are actually, or potentially, hazardous to health or the environment. This land may have been contaminated by human activities such as mining, railways, industry, chemical and oil spills, or waste disposal. Contamination can also occur naturally as a result of the geology of the area (e.g., arsenic contamination) or through agricultural use. Human exposure to contaminants can be through inhaling dust or gasses, contact with soil, or eating food grown on the land – plants can absorb the contaminants from the soil and the air. In some cases sites are so contaminated that they present an unacceptable risk to human health or the environment. When such contamination is identified, the land concerned requires remediation. Identification of contaminated land involves identification of the health effects of potential contaminants as well as modeling the pathways by which the contaminants are likely to be taken to the receptor.

## Risk-Reduction Strategy

The creation of a risk-reduction strategy involves several steps, namely, risk assessment and risk or risk-benefit evaluation (chapter "▶ Risk-Benefit Considerations in Toxicology"), identification of management options, and monitoring both of the risks and of the effectiveness of the risk management measures.

The *risk assessment*, which the risk manager receives from independent risk assessors, must be "fit for purpose." The assessment may include information on whether the statements are based on collected information or assumptions, whether extrapolation steps were included (inter- and intraspecies, route of exposure, exposure period), and, if so, what the extrapolation procedure was. Ideally it should include an identification of any specific group within a population at higher risk. *Risk-benefit* evaluations have to take into account whether benefits will be foregone as a result of the proposed risk management procedures. Occasionally, risk (or risk-benefit) evaluations will also have to take into account public opinion or, at least, opinions of affected persons ("stakeholders"), which may, in turn, be derived from controversial risk evaluations based mainly on nonscientific factors.

If the initial risk assessment results indicate to legislators that they should look further and, possibly, reduce the risk, further evaluations and adequate risk-reduction measures must be sought. A preliminary step may be to determine whether existing legislation is adequate or can be extended to cover the problem.

In REACH, the normal first step in the risk-reduction process is to perform a more specific evaluation of the risks and current management measures and, if the risks cannot be minimized to an acceptable level, a risk-benefit analysis. Technical control measures include replacement by substitution for either the chemical or the process, engineering controls – either at the design stage or by retrofitting – and use of personal protective equipment, often combined with the setting of maximum exposure standards, or restricting users or outlets. Finally, in the risk-benefit analysis, socioeconomic aspects must be born in

mind. This socioeconomic analysis requires economic and sociological expertise and includes a consideration of costs and benefits to all stakeholders of health benefits and detriments, harm to competition, and job loss. Socioeconomic analysis reduces everything to a common, usually monetary basis, and is in its infancy. Possible alternative solutions can create new markets (e.g., the use of wind energy, photovoltaics).

Any decision taken must be transparent and comprehensible even though, given the complexities and inconsistencies in the available data, this may be extremely difficult. There is always a need to review and complement existing data. The adequacy and quality of the risk management measures must be checked in time in order to confirm that the required mitigation has occurred. Decisions on classification and labeling on basis of the inherent toxicity of the substance are clearly within the realm of the regulatory toxicologist. In Europe this is done in laid down procedures in the course of chemical assessment. The decisions in this area may be subjective or a matter of judgement when qualitative factors affect the decision (e.g., extent of dermal effects) or when the relevance of animal data to humans has to be assessed (e.g., specific cancers, nephropathy: globulins).

Universally accepted decision-making patterns are emerging, even if some of these approaches are pragmatic rather than fully justified on the basis of the science.

## Voluntary Agreements and Regulatory Actions

Measures concerning restrictions or even prohibitions are usually controversial. Here, two different approaches are available: *voluntary agreements* or *regulatory actions* of the legislature. It is assumed that the voluntary agreement has the advantage that it is usually considered "common sense" and shows rapid effects, while a legislative process takes a comparatively much longer time and leaves those affected without the required protection during this period. Voluntary agreements can be made, e.g., between the specific producing industry and the responsible state agency. Although historically voluntary agreements were often preferred, they have almost universally been succeeded by regulatory actions. Examples of historically important regulatory schemes are given below.

## Thalidomide and Drugs Legislation

In combination with other agents, thalidomide was used to treat cold, cough, anxiety, migraine, and asthma and for calming children. Very quickly it reached the largest market share in its class. At the time of its introduction, regulatory requirements were minimal and sales in Germany and the UK increased rapidly. In 1960–1961 it became obvious that an increase in malformations in children was associated with maternal thalidomide intake, and the drug was withdrawn. The USA did not approve the drug because a theoretical possibility of reproductive

toxicity had not been evaluated experimentally. In the UK this disaster led to an enquiry by Sir Derek Dunlop and, both in Germany and the UK, more stringent national legislation concerning the safety of medicines. In recent years the national approval systems for medicines have been subsumed into an EU-wide scheme.

As a consequence of the thalidomide case, the UK Medicines Act of 1968 and the German Medicines Act of 1976 were enacted in order to increase drug safety. Switzerland has set up an inter-cantonal agreement in 1971. Austria followed in 1983. Generally, the legislation sets up license requirements for medicines and their producers, introduces an authorization procedure, and requires adequately conducted clinical trials. For many products a prescription is mandatory. Recent EU legislation has introduced pharmacovigilance requirements to ensure that safety in use is properly monitored.

## Smoke Control and Air Quality Legislation

The improvement of air quality with its far-reaching implications for the regulation of chemicals provides an example of successful claims regulation by new legislation.

As early as 1306, King Edward I had banned the burning of coal in furnaces in London. Almost 600 years later, the death of 1,000 inhabitants of London was reported, which was caused by "smog." The smog was caused by sulfur dioxide accumulation in the air during combustion of coal. In December 1952, there was such a dense fog for 4 days ("Killer smog") that the busses could operate only when an officer walking with a lantern showed the direction. There were 4,000 extra fatalities in London hospital. It was clear from the weekly deaths registrations in the UK that the smog was the cause of death. Based on the analysis of this incident, the "Clean Air Act" was enacted in 1956 in the UK, unfortunately not soon enough to prevent further 1,000 deaths in a smog period in 1955. The "Clean Air Act" set up "smoke control zones" within which emission of smoke was prohibited and controls were introduced on the fuels that could be burnt and on appliances used for burning. This Act was reinforced with the Clean Air Act of 1968 after another smog period in 1962, which cost 750 additional lives. These were consolidated into a Clean Air Act of 1993. Since 1968, there have been no similar smog episodes in London, and, as a result, chronic bronchitis and related disease clearly decreased and the number of sunny days increased. In the urban area of London, flora and fauna recovered, and there was a significant improvement in the quality of life of the citizens.

In New York, USA, there was also a smog period in 1953 which resulted in 170–260 additional casualties, and there were 405 additional cases in 1963 and 168 cases in 1966. This led to the founding of the EPA (Environmental Protection Agency, US EPA) in 1970 and the adoption of the American "Clean Air Act." The success of this law led in 1976 to the ratification of the "Toxic Substances Control Act," which authorizes the US EPA to control the use of toxic substances. In Germany similar legislation was made possible in 1986.

## DDT, Malaria Control and Wildlife

DDT (dichlorodiphenyltrichloroethane) belongs to the class of chlorinated hydrocarbons. Its insecticidal effect was discovered in 1939 by Paul Mueller, who was honored with the Nobel Prize in 1948. Because of its low toxicity to humans – a dose of 18 g was survived – but very good efficiency against flies, lice, and mosquitoes, which transmit malaria and other disease, its versatility and low manufacturing and application costs, DDT quickly became the world's most important insecticide, used extensively against the vector for malaria, the anopheles mosquito. In Sri Lanka (Ceylon), some 2.8 million people suffered from malaria in 1946, i.e., before DDT spraying was used to control the vector, but only 17 cases were reported in 1963, after DDT spraying. The worldwide production and application of DDT amounted to almost 100,000 t in 1963. At this time, results were published according to which DDT is toxic to fish and causes a thinning of eggshells in birds of prey, preventing their successful reproduction. This was made public in the book "Silent Spring" by R. Carson in 1962. There appeared also reports that DDT generated liver cancer in mice. This together with the accumulation of the compound in human adipose tissue and breast milk resulted in attempts to ban DDT in various countries such as the USA and Germany in 1972.

In Sri Lanka, the 2.8 million cases of malaria and more than 12,500 deaths in 1946 fell to 17, and the number of deaths fell to 1 in 1963, i.e. after spraying was introducted. But 5 years after spraying ceased, i.e. in 1969, the number of deaths had climbed to 113, and the number of cases to 500,000. Selective house spraying with DDT has restored some of the control on malaria. The WHO estimates that during the 20 years of widespread use of DDT, the lives of some 100 million people living in Africa, Asia, and South America were saved.

The DDT example shows the dimensions and the dilemma of risk management decisions. Proven and assumed chronic damage of humans and animals, especially in the USA and Europe, led to a ban, which, while it may have been appropriate for the first world where malarial treatments and expensive alternative insecticides are available, caused disease and death of people in poorer, less developed countries for whom costs are critical.

It also illustrates that, generally, prevention is better than cure when examining risk management measures. Meanwhile, the situation is further complicated by the observation that DDT-resistant insects have developed.

## Preventive Measures

Preventive measures include the assessment of possible environmental- and health impacts during the planning stage of projects involving potential exposure to chemicals and deciding on the acceptability or otherwise of the proposal, either for the projected facility or for the surrounding population. These may include

possible considerations as to where to site a manufacturing or storage facility that is a potential major accident hazard (e.g., a chemical plant) and/or whether a (usually brownfields) site is contaminated by chemicals and requires remediation. Prevention measures (e.g., "stay indoors" while the toxic chemical disperses) may also be a part of emergency planning aimed at mitigating effects should an incident involve release of substantial amounts of a hazardous chemical. It is worth noting that farm wastes, notably those from animal housing facilities, require particular care as they can result in poisonous gasses and vapors (hydrogen sulfide, carbon monoxide, and carbon dioxide) being emitted in confined spaces (e.g., slurry tanks), yet agricultural buildings may be subject to less onerous planning requirements.

A **health impact assessment** (HIA) should be performed when major construction projects involving toxic agents are planned, such as town-, traffic-, or airports projects, waste incineration plants, wind farms or tanks containing chlorine, ammonia, and phosgene.

Generally, the public health and environmental services are responsible for the assessment of health effects on humans. Health and environmental impact assessments are effective in the context of planning. Although they do not prevent preexisting dangers, they can also help in developing emergency procedures.

It is understandable that questions about scientific methods and administrative procedures used in toxicological risk analysis conducted by regulatory authorities are often controversial. Many of the methods used involve judgements. Topics of dispute may be the judgements concerning the quality of the data and its interpretation, judgements involved in the qualitative or quantitative risk characterization, judgements concerning the prognosis, and, especially, judgements in the risk-benefit evaluation and the attitudes to risk of the stakeholders. Many of the methods must be worked out on an international level and harmonized without increasing the administrative overhead. Actual criteria should be based on local conditions. Clearly, the people carrying out the risk assessment and management need to be properly educated and trained.

## Recommended Reading

European Commission (1996) Technical guidance documents in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 1488/94 on risk assessment for existing substances. CEC, Ispra

European Commission (1998) Technical guidance document on development of risk reduction strategies. Office for Official Publications of the European Communities, Luxembourg

Hertel RF (2003) Behördliche Risikokommunikation. Diskursives Verfahren. Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 46:586–591

OECD (2000) Framework for integrating socio-economic analysis in chemical risk management decision making, vol 13, Series on risk management. OECD Environmental Health and Safety Publications, Paris

OECD (2002a) Technical guidance document on the use of socio-economic analysis in chemical risk management decision making, vol 14, Series on risk management. OECD Environmental, Health and Safety Publications, Paris
OECD (2002b) Guidance document on risk communication for chemical risk management, vol 16, Series on risk management. OECD Environment, Health and Safety Publications, Paris

## Internet

Europa, Regulations. http://europa.eu.int/eur-lex/en/search_oj.html
Reach Regulation. http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm
The Presidential/Congressional Commission of risk assessment and risk management. http://www.riskworld.com

# Assessment of Limit Values in Regulatory Toxicology

Hermann H. Dieter

## Contents

Types of Exposure ..... 628
Criteria to Limit Exposure ..... 629
Spheres of Interest when Setting Legal Limit Values ..... 630
Rule of Environmental Hygiene (REH) ..... 631
REH-Based Criteria for Evaluating Exposure from Chemical Loads ..... 632
Framework or Conditions of Social Evaluation: Prevention, Optimization/Acceptance, and Minimization/Tolerance of Exposure ..... 632
Holistic Concept for Evaluating Chemical Loads or Exposure in a Triangle of Prevention, Rejection, and Acceptance/Tolerance ..... 632
Concrete Evaluation of Annoyances, Loads, and Risks Within the Triangle of Prevention, Rejection, and Tolerance/Acceptance ..... 634
Definition of Precautionary Limit Values According to Origin A–C of Compounds ..... 636
Class A, Geogenic/Biogenic: Natural Constituents Without (A1) or After Treatment (A2) of the Resource ..... 636
Class B, Anthropogenic: Additives and Their Technically Unavoidable Residues and Side or Transformation Products ..... 638
Class C, Anthropogenic: Environmental Contaminants and Their Transformation Products ..... 639
Setting and Evaluation of Legal Limit Values by Means of the REH ..... 640
Drinking Water Limit Values from the EU as an Example ..... 640
Categorization of Legal LVs in Terms of General Precaution, Early Warning, Control of Concern, or Control of Hazard ..... 643
Summary: A Short Directory to Quantify and Survey Precautionary LVs ..... 645
Conclusions ..... 646
Signs and Abbreviations ..... 647
Recommended Reading ..... 648

H.H. Dieter
Department of Toxicology of Drinking-Water, Federal Environment Agency of Germany/Umweltbundesamt (UBA), Dessau-Roßlau, Germany
e-mail: hh.dieter@t-online.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_80, © Springer-Verlag Berlin Heidelberg 2014

**Abstract**

Limit values are legal concentration limits for chemical compounds at work, in the environment, in food, in cosmetics, in medicinal products, and so on. There are different rationales for each, but all are equally binding, both negatively and positively, for concerned persons and interest groups. These values are in constant danger of being attacked as either too stringent or too lax. For this reason, only concentration limits that have gained a maximum of societal consent by means of a transparent, politically organized process should become legally enforced. Such consent is most likely to be reached on the basis of the three-dimensional rule of environmental hygiene (REH): avoid useless load/exposure, optimize functional load/exposure, and prevent harmful load/exposure.

## Types of Exposure

The criterion of "avoidability" primarily involves anthropogenic contaminants, whereas "unavoidability" concerns geogenic/biogenic exposure. With respect to tolerability (tolerance threshold T), the following regulatory differentiations must be made: on the one hand, there are the avoidable = anthropogenic exposures B and C, with their – if functional – threshold concentration $F_B > 0$ for optimal on-site technical function and $F_C \geq 0$ (remote environmental), representing the minimum concentration required to guarantee a compound's B desired on-site technical functionality (list of abbreviations for this essay is at the end of this assay). Such exposure, provided that it is kept below a (presumed) threshold Ea of concern about adverse effects, is either accepted at $F_B > 0$ or at $F_C = 0$, respectively, but only tolerated at levels of $T_B \geq F_B$ and $T_C \geq F_C$.

The situation is different for the virtually unavoidable = biogenic/geogenic exposure A from the use of a natural resource. Here, the ratio between upper limit of expected background exposure ($BG_A$) and Ea is the main criterion for whether a resource A1 ($Ea \geq BG_{A1}$) might either be usable without treatment up to a $F_{A1} \leq Ea$ or, with Ea being $< BG_{A2}$ (A2), only after reducing the compound's A2 concentration in the raw material or resource down to a tolerance threshold for its technical avoidability (elimination) of $T_{A2} < Ea$.

The following conditions for *maximum* limit values LV (maximum legal concentration limits) accepted by society as a whole can therefore be derived from the rule of environmental hygiene (REH):

*Compound class A* (*geogenic/biogenic*):

*Class A1* ($Ea \geq BG_{A1}$)

LV = threshold of the resource's usability or $LV = F_{A1} = Ea$ (A1)

*Class A2* ($BG_{A2} > Ea$)

LV = threshold of technical avoidability (elimination) or $LV = T_{A2} \leq Ea$ (A2)

*Compound class B* (*anthropogenic*, *functional*, $F_B > 0$):

LV = threshold for functionality or $LV = T_B \leq Ea$

*Compound class C* (*anthropogenic*, *nonfunctional*, $F_C = 0$):

LV = threshold for tolerance or LV = $T_C \leq \leq$ Ea (any remote $T_C$ ought to be fixed as closely as possible to $F_C = 0$)

This classification from A to C also helps to rationalize how intensely a final product needs to be surveyed on constituents (A), residues (B), and contaminants (C). Class A compounds rarely need short-time interval surveillance. On the other hand, class B compounds need continuous surveillance at the point of on-site functional and intended addition/effect, and C compounds are preferably surveyed at more or less remote points of unintended environmental penetration.

The concept of consensual legal limit values outlined here is not only a tool for a responsible environmental policy and surveillance. It is also an instrument that may help avoid "adverse effects" at the societal level by enabling the different interest groups to communicate with each other in a civilized and organized manner.

## Criteria to Limit Exposure

Limit values (LV) are legally binding limit concentrations for chemical or other parameters in technical compartments and environmental media including food and drinking water. They have proved to regulate use and handling of chemicals and of many other noxa within all compartments of the environment and human life. They eventually quantify the *societal readiness to pay* when reducing, minimizing, or avoiding useless risks or loads on a *maximum level* and admitting functional ones on a minimal but functionally unavoidable and, hence, *minimum* technical level.

Toxicologists, health professionals, ecologists, environmental technicians, and engineers propose to legislators options on how to substantiate necessity, nature, and numerical amount of legal limit values in the form of:

- Maximum values as derived based through science (e.g., toxicology, medicine, ecology)
- Maximum values as derived technically (functional technology and to avoid useless loads)

An ideal, politically set limit value:

- Represents the regulatory equivalent of a science-based maximum value
- Originates from a societal process of decision that is transparent and knowledge directed

When trying to scientifically find or define tolerable or acceptable maximum values for potentially harmful compounds (or to find minimum functional values for beneficial compounds), the following protection goals and corresponding options for management of their protection should be considered:

- Health of humans and their protection from illness
- Nonhuman organisms/intact ecosphere or compartments thereof
- Technical devices and equipment
- Cultural monuments and cultural traditions/customs
- Usability of natural resources
- Sensorial and aesthetic expectance of quality

It is clear that, within these different domains, different options are conceivable to support a maximum value for a single compound. Any concrete numerical value will vary in correspondence. As a consequence, depending on the political motivation (aim of protection), in different compartments, numerically diverse limit values are possible and reasonable to trigger regulatory interventions or political action. The nature and numerical amount of a maximum value, as well as the density and quality of knowledge that support it, may also depend upon the following four categories of environmental protection (see section below "Categorization of Legal LVs in Terms of General Precaution, Early Warning, Control of Concern, or Control of Hazard"):

1. *General precaution* to prevent any possible concern
2. *Early warning* about possible concerns in the near future
3. *Control* of present concerns
4. *Control* of imminent hazards

A maximum value may be allocated. According to circumstances, its concrete determination follows either:

- The underlying *scientific rationale* of a maximum value (background, warning about concern, threshold of concern, threshold of imminent hazard)
- The underlying *political interpretation* of a scientific maximum value (precaution, early indication of possible concern, control of concern, control of hazard)

Any scientific and politically (mis)interpreted maximum value is open to becoming judicially binding in the form of a legal limit value.

## Spheres of Interest when Setting Legal Limit Values

Only those maximal and minimal levels referring to societal acceptance of quality and quantity should be fixed as LVs. Such acceptance is only reached by relying on a transparent and knowledge-based societal process on how to decide which chemical or parameter must be regulated and what the subject (precaution/repair, intended/unintended, voluntary/involuntary exposure) of its regulation should be.

The central field of tension of Fig. 1 is the location of dispute and argument between:

– Political experts (e.g., members of parliament), representing the public interest for avoiding any load above socially (legally) accepted *thresholds of tolerance* $= T$.
– Scientific experts, representing the interest of science to specify *thresholds for concern about adverse effects* $= Ea$ (or corresponding risks) only if supported by correspondingly sufficient database(s). In human toxicology, as a rule, the Ea represents defined fraction of an ADI or TDI value (see section "Threshold of Concern for Adverse Effects, Ea").
– Experts from the private sector, representing the latter's interest for public acceptance of private benefit at technically consensual or optimized *functional thresholds* $= F$.

This complex situation is summarized by European Environment Agency (2002): "Compartmentalised science, no matter how erudite, is an insufficient base for knowing enough to anticipate or mitigate the impacts of such systems: integrated and synthesised knowledge, which pools the wisdom from many natural and social sciences, is a necessary condition for being *Homo sapiens*."

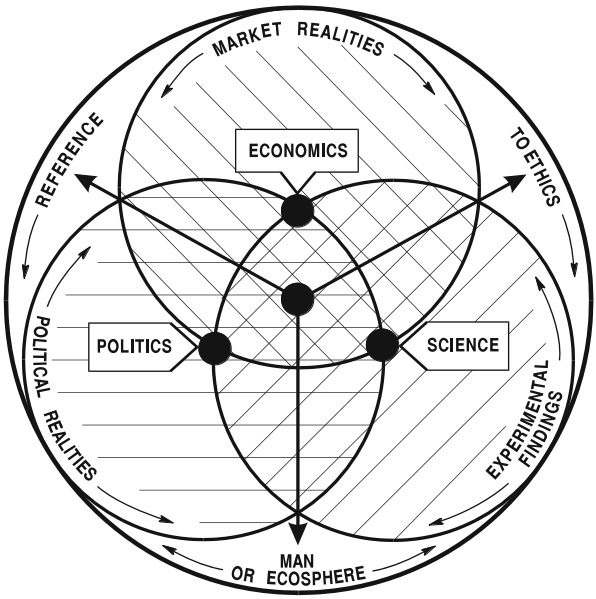



**Fig. 1** Common and separated spheres of interest (overlapping areas of circles) represented by politics, the private sector, and science regarding protection of humans (health and environment) and/or ecosphere (protection of nature and its diversity). The success of consensual societal setting of LVs is possible only in the central field of tension between experimental realities (scientific considerations), market realities (beneficial and economic considerations), and political realities (consideration of democratic minorities and majorities) (Dieter et al. 1997)

Therefore, within the dynamics of expert panels, often only lower than purely *scientifically based and effect-related thresholds of concern* are open to consensual (societal) acceptance in form of a LV. Such "lower" limit values then serve (not only) to protect human health but also to reach more ambiguous technical, aesthetic, or even nonanthropocentric aims of protection.

An optimal agreement in favor of an as-low-as-possible load on humans and the environment would correspond with the actual level of technical-scientific knowledge (LK) to avoid useless loads as a precaution. Less ambiguous limit values would reflect the state of technology (StT) or at least the generally accepted rules of technology (gaRT). Any LV should be set referring to the three-dimensional.

## Rule of Environmental Hygiene (REH)

- *Minimize useless load/exposure*. Its upper limit is *tolerated* as T = threshold of tolerance.
- *Optimize functional load/exposure*. The minimal level to guarantee intended function is F = functional threshold. F is *accepted* if set according to LK; any higher F (StT, gaRT) is *tolerated* only.
- *Prevent harmful load/exposure*. Its upper limit is Ea = threshold of concern about adverse effects.

Observance of this REH not only helps to define precautionary limit values below any Ea, thus promising not only minimal necessary or even zero exposure, but also provides a perspective on a holistic social management of environmental noxa. Its result would prove to be not only socially acceptable but also harmless from a societal point.

## REH-Based Criteria for Evaluating Exposure from Chemical Loads

### Framework or Conditions of Social Evaluation: Prevention, Optimization/Acceptance, and Minimization/Tolerance of Exposure

The regulatory framework to apply the REH and its evaluation criteria are the (social or technical) avoidability, the (technical or beneficial) functionality, and the (ecological, technical, aesthetic, or health related) concern over adverse effects of the environmental load under question. This framework must be delimited early on, before the factual risk assessment process.

Loads, if avoided, put aside the necessity to assess their risks. The first step before any balancing of social, beneficiary, or scientific interests when quantifying a limit value would be, at best, a decision on whether the load or exposure under question seems avoidable or not. Avoidable loads are rejected; unavoidable ones are either accepted or (temporarily) tolerated.

The decision on the tolerability of a useless load or the acceptance of a functional load should never be dramatized or trivialized by science nor be forced or prevented by economy. Each decision should follow a political rationale and be publicly defendable by referring to proven facts, functionalities, and identified opinions of majorities and minorities.

*Scientists and technologists*, for example, provide input to politicians with data on natural background loads, on technical or other options for avoiding exposure, or on the slope and shape of a dose/response relation.

*Sociologists or psychologists*, on the other hand, have to determine what is the best way in a democratic society to make informed decisions that then may possibly be recognized and followed by as many (but rarely all) members of society as possible. From this societal view, that is to say, not so much the potential harmfulness of a load but much more its origin and concurrent avoidability give reason for conflict, although the clashing parties often prefer to look desperately for (mostly arguable) scientific arguments in their favor.

### Holistic Concept for Evaluating Chemical Loads or Exposure in a Triangle of Prevention, Rejection, and Acceptance/Tolerance

The point of origin or source of any anthropogenic load is always bound to a functional value or intention "on-site" in close proximity of the source. The same load, after its environmental transport to a point "off-site" or distant from the source, will have lost its functional aspect there, seeming dispensable at its place of detection. In contrast, this observation does not apply on unavoidable geogenic and biogenic loads, independent of their functional value or intentional context.[1]

[1] Culturally conserved or protected loads or exposure (e.g., from natural foodstuffs, certain habitualities of feeding, or from processing of food) occupies a complicated medium position and will not be discussed here.

This *first step* of evaluating or identifying a load's origin opens the option to principally reject any dispensable anthropogenic load but to principally accept or at least tolerate any geogenic/biogenic load.

However, in a *next step*, it is necessary to ask immediately for or define the possible net functional value of any load from an anthropogenic origin since, from case to case, albeit principally avoidable, such load may have been already accepted on-site at an optimized (hopefully minimized yet fully functional) level.

Neither of these two decision steps is formally regulated at the societal level. Instead, the corresponding decisions on which potentially dangerous load may appear as avoidable and which one as functional as a rule are arbitrarily forced by economy and underlined later by science, although consensual answers on which load might be "avoidable" very often could be found much easier than (often speculative) scientific answers on concerns over the adversity in a given situation of a possibly avoidable (!) load or exposure. Such scientific decisions are sought (but not always found) in a *third step*, called risk assessment. This step, although often done too late (EEA 2002), is well established at the societal level. It describes and quantifies the harm(lessness) of two classes of compounds:

- *Class C of anthropogenic compounds*: These are found in "off-site" compartments, mostly environmental and distant from their primary functional use. They are therefore called *environmental contaminants*, minimized down to what are hoped are precautionary level(s) by LK or ALARA, respectively, of emission control and treatment/elimination. Examples of class C compounds are plant protection products if present *in drinking water*, HAMR if present in *surface water*, or industrial chemicals if present in *waste water*.
- *Class B of anthropogenic compounds*: These are found in "on-site" compartments, mostly technical, being close to their primary functional/intentional use. They comprise *workplace agents and additives*, *including their residues and side products*, hopefully minimized to a still fully functional level $F_B > 0$ by LK of functional optimization or (in case of side products) respecting such level. Examples of class B compounds are additives to conserve *food* and chlorine to disinfect *drinking water*, including unavoidable side products from disinfection or conservation.

In both classes, load or exposure are bound to *never exceed an Ea = threshold of concern about adverse effects* or to exceed an *accepted risk*, respectively. Exposure is neither allowed to damage the aim of protection nor to violate it at any possible lower level, the only exemption being a situation where a hopefully extremely high, mostly individual functional exposure is deemed to outweigh any corresponding health or functional damage or annoyance.

Close to the concrete place of their functional use and depending on the concrete binding force of exposure levels referring to LK, StT, or gaRT, respectively, *class B compounds* are tolerated by society at different minimized limits of tolerance, called *tolerance thresholds* $= \mathbf{T_B} \geq F_B > 0$. The lowest yet still technically feasible $T_B = F_B > 0$ does conform with LK and would be *accepted* as functional

by society instead of being tolerated only as would be the case if $T_B > F_B$. In any case, on this second step (see above) of the societal decision process, regulators or managers would have to allow for implementing the same or a similar functional idea only if not linked with any "unacceptable" exposure $T_B > 0$.

*Class C compounds*, despite being often structurally identical or closely related to those that are also found in class B, by definition are ascribed or linked only to places and compartments where they are devoid of any functional value ($F_C = 0$). This is the reason why they never can be *accepted* there. At best, according to the binding force of levels to be defined by LK, StT, or gaRT, they may or may not even be *tolerated* at minimized limits *called tolerance thresholds* $T_C > 0$ *or even* $T_C = 0$ at/in such places and compartments.

A third class of compounds that should be clearly differentiated by management criteria from classes B and C with regard to their avoidability in a given raw material or resource are geogenic/biogenic loads or exposure. They are encompassed in the following as:

– *Class A compounds*, comprising natural constituents and their technically unavoidable transformation products.

Natural constituents, if identified or proven as potentially harmful and present at levels > Ea, are reduced by treatment down to a (technical) *tolerance threshold* $T_A <$ Ea. The numerical value of $T_A$, however, depends strongly on societal willingness to pay either for applying LK, StT, or simply gaRT. Examples are the elimination of inorganic arsenic from drinking water to levels far below its Ea = 10 μg/l, measures to avoid, by appropriate storage and preparation of food, the formation there of analytically detectable levels of aflatoxins, nitrosamines, or acrylamide, or the minimization of natural radioactivity , especially radon, in buildings by technical or structurally engineered measures. The *threshold of usability* $F_A$ of such resource or space is reached at the latest if $T_A = \text{Ea} = F_A$; values of $F_A >$ Ea (and the corresponding resource) are then rejected.

The definitions material to understanding the process of defining and quantifying precautionary limit values are assembled in Table 1.

## Concrete Evaluation of Annoyances, Loads, and Risks Within the Triangle of Prevention, Rejection, and Tolerance/Acceptance

The numerical amount for the threshold Ea of concern, in contrast to numerical amounts of the different categories of F and T, should, in principle, be based on strictly scientific data. This is why Ea, if either the societal tolerance of a load or exposure or the acceptance of its functional value would cease to exist, never shifts downwards nor upwards, whereas such shifting of Ea could happen when "changing" or reevaluating the protection goal's sensitivity or its societal valuation.

It is to be noted here that individual perception and evaluation of any "objective" risk, be this a merely supposed or an actually measured one, varies strongly with the absence or presence of a personal benefit from the same exposure and its subjective evaluation. Any subjectively correct Ea would vary accordingly, more so in cases

**Table 1** Decisive maximal (max) and minimal (min) concentrations or doses of chemicals to be considered when setting precautionary limit values

| Designation of maximal or minimal value | Symbol | Differentiation according to class of origin A, B, or C (see text) A | B | C | Definition |
|---|---|---|---|---|---|
| Threshold (max or min) to suboptimal functionality | **F** | | $F_B$ | | Anthropogenic *on-site* concentration $F_B > 0$ of a functional chemical or its residues in a final product |
| | | | | $F_C$ | Anthropogenic *remote* concentration $F_C \geq 0$ of a contaminant C or its metabolites in a final product |
| | | | | | (a) *Max*: *above* which a corresponding B compound could not be allowed for use *on-site* even when applying there StT |
| | | | | | (b) *Min*: *below* which a corresponding B compound would be excluded for use *on-site* even when applying there StT, the lowest desirable $F_C$ being 0 |
| **Threshold** (max) **to non-usability** | | $F_A$ | | | Geogenic concentration of a natural constituent A *above* which a resource would not be usable prior to treatment since $BG_A > Ea$ |
| **Threshold** (max) **to non-tolerability** | **T** | | | | Tolerated and/or accepted |
| | | $T_A$ | | | • *Supraregional* geogenic background concentration of a natural constituent ($T_A \geq BG_A > 0$)[a] |
| | | | $T_B$ | | • Anthropogenic o*n-site* ($T_B \geq F_B > 0$) concentration of a functional chemical or residue from it |
| | | | | $T_C$ | • Anthropogenic *off-site* ($T_C > F_C \geq 0$) concentration of a functional chemical or its metabolites/transformation products if presenting, e.g., at the same time as a contaminant of drinking water |
| **Threshold** (max) **for concern about adverse effects**[b] | **Ea** | No differentiation between classes of origin A, B, and C | | | Concentration or dose threshold above which the usability of a chemical B or of a natural resource containing a constituent A or a contaminant C would give reason for concern about adverse effects in the goal of protection[b] |

[a]$BG_A$ represents a background concentration below $T_A$ in any *regional* resource under specific consideration. In order to become usable, such regional resource would need elimination of a class A compound down to $\leq$Ea only if $BG_A > T_{A1}$ with $T_{A1} = Ea$. As long as treatment down to at least $T_{A2} = Ea$ would not seem possible or affordable, respectively, such resource remains not usable

[b]This chapter deals preferentially with potentials of threshold adversity as quantified by human toxicology. There exist, however, effect thresholds the exceeding of which could result in technical or esthetic (color, odor, taste, purity) adversity or impairments/discomfort/annoyances

where its scientific database appears vulnerable to being denunciated as not sufficient. Therefore, if a load needs to be evaluated on an insufficient or "patchy" database, the only consensual way to proceed is to look for a level of Ea being not higher (albeit possibly lower) than a level seeming eventually quantifiable later on a then-sufficient database.

In most cases, exposure to class B compounds results in relatively high levels that are similar to exposure to class A compounds. In the absence of formal requirements to evaluate class A compounds, their toxicological database as a consequence, similar to class C, is often incomplete. Possible A risks are correspondingly often difficult to quantify but accepted more easily than B and C risks inasmuch as they appear more difficult to avoid than the latter.

Moreover, surveillance of compliance of LVs for A compounds is not necessary at the same (and high) frequency as advisable for B compounds. If the raw material or resource has been selected properly, concentrations of A compounds can be supposed to be constant, whereas concentrations of B compounds in a final product may easily be subject to technical change and failure. Finally, surveillance of C compounds should preferably be performed at the point of their environmental input and be eliminated there and not only after having reached a critical raw material or resource.

In any case, Ea turns out to be the only but at the same time also the maximal point of reference (health-related guide value, HRGV) to be considered when looking for a decision about which numerical amount of the different lower and much lower $F_A$, $T_A$, $F_B$, $T_B$, $F_C$, or $T_C$ might appear as acceptable or tolerable to be set and surveyed in whichever frequency as a legal limit value. The numerical amount of Ea is the only merely scientific one of all these levels. It depends neither on an exposure's or loads' anthropogenic or geo/biogenic origin nor on whether such load or exposure may be functionally accepted or just tolerated as nonfunctional but (temporarily) unavoidable.

## Definition of Precautionary Limit Values According to Origin A–C of Compounds

### Class A, Geogenic/Biogenic: Natural Constituents Without (A1) or After Treatment (A2) of the Resource

Group A compounds, according to their natural (perceived or analyzed) background concentration $BG_A$, the defensible effort for treatment, and the technical or health-related benefit resulting from such effort, are either eliminated from a natural resource or tolerated and even accepted, respectively.[2]

[2] The criteria for whether and how to perceive geogenic/biogenic loads as harmful or harmless refer not only to their scientific evaluation but also to value systems coming from society or cultural history.

*By using the respective conceptual definitions from* Table 1, *two relations are obtained to define precautionary limit values* (*LV*) *for class A compounds in a natural raw material or resource*: *one relation A1 for geogenic/biogenic compounds in resources without treatment and a second one* (*A2*) *after their treatment to eliminate class A compounds*.

*Definition of precautionary limit values for class A1 compounds* ($LV_{A1}$; *without treatment*):

$$0 < BG_{A1} \leq F_{\mathbf{A1}} = \mathbf{T}_{A1} \equiv \mathbf{LV_{A1}} = Ea$$

The sign "≡" in this relation denominates the conceptual and numerical identity of $T_{A1}$ from remark a) under Table 1 with the compound's legally tolerated (politically set) and mainly *natural background/health-related* limit value $LV_{A1} = Ea$ in the untreated final product. The compliance of the compound's natural concentration $T_{A1}$ in the final product with $LV_{A1}$ indicates social acceptance ($T_{A1} = BG_{A1}$) or at least tolerance ($T_{A1} = F_{A1}$) for utilization of natural raw material or resource, even if not treated to eliminate the critical compound.

The maximum admissible load, that is to say, the *highest possible limit value* $LV_{A1}$ for accepting the exploitation of a natural resource without treatment, is reached if $T_{A1} = F_{A1} = LV_{A1} = Ea$.

On the other hand, by scaling the environmental quality of the $LV_{A1}$ using the criteria offered by the REH, the decisive maximal concentration to be consensually chosen for $LV_{A1}$ from this relation is the lowest possible concentration placed left from the sign "≡." Therefore, only if $LV_{A1} = BG_{A1}$ may the former be denominated the *lowest possible precautionary limit value* $LV_{A1}$ to limit legally the compound's concentration in the untreated resource on its natural 50-, 90-, or any other percentile of background level (percentiles to be calculated by science and set by politics).

If weighting the relations "<" and "≤" and "=" left from any LV by 2 or 1 or zero points, respectively,[3] the left $WS_{A1}$ takes a fraction of 3/3. This 100 % *left WS fraction*, if evaluating class A1 compounds according to the REH, as a rule anticipates numerical identity with Ea of a REH-compatible *precautionary* $LV_{A1}$.

*Definition of precautionary limit values for class A2 compounds* ($LV_{A2}$; *after treatment*):

[3] This "rule of weighting" implies the condition that in this and the following relations, the total weighting sum WS of all signs left and right from the LV is set on 100 %, respectively. The differential distribution of the signs left and right the LV describes the expected numerical differences between the respective concentrations of exposure if regulated according to the REH.

$0 \leq F_{\mathbf{A2}} = \mathbf{T}_{A2} \equiv \mathbf{LV}_{\mathbf{A2}} \leq Ea < BG_{A2}$

The sign "≡" in this relation denominates the conceptual and numerical identity of $T_{A2}$ from remark a) under Table 1 with the compounds legally tolerated (politically set) and mainly *technical/treatment-related* limit value $LV_{A2} \leq Ea$ in the treated final product. The compliance of the compound's concentration $T_{A2}$ after treatment in the final product with $LV_{A2}$ indicates social acceptance ($T_{A2} = 0$) or at least tolerance ($T_{A2} = F_{A2}$) for the latter's utilization only if treated to eliminate the critical compound down to $T_{A2}$ or lower.

The maximum admissible load, that is to say, the *highest possible limit value* $LV_{A2}$ for accepting the exploitation of a treated natural resource, is $T_{A2} = F_{A2} = LV_{A2} \leq Ea$. On the other hand, by scaling the environmental quality of the $LV_{A2}$ using the criteria offered by the REH, the decisive maximal concentration to be consensually chosen for $LV_{A2}$ from this relation is the lowest possible concentration placed left from the sign "≡." Therefore, only if $LV_{A2} = 0$ may the former be denominated the *lowest possible precautionary limit value* $LV_{A2}$ to limit legally the compound's concentration in the treated resource, the technically lowest possible value of $T_{A2}$ being a function of whether the raw material or resource was treated according to *accepted* LK or *tolerated* StT and gaRT, respectively.

The *rule of weighting* (*see with definition A1*) results in a $WS_{A2}$ left fraction of $1/4 = 25$ %. This *very low left fraction of* WS, if evaluating A2 compounds by applying the REH and LK for their technical elimination, anticipates only a rare numerical identity with Ea of a REH-compatible *precautionary* $LV_{A2}$.

## Class B, Anthropogenic: Additives and Their Technically Unavoidable Residues and Side or Transformation Products

These compounds deliver in their intentional and mostly technical target compartments either an *accepted* function or their presence there is linked with such function in a technically unavoidable manner.

*By using the respective conceptual definitions from* Table 1, *the following relation to define precautionary limit values* (*LV*) *for class B compounds in their mostly technical target compartments is obtained*:

$0 < F_B \leq \mathbf{T_B} \equiv \mathbf{LV_B} \leq Ea$

The sign "≡" in this relation denominates the conceptual and numerical identity of $T_B$ from Table 1 with the compound's legally tolerated (politically set) and mainly *technical/function-related* limit value $LV_B \leq Ea$ in the final product or technical compartment. The compliance of the compound's concentration $T_B$ with its $LV_B \leq Ea$ in the final product or technical compartment implies social *tolerance* for the compound's functional use if $T_B > F_B$ and *acceptance* if $T_B = F_B$.

The maximum admissible load, that is to say, the *highest possible limit value* $T_B = LV_B \leq Ea$ for tolerating the functional value of a class B additive/residue/side product, is reached if $T_B = Ea$. On the other hand, by scaling the environmental quality of the $LV_B$ using the criteria offered by the REH, the decisive maximal concentration to be consensually chosen for $LV_B$ from this relation is the lowest possible concentration placed left of the sign "≡"; hence, $F_B = T_B \leq LV_B \leq Ea$. The $F_B$ below which the compound's *accepted* function would no longer be realizable corresponds to LK. An $LV_B = F_B$ is the *lowest possible precautionary legal value* to limit a B compound's concentration in the final product. Values between $F_B$ and Ea are called $T_B$ and *tolerated* in case of applying only StT or gaRT.

Applying the *rule of weighting* (*see with definition A1*) results in a $WS_B$ left fraction of 3/4 = 75 %. This *high left WS fraction*, if evaluating class B compounds by applying the REH and LK for their functional use on-site, anticipates a frequent (between A1 and A2) numerical identity with Ea of a REH-compatible *precautionary* $LV_B$.

## Class C, Anthropogenic: Environmental Contaminants and Their Transformation Products

These compounds deliver in their mostly environmental yet unintentional target compartments neither an *accepted* function nor is their presence there linked directly with such function.

*By using the respective conceptual definitions from* Table 1, *the following relation to define precautionary limit values* (*LV*) *for class C compounds in environmental compartments is obtained*:

$0 = F_C \leq T_C \equiv \mathbf{LV_C} \leq \leq Ea$

The sign "≡" in this relation denominates the conceptual and numerical identity of $T_C$ from Table 1 with the compound's legally tolerated (politically set) limit value $LV_C \leq \leq Ea$. The compliance of the compound's concentration $T_C$ with its $LV_C \leq \leq Ea$ in the environmental yet unintentional target compartment implies only social *tolerance* for the compound's presence there ($T_C > F_C = 0$) and *acceptance* only for its absence ($T_C = F_C = 0$).

The maximum admissible load, that is to say, the *highest possible limit value* $T_C = LV_C \leq \leq Ea$ for tolerating the presence of a class C compound in a "remote" environmental compartment, is reached if $T_C = Ea$. On the other hand, by scaling the environmental quality of the $LV_C$ using the criteria offered by the REH, the decisive "off-site" maximal concentration to be consensually chosen for $LV_C$ from this relation is the lowest possible concentration placed left of the sign "≡"; hence, $F_C = 0 = LV_C \leq \leq Ea$. If, with a $LV_C = 0$ in a remote compartment, the compound's *accepted* on-site function would no longer be realizable even by using LK, any values of $T_C$ between $N_C$ and Ea may be *accepted* if applying on-site LK but would be only *tolerated* if only StT or gaRT would be applied on-site.

Applying the *rule of weighting* (*see with definition A1*) results in a $WS_C$ left fraction of 1/3 = 33 %. This *low left* WS fraction, if evaluating class C compounds by applying the REH and LK for their functional use on-site as B compounds, anticipates only an occasional numerical identity with Ea of an REH-compatible *precautionary* $LV_C$.

## Setting and Evaluation of Legal Limit Values by Means of the REH

### Drinking Water Limit Values from the EU as an Example

Using drinking water as an example, Table 2 demonstrates how to become informed on whether or not a real limit value was set and quantified directly as a precautionary value by using the REH-based relations above. The legal limit values in column 4 represent a society's eventual expression of tolerance or acceptance (LV ≡ T, column 4) of exposure (column 1). These LV ≡ T are compared respectively and in accordance with the compounds' class of origin, with their Ea in column 5 and the author's proposal for their numerical $F_B$ or their $F_C$ in column 6. Two partial evaluations in columns 5a and 6a of the LV in column 4 are obtained, their absolute amount depending on whether the LV under evaluation is (much) larger, similar, or (much) smaller than the respective compound's value of Ea and of $F_B$ or $F_C$. Each sign ">" ("<") counts for 2 negative (positive) weighting points, each "≥" ("≤") for 1 negative (positive) point, and "=" counts for ± 0. The net total sum of all partial

**Table 2** Examples of REH-based evaluation (col. 7) of parametric values = limit values LV (col. 4) from EU "Drinking-Water" Directive 98/83/EEC. For details see definition A, A1, B, and C of LVs in the text and remarks at end of table. Numbers for T- and F-values as proposed by author

| **Evaluation** → / ↓ **Compound** (class of origin) | Column 2 **highest allowable threshold = Ea** (here: health-related guide value[a]) for drinking-water) | Column 3 **lowest possible threshold F** according to LK and class of compounds *Dimension: col. 1* | Column 4 **tolerance threshold T** as given by EU-Directive in form of **LV≡ T** *Dimension: col. 1* | **Evaluation of LV ≡ T from column 4** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Column 5 **comparison** of Ea (col. 2) with LV (col. 4) | Column 5a **+bonus/-malus** for LV in col. 4 by comparison with T in col. 5 | Column 6a **+bonus/-malus** for LV in col. 4 by comparison with F in col. 6 | Column 6 **comparison** of F (col. 3) with LV (col.4) | Column 7 **Total bonus (+) or malus (–)** from evaluations **in col. 5a + 6a** of **LV≡T** (col. 4) | |
| | | | | | | | | **Bonus *plus* Malus** from summing col.s 5a and 6a | **corresponding qualitative evaluation of the LV in col. 4** |
| **Arsenic** (A2) | 10 µg/l | $F_{A2}$[b] = **Ea**= 10 | T ≡ **LV** = 10[c] | **Ea** = T → | **±0** | **±0** | ← F = T | **±0** | **LV✓?** |
| **Lead** (B) | 10 µg/l | $F_B$ = 40 | T ≡ **LV** = 40[d] | **Ea** << T → | **–2** | **±0** | ← F = T | **–2** | **LV ↓↓!**[d] |
| **Bromate** (B) | 0.3 µg/l | $F_B$ = 10 | T ≡ **LV** = 10 | **Ea** << T → | **–2** | **±0** | ← F = T | **–2** | **LV ↓↓!** |
| **Cadmium** (A1) | 3 µg/l | $F_{A1}$[b] = **Ea** = 3 | T ≡ **LV** = 5[a] | **Ea** < T → | **–1** | **–1** | ← F < T | **–2** | **LV ↓↓!**[a] |
| **Cyanide** (A1) | 50 µg/l | $F_{A1}$ = $BG_{A1}$[e] < **Ea** | T ≡ **LV** = 50 | **Ea** = T → | **±0** | **–1** | ← F < T | **–1** | **LV ↓!** |
| **1.2-Dichloro-ethane** (C) | 3 µg/l | $F_C$ = 0.00 | T ≡ **LV** = 3 | **Ea** = T → | **±0** | **–1** | ← F < T | **–1** | **LV ↓!** |
| **Fluoride** (A1) | 1.5 mg/l | $F_{A1}$[b] = **Ea** = 1.5 | T ≡ **LV** = **Ea** | **Ea** = T → | **±0** | **±0** | ← F < T | **±0** | **LV✓?** |
| **Copper** (B) | 2 mg/l | $F_B$ = 2 | T ≡ **LV** = 2 | **Ea** = T → | **±0** | **±0** | ← F = T | **±0** | **LV✓?** |
| **Chlorinated solvents** (C) | 20←→100 µg/l | $F_C$ = 0.00 | T ≡ **LV** = 10.0 | **Ea** > T → | **+1** | **–1** | ← F < T | **+0** | **LV✓?** |
| **Manganese** ($Mn^{+2}$) (A2) | 1.0 mg/l (E) 0.2 mg/l (**Ea**) | $F_{A2}$[b] = **Ea**[f]= 0.05 | T ≡ **LV** = 0.05[c] | **Ea** = T → | **±0** | **±0** | ← F = T | **±0** | **LV✓?** |
| **Nitrate** (C) | 50 mg/l | $F_C$= 20 | T ≡ **LV** = 50 | **Ea** = T → | **±0** | **–1** | ← F < T | **–1** | **LV ↓!**[g] |
| **PAH** (A1; B)[h] | ≥ 0.1 µg/l | $F_{A1}$ = 0.1[e] ≤ **Ea** | T ≡ **LV** = 0.10 | **Ea** ≥ T → | **+0.5** | **±0** | ← F = T | **+0.5** | **LV✓!** |
| **Pesticides** (C) | 1←→1,000 µg/l | $F_C$= 0.00 | T ≡ **LV** = 0.10 | **Ea** >> T → | **+2** | **–1** | ← F < T | **+1** | **LV✓!** |
| **THMs** (B) | 60–200 µg/l | $F_B$= 10[i] | T ≡ **LV** = 10[i] | **Ea** > T → | **+1** | **±0** | ← F = T | **+1** | **LV✓!** |

(*continued*)

**Table 2** (continued)

✓! **means**: **Bonus + Malus** $> \mathbf{0}$. The actual **LV** $\equiv$ T for these compounds may be evaluated without any restriction as a precautionary maximum far value below any Ea

! **means**: **Bonus + Malus** $< \mathbf{0}$. The actual **LV** $\equiv$ T for these compounds is not a precautionary maximum value. It is even too high (↓↓!) if interpreted as adverse effect-related LV (its lowering seems necessary)

✓? **means**: **Bonus + Malus** $= \mathbf{0}$. **LV** $\equiv$ T for these compounds may yet be interpreted as a precautionary maximum value but also to be adverse effect related. Its compliance therefore needs regular short-time-interval surveillance

[a]From WHO (2011), **LV** $\equiv$ T for cadmium should be lowered for health reasons to Ea $= 3$ µg/l (guide value of WHO 2011)

[b]Threshold to usability of a raw water if not treated accordingly to eliminate potentially harmful geogenic/biogenic constituents.

[c]After treatment

[d]EU-LV since November 1998 (with moratorium): 10 µg/l, to be met only after exchange of lead pipes by pipes made from better adapted materials

[e]Upper limit of geogenic load (cyanide) or of geogenic background (PAH) plus unavoidable load from using old coal tar-coated pipes (numbers estimated by author)

[f]Threshold to *technical* adversity

[g]The LV for nitrate is the only (!) for an environmental contaminant C in drinking water with an exclusively health-related rationale

[h]Sum of four polycyclic aromatic hydrocarbons (PAH, without benzo[a]pyrene) originating from old coal tar-coated pipes and/or geogenic/biogenic background

[i]To be complied with an outlet of waterworks

evaluations, $TS_E$, in column 7 eventually gives the information on whether the LV under question is a precautionary limit value ($WS > 0$) or whether it stands merely for nothing better than simply to defend the integrity of human health or of a technical device ($WS < 0$).

The method presented here to evaluate, from the aspect of environmental hygiene, legally fixed limit values for any environmental or technical medium could easily also be applied at the beginning of any societal discourse between science, politics, and the private sector to define in advance the precautionary character of limit values to be sought in a societal consensus.

## Categorization of Legal LVs in Terms of General Precaution, Early Warning, Control of Concern, or Control of Hazard

The expression *limit value* (*LV*) should only be used to denominate legally binding maximum concentrations. Legal LVs for a specific compound exhibit a strong numerical variance in correspondence with the underlying societal consensus about which protection aim should be considered and to what extent it should be eventually protected as early as possible.

With increasing numerical ratio (hazard index) between an actual LV and an Ea value of the same compound, the setting and observation of the former departs step by step from the (1) precautionary principle over (2) early warning to enable (3) control of concern and, eventually, (4) control of hazard (see section "Criteria to Limit Exposure").

This means that risk management, in case a LV would be exceeded, should be organized in accordance with the motivation underlying this LV to safeguard the aim of protection when allowing for overexposure during the LV's exceedance.

### General Precautionary Values, $PV_g$

*General precautionary values* are the numerically lowest of all possible LVs. They help to avoid from the start loads and annoyances, not just when looking at specific protection aims but rather in general for present and future generations. For compounds of classes A1 and C, such PVs ideally ask for not exceeding a compound's natural (regional?) background concentration. For class C compounds, this condition mostly would mean a (analytical) level "zero," realizable at best by LK or ALARA, whereas precautionary LVs for class B or A2 compounds, as a rule, are to be found between classes C and A1 since they are situated close to or are identical with the lowest level of technical feasibility (of treatment or function, respectively).

The best rationale of a broadly accepted PV would be an equal balance between scientific quantification, technical/functional benefit, and political acceptance/tolerance of any corresponding risk or load. Their scientific part, as a rule, is confined to quantifying natural background concentrations or high-quality analytical criteria to allow for reliable detection or definition, now and in future, of any deviation of load/exposure from a legally accepted (e.g., "background") $PV_g$.

Only a few of the LVs in Table 2 for A1 and C compounds or A2 and B compounds, respectively, are close to meeting their ideal (optimal precautionary) maximum contaminant level goal as defined by this condition.

Sustainable repair of a precautionary LV's exceedance to achieve legal recompliance normally is not adequately feasible by means of short-time measures to protect persons or technical devices from immediate hazards or risks. Instead, such sustainable repair ought to be considered on a medium to long-term time scale by using the scope of sustainable action, as it should be part of any precautionary LV concept. For the time of repair, so-called health-related "maximum action values" may be functional to rationalize and avoid risks from possible but temporarily limited exposure $>$ LV.

Within the framework of this discussion, general PVs are conceptually and numerically identical, respectively, with $F_C$, $F_B$, or $BG_{A1}$.

### Warning Values, WV

The next higher and, from a scientific point of view, sounder category of maximum values is the category of *warning values*, *WVs*. Their exceedance should indicate as early as possible, on the basis of scientific data, that the normal state of a system or organism could be undergoing a switch to instability or nonnormality. The database of a WV is more informative than that of a general precautionary value; therefore, WVs are also called specific (health related, technical, sensorial/aesthetic) precautionary values. As such, they do not need to be "LK," but should at the same time never be higher than any science-based threshold of concern able to replace it and possibly being derived later on a sufficient database.

Within the context of this discussion, WVs are conceptually and numerically identical, respectively, with $T_C$, $T_B$, or $T_{A1}$. For the special case of human toxicology, such WVs have been denominated in Germany as *GOW = Gesundheitlicher Orientierungswert* (in English, *HRIV = health-related indication value*).

### Threshold of Concern for Adverse Effects, Ea

The exceedance of a scientifically based *threshold of concern for adverse effects*, Ea, would not just warn about a possible concern for adverse effects in the future but should also directly trigger such concern. Toxicologists derive an Ea in a way that the protection aim is unlikely to be harmed as long as the measured load/exposure is in compliance with the same compounds' Ea.

In human toxicology, an Ea, as a rule, is derived from an ADI or TDI value by allocating a certain percentage from the latter to the amount in kilograms or liters of daily personal consumption (e.g., 2 L of drinking water or 1 kg of food). In the case of drinking water, such aliquot concentration normally represents 10 % of an ADI or TDI and is usually called a compound's health-related guide value, HRGV, for drinking water.

### Hazard-Linked Action Values, AV

Scientifically based maximum values whose exceedance in a standard scenario would trigger with sufficient probability a hazard from toxic exposure are

called *hazard-linked action values*, AV. They are higher by a compound-specific "interpolation factor" (IF) than the scientific Ea of the same compound.

In Germany, as a rule, AVs are calculated as being 3–10-fold higher than a corresponding Ea. On a logarithmical scale, the interpolation into the margin of safety (space of adverse effect extrapolation from experimental conditions on humans) places the AV halfway between the selected point of departure and the protection aim's Ea.

## Summary: A Short Directory to Quantify and Survey Precautionary LVs

The REH helps to organize the following steps to fix socially consensual (tolerated or accepted) limit values and criteria for their surveillance:

A: Geogenic/biogenic constituents (hardly avoidable):

A1: If no single threshold of concern Ea is exceeded in the raw material or resource, respectively, treatment to eliminate class A compounds would neither be necessary nor indicated. Any $LV_{A1}$ to be chosen accordingly would appear as tolerable, if representing rather an "upper" percentile, or as acceptable, if representing rather a "lower" percentile of regional $BG_{A1}$ levels.

As a rule, any $LV_{A1} \equiv BG_{A1}$ would need only a longtime interval surveillance to safeguard compliance.

A2: If one or more thresholds of concern Ea are exceeded in the raw material or resource, respectively, treatment(s) to eliminate class A compounds down to a technically tolerable (gaRT) or acceptable (StT or LK) level of $LV_{A2} \equiv T_{A2} \leq Ea$ is indicated only if a resource to need no such treatment would not be readily (e.g., regionally) available.

As a rule, any $LV_{A2} \equiv T_{A2}$ would need a short-time interval surveillance in the treated resource to safeguard compliance.

B: Anthropogenic additives and their related residues being functional in their intended compartments:

B compounds ought to exert their intended function as far as possible below their on-site threshold of concern Ea. Any $LV_B$ to be chosen accordingly would appear as being tolerable at a level of $LV_B \equiv T_B \leq Ea$ (gaRT) or as acceptable as a precautionary maximum value at a level of $LV_B \equiv T_B = F_B < Ea$ (StT or LK). The societal readiness to *accept* any levels $T_B > F_B$ below Ea increases with optimization of functional efficiency and increasing readiness to pay for them.

As a rule, any $LV_B \equiv T_B$ would need a short-time interval surveillance in the finished product to safeguard compliance.

C: Anthropogenic environmental contaminants and degradation products devoid of intended function but potentially harmful at or in remote places and compartments:

C compounds dissipate unintentionally from the on-site use of B compounds onto remote places or compartments of the environment. If there is no single or

sum threshold of concern Ea exceeded in the raw material or resource, respectively, its treatment to eliminate class C compounds would neither be necessary nor indicated. A $LV_C$ to be chosen accordingly would appear as tolerable at a level of $LV_C \equiv T_C < Ea$ (gaRT) as acceptable as a precautionary maximum value at a level of $LV_C \equiv T_C = F_C = 0$ (StT or LK). The societal readiness to *tolerate* any remote levels $T_C > F_C$ below Ea increases with optimization of functional compartmentalization of the use on-site of the respective class B compounds and increasing readiness to pay for.

As a rule, any $LV_C \equiv T_C$ would ask for a longtime interval surveillance to safeguard compliance in any remote contaminated raw material or resource if this is done in alliance with the respective class B compound's short-time interval on-site surveillance of emission and degradation/dissipation.

## Conclusions

The three-dimensional *rule of environmental hygiene* (REH) serves to ascertain in each individual case whether a limit value is, in fact, necessary to avert contamination and, if so, which component of the overall rationale determines or should determine the level at which it is set. This assessment has to take place by way of a rational public discourse befitting democratic forms of government, with the participation of all societal players (cf. AdW 1992; EEA 2002). Only in this way is it possible to avoid exposure inequities, differences in acceptance, and uncertainty as to the conditions governing economic activities within our societies. Violators of limit values thus established rightfully face society's sanctions.

Limit values also serve to ensure an overall social compatibility to use concepts whose implementation is useful to one party, of no particular interest to another, and possibly detrimental to a third. This should be in place before a question of whether an adverse effect threshold is reached or exceeded can or must be answered by science in a reliable manner.

The multidimensional limit value derivation concept outlined here is an instrument that may also be helpful in limiting "adverse effects" at the societal level by enabling different interest groups to communicate with each other in a civilized, organized, tolerant, and, hence, acceptable manner.

Those who permanently or temporarily withdraw from such rational, civilized discourse in order to push through, out of self-interest, goals lacking general acceptance deliberately act in an anti-democratic or a politically short-sighted manner and are in danger of losing their credibility.

On the other hand, it is only in rare cases that the temporary exceedance of a limit value directly results in immediate danger. Information on such "dangerous" values can be obtained from toxicologists and ecologists, who should have no reservations about divulging it. The overwhelming majority of limit and guide values, however, have been derived on the basis of criteria relating to the functional value of useful exposure and the avoidability of unnecessary exposure – at levels usually far below those based on the criterion of "imminent danger."

Thus, the often-heard slogan of "poisoning sanctioned by limit values" is quite out of place. All the more care must be taken to ensure that limit values are complied with and that remedial measures are performed where necessary, because carelessness spreads when there is no threat of impending or immediate danger.

Even farther off the mark is the common claim that the application of StT and gaRT (LK usually plays a role in pilot projects only) and the concomitant limit values would be a threat to the industrial base of high-tech nations and meaningful employment for millions of people. Many of the case studies in EEA (2002) suggest that wider use of the precautionary principle can help stimulate both innovation and science, replacing nineteenth-century technologies and the simple science of the first industrial revolution with the "eco-efficient" technologies and systems science of the third.

More important than the negotiation of and the compliance with limit values is, however, to never stop questioning the necessity and functional value of pollution- and exposure-intensive use concepts. It is the exposure that is avoided that is the most compatible with human health, its environments, and nature as a whole.

Given this and the observance of the four conditions for limit values acceptable from the viewpoint of environmental hygiene, the limit value concept will also in the future be accepted by society as a multidimensional concept to take care of the environment.

## Signs and Abbreviations

. . . ≡ . . . "defined/fixed as" or "identical with"
**A** Class of biogenic/geogenic compounds
**ADI** See TDI
**B** Class of functional loads/exposure (compounds with intended function on-site)
**BG** (Natural) background load/exposure
**C** Class of nonfunctional loads/exposure (mostly environmental contaminants with no function where detected)
**Ea** Threshold of concern about adverse effects
**F** Threshold of optimized functionality (class B compounds; class C compounds)/ threshold of usability (class A) compounds
**gaRT** Generally accepted rules of technology
**LK** Level of technical-scientific knowledge
**LV** Limit value (a maximum concentration fixed by law)
**PoD** Threshold (dose or concentration) of adverse effect chosen for being extrapolated on humans as part of a toxicological evaluation of experimental or epidemiological data to derive an ADI or TDI
**REH** Three-dimensional rule of environmental hygiene
**StT** State of technology
**T** Tolerance threshold
**TDI** (ADI) Tolerable (acceptable) daily intake of a chemical considered to be safe for lifelong exposure, mostly given in mg/kg of body mass

## Recommended Reading

AdW (Akademie der Wissenschaften zu Berlin) (1992) Forschungsbericht 2: Umweltstandards. Walter de Gruyter, Berlin/New York

Dieter HH (1999) Ableitung von Grenzwerten (Umweltstandards) - Wasser. *in*: *Handbuch der Umweltmedizin* (Wichmann, E., et al., Hrsgg.), Kapitel III-1.3.5. ecomed, Landsberg

Dieter HH (2011) Drinking water toxicology in its regulatory framework. In: Wilderer P (ed) Treatise on water science, vol 3. Academic, Oxford, pp 377–416

Dieter HH, Konietzka R (1995) Which multiple of a safe body dose derived on the basis of default factors would probably be unsafe? Regul Toxicol Pharmacol 22:262–267

Dieter HH, Grohmann A, Thompson D (1997) Specific contributions of politics, economics, and toxicology in setting socially consensual limit values. Environ Manage 21(4):505–515

European Environment Agency (2002) Late lessons from early warnings: the precautionary principle 1896–2000. Environmental issue report No 22/2001 (introduction, p 4). http://www.eea.europa.eu/publications/environmental_issue_report_2001_22

Konietzka R, und Dieter HH (1998) Ermittlung gefahrenbezogener chronischer Schadstoffdosen zur Gefahrenabwehr beim Wirkungspfad Boden-Mensch. *Bodenschutzhandbuch (27. Lieferung X/98, Ziff.3530).* Erich Schmidt Verlag, Berlin

World Health Organization (WHO) (2011) Guidelines for drinking-water quality, third edition, incorporating first and second addenda. http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/index.html

# Limit Value Setting in Different Areas of Regulatory Toxicology

Klaus-Michael Wollin and H. Paul A. Illing

## Contents

Exposure Limit Setting in the Context of the Regulatory Framework ..... 650
The Setting of Occupational Exposure Limits ..... 651
Health-Based Limit Values for Environmental Contaminants ..... 654
Air Pollutants ..... 654
Water Quality Criteria and Standards ..... 655
Soil Values (Contaminated Land) ..... 657
Future Perspectives ..... 658
References ..... 658
Resources ..... 659

**Abstract**

Standards for the protection of human health are important tools used for risk management. They represent the *limit value*, the maximum level of exposure deemed acceptable or tolerable, under the particular exposure circumstances for which they are set. Usually, there is a formal assessment process by which the standard is set. From a toxicological point of view, limit values reflect a *risk characterization* for an available database. Because assessments by individual scientists can differ, limits are usually based on a consensus. Although they must meet a scientific rationale, limit values also have to take into account political considerations, technical feasibility, and economic consequences.

K.-M. Wollin (✉)
Lower Saxony Governmental Institute of Public Health, Hannover, Germany
e-mail: klaus-michael.wollin@nlga.niedersachsen.de

H.P.A. Illing
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_81, 

## Exposure Limit Setting in the Context of the Regulatory Framework

According to the NAS/NRC (and IOMC) risk assessment/management paradigm, the risk characterization (the qualitative and, wherever possible, quantitative determination of the probability of occurrence of known and potential adverse effects of an agent under defined exposure condition) is the final stage of the risk assessment. The development of alternative regulatory options and the weighing of their economic, social, and political consequences are elements of risk evaluation (IOMC), which is the first stage of risk management. The US NAS/NRC report was concerned principally with risk assessment, and the EPA does not break down risk management into the three components identified by IOMC, namely, risk evaluation, emission and exposure control, and risk monitoring.

A principle way to regulate harmful substances is to manage exposures in order to prevent the exceedance of an acceptable or tolerable level of risk. For the regulator this includes setting and enforcing limit values. In this context, risk assessment and risk management are two related but independent processes. The *risk assessment* is based exclusively on scientific principles, while risk management (and, in particular, the risk evaluation) has to balance problems of socioeconomic costs and benefits, technical feasibility, societal perception, and public policy. The *risk management* process includes identification of the procedures that should be adopted to control exposure (engineering controls, use of protective equipment, remediation, etc.), the setting of limits, and the enforcement of the procedures and limits. Decoupling of political management and scientific analysis ensures clear responsibilities.

In the narrowest sense, limit values are measurable, quantitative thresholds representing uptake at the receptor or site of action within the body for hazardous substances. In practice the human's body burden of toxic chemical compounds, elements, or their metabolites is measured in biological samples (exhaled air, blood, urine, sweat, hair) or is estimated by extrapolation from measurements on exposure in various media such as air, water, soil, or food. The limit values have been recommended by the regulatory body established under the appropriate legal framework. *Legal limits* represent "tolerable" or "acceptable" risks, depending on their definition and the framework within which they are utilized.

The general public uses a very general understanding of the generic term *limit value*. Its scope is extended to guidance values, threshold values, ceiling values, etc. (see chapter "► The Regulatory Process in Toxicology" in this book), many of which are not enforceable. In contrast, if limit values are treated as values set within a legal framework established by the state, binding thresholds are defined and exceeding these thresholds triggers specific consequences. In contrast, normally adherence to *guidance values* (whether from nongovernmental organizations or from government) is voluntary.

The approach used for establishing limit values generally distinguishes between populations. It may also distinguish different levels of protection. A clear definition of the group "at risk" and of the type and level of risk being addressed is one of the

most important requirements when setting limits. Thus, health-based limit values can protect different groups of people (to different extents) depending on the circumstances of the exposure; these include:

- Workers.
- Consumers.
- The general public via environmental exposure (including human health-based standards aimed at protection of the environment as a whole or specific compartments (soil, groundwater/surface water, ambient air) within the environment).

The general methodology for establishing health-based limits should be equally applicable both in workplace and non-workplace scenarios. There should be a clear distinction between scientific and other aspects in the practice of setting limit values. Transparency of derivation, flexibility and ease of use, and defined rules for reevaluation and updating all help to build public acceptance of governmental limit values for the regulation of toxic chemicals. It should be noted that, although apparently different approaches for the risk assessment of chemicals in the workplace and in other scenarios have emerged on the international and the national level, these differences are due to, *inter alia*, the standards being for different populations (healthy workers, without children or the elderly and with the possibility of excluding the more susceptible individuals, versus everyone), often with different attitudes to risk, and different exposure scenarios (8-h workplace shifts versus continuous).

## The Setting of Occupational Exposure Limits

*Occupational Exposure Limit values* (OELs) are set by national authorities or national institutions as limits for concentrations of hazardous compounds in the workplace air. Most of the industrialized countries establish and maintain OEL lists that regulate hazardous substance concentration levels to which workers may be exposed via inhalation, ingestion, or skin contact for specified time periods without being at risk over a working lifetime. These limits can be binding or indicative. For workplace airborne exposures to gases, vapors, and particulates, there are three principal limits in widespread use. They are based on different durations of exposure:

- The 8-h *time-weighted average* (*TWA*) *exposure limit* – the maximum average concentration of a chemical in air for a 8-h working day and 40-h week
- The *short-term exposure limit* (*STEL*) – the maximum average concentration to which workers can be exposed for a short period (usually 15 min)
- The ceiling value – a concentration that should not be exceeded at any time

In addition, *Biological Exposure Indices* (*BEIs*) represent the body burden, i.e., the concentration of chemicals in the body that would correspond to inhalation exposure at a specific concentration in air. Theoretically, biological effects indices are also possible, but they are unlikely to be set on the grounds that the aim is to prevent harmful effects occurring, and harmful effects are occurring if the measure is one of minimal harm.

Fundamental work to develop a systematic and comprehensive approach to setting occupational exposure limits was done by the American Conference of Governmental Industrial Hygienists (ACGIH). The conception of the ACGIH to derive Threshold Limit Values (TLVs) is one of the earliest developments aimed at managing workplace exposures. The ACGIH first published Maximum Allowable Concentrations (MACs) in 1946. These were later renamed TLVs and are republished annually by the ACGIH. TLVs are subject to a health-based view only and are not legally binding. ACGIH is not a regulatory authority. The US Occupational Safety and Health Administration (OSHA), which is a regulatory body, adopts mandatory limits, the *Permissible Exposure Limits* (*PELs*), and OSHA is supported in this process by the National Institute for Occupational Safety and Health (NIOSH). NIOSH develops its own health-based Recommended Exposure Limits (RELs). Together with ACGIH's TVLS, the RELs of NIOSH contribute to the setting of PELs by the OSHA; however, OSHA makes its own independent judgment regarding the final value of PEL. PELs arise from a comprehensive and well-documented rule making that takes into account significant health risks, sampling and analytical procedures, as well as technological and economic feasibility.

Similar approaches to that of ACGIH and NIOSH were adopted by the Deutsche Forschungsgemeinschaft (DFG) in Germany (non-enforceable maximum workplace concentration, MAK), the Netherlands, and Scandinavia.

The UK Health and Safety Executive (HSE, a regulatory authority with enforcement responsibilities) pursued a dual system of maximum exposure limits (MELs) and occupational exposure standards (OESs), each of which carried different exposure management requirements, until 2005. In 2005, UK's two-OEL system has nominally been replaced by a single-OEL system of *workplace exposure limits* (*WELs*), in which most of the existing MELs and OELs have been converted to WELs, but the different management approaches previously applicable to MELs and OESs have been maintained using EU classification and labelling requirements to identify which management approach is appropriate. The list of approved workplace exposure limits, which have been approved by the Health and Safety Executive (HSE), is legally binding.

On the European scale, the European Commission decided to set up a formal base for the work on the scientific evaluation of the health risks posed by exposure to chemical substances in the workplace with its Decision 95/320/EC of 12 July 1995 to encourage OELs. OELs are proposed by the Scientific Committee on Occupational Exposure Limits (SCOEL). The major task of the SCOEL is to give advice on the setting of OELs based on scientific data and, where appropriate, propose values. SCOEL's approach is documented in its *Methodology for the Derivation of Occupational Exposure Limits*: *Key Documentation* (2009).

The SCOEL may recommend OELs, which can be supplemented by further notations as:

- Eight-hour time-weighted average (TWA – 8 h)
- Short-term exposure limits (STEL)
- Biological limit values (BLVs)

The SCOEL aims to give health-based OELs that can be recommended when the available scientific data suggest that a clear threshold value can be identified for the adverse effects of the substance in question.

For some adverse effects (in particular genotoxic carcinogenicity, respiratory sensitization, and genotoxicity), it is deemed that, according to current knowledge, it is not possible to identify thresholds. In these cases, the SCOEL recommends a pragmatic OEL, which is established at levels considered implying sufficiently low risk. Since the late 1990s, SCOEL has developed the concept of "practical thresholds" in the derivation of OELs for carcinogens (Bolt 2008). For some carcinogens health-based OELs have been recommended, while a quantitative assessment of the substance-related carcinogenic risk is made for others. Non-genotoxic carcinogens and/or non DNA-reactive carcinogens are deemed to have a true threshold associated with a clearly founded NOAEL. The remaining carcinogens are categorized into three groups: genotoxic carcinogens for which a practical threshold is supported by studies on mechanisms and/or toxicokinetics and a health-based OEL can be derived based on an established NOAEL; genotoxic carcinogens, for which the existence of a threshold cannot be supported currently and the linear non-threshold model is applied as a default assumption; and non-threshold carcinogens for which a linear non-threshold model appears appropriate.

For respiratory sensitizers, the SCOEL evaluates data on a case-by-case basis and provides further information to the Commission.

An overview of existing OELs in the EU is given on the website of the European Agency for Safety and Health at Work (EU-OSHA). The so-called *Indicative Occupational Exposure Limit Values* (*IOELVs*) are health-based limits set under the Chemical Agents Directive (98/24/EC). IOELVs are listed in Directives which Member States are obliged to take into account when implementing by introducing national limits for the chemical agents in question, taking into account the European values. For chemicals for which a *binding OEL value* (*BOELV*) is established at Community level, Member States have to introduce a corresponding national binding limit based on, but not exceeding (i.e., higher than), the BOELV value.

When carrying out an assessment of human health effects for the chemical safety assessment under Regulation (EC) No 1907/2006 (REACH), the regulation requires the derivation of a "*Derived No Effect Level*" (*DNEL*) or "*Derived Minimal Effect Level*" (*DMEL*) by the registrant. DNEL or DMEL should be derived for all relevant routes of exposure (inhalation, dermal, or oral). Inhalation is usually considered an important potential route of exposure in the workplace. A (generic) maximum "safe" inhalation exposure level can be developed from the appropriate DNEL/DMEL using the recommended (in Guidance from ECHA) standardized procedure and assessment factors. If no OEL is available, the adequacy of the protective measures used in the workplace can be assessed by comparing the predicted or actual exposure levels with the maximum "safe" exposure level derived from this REACH-based procedure.

## Health-Based Limit Values for Environmental Contaminants

### Air Pollutants

The World Health Organization (WHO) defines air pollution as "contamination of the indoor or outdoor environment by any chemical, physical, or biological agent that modifies the natural characteristics of the atmosphere." Effects of air pollutants can impair human health either directly via inhalation exposure or indirectly via atmospheric deposition on edible plants and thus entering the food chain. Outdoor (ambient) and indoor air quality are usually considered separately.

WHO's *air quality guidelines* (for ambient air quality) were first published as "Air Quality Guidelines for Europe" in 1987 (WHO 1987), followed by the "Guidelines for Air Quality" in 2000. WHO emphasizes that these guidelines are not intended as standards. In moving from guidelines to standards, the prevailing exposure levels and environmental, social, economic, and cultural conditions in a country or region should be taken into account. The guideline setting process has been described in detail in the "Guidelines for Air Quality" (WHO 2000). In short, toxic effects are considered to be of two types, threshold and non-threshold. For substances where the critical effect is considered to have a threshold (including non-genotoxic carcinogenesis for which there is adequate mechanistic data), a *Tolerable Intake* (TI) expressed as airborne concentrations (i.e., μg or mg/$m^3$) is developed usually on the basis of an NOAEL. The derivation of guidance values for compounds present in other environmental media than air will require the allocation of proportions of the TI to such as air, food, and water, which will be based on sound information on relative exposure via different routes. A default approach, low-dose risk extrapolation, was conducted for carcinogens of IARC classification groups 1 and 2A, and an uncertainty factor approach applied in the case of substances in groups 2B and 3. The mechanism of action was the determining factor for the method of assessment. Hence, it was decided that compounds classified under 1 or 2A could be assessed using uncertainty factors, if evidence for a *threshold mechanism* of carcinogenicity existed. In contrast, compounds classified under 2B could be assessed by low-dose extrapolation methods, if a *non-threshold mechanism of carcinogenicity* in animals was proven.

WHO has revised its air quality guidelines in 2005 for key parameters of contamination (particulate matter, ozone, nitrogen dioxide, and sulphur dioxide). Whereas the previous guidelines (published in 1987 and 1997) concentrated on Europe, the 2005 revision included information from low- and middle-income countries worldwide. They are designed to offer global guidance on reducing adverse health impacts of air pollution. WHO air quality guidelines are not legally binding, but constitute an important basis for the regulation of air pollution. National air quality *standards* will vary from country to country. They depend on each country's attitude to health risk and its specific approaches to balancing risks to health and technological feasibility. They also take into account economic considerations and political and social factors.

Recently, WHO proposed its guidelines for selected indoor air pollutants (WHO 2010). The substances considered, i.e., benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, benzo(a)pyrene, radon, trichloroethylene, and tetrachloroethylene, have indoor sources or sources sub-adjacent to the building and are often found indoors in concentrations of health concern. WHO's guidelines for indoor air quality provide the scientific basis for legally enforceable standards.

The US *National Ambient Air Quality Standards* (NAAQS) are standards established by the US EPA under authority of the Clean Air Act (CAA) that apply to outdoor air. EPA has set NAAQS for the following principal pollutants: carbon monoxide, lead, nitrogen dioxide, ozone, particulate matter (PM), and sulphur dioxide. The standards are listed in Title 40 of the Code of Federal Regulations Part 50. CAA established two types of national air quality standards. Primary standards set limits to protect public health with an adequate margin of safety to allow for the health of vulnerable populations such as individuals suffering from respiratory disorders, children, and the elderly. Secondary standards set limits to protect public welfare, including protection against visibility impairment and damage to animals, crops, vegetation, and buildings.

The European Union has legislation concerned with ambient air quality. Directive 2008/50/EC of 21 May 2008 on ambient air quality consolidated as much existing legislation on objectives for ambient air quality in relation to sulphur dioxide, nitrogen dioxide and oxides of nitrogen, particulate matter (PM10, PM2.5), lead, benzene, carbon monoxide, and ozone, and Directive 2004/107/EC (which was not included in the consolidation) set objectives for arsenic, cadmium, mercury, nickel, and polycyclic aromatic hydrocarbons (PAHs) in ambient air.

## Water Quality Criteria and Standards

Quality standards for ground and surface water may reflect either or both ecological criteria and quality criteria for drinking water. Either water resources used as sources of drinking water, and their related water ecosystems, should be protected from pollution, or they have to be purified during supply.

The European Union has implemented the Water Framework Directive (EU Directive 2000/60/EC) establishing a framework for Community action in the field of water policy. Its ultimate objective is to achieve a "good ecological and chemical status" for all community waters by 2015. The Directive establishes a list of 33 priority substances, including cadmium, lead, mercury, nickel, and its compounds, benzene, PAHs, and DDT, for action. The corresponding *environmental quality standards* (EQS) for priority substances and certain other pollutants have been laid down in Annex I of the Directive 2008/105/EC on environmental quality standards in the field of water policy. Generally, groundwater is the most sensitive and the largest body of freshwater and, in particular, is a main source of public drinking water supplies. The Directive 2006/118/EC on the protection of groundwater against pollution and deterioration comprises *groundwater quality standards* for nitrates and active substances in pesticides, including their relevant metabolites,

degradation, and reaction products. It also requires Member States to establish threshold values for groundwater pollutants and indicators of pollution on the basis of a minimum list of pollutants and their indicators (arsenic, cadmium, lead, mercury, ammonium, chloride, sulfate, trichloroethylene, tetrachloroethylene, and conductivity [which is indicative of saline or other intrusions]) considering the guidelines outlined in Annex II/Part A.

Section 304(a) (1) of the US Clean Water Act is the legal basis for the development of criteria for water quality for the protection of aquatic life as well as for human health (including organoleptic effects) in the USA. US EPA's *National Recommended* ***Water Quality Criteria*** defines the human health criterion as the highest concentration of a pollutant in water that is not expected to pose a significant risk to human health (US EPA 2013). The criteria consider human health for the consumption of water and organisms or organisms only. The methodology for deriving *Ambient Water Quality Criteria for the Protection of Human Health* has been revised in 2000 with revisions in the assessment of exposure to carcinogens, exposure to noncarcinogens, and exposure assessment and bioaccumulation. For noncarcinogens the effective EPA guidance on assessing noncarcinogenic effects of chemicals and for the Reference Dose (RfD) derivation should be used. More sophisticated methods are recommended for cancer risk assessment, including identification of the likely mechanism of human carcinogenicity and use of the most appropriate low-dose extrapolation.

WHO's water-related activities cover a broad range of activities, including water and drinking-water quality and infectious agents, toxic chemicals, and radiological hazards and general aspects of water supply and sanitation as well. A comprehensive framework, the *Guidelines for Drinking-Water Quality* (GDWQ), has been published regularly by the WHO. Two approaches to derive guideline values are used: one for "*threshold chemicals*" and the other for "*non-threshold chemicals*" (mostly genotoxic carcinogens). In establishing GDWQ, the IARC evaluation of carcinogenic compounds, where available, is taken into consideration. The principles in the derivation of ADIs (acceptable daily intakes) developed by FAO, JECFA, and JMPR have been adopted, where appropriate, in the derivation of TDIs used in developing guideline values for drinking-water quality. GDWQ are kept up to date through an ongoing "*rolling revision*" process. Increasingly the preferred approaches for the derivation of TDIs/ADIs for threshold chemicals include the benchmark dose (BMD) or the benchmark dose lower confidence limit (BMDL) and chemical specific adjustment factors. In order to make the distinction with respect to the underlying mechanism of carcinogenicity, compounds that have been shown to be a carcinogen (i.e., chemicals classified in group 1 or group 2A by IARC) are evaluated on a case-by-case basis. The evidence of genotoxicity, the range of species affected, the relevance of the tumors observed in experimental animals to humans, and the toxicokinetics of the substance are considered when determining the mode of action and therefore the approach taken. For carcinogens for which there is evidence to suggest a non-genotoxic mechanism or to suggest that detoxification mechanisms require to be overwhelmed by high doses, guideline values are derived using the threshold chemicals approach. WHO's normal allocation of 20 % of the TDI/ADI to

drinking water has changed from the allocation of 10 % used in the third edition of the GDWQ. The latter was found to be excessively conservative and the new value will be incorporated in new guidelines and revisions of existing guidelines (WHO 2011).

The current EU binding framework for Member State national standard setting for the quality of *water intended for human consumption at the point of deliver* is contained in the revised Council Directive 98/83/EC. The numerical values for chemical parameters in Annex I are generally those of WHO's GDWQ. The Commission must review Annex I at least quinquennially and has to make proposals for amendments in the light of scientific and technical progress.

***Drinking Water Standards*** and *Health Advisories* (DWSHA) are issued periodically by US EPA. The Health Advisory (HA) Program publishes concentrations of drinking-water contaminants at Drinking Water Specific Risk Level Concentration for cancer ($10^{-4}$ cancer risk) and concentrations of drinking-water contaminants at which noncancer adverse health effects are not anticipated to occur over specific exposure durations – one-day, ten-day, and lifetime. The *lifetime HA* for the drinking-water contaminant is calculated from its associated *Drinking Water Equivalent Level* (DWEL), obtained from its Reference Dose (RfD), and incorporates a drinking-water Relative Source Contribution (RSC) factor of contaminant-specific data or a default of 20 % of total exposure from all sources. *One-day HAs*, *ten-day HAs*, and *lifetime HAs* are not to be construed as legally enforceable federal standards. In contrast, an enforceable *Maximum Contaminant Level* represents the highest level of a contaminant that is allowed in drinking water. MCLs are set as close as feasible to the *Maximum Contaminant Level Goal* (MCLG) using the best available analytical and treatment technologies and taking cost into consideration (US EPA 2012).

## Soil Values (Contaminated Land)

Land contamination may occur naturally or through anthropogenic activities. A distinction is often made between soil contamination originating from clearly confined sources (local or point source contamination, e.g., abandoned hazardous sites) and that caused by diffuse sources. In general, land contamination and remediation is a newer field of environmental legislation, and control is currently mainly through land use planning legislation. Different policies (e.g., on water, waste, chemicals, industrial pollution prevention, pesticides, agriculture) have contributed to preventing land being contaminated. However, as these policies have other aims, they are not sufficient to ensure an adequate level of protection. On the European scale, a proposal for a framework Directive (COM (2006) 232) exists which sets out common principles for protecting soils across the EU. Within this common framework, the Member States will be in a position to decide how best to deal with issues associated with contaminated land, its potential, uses, and its remediation. According to Article 11 of COM (2006) 232, a soil status report shall be issued including the concentration levels at which there are sufficient reasons to believe that the dangerous substances concerned pose a significant risk to human

health or to the environment, but special soil trigger values have not been proposed. Specific *soil trigger values* have been set in recent times at the national level, notably in Canada, Germany, the Netherlands, Switzerland, and United Kingdom.

The US EPA developed the Soil Screening Guidance to help standardize and accelerate the evaluation and cleanup of contaminated soils. This guidance provides a methodology to calculate risk-based and site-specific *Soil Screening Levels* (SSLs) for contaminants in soil. To calculate SSLs, the exposure equations and pathway models are run in reverse to back calculate an "acceptable level" of a soil contaminant. For ingestion, dermal, and inhalation pathways, toxicity criteria are used to define an acceptable level of contamination in soil, based on a $10^{-6}$ individual excess cancer risk for carcinogens and a hazard quotient (HQ) of 1 for noncarcinogens. SSLs are back calculated for migration to groundwater pathways using groundwater concentration limits (MCLGs, MCLs, or health-based limits (HBLs) ($10^{-6}$ cancer risk or a HQ of 1, where MCLs are not available)). Generic SSLs are not national cleanup standards.

## Future Perspectives

Increasingly, scientific quantitative risk assessment succeeds in identifying and reducing uncertainties that are inherent in all stages of the risk analysis. For substances with adverse health effects, alternative methods such as the benchmark dose method are being incorporated into the determination of dose–response relationships. These alternatives can reduce the shortcomings of the classical concept of determining tolerable body doses based on a NOAEL or LOAEL. Recent assessments of carcinogenicity are based on the complete analysis of all available biological information, including that on the mechanism of action. This is an improvement on the older risk quantification in the low-dose range using the linearized multistage model, which often led to an overestimation of risk. Exposure assessment methods are beginning to allow a more realistic description of exposure. However, better exposure models require an expanded database. Current issues include the use of multiple "worst case" (or "reasonable worst case") assumptions by regulatory authorities, leading to unrealistically precautionary overall risk assessments. Probabilistic approaches, such as Monte Carlo analysis, yield more realistic overall risk assessments. Emerging issues include approaches to considering the extrapolation to low doses in a sound manner, low-dose effects in toxicology/non-monotonic dose–response, and the development of scientific state-of-the-art approaches to mixtures of chemicals.

## References

Bolt HM (2008) The concept of "practical thresholds" in the derivation of occupational exposure limits for carcinogens by the scientific committee on occupational exposure limits (SCOEL) of the European Union. Genes Environ 30:114–119

EU Directive 2000/60/EC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:327:0001:0072:EN:PDF
European Commission (2006) Proposal for a directive of the European parliament and of the council establishing a framework for the protection of soil and amending Directive 2004/35/EC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2006:0232:FIN:EN:PDF
European Commission Employment, Social Affairs and Inclusion (2009) Methodology for the derivation of occupational exposure limits methodology for the derivation of occupational exposure limits. Key documentation (version 6). http://ec.europa.eu/social/main.jsp?catId=148&langId=en&internal_pagesId=684&moreDocuments=yes&tableName=INTERNAL_PAGES
US EPA (2012) 2012 edition of the drinking water standards and health advisories. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf
US EPA (2013) National recommended water quality criteria. Last updated on Tuesday, June 11, 2013. http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm#hhtable
World Health Organization (1987) Air quality guidelines for Europe. Copenhagen, WHO Regional Office for Europe, 1987 (WHO Regional Publications, European Series, No. 23)
World Health Organization (2000) Guidelines for air quality. Geneva, 2000. http://www.airimpacts.org/documents/local/AQGUIDE.pdf
World Health Organization (2005) WHO air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide Global update 2005. http://whqlibdoc.who.int/hq/2006/WHO_SDE_PHE_OEH_06.02_eng.pdf
World Health Organization (2010) WHO guidelines for indoor air quality: selected pollutants. http://www.euro.who.int/__data/assets/pdf_file/0009/128169/e94535.pdf
World Health Organization (2011) Guidelines for drinking-water quality, 4th edn. http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/index.html

## Resources

European Agency for Safety and Health at Work (EU-OSHA). http://osha.europa.eu/en/topics/ds/oel/members.stm
Summaries of EU legislation > Environment. http://europa.eu/legislation_summaries/environment/index_en.htm
UK Health and Safety Executive. http://www.hse.gov.uk/
U.S. Department of Labor, Occupational Safety & Health Administration. http://www.osha.gov/
US Environmental Protection Agency. http://www.epa.gov/
World Health Organization. http://www.who.int/en/

# Registration and Approval in Regulatory Toxicology

Thomas Wallenhorst

## Contents

Chemical Regulations ..... 662
Pesticides ..... 665
Plant Protection Products ..... 665
Biocides ..... 666
Food Contact Materials ..... 669
Toys ..... 670
Cosmetics ..... 671
Medical Devices ..... 671
Medicines ..... 673

**Abstract**

In daily life, consumers are exposed directly or indirectly to all kinds of chemical substances, products, and articles including chemicals. The different application fields of the various products and articles are regulated in different ways and with various processes to insure the safety of man and environment.

Application areas in which consumers are exposed to chemical substances are subject to regulated approval processes e.g., chemicals, pesticides, plant protection, biocides, consumer goods e.g., cosmetics and toys as well as areas for human health e.g., medical devices and medicines. These areas are some of the most important and most relevant regulated fields without the claim of completeness. The focus will be on areas related to chemicals, in which chemical substance exposure is expected for humans and the environment and therefore needs to be evaluated. Most recent developments in chemical laws and for the regulation of biocides are presented.

T. Wallenhorst
Sanitized AG, Burgdorf, Switzerland
e-mail: thomas.wallenhorst@sanitized.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_82, 

Substances, products or articles need to pass different regulated approval processes depending on their application area and intended uses before they can enter the market.

The key point of every approval process is to evaluate the potential risk for man and the environment depending on the intended use of the final article or product, a risk-benefit analysis needs to be performed to evaluate if the correlated risk of the use of the article or product is acceptable or not.

Authorization, registration or notification of substances, products or articles for the various application areas is different from country to county and from region to region. The regions with the most advanced and developed registration requirements are Europe, USA, Canada, and Japan. However, especially Asian counties are implementing more and more regulation processes, very often the requirements are a mixture of the US and the European system combined with some national demands.

On an international basis it is the aim to harmonize the requirements and standards for the regulations between the different regions and counties. On an international intercontinental level OECD standards bring a certain level of harmonization. In Europe, the EU Directives and EU Regulation are aiming for a harmonization between the European member states. Europe is the region with the fasted development in harmonization of the various regulated areas. European regulations and directives of the above mentioned areas are the focus of the following outlines.

## Chemical Regulations

Chemicals are usually regulated through national chemical inventories. The requirements for inclusion of chemicals in the different national chemical inventories vary greatly from country to country. The chemical regulation in the EU went through a dramatic and fundamental change in the past years. The new chemical regulation in the EU is probably the one with the deepest impact on chemical-related industries since the existence of the EU.

The new chemical regulation in the EU is known as REACH with the official number EC No 1907/2006. REACH stands for Registration, Evaluation, Authorization and Registration of Chemicals. It replaces the former EINECS (European Inventory of Existing Commercial Substances) and the ELINCS (European List of New Chemical Substances) Lists.

REACH had a tremendous impact on manufactures, importers as well as on the so-called downstream users of chemicals. REACH is a very complex registration system only the basic points and the frame idea are outlined below.

The REACH regulation became effective on 1 June 2007. The aim of this regulation is to control all manufactured or imported chemicals into the EU in order to improve the protection of human health and environment. The newly established European Chemical Agency (ECHA), located in Helsinki, was appointed to organize and manage the registration processes and to handle

**Table 1** Timelines according to article 23 of the REACH regulation

| Amount places on the market | Timelines |
|---|---|
| ≥ 1,000 t/a | 1 December 2010 |
| CMR substances ≥ 1 t/a | 1 December 2010 |
| Substances dangerous to the environment ≥ 100 t/a | 1 December 2010 |
| Notification Art 7 (4) | 1 June 2011 |
| ≥ 100 t/a | 1 June 2013 |
| ≥ 1 t/a | 1 June 2018 |

a central database where all necessary data is stored and which is accessible for national competent authorities, professionals as well as for consumers.

REACH affects all chemicals manufactured or imported with a quantity over 1 t/year per producer or importer. The regulation differs between phase-in and phase-out substances. Phase-in substances are chemicals already listed in the EINECS or "no long polymers" as well as chemicals produced 15 years before REACH was in force but were never sold in the market e.g., production internal chemicals.

Not under the scope of REACH are polymers (not the monomers), radioactive substances, substances for research and substances in transit, additionally substances which are controlled by other regulations, e.g., medicines for human and animal health, plant protection, and biocide active substance which are considered as registered, substances for food and feed, as well as reimported substance into the EU. Also substances mentioned in Annex IV and V e.g., water, limestone and natural substances classified as not dangerous.

The whole REACH system is divided into different notification and registration phases with different deadline. In principle, since 1 December 2008, only registered chemicals are allowed on the market. The basic principle is no data, no market. However, if a manufacturer or importer has preregistered the chemical, transitional periods will apply. Only the "phase-in" chemicals are under the scope of the preregistration. The advantage of the preregistration is the extended timelines depending on the amount of chemicals placed on the market (Table 1) and the participation in a so-called SIEF forum. SIEF stands for "Substance Information Exchange Forum." In this forum, different manufacturers and importers can exchange data about the same chemicals and can also share costs for the data needed for the registration.

ECHA has published a list with the preregistered chemicals. The list can be downloaded on the ECHA website (http://echa.europa.eu/web/guest/information-on-chemicals/pre-registered-substances). Under certain circumstances, even if the preregistration phase is over, it is still possible for a late preregistration e.g., if an importer decides to import a certain chemical.

The requirements for submission of data to a certain timeline depend on the total amount of the chemical placed on the EU market. The higher the amount of

chemical manufactured or imported the more data is required for the registration and the shorter is the deadline to submit the data. All chemicals above 1 t/year on the EU market are under the scope of the regulation and need to go through the REACH processes. Chemicals classified as carcinogenic, mutagenic or reprotoxic (CMR) have special more strict requirements in respect of registration timelines and requested data.

For all phase-in and non-phase-in substances a submission of a technical dossier is required. Beside the physical/chemical properties and toxicological- and oekotoxicological properties, the dossier must also include information on classification and labeling, manufacturing and intended uses as well as instructions for the safe usage of the substance.

The quantitative and qualitative amount of data is dependent on the amount of sold or imported substance on the market in 1 year. The higher the amount the more data is requested. The technical dossier needs to be submitted via the latest IUCLID (http://iuclid.eu) (International Uniform Chemical Information Database) database software version.

Substances may be recognized as substances of very high concern, so-called SVHC (Substances of Very High Concern). If a substance is identified as a SVHC, because of its potential negative impacts on human health or the environment, a substance may be included on the authorization list (Annex XIV of the REACH Regulation) and become subject to authorization. If an SVHC is placed on the authorization list, the use of this substance needs an authorization by ECHA. Candidates for authorization are included on the candidates list of the SVHC. This list is continually updated and can be downloaded from the ECHA website (http://echa.europa.eu). From 1 June 2011, the ECHA must be notified of the presence of SVHCs in articles if the total quantity used is over 1 t/year and the SVHC is present on more than 0.1 % of the mass of the object.

An additional important REACH element is the communication along the user downstream. Manufactures as well as users need to communicate and exchange data on the supported use and the use of the chemical. In this sense, the whole industry is affected from the big chemical industry to the medium-sized and small-sized companies using the chemical.

The REACH regulation is complemented by the GHS (Globally Harmonized System) Regulation or also called CLP regulation (EC No. 1272/2008) on classification, labeling, and packaging of substances and mixtures. The CLP regulation became effective on 20 January 2009 and substitutes the EU Directives 67/548/EEC and 1999/45/EC for labeling and classification for on substances and on mixtures, respectively.

Information on REACH is available on the ECHA website (http://echa.europa.eu), in particular in the guidance documents. Also national helpdesks provide support for REACH and CLP questions (http://www.reach-clp-helpdesk.de/de/Startseite.html).

A special situation occurs in Switzerland. Switzerland, not a member of the EU, adopted the REACH regulation in the national "Chemikalienverordnung" under the chemical law "Chemikaliengesetz (ChemG)." However, there are essential

differences between the Swiss legislation and the REACH regulation. Information is available on the Swiss Chemical website (http://www.bag.admin.ch/anmeldestelle/13023/index.html?lang=de).

## Pesticides

Pesticides are essential for agriculture and high hygiene standards in our society. Pesticides are chemical compounds intended to kill, repel, control pests, to protect crops before and after harvest, to destroy weeds prevent their growth as well as to influence and preserve plant products. Pesticides cover a broad range of specific protection products like insecticides, acaricides, herbicides, fungicides, plant growth regulators, rodenticides, biocides, and veterinary medicines.

The EU regulates pesticides strictly to insure safety of human and environment and to ensure the efficacy of the used products.

The EU established an approval and authorization system on pesticides, especially on plant protection products and biocidal products in a two-step approach:

1. The Commission approves the active substances contained in the products.
2. EU member states authorize the products on their territory and ensure compliance with EU rules.

## Plant Protection Products

The Plant Protection Products Directive was introduced in 1991 with the Council Directive 91/414/EEC, which regarded the placing of plant protection products on the EU market. The Directive lays down rules and procedures for approval of active substances at EU level and for the authorization at Member State level of plant protection products (PPPs) containing these active substances. The system for the approval of an active substance on the EU level and then the approval of the products on the Member State level was the example for the BPD (98/8/EC) introduced 8 years later. The Directive 91/414/EEC was replaced by Regulation (EC) No. 1107/2009.

The regulation intents to ensure a high level of human, animal, and environmental protection and to provide clear rules to make the approval process for plant protection products more effective.

A plant protection product contains one or usually more active substances. Before companies can use an active substance in a plant protection product, the active substance needs to be approved by the responsible EU Committee.

To find out which active substances are approved in the European Union, a database can be consulted on the website of the European Commission (http://ec.europa.eu/sanco_pesticides/public/index.cfm). For each substance, there is a reference to the EU legislation, including the relevant toxicological information and the maximum residue levels in food and feed.

More general information concerning active substances and plant protection products and different guidance documents can be found on the EU website (http://ec.europa.eu/food/plant/protection/evaluation/index_en.htm).

In correlation to Regulation (EC) No. 1107/2009, two related topics need to be mentioned, one is the Regulation (EC) No. 396/2005 which regulates the residues of pesticides in food and the Directive 2009/128/EC which regulates the sustainable uses of pesticides.

In Switzerland, not an EU Member State, plant protection products are regulated by the "Pflanzenschutzmittelverordnung" (PSMV). This regulation has many similarities to the EU regulation. Further information is available at the website of the BAG (Bundesamt für Gesundheit) (http://www.admin.ch/ch/d/sr/c916_161.html).

## Biocides

Biocides are essential for the health and hygiene standards in our societies. Biocides are used in various application fields like disinfections, insecticides, or as preservatives to protect perishable materials. The regulation of biocides is one of the most recent and most comprehensive regulation development in the EU.

Biocides are regulated in the EU under the current Biocidal Products Directive (BPD) 98/8/EC, which will be replaced on 1 September 2013 by the Biocidal Products Regulation (BPR) (EU) No. 528/2012. Before the BPD biocides were regulated individually by the national Member States with different registration or notification systems or were not regulated at all. With the BPD (98/8/EC), the EU tried to implement a harmonized framework on biocidal products.

Essential for understanding of the authorization processes is the differentiation between an active substance and a biocidal product. A chemical or a microorganism that has an action on or against harmful organisms is defined as an active substance. Formulations with an active substance are defined as biocidal products, which are usually used for various biocidal purposes.

The definition of a biocidal product includes all substances or mixtures containing or generating active substances with the intention to destroy, deter, render harmless, prevent the action, or exert otherwise a controlling effect on any harmful organism by any means other than mere physical or mechanical action (definition according the Biocidal Products Regulation (EU) No. 528/2012).

The BPD as well as the BPR determines a two-step approach. In the first step, the approval of the active substances takes place at EU level. The assessment of the single active substance for the intended uses, so-called product type (PT), has been allocated to the competent authorities of an EU Member State. The appointed EU Member State reports the results of the evaluation, which are discussed at the competent authority meetings. The responsible EU Committee then decides on the inclusion on the positive list for active substances (Annex 1). In the second step, the subsequent authorization of the biocidal product is on the single EU Member State, meaning that an application for authorization needs to be submitted in the Member State in which the biocidal product is to be marketed.

One key point of the BPD and the BPR is the assignment of a biocidal product to one of the 22 different application fields for the intended uses outlined in the 22 product types (PT) as described in Annex V of BPR.

There are four main groups of PTs, which are further divided in subgroups adding up to 22 PTs. The four main PTs are:

- Disinfectants and general biocidal products e.g., human hygiene, private and public health area, veterinary hygiene, food and feed area and drinking water disinfectants
- Preservatives e.g., in-can or film preservatives, wood preservatives, preservatives for leather, rubber, masonry, preservatives for liquid-cooling and liquid-processing systems, slimicides e.g., for paper production and metalworking fluid preservatives
- Pest control e.g., rodenticides, avicides, molluscicides, piscicides, insecticides, repellents, and attractants
- Other biocide products e.g., preservatives for food or feedstock, antifouling products (e.g., for marina use)

With the assignment to a PT, a clear intended use and application field is defined. Any uses in other applications are clearly separated. Other uses are not under the scope of the BPD/BPR if these intended uses are controlled by their own regulations, e.g., pharmaceuticals for human and animals, plant protection, cosmetic, medical devices, food and feed.

The impending BPR intends to improve and simplify the registration and authorization processes compared to the BPD.

The main differences between the BPD and the BPR among other points are the involvement of ECHA (European Chemical Agency). ECHA will coordinate the submitted dossier of an active substance. After completeness check, the evaluation is allocated to a national competent authority. ECHA will then prepare an opinion on which the European Commission will make a decision. A new tool of communication will be a database for biocidal products, the so-called Register for Biocidal Products (R4BP). The R4BP database will be maintained by ECHA and is used as a submitting and exchanging platform between applicants, ECHA, Member States, competent authorities, and the European Commission.

New elements in the BPR are also exclusion and substitution criteria for active substances. If a substance meets the exclusion criteria the substance will not be approved. Exclusion criteria are e.g., carcinogens, mutagens and reprotoxic substances category 1A or 1B according to CLP classification, endocrine disruptors, persistent, bioaccumulative and toxic (PBT) substances as well as very persistent and very bioaccumulative (vPvB) substances.

Active substances falling under the substitution criteria will be candidates for substitution during the approval processes.

Under the BPR, the authorization of the biocidal product is still mainly at the national competent authority level. That means if an active substance is approved and a company is using the approved active substance for a biocidal product, the company needs to apply for authorization in a Member State. However, if an authorization is granted in a Member State, the applicant has the possibility to

ask for recognition of the authorization in another Member State. This so-called mutual-recognition procedure can be performed in sequence or in parallel. In addition, to the mutual-recognition of an authorization, a union authorization for all Member States is now possible under the BPR. The union authorization is organized by the ECHA and is only possible for single PTs currently.

Another main new subject under the BPR is the regulation of so-called treated articles. Treated articles are articles which have been treated with or intentionally incorporated one or more biocidal products. Treated articles do not fall under the definition of a biocidal product and do not need an authorization. However, it will be required to label the article with certain information, e.g., that the article was treated with a biocidal product and which active substance the article includes. In the past treated articles were not defined and were not subject under the BPD, this lack of regulation was recognized. Treated articles are widely spread in articles used by consumers in daily life e.g., treated wood, plastic and leather products as well as in technical textiles and apparel, furniture, and building material.

Under BPD as well as under the BPR transitional periods exist and will continue to exist especially for active substances, which are still in the evaluation phase for inclusion in Annex 1 of the BPD or to the corresponding Union List of the BPR. Originally, the evaluation of all actives substances was planned for 2010. However, the necessary administrational efforts and organizational needs were greatly underestimated by the EU Commission and the competent Authorities. The time for the evaluation of an active substance was prolonged several times and it is still not completely clear at what time all active substance will be evaluated. This situation revels in transitional period for those substances which are still in the evaluation phase. Biocidal products containing substances still in the evaluation phase are subject to national regulation. The nation regulations are very different form Member State to Member State. In some Member States, a simple notification is requested e.g., Germany. In other member States a complete BPD dossier is required e.g., in The Netherlands or a time-consuming complex notification and registration system was implemented e.g., in Belgium.

It needs to be mentioned that active substance registered or in the registration phase under the BPD or BPR is excluded from the REACH requirements. These substances are considered as registered. However, other chemical substances included in the biocidal products e.g., solvents, stabilizers and emulsifiers are still subjects to REACH.

Information about biocides is available on the EU Commission biocide website (http://ec.europa.eu/environment/biocides/index.htm) or on the website of the national authorities usually under the chemical section, e.g., in Germany (http://www.baua.de/de/Startseite.html).

Switzerland, not a member of the EU, basically adapted the EU legislation for biocides and has implemented the “Biozidprodukteverordnung” (VBP) under the chemical law. Information is available on the chemical website of the BAG (http://www.bag.admin.ch/anmeldestelle/index.html?lang=de).

## Food Contact Materials

Food contact materials are intended to come into contact with food e.g., packaging material, containers, cutlery and dishes, material in contact with water for human consumption. The regulation of food contact materials in the EU is a complex system of regulations and directives. The frame Regulation No 1935/2004 describes the general requirements for all food contact materials. In principle, material intended to come into contact with food shall not release chemicals into food in unacceptable quantities. Furthermore, the material shall not change the food in composition and in quality and it shall not impact taste, smell, or appearance of the food. The overall migration limit for plastic materials is set to 10 mg of substances/$dm^2$ of food contact surfaces, which is equivalent to 60 mg/kg foodstuff for all substances that can migrate from the contact material into food. Specific migration limits (SML) are also established for individual substances based on ADI (acceptable daily intake) or TDI (tolerable daily intake) values established by the Scientific Committee on Food. A relatively new regulation is Regulation EU No 10/2011, the plastic regulation on plastic materials and articles intended to come into contact with food. Regulation EU No 10/2011 replaces the EU Directive 2002/72/EC and includes a list of approved substances (Annex I) for food contact materials. This regulation also outlines the test methods and conditions for the measurement of migration.

An overview of the related regulations and directives for food contact materials is outlined and illustrated in the picture below.

Frame legislation:

- Regulation EC No 1935/2004: Frame regulation on materials and articles intended to come into contact with food
- Regulation EC No 2023/2006: Good manufacturing practice

Legislation on specific materials:

- Regulation EU No 10/2011, updated by Regulation EU No 1282/2011: Plastic materials and articles intended to come into contact with food
- Regulation EC No 450/2009: Active and intelligent materials and articles intended to come into contact with food
- Regulation EC No 282/2008: Recycled plastic material and articles intended to come into contact with food
- Directive 2007 /42/EC: Materials and articles made of regenerated cellulose film intended to come into contact with food
- Directive 84/500/EEC, amended by Directive 2005/31/EC: Sets migration limits for cadmium and lead which might be released from decoration or glazing

Legislation on specific substances:

- Regulation No 1895/2005/EC: Restriction of use for certain epoxy derivatives
- Regulation EU No 321/2011: Restriction on bisphenol A use in plastics for infant feeding
- Regulation EU No 284/2011: On import procedures for polyamide and melamine plastic kitchenware from China and Hong Kong

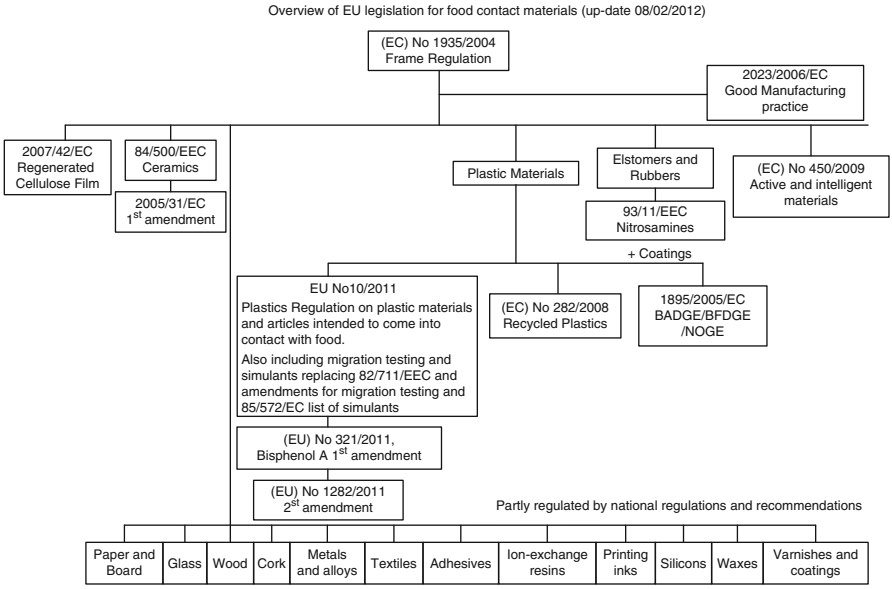



**Fig. 1** Overview of EU legislation on food contact material (Source: References of the European and National Legislations, European Commission, 2012 (http://ec.europa.eu/food/food/chemicalsafety/foodcontact/docs/ReferencesEurNatLeg_20091026.pdf))

- Directive 93/11/EEC: Release of N-nitrosamines and N-nitrosatable substances from rubber teats and soothers (Fig. 1)

Further information can be found on the EU website (http://ec.europa.eu/food/food/chemicalsafety/foodcontact/index_en.htm).

## Toys

Toys are a source for exposure of chemicals to the consumer. Therefore, toys must meet high safety standards to ensure the safety of consumers in general and for children in specific. Toys are regulated by the "Toy Safety Directive" 2009/48/EC, which replaces the old Directive 88/378/EEC.

The Directive lays down the basic safety criteria toys must meet before being placed on the market. Technical details are described in technical harmonized standards which are suitable to ensure the safety of toys, e.g., Standard EN 71-2 for flammability or Standard EN 71-3, migration of certain elements, e.g., metals.

Toys are not subject to registration processes. However, toys underlie the conformity assessment in which the conformity with the applicable safety standard needs to be confirmed. The conformity is identified by the CE mark on toy articles.

Details and further information can be found on the EU website (http://ec.europa.eu/enterprise/sectors/toys/index_en.htm).

## Cosmetics

Cosmetic products are products only intended to be used for contact with the various external parts of the human body e.g., epidermis, hair system, nails, lips or with the teeth, and the mucous membranes of the oral cavity with the intention to cleaning them, perfuming them, changing their appearance and/or correcting body odors, and/or protecting them or keeping them in good condition.

In comparison to the already discussed previous regulated areas, substances used for cosmetic products are intended for the exposure to consumers.

Cosmetic products are regulated by the Council Directive 76/768, which was amended into the new EU Regulation No 1223/2009. The new Cosmetic Regulation came into force in January 2010 and will be effective on 1 July 2013, except for some parts concerning CMR substances and nanomaterial, which are effective earlier.

The general structure of the former Cosmetic Directive is also reflected in the new Cosmetic Regulation. There is no registration requirement for cosmetic products before placing them on the market. However, substances included in cosmetic products are regulated depending on their function. The Cosmetic Directive as well as the Regulation mention positive and negative lists of substances for specific uses.

On the negative list are substances that are not allowed in cosmetic products, e.g., CMR substances. On the positive list are substances for the specific intended use as preservatives, UV filters and dyes with concentration limits.

Substances, which are not regulated by negative or positive lists, are permitted as ingredients in cosmetic products as long as they are safe for consumers. Therefore, a key element of a cosmetic product is the dossier with the safety assessment that needs to be available on demand for the Competent Authority. The new Regulation describes more precisely the requirements of the safety assessment than the former Directive.

Responsible for the safety of cosmetic products is the importer or manufacturer placing the product on the market.

More information is provided at the EU website for cosmetics (http://ec.europa.eu/consumers/sectors/cosmetics/index_en.htm).

## Medical Devices

According to the definition in the Directive 2007/47/EC, medical devices are instruments, apparatuses, implants, in vitro reagents, or related article that are used to diagnose, prevent or treat disease or other conditions and do not achieve their purposes through chemical action within or on the body. Medical devices act

by physical, mechanical or thermal means. Medical devices vary greatly in complexity and application. They cover a vast range of equipment from simple tongue depressors to hemodialysis machines and pacemakers.

Rules that relate to safety and performance of medical devices were harmonized in the EU. The core legal framework consists of 3 basic Directives:

- Directive 90/385/EEC regarding active implantable medical devices
- Directive 93/42/EEC regarding medical devices
- Directive 98/79/EC regarding in vitro diagnostic medical devices

The aim of a harmonized Medical Device Directive in the EU is to ensure a high level of protection of human health and safety. These 3 main Directives have been supplemented and amended over time by several modifying and implementing directives. Directive 2007/47 EC introduced the latest technical revision. However, a new revision of the regulatory framework for medical devices in the EU is in preparation. The European Commission adopted a proposal for a regulation on medical devices as well as a proposal for a regulation on in vitro diagnostic medical devices which will, once adopted by the European Parliament and by the Council, replace the existing three medical device directives.

Medical device regulation is based on a compliance system. The responsibility for safety and compliance with the EU Directives or the according national regulations reflecting the EU Directives is on the side of the medical device manufacturer or importer. Manufacturers guarantee the authorization of medical devices through a Declaration of Conformity. Medical devices belonging to class I can be marketed by self-certification of the manufacturer. Certification of higher-risk products (classes IIa, IIb, or III) must be verified by a Certificate of Conformity issued by a Notified Body. A Notified Body is a public or private organization that has been accredited to validate the compliance with the according regulations. All medical devices must be identified with the CE mark.

Medical devices are divided into different classes based on their design complexity, their use characteristics and their potential for harm if misused. A combination of medical devices and drugs, so-called combination products, needs to follow a special regulatory process before being marketed.

The classification of medical devices in the European Union is outlined in the Directive 93/42/EEC. There are basically four classes, ranging from low risk, class I, to high risk, class III:

- Class I (e.g., wound bandages)
- Class Ila (e.g., surgical suture)
- Class IIb (e.g., lung ventilator)
- Class III (e.g., pacemaker)

The European classification depends on rules that involve the medical device duration of body contact, invasive character, use of an energy source, effect on the central circulation or nervous system, diagnostic impact, or incorporation of a medicinal product.

More detailed information is available on the EU website (http://ec.europa.eu/health/medical-devices/index_en.htm), the MEDDEV guidelines (http://ec.europa.eu/health/medical-devices/documents/guidelines/index_en.htm), and the national

competent authorities, e.g., BfArM, (http://www.bfarm.de/DE/Medizinprodukte/medizinprodukte-node.html).

## Medicines

Medicine products, also called pharmaceuticals, achieve their principal action by pharmacological, metabolic, or immunological means, the action is different than for medical devices. Medicines are intended to have an effect on humans and animals. An exposure of the chemical substance on humans or animals is intended. The authorization of medicines is the most complex and most expensive regulated field compared to other regulated areas.

To ensure a high safety standard and an acceptable risk-benefit ration, medicines need to pass a complex authorization process.

The requirements for medicines are basically very similar in all countries with an authorization system for medicines and are very much harmonized in Europe, North America, and Japan. The basic requirement for an authorization of a medicine is the acceptable pharmaceutical quality and pharmaceutical efficacy, safety for the patient, and an acceptable risk-benefit ration.

The medicine must have an acceptable quality according to pharmaceutical rules. The guidelines and rules of pharmaceutical quality are described, e.g., in the Pharmacopoeia monographs. The pharmaceutical quality covers the composition of a medicine, the manufacturing processes, quality control of the raw materials, the intermediates and the final product as well as storage stability studies. The manufacturing processes need to comply with the rules for "good manufacturing practice" (GMP).

The efficacy of a medicine is a basic requirement for the authorization. The efficacy is the intended effect to heal diseases or improve the health of a patient.

Safety can only be considered in relative terms. All medicines carry a certain degree of risk and could cause problems in specific circumstances. The safety of a medicine needs to be demonstrated by nonclinical and clinical studies. In order to obtain a complete safety profile of the medicine, observations of the continuously collected "pharmacovigilance" reports are included in the safety evaluation of the competent authorities. In this way also long-term effects, which could not be discovered during the clinical studies, can be recognized.

Pharmacovigilance is the pharmacological science relating to the detection, assessment, understanding and prevention of adverse effects particularly long-term and short-term side effects of medicines (http://apps.who.int/medicinedocs/en/d/Js4893e/).

The benefit-risk ratio reflects the possible benefit of the medicine for the human or the animal in relation to the possible risks, which could occur, e.g., possible side effects. The benefit risk is also constantly under observation using the pharmacovigilance reporting.

For the authorization of a medicine, all necessary data and studies need to be submitted to the competent authorities for evaluation. In order to harmonize the

**Table 2** Different ways of authorization in the EU

| | Centralized procedure | Decentralized procedure | National procedure | Mutual- recognition procedure |
|---|---|---|---|---|
| Procedure | Authorization in all EU member states | Authorization in several EU member states simultaneously | Only nation authorization | Authorization on the base on an existing authorization in another member state |
| Competent authority (CA) | EMEA | EMEA and national CA | National CA | National CA |
| Validity | All EU member states including Iceland, Norway, Lichtenstein | All member states in which the authorization is accepted | In the concerning national state | In the member states who accepted the application |

submitted data, a standard application format dossier was developed by the ICH (International Conference on Harmonization). The standard application format is known as the Common Technical Document (CTD). The CTD dossier consists of five modules, which include all the necessary information. Module 1 is about regional and specific information. Module 2 includes an overview and summary of modules 3, 4, and 5. Module 3 includes the quality part, describing manufacturing and analytics of the medicines. Module 4 includes the preclinical pharmacological and toxicological studies. Module 5 includes the clinical studies.

More information is available on the ICH website (http://www.ich.org).

The approval procedures are regulated by national regulations, international regulations, and international mutual-recognition procedures.

In the EU, the authorization of medicines was regulated in the beginning by Directive 65/65/EEC, which has been replaced by the Directive 2001/83/EC. The Directive is integrated in the different national laws for the authorization of medicines.

The regulation in the EU offers several different ways of how a company can apply for the authorization of medicines.

The national procedure was the only way in the EU until 1995. Since then the European procedure gained importance and the national procedures were preplaced by the European ones. Nowadays the national procedure is only possible in one Member State. Multiple national procedures are not possible any more. Nevertheless, national competent authorities still play a major role in the authorization process.

The centralized authorization procedure for human and veterinary medicine is managed by the EMEA (European Medicines Agency) in London and the EU Commission grants the authorization. The authorization is valid in all EU Member States as well as in Iceland, Lichtenstein, and Norway. The centralized procedure is mandatory for:

- Human medicines for the treatment of cancer, diabetes, HIV/AIDS, neurodegenerative diseases, autoimmune and other immune dysfunctions, and viral diseases

- Veterinary medicines for use as growth or yield enhancers
- Medicines derived from biotechnology processes, e.g., genetic engineering
- Gene therapy, somatic cell therapy, or tissue-engineered medicines
- Officially designated "orphan medicines" (medicines used for rare human diseases)

Decentralized procedure is used when an authorization is intended simultaneously in several EU Member States if the medicine does not already have an authorization in an EU Member State.

Mutual-recognition procedure needs to be used if a national authorization has already been granted and additional authorization in other Member States is intended (Table 2).

Further and more detailed information is available on the websites of the EMEA (http://www.ema.europa.eu) and the national competent authorities responsible for medicinal products e.g., Germany (http://www.bfarm.de).

# Health Hazards Classification and Labeling

Herbert Desel, Pieter Brekelmans, and Ronald de Groot

## Contents

European Chemicals Legislation ..... 678
Health Hazard Classification and Labelling ..... 678
Safety Data Sheet ..... 681
Poisons Centers Perspective ..... 682
References ..... 682
Resources ..... 683

**Abstract**

The European Union CLP Regulation (EC No 1272/2008) brings the UN Globally Harmonized System of Classification and Labelling of Chemicals (UN-GHS) into force in the European Union. The structure of the CLP hazard classification and the rules for labelling hazardous substances and mixtures are described. The structure and uses of Safety Data Sheets (emergencies/safe use scenarios) are described.

The authors are active participants in the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) Working Group on Poisons Centres' Activities/European Regulatory Issues, and as poisons center experts, they take part in discussions on harmonization of product notification for poisons centers with the European Commission, industry, and other stakeholders.

H. Desel (✉)
GIZ-Nord Poisons Center, University Medical Center Göttingen - Georg-August-Universität, Göttingen, Lower Saxony, Germany
e-mail: hdesel@med.uni-goettingen.de; hdesel@giz-nord.de

P. Brekelmans • R. de Groot
National Poisons Information Center (NVIC), University Medical Center Utrecht, Utrecht, GA, The Netherlands
e-mail: P.Brekelmans@umcutrecht.nl; R.degroot-5@umcutrecht.nl

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_83, 

## European Chemicals Legislation

Several regulations of the European Union (EU) aim to ensure a high level of protection of human health from the risks that can be posed by chemicals on the EU market.

Due to the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation (EC No 1907/2006, European Parliament and Council 2006), industry is responsible for assessing and managing the risks and to gather all relevant substance information for registration at the European Chemicals Agency (ECHA). The *REACH Regulation* (see chapter "▶ Reach (and CLP). Its Role in Regulatory Toxicology") also incorporates updated requirements for the Safety Data Sheet, an important document informing professionals on safe use of a substance or mixture ("product").

Classification and labelling of hazardous substances and mixtures is important in communicating the potential hazards and providing the basis to describe and plan for safe use. Furthermore, classification supports the poisoning risk assessment if persons have been exposed in an unsafe way. To harmonize hazard classification criteria and communication elements worldwide, the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (UN-GHS) was developed. The *CLP Regulation* on Classification, Labelling and Packaging of substances and mixtures (EC No 1272/2008, European Parliament and Council 2008) implements UN-GHS (UNCED 1992) in the EU. The CLP Regulation has entered into force on 20 January 2009 and has replaced the existing rules on classification, labelling, and packaging of substances since 1 December 2010 and will replace those for mixtures from 1 June 2015.

## Health Hazard Classification and Labelling

The framework of the CLP health hazard classification (CLP, Annex I, Part 3) is mainly based on health hazard classes, describing the quality of action of the hazard in focus (e.g., "acute toxicity," "specific target organ toxicity"). The quantity measure (strength or potency) of a hazard quality is described using numeric hazard categories, where higher category numbers (1 to a maximum of 4,) indicate lower toxicity. For some classes, category 1 is subdivided into 1A, 1B, and 1C. An overview of CLP health hazard classes and categories is presented in Table 1.

Each hazard category is linked to four groups of specific hazard communication elements: *signal words*, *hazard pictograms*, *hazard statements*, and *precautionary statements*.

The signal word "Danger" is associated with categories of higher hazard and "Warning" with those of lower hazard.

Four health hazard pictograms are used in the hazard communication (see Fig. 1). Carried over from the preceding legislation (with some graphical amendments) are the "skull and crossbones" pictogram and the "corrosion" pictogram. The "health hazard" pictogram and "exclamation mark" pictogram have no preceding equivalent.

**Table 1** CLP health hazard classes

| CLP Annex I chapter | CLP health hazard class | Differentiation |
|---|---|---|
| 3.1 | Acute toxicity | Oral, dermal, inhalation |
| 3.2 | Skin corrosion/ irritation | |
| 3.3 | Serious eye damage/eye irritation | |
| 3.4 | Respiratory or skin sensitization | Respiratory, skin |
| 3.5 | Germ cell mutagenicity | |
| 3.6 | Carcinogenicity | |
| 3.7 | Reproductive toxicity | Sexual function and fertility, development of the offspring |
| 3.8 | Specific target organ toxicity – single exposure | |
| 3.9 | Specific target organ toxicity – repeated exposure | |
| 3.10 | Aspiration hazard | |

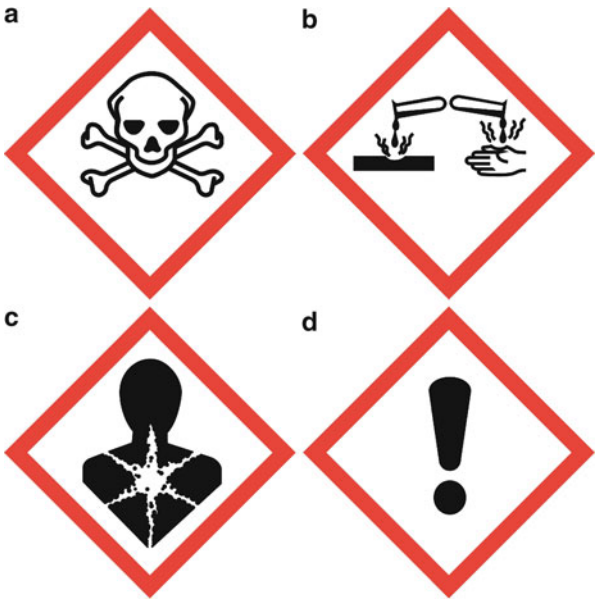



**Fig. 1** Pictograms for health hazards according to the CLP Regulation (EC) No 1272/2008. (**a**) skull and crossbones, (**b**) corrosion, (**c**) health hazard, (**d**) exclamation mark (From UNECE 2012, with permission)

An overview of the fixed connection of health hazard classes and categories with signal words and hazard pictograms is presented in Fig. 2.

Hazard (H)-statements are used to describe the character of the hazard often in combination with the route of exposure (see Table 2 for some examples). Precautionary (P)-statements advise about the correct handling of chemical substances and mixtures. A complete list of hazard and precautionary statements (with translations in all EU languages) is included, respectively, as Annex III and Annex IV to the CLP Regulation.

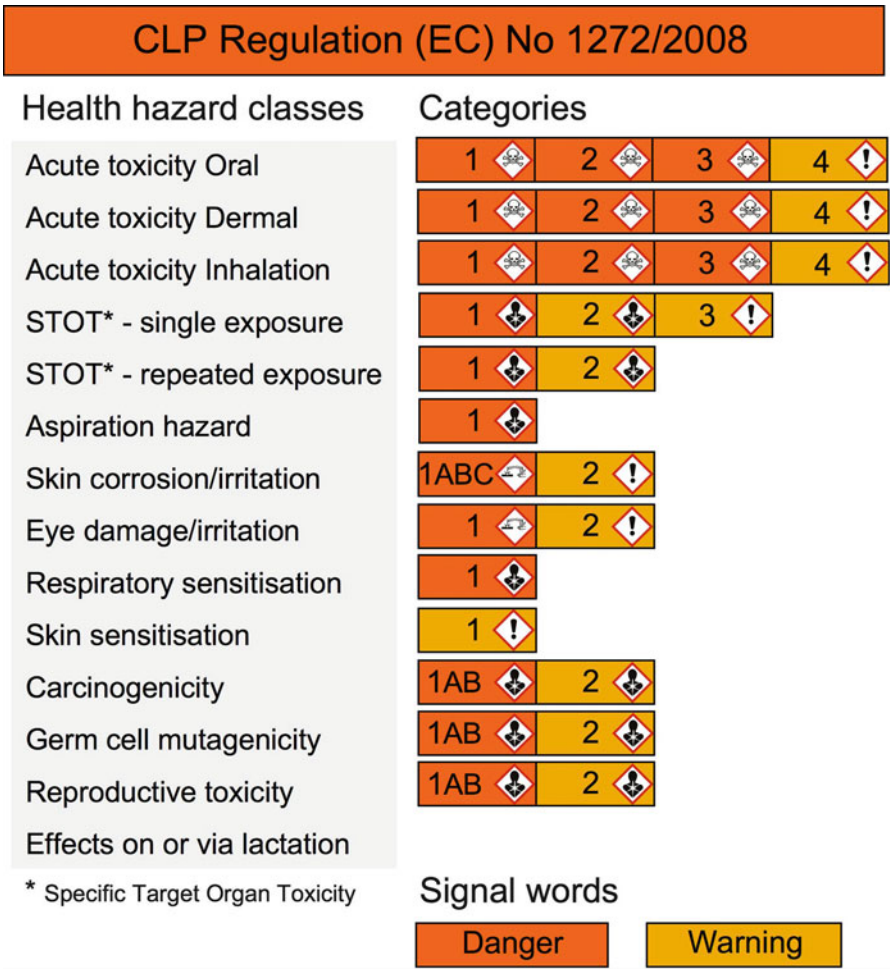



**Fig. 2** Health hazard classification of substances and mixtures according to the CLP Regulation (EC) No 1272/2008 with corresponding signal words and hazard pictograms (Adapted from Clinical Toxicology (2010) 48, 28–33)

Classification and labelling information on substances is made available online in the Classification & Labelling Inventory (maintained by ECHA). This database includes all substances with a harmonized (and legally binding) hazard classification as listed in Annex VI of the CLP Regulation and substances registered under REACH for which the manufacturer or importer is responsible for correct classification and labelling.

For mixtures, data indicating their (toxic) hazard profile are only rarely available. If there are no data on a mixture to be classified, then procedures listed in Annex I of the CLP Regulation can be used to calculate or evaluate its hazard

**Table 2** Hazard statements for acute toxicity (selected examples for oral and dermal exposure)

| Category of acute toxicity | Route | Hazard statement code | Hazard statement |
|---|---|---|---|
| 1 | Oral | H300 | Fatal if swallowed |
| 2 | Oral | H300 | Fatal if swallowed |
| 3 | Oral | H301 | Toxic if swallowed |
| 4 | Oral | H302 | Harmful of swallowed |
| 1 | Dermal | H310 | Fatal in contact with skin |
| 2 | Dermal | H310 | Fatal in contact with skin |
| 3 | Dermal | H311 | Toxic in contact with skin |
| 4 | Dermal | H312 | Harmful in contact with skin |

**Table 3** Structure of Safety Data Sheet according to REACH

| Section number | Content |
|---|---|
| 1 | Company name and address and emergency telephone number |
| 2 | Description of the hazards of the substance or mixture and the appropriate warning information associated with those hazards |
| 3 | Composition/information on ingredients. Listing all ingredients classified as hazardous (above specified concentration thresholds) and their concentration (either exact or ranges) |
| 4 | First aid measures by relevant routes of exposure |
| 11 | A description of the various toxicological (health) effects and the available data used to identify those effects, including where appropriate information on toxicokinetics, metabolism, and distribution |

(*these bridging principles are described in chapter* "► *Bridging. The Regulation of Toxic Mixtures*" *of this monograph*). The most important tools are calculation methods that allow deduction of the mixture classification from classification of its ingredients.

## Safety Data Sheet

For a more detailed risk assessment, especially in emergency situations and for development of scenarios for safe use of hazardous substances and mixtures at the workplace, the communication elements on the label are not sufficient. Additional information is provided in the *Safety Data Sheet* (SDS). The SDS has a fixed structure with 16 sections. The content of the sections with important use for toxicology are listed in Table 3.

The toxicological information in section 11 shall apply to the substance or mixture as placed on the market. If available, the relevant toxicological properties of the hazardous substances in a mixture shall also be provided. For every relevant health hazard class (for mixtures for every "relevant effect" until from 1 June 2015, the new hazard classification applies), toxicological information should be included, and if available, human data should be provided.

For substances, section 11 of the SDS will include (a summary consistent with) the toxicological information which is supplied for the registration of the substance according to the REACH Regulation.

For some substances, a Chemical Safety Report (CSR) is compiled for the REACH registration which includes *exposure scenarios* giving a.o. information on how the mixture will be used by professional users or consumers (e.g., duration and frequency) and risk management measures to reduce or avoid direct and indirect exposure. These exposure scenarios will be made available as an Annex to the SDS.

## Poisons Centers Perspective

Consumers are informed about the hazards and safe use of a product by communication elements on the product label, professional users have access to additional information on the SDS, but in case of incidents (unsafe exposures), the SDS is only a starting point and more detailed information is necessary for medical management in many cases.

When exposure cases are treated in the medical system, most often in a hospital, *poisons centers* can be consulted for toxicological support. Poisons centers often have to deal with unusual exposures, e.g., intake of large doses, untypical exposure pathways (intravenous application, ingestion), or special patient groups (e.g., pregnant, child, immunosuppressed patients, or patients with reduced mental capacity).

Although the improved toxicological information on the SDS will be helpful, an important shortcoming of the SDS for poisons centers practice is that only substances that are classified as hazardous have to be mentioned and only above specified threshold concentrations. Furthermore, as guidelines on the notification of the concentration of ingredients are not available in practice, wide concentration ranges are often used. To perform a risk assessment in individual poisoning cases, poisons centers need and have access to a detailed product composition of all hazardous products.

Notification of product information for poisons centers is described in chapter "► Notification of Cosmetic Products and Dangerous Mixtures in Regulatory Toxicology."

## References

European Parliament and Council (2006) Regulation (EC) No 1907/2006 of the European parliament and of the council of 18 December 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH). Off J Eur Union (EN) L. 396:1–849

European Parliament and Council (2008) Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures. Off J Eur Union (EN) L 353:1–1355

United Nations Conference on Environment and Development (UNCED, 1992) Agenda 21, chapter 19: environmentally sound management of toxic chemicals, including prevention of illegal international traffic in toxic & dangerous products. http://sustainabledevelopment.un.org/content/documents/Agenda21.pdf. Accessed 21 Aug 2013

United Nations Economic Commission for Europe (UNECE 2012) Globally harmonized system of classification and labelling of chemicals (GHS) - GHS pictograms. http://www.unece.org/trans/danger/publi/ghs/pictograms.html. Accessed 21 Aug 2013

## Resources

De Groot R et al (2010) The changes in hazard classification and product notification procedures of the new European CLP and Cosmetics Regulations. Clin Toxicol 48:28–33. http://informahealthcare.com/doi/abs/10.3109/15563650903376097. Accessed 21 Aug 2013

De Groot R et al (2011) Implications of the new EU legislation on chemicals for poisons centres. Clin Toxicol 49:794–798. http://informahealthcare.com/doi/abs/10.3109/15563650.2011.623129. Accessed 21 Aug 2013

European Chemicals Agency (ECHA, 2013) Classification & Labelling Inventory. http://echa.europa.eu/information-on-chemicals/cl-inventory. Accessed 20 Aug 2013

United Nations (2003) Globally harmonized system of classification and labelling of chemicals (GHS), see e.g.: http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html. Accessed 21 Aug 2013

# Human Biomonitoring. Its Importance in Toxicological Regulation

Michael Wilhelm

## Contents

Introduction ..... 686
Environmental Monitoring ..... 686
Chemicals (Biomarkers of Internal Exposure) ..... 687
Biochemical Effect Monitoring ..... 687
DNA Adducts ..... 687
8-Hydroxy-2′-deoxyguanosin (8-OHdG) ..... 691
Hemoglobin Adducts (Hb Adducts) ..... 691
Biological Effects (Biomarker of Effect) ..... 692
Other Markers ..... 692
General Population HBM Programs ..... 693
Evaluation of HBM Results (Internal Exposures) ..... 693
Reference Values ..... 693
Reference Value and Risk Assessment ..... 694
Health-Based Values ..... 697
HBM Values ..... 697
Biological Equivalents (BEs) ..... 700
Risk Assessment in Context with Surveys/Tool in Risk Management ..... 701
References ..... 702
Resources ..... 702

**Abstract**

Human biomonitoring (HBM) aims to determine internal exposure to chemicals and related effects. Similar to environmental monitoring, HBM is a basic method for the protection of human health in case of exposure to chemical substances. About 200 chemicals can be analyzed by HBM. In many countries broad general

M. Wilhelm
Department of Hygiene, Social and Environmental Medicine, Ruhr-University Bochum, Bochum, Germany
e-mail: wilhelm@hygiene.ruhr-uni-bochum.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_84, 

population HBM programs have been established. HBM data are evaluated by reference values and health-based values. Interpretation of HBM data in exposure assessment and health risk context increases its utility and input into risk assessment and risk management.

## Introduction

*Human biomonitoring* (HBM) is defined as the measurement of concentrations of chemicals or their metabolites in human biological media such as blood, urine, or breast milk. Application of hair, pulmonary air, teeth, nails, and saliva in HBM is limited to specific issues. HBM also includes chemical and biological parameters (biochemical effect monitoring, biological effect monitoring) which allow inferences about the pollutants' biological effect. HBM is considered the method of choice for determining internal exposures in the population, population groups, or individuals. Similar to environmental monitoring (EM), HBM is a basic method for the protection of human health in case of exposure to chemical substances. HBM of dose and biochemical effect is an efficient and cost-effective tool to assess human exposure to chemical substances. HBM considers all routes of uptake and all sources which are relevant. HBM is an ideal instrument for risk assessment and risk management. HBM can identify new chemical exposures (merging chemicals), trends, and changes in exposure, establish distribution of exposure among the general population, identify vulnerable groups and populations with higher exposures, and identify environmental risks at specific contaminated sites. The focus of this paper is on HBM related to environmental and not to occupational exposures. Several overviews on HBM are available (e.g., Angerer et al. 2007, 2011; Needham et al. 2007; Schulz et al. 2011). Figure 1 shows the relation between exposure and health impairment and how EM and HBM are integrated in the scheme.

## Environmental Monitoring

Environmental monitoring (EM), also called ambient monitoring, is the determination of chemical substances in environmental samples such as water, air, soil, indoor air, dust, or food (food monitoring). HBM is considered to supplement EM. EM is especially necessary to identify the sources of exposure and to facilitate measures for minimizing emissions. The purpose of EM is similar to HBM to show how well environmental objectives are met and to help detect new environmental issues. The results are also of fundamental importance to environmental management in general, as the drafting and prioritization of environmental policies is based on the findings of EM.

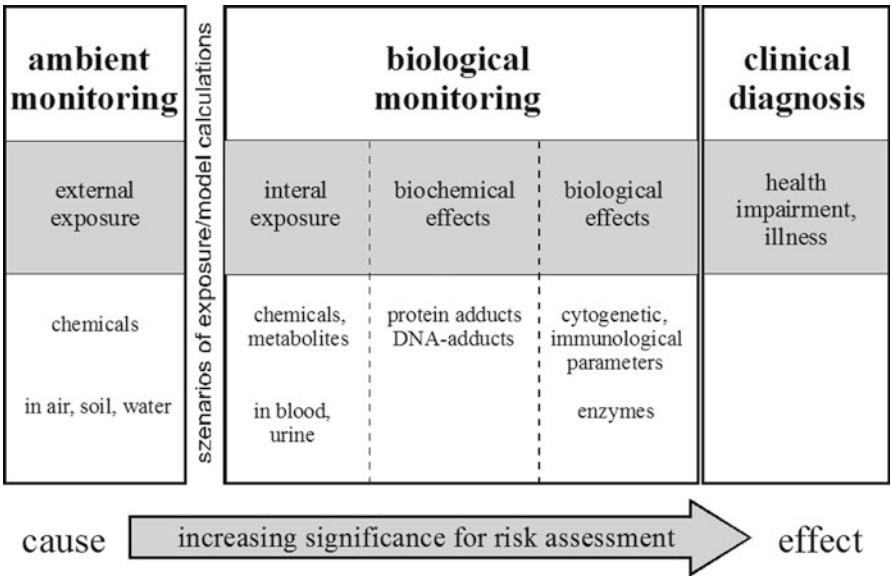



**Fig. 1** Relation between exposure and health impairment (Modified from Angerer et al. 2007)

## Chemicals (Biomarkers of Internal Exposure)

Nowadays, about 200 chemicals can be analyzed by HBM (Table 1). The number is steadily increasing.

## Biochemical Effect Monitoring

### DNA Adducts

*DNA adducts* are markers of exposure to carcinogenic substances showing the intake of carcinogens and metabolic activation by forming an ultimate carcinogen which can covalently interact with cellular DNA (details in Angerer et al. 2007). DNA adducts represent key events in mutagenesis and carcinogenesis. For the determination of adducted nucleosides, mostly white blood cells and lymphocytes, in some cases sputum and exfoliated urothelial cells, have been used as surrogate tissues. DNA adduct monitoring has been performed in relation to substances like PAH, aromatic amines, dietary heterocyclic amines, and others. Though there are very sensitive techniques for DNA adduct monitoring available, they lack specificity. DNA adducts seem to be a promising tool within molecular epidemiology in population studies; interpretation on an individual level is currently not possible.

**Table 1** Biomarkers of internal exposure in environmental health

| Classes of chemical exposure | Chemical, metabolites |
|---|---|
| Aromatic amines | Aniline, o-toluidine, m-toluidine, p-toluidine, o-anisidine, 3-chloroaniline, 4-chloroaniline, 3,5-dichloroaniline, 4-dichloroaniline, 2-aminonaphthalene, 4-aminobiphenyl |
| Carbamates | Carbofuranphenol, 2-isopropoxyphenol |
| Chlorophenols | 2-Monochlorophenol, 4-monochlorophenol, 2,4-dichlorophenol, 2,5-dichlorophenol, 2,6-dichlorophenol, 2,3,4-trichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachloro-phenol, pentachlorophenol (PCP) |
| Disinfection by-products | Bromodichloromethane, dibromochloromethane, tribromomethane (bromoform), trichloromethane (chloroform) |
| Fungicides | Ortho-phenylphenol, ethylene thiourea, pentachlorophenol, propylene thiourea |
| Herbicides | 2,4-Dichlorophenol, 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, various sulfonylurea herbicides |
| Metals/metalloids | Aluminum, antimony, arsenic and arsenic compounds/species, barium, beryllium, cadmium, cesium, cobalt, chromium, copper, lead, mercury and mercury compounds/species, molybdenum, nickel, platinum, selenium, thallium, tungsten, vanadium, uranium, zinc |
| Organochlorine pesticides | Aldrin and dieldrin, oxychlordane, heptachlor epoxide, *trans*-nonachlor, dichlorodiphenyltrichloroethane (DDT), p,p′-dichlorodiphenyltrichloroethane (DDT), p,p′-dichlorodiphenyldichloroethene (DDE), o,p′-dichlorodiphenyltrichloroethane, endrin, hexachlorobenzene, hexachlorocyclohexane, beta-hexachlorocyclohexane, gamma- hexachlorocyclohexane (lindane), 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and other pesticide metabolites |
| Organophosphorus insecticides, dialkyl phosphate metabolites | Diethylphosphate, dimethyl phosphate, diethylthiophosphate, dimethylthiophosphate, diethyldithiophosphate, dimethyl dithiophosphate and specific metabolites: urinary acephate, urinary dimethoate, urinary omethoate, urinary methamidophos |
| Parabens | Butylparaben, ethylparaben, methylparaben, *n*-propylparaben |
| Perchlorate and other anions | Nitrate, perchlorate, thiocyanate |
| Perfluorinated compounds | Perfluorobutanesulfonic acid, perfluorodecanoic acid, perfluorodecanoic acid, perfluoroheptanoic acid, perfluorohexane sulfonic acid, perfluorononanoic acid, perfluorooctanoic acid, perfluorooctanesulfonic acid, perfluorooctanesulfonamide, 2-(*N*-ethyl-perfluorooctane sulfonamido) acetic acid, 2-(*N*-methyl-perfluorooctane sulfonamido) acetic acid, perfluoroundecanoic acid |
| Phenols | Benzophenone-3, bisphenol A, 4-tert-octylphenol, triclosan |

(*continued*)

**Table 1** (continued)

| Classes of chemical exposure | Chemical, metabolites |
|---|---|
| Phthalates | Monobenzyl phthalate, monoisobutyl phthalate, mono-*n*-butyl phthalate, mono-cyclohexyl phthalate, mono-ethyl phthalate, mono-2-ethylhexyl phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-oxohexyl) phthalate, mono-(2-ethyl-5-carboxypentyl) phthalate, mono-(carboxynonyl) phthalate, monoisononyl phthalate, mono-(carboxyoctyl) phthalate, mono-methyl phthalate, mono-(3-carboxypropyl) phthalate, mono-*n*-octyl phthalate |
| Phytoestrogens | Daidzein, enterodiol, enterolactone, equol, genistein, o-desmethylangolensin |
| Polyaromatic hydrocarbons | 1-,3-,9-Hydroxybenz[a]anthracene; 1-,2-,3-hydroxybenzo[c]phenanthrene, 1-,2-,3-,4-,6-hydroxychrysene, 3-hydroxyfluoranthene, 2-,3-,9-hydroxyfluorene, 1-,2-,3-,4-,9-hydroxy-phenanthrene, 1-hydroxypyrene, 3-hydroxybenzo[a]pyrene, 1-,2-hydroxynaphthalene |
| Polybrominated diphenyl ethers | 2,2′,4,4′,5,5′-Hexabromobiphenyl (BB-153), 2,2′,4-tribromodiphenyl ether (BDE 17), 2,4,4′-tribromodiphenyl ether (BDE 28), 2,2′,4,4′-tetrabromodiphenyl ether (BDE 47), 2,3′,4,4′-tetrabromodiphenyl ether (BDE 66), 2,2′,3,4,4′-pentabromodiphenyl ether (BDE 85), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE 99), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE 100), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE 153), 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE 154), 2,2′,3,4,4′,5′,6-heptabromodiphenyl ether (BDE 183), 2,2′,4,4′,5,5′-hexabromobiphenyl (BB 153) |
| Polychlorinated biphenyls, non-dioxin-like | 2,4,4′-Trichlorobiphenyl (PCB 28), 2,2′,3,5′-tetrachlorobiphenyl (PCB 44), 2,2′,4,5′-tetrachlorobiphenyl (PCB 49), 2,2′,5,5′-tetrachlorobiphenyl (PCB 52), 2,3′,4,4′-tetrachlorobiphenyl (PCB 66), 2,4,4′,5-tetrachlorobiphenyl (PCB 74), 2,2′,3,4,5′-pentachlorobiphenyl (PCB 87), 2,2′,4,4′,5-pentachlorobiphenyl (PCB 99), 2,2′,4,5,5′-pentachlorobiphenyl (PCB 101), 2,3,3′,4′,6-pentachlorobiphenyl (PCB 110), 2,2′,3,3′,4,4′-hexachlorobiphenyl (PCB 128), 2,2′,3,4,4′,5′and 2,3,3′,4,4′,6-hexachlorobiphenyl (PCB 138 and 158), 2,2′,3,4′,5,5′-hexachlorobiphenyl (PCB 146), 2,2′,3,4′,5′,6-hexachlorobiphenyl (PCB 149), 2,2′,3,5,5′,6-hexachlorobiphenyl (PCB 151), 2,2′,4,4′,5,5′,-hexachlorobiphenyl (PCB 153), 2,2′,3,3′,4,4′,5-heptachlorobiphenyl (PCB 170), 2,2′,3,3′,4,5,5′-heptachlorobiphenyl (PCB 172), 2,2′,3,3′,4,5′,6′-heptachlorobiphenyl (PCB 177), 2,2′,3,3′,5,5′,6-heptachlorobiphenyl (PCB 178), 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180), 2,2′,3,4,4′,5′,6-heptachlorobiphenyl (PCB 183), 2,2′,3,4′,5,5′,6-heptachlorobiphenyl (PCB 187), |

(*continued*)

**Table 1** (continued)

| Classes of chemical exposure | Chemical, metabolites |
|---|---|
| | 2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl (PCB 194), 2,2′,3,3′,4,4′,5,6-octachlorobiphenyl (PCB 195), 2,2′,3,3′,4,4′,5,6′ and 2,2′,3,4,4′,5,5′,6-octachlorobiphenyl (PCB 196 and 203), 2,2′,3,3′,4,5,5′,6-octachlorobiphenyl (PCB 199), 2,2′,3,3′,4,4′,5,5′,6-nonachlorobiphenyl (PCB 206), 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl (PCB 209) |
| Polychlorinated dibenzo-p-dioxins | 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin (HpCDD), 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HxCDD), 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (HxCDD), 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HxCDD), 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (OCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) |
| Polychlorinated dibenzofurans | 1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF), 1,2,3,4,7,8,9-heptachlorodibenzofuran (HpCDF), 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,6,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,7,8,9-hexachlorodibenzofuran (HxCDF), 2,3,4,6,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF), 1,2,3,7,8-pentachlorodibenzofuran (PeCDF), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF) |
| Polychlorinated biphenyls, coplanar | 3,4,4′,5-Tetrachlorobiphenyl (PCB 81), 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), 3,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 169) |
| Polychlorinated biphenyls, mono-ortho-substituted | 2,3,3′,4,4′-Pentachlorobiphenyl (PCB 105), 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118), 2,3,3′,4,4′,5-hexachlorobiphenyl (PCB 156), 2,3,3′,4,4′,5′-hexachlorobiphenyl (PCB 157), 2,3′,4,4′,5,5′-hexachlorobiphenyl (PCB 167), 2,3,3′,4,4′,5,5′-heptachlorobiphenyl (PCB 189) |
| Pyrethroid pesticides | 4-Fluoro-3-phenoxybenzoic acid, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid, *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid, 3-phenoxybenzoic acid |
| Tobacco smoke | Cotinine, (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol) |
| UV filters, benzophenone-type | 2-Hydroxy-4-methoxybenzophenone (2OH-4MeO-BP), 2,4-dihydroxybenzophenone (2,4 OH-BP), 2,2′-dihydroxy-4-methoxybenzophenone (2,2′OH-4MeO-BP), 2,2′,4,4′-tetrahydroxybenzophenone (2,2′,4,4′OH-BP), 4-hydroxybenzophenone (4OH-BP) |

(*continued*)

**Table 1** (continued)

| Classes of chemical exposure | Chemical, metabolites |
|---|---|
| Volatile organic compounds | 1,1,1-Trichloroethane (methyl chloroform), 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,1-dichloroethane, 1,1-dichloroethene (vinylidene chloride), 1,2-dibromo-3-chloropropane, 1,2-dichlorobenzene, 1,2-dichloroethane (ethylene dichloride), *cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene, 1,2-dichloropropane, 1,3-dichlorobenzene, 1,4-dichlorobenzene (para-dichlorobenzene), 2,5-dimethylfuran, benzene, chlorobenzene, dibromomethane, dichloromethane (methylene chloride), ethylbenzene, hexachloroethane, methyl tert-butyl ether (MTBE), nitrobenzene, styrene, tetrachloroethene (perchloroethylene), tetrachloromethane (carbon tetrachloride), toluene, trichloroethene (trichloroethylene), m-/p-xylene, o-xylene |

## 8-Hydroxy-2′-deoxyguanosin (8-OHdG)

Besides substance-specific DNA adducts, biomarkers of DNA oxidation are increasingly used in HBM (details in Angerer et al. 2007). Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to DNA. Oxidative DNA damage is always present and can be physiologically compensated. Additionally, oxidative DNA damage occurs due to exogenous causes, such as inorganic and organic pollutants or their metabolites. *8-OHdG* levels in blood and urine are used in HBM as a biomarker of oxidative stress in relation to exposures to chemicals, physical stress, or tobacco smoking. However, though valid methods for the determination of 8-OHdG are available, diagnostic reliability of this marker is still in debate. It is a marker which is unspecific for the substance taken up. Furthermore, there is a lack of well-established dose–response relations between environmental exposures and the induction of 8-OHdG. Thus, interpretation has to be undertaken with caution and the biomarker is not suitable for individuals.

## Hemoglobin Adducts (Hb Adducts)

Many reactive electrophilic intermediates of mutagenic substances bind to nucleophilic sites of proteins forming protein adducts. The preferred sites are the sulfhydryl group of cysteine, nitrogen of histidine, and N-terminal valine (Angerer et al. 2007). Hemoglobin (Hb) and serum albumin are the preferred monitor molecules because they are readily accessible in large amounts. Considering the life span of Hb (120 days), Hb adducts cumulate in the body. The reaction products of chemical substances with Hb indicate genotoxic properties of that special substance. Since protein adducts are stable and are not removed by active repair processes, they are a more precise HBM tool when compared with DNA adducts.

The level of Hb adducts in blood enables the estimation of internal exposure as well as biochemical effects. Examples for chemicals and their adducts are:

Alkylating agents: ethylene, -oxide → -hydroxyethylvaline, butadiene, -oxide → *N*-(2-hydroxy-3-butenyl)valine, acrylonitrile → cyanoethylvaline, *acrylamide* → *N*-2-carbamoylethylvaline, glycidamide → *N*-(R,S)-2-hydroxy-carbamoylethylvaline.

Nitro aromatic compounds: 2,6-dinitrotoluene → 2-amino-6-nitrotoluene, 2,4,6-trinitrotoluene → 2-amino-4,6-dinitrotoluene, 1-nitropyrene → 1-aminopyrene

Further examples are Hb adducts for aromatic amines: aniline o-toluidine, m-toluidine, p-toluidine, o-anisidine, 2-aminonaphthalene, and 4-aminobiphenyl.

## Biological Effects (Biomarker of Effect)

Markers for nephrotoxic effects, such as proteins in urine of subjects exposed to solvents or metals, have been well established. In HBM studies with exposure to genotoxic chemicals, especially the measurement of DNA strand breaks (comet assay) in lymphocytes in white blood cells has become very popular (Angerer et al. 2007). The *comet assay* may be effective in distinguishing exposed from non-exposed groups at high exposure. As with 8-OHdG, the biomarker is not specific, there is still a lack of well-established dose–response relations between exposures and the formation of strand breaks which limits the applicability of this marker in HBM. It may be useful as group indicators, but not for interpretation on an individual level.

Cytogenetic biomarkers currently applied in molecular epidemiologic studies include chromosomal aberrations, micronuclei, and sister chromatid exchange. This important group of genotoxicity biomarkers has been developed in animals, even in vitro, and is now increasingly applied to exposed populations. However, these biomarkers are currently inadequate to HBM purposes, especially for interpretation in individuals.

## Other Markers

The concept of individual variability has led to discuss the suitability of *biomarkers of susceptibility*. Of special interest is polymorphism in enzymes such as cytochrome P450 families and the glutathione transferases. Despite the intense work ongoing and the promising results achieved on the pharmacological and toxicological significance of polymorphic metabolizing enzymes, their routine use as HBM biomarkers in environmental health is yet not be validated.

"*Omic*" technologies include genomics, transcriptomics (gene expression profiling), proteomics, and metabolomics. These new techniques are increasingly utilized in an effort to develop novel biomarkers of exposure, susceptibility, and response to chemicals. The application in the prediction of risks and the prevention of diseases related to chemical exposures is promising, but yet not established in HBM.

## General Population HBM Programs

Broad general population HBM programs are established or planned by international, national, and state organizations in a number of countries. One of the most recognized programs is the US *National Health and Nutrition Examination Survey* (NHANES, http://www.cdc.gov/nchs/nhanes.htm). Four surveys have been conducted between 1971 and 1994. In 1999, NHANES became a continuous survey. NHANES includes a physical examination and collecting of biological specimen and a detailed medical history. Approximately 7,000 residents participate each year. Biological specimen is used for clinical and nutritional testing as well as to assess exposure of the noninstitutionalized civilian US population to environmental chemicals. In Germany (Kolossa-Gehring et al. 2012), the nationwide population representative study on exposure to environmental chemicals and its sources comprises of four surveys (*German Environmental Surveys*, GerES I–IV) conducted since 1985 (http://www.umweltbundesamt.de/gesundheit-e/survey/index.htm). GerES IV (2003–2006) was the first survey exclusively on children. A further HBM tool in Germany is the *German Environmental Specimen Bank* (ESB). The ESB is a permanent monitoring instrument and an archive for human species (http://www.umweltbundesamt.de/gesundheit-e/gbub/hpb.htm). The German HBM activities include the *German Human Biomonitoring* Commission (http://www.umweltbundesamt.de/gesundheit-e/monitor/index.htm). The commission provides general HBM concepts and derives values for interpretation of HBM data since 1992 (Schulz et al. 2011). Other countries with HBM programs include Belgium, Canada, the Czech Republic, Denmark, France, Israel, Japan, and South Korea (for overview see Special Issue, Berlin International Conference on Human Biomonitoring. Int J Hyg Environ Health 215 2012). A more broadly harmonized HBM program has been started throughout the European Union in 2011(COPHES, http://www.euhbm.info/about-cophes).

## Evaluation of HBM Results (Internal Exposures)

Leading concepts for the evaluation of HBM data in the general population have been given by the German HBM Commission (Schulz et al. 2011; http://www.umweltbundesamt.de/gesundheit/monitor/index.htm) as well as by Hays and Aylward (2012; Summit Toxicology, http://www.summittoxicology.com).

## Reference Values

The German HBM Commission has established the concept of *reference values*. The reference values ($RV_{95}$) are statistical descriptions of the ranges of concentrations typically seen in a specified reference population but which have no direct relationship to health effects or risk assessment. They are based on the 95th percentile. The reference values derived by the German HBM Commission for

**Table 2** gives an overview on HBM guidance values in environmental (nonoccupational) exposures

| Value | Basis | Reference |
|---|---|---|
| Reference value $RV_{95}$ | Population studies (not always strictly representative) | German HBM Commission |
| Human biomonitoring value I HBM I | Epidemiological data | German HBM Commission |
| | Toxicological data | |
| | Tolerable daily intake (TDI) | |
| Human biomonitoring value II HBM II | Epidemiological data | German HBM Commission |
| | Toxicological data | |
| Biomonitoring equivalent BE | Reference dose (RfD) | Summit Toxicology |
| | Reference concentration (RfC) | |
| | Tolerable daily intake (TDI) | |
| | Acceptable daily intake (ADI) | |
| | Minimal risk level (MRL) | |
| | Risk-specific doses (cancer) | |

various substances are summarized in Tables 2–11. Many data for adults are based on the GerES III performed in 1997–1999. The exposure to most of the substances shown in the following tables has been decreased since then. Striking examples are lead in blood and PCB in blood.

For describing background exposure in the nonsmoking general population to acrylamide (AA) through the acrylamide hemoglobin adduct (*N*-2-carbamoylethylvaline: AAVal) in the blood, the following levels were derived:

- 1.8 µg AAVal/l for nonsmoking children
- 1.2 µg AAVal/l for nonsmoking adults (Schulz et al. 2011)

Hb adduct of acrylamide (AAVal) reflects the acrylamide dose taken up in the previous 4 months.

## Reference Value and Risk Assessment

$RV_{95}$ is a strictly statistically derived value and has per se no health relevance. However, $RV_{95}$ is an important tool for prevention to assess whether populations or individuals are more exposed when compared to the environmental background exposure. In case of exposures above $RV_{95}$, the recommendation is to clarify whether a conspicuous source exists and if it can be avoided. From the perspective of environmental hygiene and preventive medicine, it should be considered whether this exposure can be reduced as far as reasonably possible. Furthermore, for substances which are considered carcinogenic (genotoxic), no health-based HBM values can be derived; $RV_{95}$ may be also used for risk

**Table 3** Reference values ($RV_{95}$) for antimony, arsenic, cadmium, lead, mercury, nickel, thallium, platinum, and uranium in urine or blood (Schulz et al. 2011)

| Parameter and matrix | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| Antimony in urine | Children (3–14 years) | 2003–2006 | 0.3 µg/l |
| Arsenic in urine | Children (3–14 years) | 2003–2006 | 15.0 µg/l |
| | Adults (18–69 years) | 1997–1999 | |
| Cadmium in urine | Nonsmoking children (3–14 years) | 2003–2006 | 0.2 µg/l |
| | Nonsmoking adults (18–69 years) | 1997–1999 | 0.8 µg/l |
| Cadmium in blood | Nonsmoking children (3–14 years) | 2003–2006 | <0.3 µg/l |
| | Nonsmoking adults (18–69 years) | 1997–1999 | 1.0 µg/l |
| Lead in blood | Children (3–14 years) | 2003–2006 | 35 µg/l |
| | Women (18–69 years) | 1997–1999 | 70 µg/l |
| | Men (18–69 years) | 1997–1999 | 90 µg/l |
| Mercury in urine | Children without dental amalgam fillings (3–14 years) | 2003–2006 | 0.4 µg/l |
| | Adults without dental amalgam fillings (18–69 years) | 1997–1999 | 1.0 µg/l |
| Mercury in blood | Children who ate fish ≤ 3 times per month (3–14 years) | 2003–2006 | 0.8 µg/l |
| | Adults who ate fish ≤3 times per month (18–69 years) | 1997–1999 | 2.0 µg/l |
| Nickel in urine | Children (3–14 years) | 2003–2006 | 4.5 µg/l |
| | Adults (not strictly representative) | Not specified | 3 µg/l |
| Platinum in urine | Adults without platinum dental material (18–69 years) | 1997–1999 | 0.01 µg/l |
| Thallium in urine | Children (3–14 years) | 2003–2006 | 0.6 µg/l |
| | Adults (20–29 years) | 2000–2008 | 0.5 µg/l |
| Uranium in urine | Children (3–14 years) | 2003–2006 | 0.04 µg/l |
| | Adults (not strictly representative) | 2001–2003 | 0.03–0.06 µg/l |

assessment and risk management. This also applies for other substances for which no threshold is known. For example, the German HBM Commission recently rescinded the HBM values for lead in blood of children and adults (Wilhelm et al. 2010). For reasons of preventive health protection, the Commission recommends using the $RV_{95}$ for the assessment of lead exposure.

For occupational health purposes, BAR values (Biologischer Arbeitsstoff-Referenzwert) are established by the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission). These BAR values are similar to the reference values of the German HBM Commission. However, in risk communication two kinds of values with the same meaning may contribute to confusion.

**Table 4** Reference values ($RV_{95}$) for chlorophenols in urine of children and adults and pentachlorophenol in serum of adults (Schulz et al. 2011)

| Parameter | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| 2-Monochlorophenol | Children (3–14 years) | 2003–2006 | 7.0 µg/l |
| 4-Monochlorophenol | Children (3–14 years) | 2003–2006 | 15.0 µg/l |
| | Adults (18–69 years) | 1997–1999 | |
| 2,4-Dichlorophenol | Children (3–14 years) | 2003–2006 | 2 µg/l |
| | Adults (18–69 years) | 1997–1999 | 3 µg/l |
| 2,5-Dichlorophenol | Children (3–14 years) | 2003–2006 | 6 µg/l |
| | Adults (18–69 years) | 1997–1999 | 20 µg/l |
| 2,6-Dichlorophenol | Children (3–14 years) | 2003–2006 | <0.3 µg/l |
| | Adults (18–69 years) | 1997–1999 | |
| 2,3,4-Trichlorophenol | Children (3–14 years) | 2003–2006 | <0.3 µg/l |
| | Adults (18–69 years) | 1997–1999 | |
| 2,4,5-Trichlorophenol | Children (3–14 years) | 2003–2006 | 0.5 µg/l |
| | Adults (18–69 years) | 1997–1999 | 1 µg/l |
| 2,4,6-Trichlorophenol | Children (3–14 years) | 2003–2006 | 0.7 µg/l |
| | Adults (18–69 years) | 1997–1999 | 1.5 µg/l |
| 2,3,4,6-Tetrachlorophenol | Children (3–14 years) | 2003–2006 | <0.3 µg/l |
| | Adults (18–69 years) | 1997–1999 | 1 µg/l |
| Pentachlorophenol in urine | Children (3–14 years) | 2003–2006 | 2.0 µg/l |
| | Adults (18–69 years) living in homes without wood preservatives | 1997–1999 | 5 µg/l |
| Pentachlorophenol in serum | Adults (not strictly representative) | 1995–1996 | 12 µg/l |

**Table 5** Reference values ($RV_{95}$) for metabolites of organophosphorus insecticides (DMP, DMTP, DMDTP, DEP, DETP) in urine (Schulz et al. 2011)

| Parameter | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| Dimethylphosphate DMP | Children (3–14 years) | 2003–2006 | 75 µg/l |
| | General population (not strictly representative) | 1998 | 135 µg/l |
| Dimethylthiophosphate DMTP | Children (3–14 years) | 2003–2006 | 100 µg/ |
| | General population (not strictly representative) | 1998 | 160 µg/l |
| Dimethyl dithiophosphate DMDTP | Children (3–14 years) | 2003–2006 | 10 µg/l |
| Diethylphosphate DEP | Children (3–14 years) | 2003–2006 | 30 µg/l |
| | General population (not strictly representative) | 1998 | 16 µg/l |
| Diethylthiophosphate DETP | Children (3–14 years) | 2003–2006 | 10 µg/l |

**Table 6** Reference values ($RV_{95}$) for metabolites of pyrethroid insecticides (*cis*-$Cl_2CA$, *trans*-$Cl_2CA$, 3-PBA) in urine (Schulz et al. 2011)

| Parameter | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (*cis*-$Cl_2CA$) | Children (3–14 years) | 2003–2006 | 1 µg/l |
| | General population (not strictly representative) | 1998 | |
| *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (*trans*-$Cl_2CA$) | Children (3–14 years) | 2003–2006 | 2 µg/l/ |
| | General population (not strictly representative) | 1998 | |
| 3-Phenoxybenzoic acid (3BPA) | Children (3–14 years) | 2003–2006 | 2 µg/l |
| | General population (not strictly representative) | 1998 | |

**Table 7** Reference values ($RV_{95}$) for metabolites of polycyclic aromatic hydrocarbons in urine of nonsmoking children and of nonsmoking adults (Schulz et al. 2011)

| Parameter | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| 1-Hydroxypyrene | Nonsmoking children (3–14 years) | 2003–2006 | 0.5 µg/l |
| | Nonsmoking adults (18–69 years) | 1997–1999 | |
| 1-Hydroxy-phenanthrene | Nonsmoking children (3–14 years) | 2003–2006 | 0.6 µg/l/ |
| 2/9-Hydroxy-phenanthrene | Nonsmoking children (3–14 years) | 2003–2006 | 0.4 µg/l |
| 3-Hydroxy-phenanthrene | Nonsmoking children (3–14 years) | 2003–2006 | 0.5 µg/l |
| 4-Hydroxy-phenanthrene | Nonsmoking children (3–14 years) | 2003–2006 | 0.2 µg/l |
| ∑Hydroxy-phenanthrene (1,2/9,3,4) | Nonsmoking children (3–14 years) | 2003–2006 | 1.5 µg/l |
| 1-Naphthol | Nonsmoking adults (not representative) | | <30 µg/l[a] |
| 2-Naphthol | Nonsmoking adults (not representative) | | <20 µg/l[a] |

[a]Background exposure values, no strict reference value

## Health-Based Values

### HBM Values

The health-related biological exposure limits established by the German Human Biomonitoring Commission are called the *HBM values*. Two levels were defined: the HBM-I value and the HBM-II value. The HBM-I value is a control value, while the HBM-II value is defined as an action level. The HBM-I value describes the concentration in the body matrix of a substance below which no adverse health effect should be expected. At a concentration level higher than the HBM-I and lower than the HBM-II value, an investigation of potential sources of exposure should be undertaken. Exposure to such sources should be minimized, or relevant sources should be eliminated where necessary and achievable with an acceptable

**Table 8** Reference values ($RV_{95}$) for polychlorinated biphenyls (*PCB*), alpha-hexachlorocyclohexane (*α-HCH*), beta-hexachlorocyclohexane (*β-HCH*), hexachlorobenzene (*HCB*), and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*DDE*) in whole blood (Schulz et al. 2011)

| Parameter | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| PCB 28 | Children (7–14 years) | 2003–2006 | 0.01–0.1 µg/l[a] |
| PCB 52 | Children (7–14 years) | 2003–2006 | 0.01–0.1 µg/l[a] |
| PCB 101 | Children (7–14 years) | 2003–2006 | 0.01–0.1 µg/l[a] |
| PCB138 | Children (7–14 years) | 2003–2006 | 0.4 µg/l |
| | Adults (18–69 years) | 1997–1999 | 0.4–2.2 µg/l[b] |
| PCB 153 | Children (3–14 years) | 2003–2006 | 0.6–3.3 µg/l[b] |
| | Adults (18–69 years) | 1997–1999 | |
| PCB 180 | Children (3–14 years) | 2003–2006 | 0.3–2.4 µg/l[b] |
| | Adults (18–69 years) | 1997–1999 | |
| ∑PCB (138 + 153 + 180) | Children (3–14 years) | 2003–2006 | 1.0 µg/l |
| | Adults (18–69 years) | 1997–1999 | 1.1–7.8 µg/l[b] |
| α-HCH | Children (7–14 years) | 2003–2006 | <0.1 µg/l |
| | Adults (18–69 years) | 1997–1999 | |
| β-HCH | Children (7–14 years) | 2003–2006 | 0.3 µg/l |
| | Adults (18–69 years) | 1997–1999 | 0.3–0.9 µg/l[b] |
| HCB | Children (7–14 years) | 2003–2006 | 0.3 µg/l |
| | Adults (18–69 years) | 1997–1999 | 0.4–5.8 µg/l[b] |
| DDE | Children (7–14 years) | 2003–2006 | 0.7–1.4 µg/l[c] |
| | Adults (18–69 years) | 1997–1999 | 1.5–31 µg/l[c] |

[a]Reference values had been originally derived related to the detection limit of 0.1 µg/l. Meanwhile, detection limit for PCB 28, 52, and 101 is about 0.01 µg/l. Levels above 0.01 µg/l may indicate an exposure above background exposure

[b]Levels increase between age groups 18 and 69 years continuously. Due to the general decrease of PCB exposure and considering that samples were collected in 1997–1999, the current reference values should be lower at least by a factor of 0.5

[c]Levels increase between age groups 18 and 69 years continuously. Furthermore, data include the comparison between samples collected in West and East Germany. Levels of participants from East Germany were 2–3 times higher compared to those from West Germany

Reference values for PCBs, HCB, ß-HCH, and DDT in breast milk (sampled 2003–2005) are 0.5 mg/kg fat for total DDT and ∑PCB (1.64 × (138 + 153 + 180)), 0.06 mg/kg fat for HCB, and 0.07 mg/kg fat for ß-HCH

**Table 9** Reference values ($RV_{95}$) for the perfluorinated compounds perfluorooctanoic acid (*PFOA*) and perfluorooctanesulfonic acid (*PFOS*) in plasma (Schulz et al. 2011)

| Parameter | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|
| PFOA | Women, men, children < 10 years | 2003–2007 | 10 µg/l |
| PFOS | Women (not strictly representative) | 2003–2007 | 20 µg/l |
| | Men (not strictly representative) | 2003–2007 | 25 µg/l |
| | Children < 10 years (not strictly representative) | 2003–2007 | 10 µg/l |

**Table 10** Reference values ($RV_{95}$) for aromatic amines in urine of nonsmoking adults (Schulz et al. 2011). The data are based on samples which are not strictly representative. Study period was 2003–2004

| Parameter | $RV_{95}$ |
|---|---|
| Aniline | 14.5 µg/l |
| o-Toluidine | 0.20 µg/l |
| m-Toluidine | 0.25 µg/l |
| p-Toluidine | 1.25 µg/l |
| o-Anisidine | 1.10 µg/l |
| 3-Chloroaniline | 0.25 µg/l |
| 4-Chloroaniline | 1.00 µg/l |
| 3,4-Dichloroaniline | 0.45 µg/l |
| 3,5-Dichloroaniline | 4.30 µg/l |

**Table 11** Reference values ($RV_{95}$) for metabolites of phthalates in urine of children and adults (Schulz et al. 2011). Adults were students from Münster, West Germany

| Phthalate | Metabolite | Population group (age range) | Study period | $RV_{95}$ |
|---|---|---|---|---|
| DnBP | MnBP | Children (3–14 years) | 2003–2006 | 300 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 70 µg/l |
| DiBP | MiBP | Children (3–14 years) | 2003–2006 | 300 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 140 µg/l |
| BBzP | MBzP | Children (3–14 years) | 2003–2006 | 75 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 15 µg/l |
| DEHP | ∑5-OH-MEHP +5-oxo-MEHP | Children (3–14 years) | 2003–2006 | 280 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 50 µg/l |
| | 5-OH-MEHP | Children (3–14 years) | 2003–2006 | 160 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 30 µg/l |
| | 5-oxo-MEHP | Children (3–14 years) | 2003–2006 | 120 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 20 µg/l |
| | 5-cx-MEPP | Children (3–14 years) | 2003–2006 | 200 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 30 µg/l |
| | ∑3 Metabolites of DiNP | Children (3–14 years) | 2003–2006 | 140 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 60 µg/l |
| DiNP | OH-MiNP | Children (3–14 years) | 2003–2006 | 50 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 20 µg/l |
| | Oxo-MiNP | Children (3–14 years) | 2003–2006 | 30 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 15 µg/l |
| | cx-MiNP | Children (3–14 years) | 2003–2006 | 60 µg/l |
| | | Adults (20–29 years) | 2006 and 2008 | 15 µg/l |

*DnBP* di-*n*-butyl phthalate, *MnBP* mono-*n*-butyl phthalate, *DiBP* diisobutyl phthalate, *MiBP* monoisobutyl phthalate, *BBzP* butyl benzyl phthalate, *MBzP* monobenzyl phthalate, *DEHP* di(2-ethylhexyl) phthalate, *5-OH-MEHP* mono(2-ethyl-5-hydroxyhexyl) phthalate, *5-oxo-MEHP* mono(2-ethyl-5-oxohexyl) phthalate, *5-cx-MEPP* mono(2-ethyl-5-carboxypentyl) phthalate, *DiNP* diisononyl phthalate, *MiNP* monoisononyl phthalate, *OH-MiNP* monohydroxylisononyl phthalate, *oxo-MiNP* monooxoisononyl phthalate, *cx-MiNP* monocarboxylisononyl phthalate

**Table 12** Human biomonitoring (HBM) values derived by the German HBM Commission

| Parameter and medium | Population group/age groups | HBM-I value | HBM-II value |
|---|---|---|---|
| Bisphenol A in urine | Children | 1.5 mg/l | |
| | Adults | 2.5 mg/l | |
| Cadmium in urine | Children and adolescence | 0.5 µg/l | 2 µg/l |
| | Adults | 1 µg/l | 4 µg/l |
| ∑Metabolites of di(2-ethylhexyl)phthalate DEHP: 5oxo- and 5OH-MEHP in urine | Children (6–13 years) | 500 µg/l | |
| | Women of reproductive age | 300 µg/l | |
| | Men ≥14 years, general population | 750 µg/l | |
| Mercury in urine | Children and adults | 7 µg/l | 25 µg/l |
| | | 5 µg/g creatinine | 20 µg/g creatinine |
| Mercury in blood | Children and adults | 5 µg/l | 15 µg/l |
| Pentachlorophenol in serum | General population | 40 µg/l | 70 µg/l |
| Pentachlorophenol in urine | General population | 25 µg/l | 40 µg/l |
| | | 20 µg/g creatinine | 30 µg/g creatinine |
| Thallium in urine | General population | 5 µg/l | |
| ∑PCB (138 + 153 + 180) in serum x 2 | Infants, children, women of reproductive age | 3.5 µg/l | 7 µg/l |

level of input. HBM values are derived from toxicological and epidemiological data as well from existing health-based exposure guidance values such as the tolerable daily intake (Table 2). The protection levels intended by the tolerable intake values described above correspond to the protection level intended by the HBM-I value. The HBM-II value describes the concentration in the body matrix of a substance above which relevant adverse health effects may occur, and hence, immediate action to reduce exposure must be taken and expert care in environmental medicine will be required. HBM values are summarized in Table 12.

## Biological Equivalents (BEs)

*Biomonitoring equivalents* (BEs) are defined as the concentration of a chemical or metabolite in a biological medium (blood, urine, human milk, etc.) consistent with defined exposure guidance values or toxicity criteria, including reference doses and reference concentrations (RfD and RfCs), minimal risk levels (MRLs), and tolerable daily intakes (TDIs) (Hays and Aylward 2012). Thus, the definition of BE is functionally similar to the HBM-I value of the German HBM Commission (Angerer et al. 2011). BE values have been derived for more than 80 chemicals (Table 13).

A second BE level has also been defined, the $BE_{POD}$. This is the BE value corresponding to an exposure level which incorporates uncertainty factors

**Table 13** Chemicals for which BE values corresponding to current risk assessment-based exposure guidance values have been derived (Hays and Aylward 2012)

| | |
|---|---|
| Cadmium | 2,4-Dichlorophenoxyacetic acid |
| Arsenic, inorganic | 1,2,3-Trichloropropane |
| 2,4-D Cyfluthrin | Styrene |
| Deltamethrin | Ethylbenzene |
| DDT/DDE/DDD | 1,2-Dibromoethane |
| Dioxin TEQ (dioxin, furan, and coplanar PCB compounds) | 1,2-Dichloroethane |
| | Acrylonitrile |
| Carbon tetrachloride | Toluene |
| Chloroform | Methyl isobutyl ketone (MIBK) |
| Hexachloroethane | Furan |
| 1,1,1-Trichloroethane | Tetrahydrofuran |
| Benzene | *n*-Hexane |
| Dibromomethane | *n*-Octane |
| Bromoform | *n*-Nonane |
| Bromodichloromethane | Hexachlorobenzene |
| Methylene chloride | 1,4-Dioxane |
| 1,1-Dichloroethane | Dibromochloromethane |
| 1,1-Dichloroethene | *n*-Decane |
| Acrylamide | Tetrachloroethene |
| 1,1,2-Trichloroethane | *cis*-1,2-Dichloroethene |
| 1,1,2,2-Tetrachloroethane | *trans*-1,2-Dichloroethene |
| Trichloroethene | *n*-Heptane |
| Bisphenol A | 1,1,1,2-Tetrachloroethane |
| Di(2-ethylhexyl)phthalate | Xylenes, mixed |
| Diethyl phthalate | Methyl tert-butyl ether (MTBE) |
| Dibutyl phthalate | Triclosan |
| Benzyl butyl phthalate | Hexabromocyclododecane |
| Diisononyl phthalate | PBDE 99 |

associated with NOAEL or LOAEL, duration adjustment, as well as interspecies extrapolation but which omits uncertainty factors which address intraspecies factors or other database uncertainty factors.

The various HBM guidance values used in occupational health will not be mentioned here.

## Risk Assessment in Context with Surveys/Tool in Risk Management

Using BEs, hazard quotients are calculated as the ratio of the biomarker concentration to the BE as proposed by Hays and Aylward (2012). *Hazard quotients* $< 1$ indicate that the measured concentration of a chemical in a biological medium is below BE. In this case the exposure is expected to be below the corresponding

exposure guidance value. Applying health-based guidance values (HBM-I, HBM-II, BEs) to the NHANES data (geometric mean and 95th percentile population biomarker concentrations) from the report 2012, Aylward et al. (2012) calculated hazard quotients. Most analytes showed hazard quotients below 1. Hazard quotients approaching or exceeding 1 or cancer risks greater than $1 \times 10^{-4}$ were found for acrylamide, dioxin-like chemicals, benzene, xylene, several metals, di-2-(ethylhexyl)phthalate, and some legacy organochlorine pesticides suggesting that exposure levels may exceed published human health benchmarks. This approach is very useful to assist risk managers in the prioritization of chemicals for more detailed chemical-specific evaluation and risk assessment follow-up.

## References

Angerer J, Ewers U, Wilhelm M (2007) Human biomonitoring: state of the art. Int J Hyg Environ Health 210:201–228

Angerer J, Aylward LL, Hays SM, Heinzow B, Wilhelm M (2011) Human biomonitoring assessment values: approaches and data requirements. Int J Hyg Environ Health 214:348–360

Aylward LL, Kirman CR, Schoeny R, Portier CJ, Hays SM (2012) Evaluation of biomonitoring data from the CDC National Exposure Report in a risk assessment context: perspectives across chemicals. Environ Health Perspect. http://dx.doi.org/10.1289/ehp.1205740. Online 11 Dec 2012

Hays SM, Aylward LL (2012) Interpretation human biomonitoring data in a public health risk concept using biomonitoring equivalents. Int J Hyg Environ Health 215:145–148

Kolossa-Gehring M, Becker K, Conrad A, Schröter-Kermani C, Schulz C, Seiwert M (2012) Environmental surveys, specimen bank and health related environmental monitoring in Germany. Int J Hyg Environ Health 215:120–126

Needham LL, Calafat AM, Barr DB (2007) Uses and issues of biomonitoring. Int J Hyg Environ Health 210:229–238

Schulz C, Wilhelm M, Heudorf U, Kolossa-Gehring M (2011) Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int J Hyg Environ Health 215:26–35

Wilhelm M, Heinzow B, Angerer J, Schulz C (2010) Reassessment of critical lead effects by the German Human Biomonitoring Commission results in suspension of the human biomonitoring values (HBM-I and HBM-II) for lead in blood of children and adults. Int J Hyg Environ Health 213:265–269

## Resources

COPHES. http://www.eu-hbm.info/cophes

Federal Environmental Agency, Germany. http://www.umweltbundesamt.de

German Environmental Specimen Bank (ESB). http://www.umweltbundesamt.de/gesundheit-e/gbub/hpb.htm

German Environmental Survey (GerES). http://www.umweltbundesamt.de/gesundheit-e/survey/index.htm

German Human Biomonitoring Commission. http://www.umweltbundesamt.de/gesundheit/monitor/index.htm

National Health and Nutrition Examination Survey (NHANES). http://www.cdc.gov/nchs/nhanes.htm

Summit toxiocology. http://www.summittoxicology.com/

# Restrictions and Prohibitions as Tools in Regulatory Toxicology

Ullrich Kleeberg, Hans Jochen Kittel, and Alfred Georg Hildebrandt

## Contents

Risk Management, Restrictions and Prohibitions ..... 704
Chemicals ..... 705
Pesticides ..... 707
Occupational Health and Safety ..... 708
Poisoning by Chemicals for General Use: Especially in Children ..... 708
Food and Feed ..... 711
Food Additives, Sweeteners, and Contaminants ..... 711
Food Supplements and Health Claims ..... 712
Cosmetics ..... 712
Drugs: Approval, Certification, and Safety ..... 713
Narcotic and Psychotropic Substances ..... 713
Doping ..... 714
Self-commitments of the Chemical Industry ..... 716
References and Recommended Reading ..... 717
Chemicals ..... 717
Chemicals/Biocides ..... 717
Food and Feed ..... 718
Drugs ..... 718
Narcotic and Psychotropic Substances ..... 718

U. Kleeberg (✉)
Berlin Medical Association – Berliner Medizinische Gesellschaft, Berlin, Germany
e-mail: u.kleeberg@gmx.de

H.J. Kittel
Institute for European Projects/Institut für Europaprojekte e.V, Berlin, Germany
e-mail: ipe-eu@t-online.de

A.G. Hildebrandt
(former) Head of Federal Institute of Drugs and Medicinal Products, Bonn, Germany

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_85, 

**Abstract**

Restrictions and prohibitions or the assessment of prescriptive limits are the appropriate measures to save men and environment from reversible or irreversible hazards. They are based on conventions, regulatory decisions, or normative conventions. Risk management by the authorities is subject to constitutional principles. Focused on the chemicals in environment and industrial processes, the REACH regulation plays a key role in occupational health as well as in poisoning prevention. The hazardous potential of long-acting toxicants ranges over next generation(s) (developmental toxicology). Along the food chain, the consumers are implicated in the incorporation of contaminants, additives, and supplements. The Drug Law represents the strictest legislative directives protecting the consumer in its exceptional situation as a patient. Regulations by the EMA and international harmonization guidelines should protect the individuals confronted with special drugs, combined side-effects, and possible residues from veterinary medical substances. A particular paragraph is dedicated to the prohibition of doping.

This chapter provides examples for restrictions and prohibitions as regulatory tools in different application fields. Taking Germany as an example, it also shows how international directives are harmonized with national law.

## Risk Management, Restrictions and Prohibitions

Most chemicals are subject to some kind of regulatory restriction with regard to synthesis, transport, handling, application fields, maximal concentrations in the final product, and others. Restrictions are often legitimated by toxicological and safety arguments, although technical considerations may also play a role. In cases, where a chemical is considered to pose a high risk, restrictions may become manifold and finally result in a ban or prohibition. Prohibition is often the endpoint of a long-lasting management process. Prohibition is facilitated when alternative compounds with lower risks are available. Different types of prohibition exist, such as prohibition of production, trade, or usage.

Risk management means the perception of risks, the handling of risk evaluation, and strategic conceptions of the government and the public with regard to avoidance or limitation of hazards and risks. Therefore, appropriate information, restrictions, or prohibitions will be implemented. In case of emergency, the authority has to enforce the decision against interested pressure groups. According to our comprehension, risk is defined in terms of the probability and severity of hazardous events caused by chemical compounds, technical procedures, and by-products during manufacture.

Production or use of toxic chlororganic compounds such as DDT and PCBs is banned in various countries. It is strictly forbidden to produce or to apply these toxic substances, that extremely endanger men and environment. In the international Stockholm convention in 2004, 12 persistent organic pollutants

(POPs; cf. Lit.) had been defined as unintentional by-products of industrial production, especially dioxines and furans. New POP substances can be identified under the criteria of REACH concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (cf. Lit.). REACH applies to all chemicals imported or produced in the European Union according to the general principle "no data, no market." The order of magnitude of 143,000 chemical substances actually traded on the European market demonstrates the potential impact on both human health and the environment. Risk has to be reduced by the chemical industry in cooperation with the governmental administration.

## Chemicals

The law of chemical substances (germ.: Chemikaliengesetz (ChemG)) defines in various paragraphs substances, hazardous compounds, toxic for men and the environment; furthermore, terms such as preparations, manufacturing, putting on the market, and hazard classification are included (GHS – Globally Harmonized System of Classification and Labelling of Chemicals; cp. UNECE). Parts 3 and 4 are directed in detail on health and environmental hazards, respectively. The legal bases of the European Union are the Guidelines No. 79/831 EEC and the 6. Amendment of Guideline No. 67/548 EEC to fit the laws, regulations, and the administrative requirement for the classification, labeling, and packaging of dangerous substances (cf. "CLP" – Literature/I-Net). Therefore, companies are required to classify, label, and package their hazardous products before placing them on the market. Accordingly, the protection of workers, consumers as well as of the environment against possible hazardous effects is the aim of these regulations.

The European Commission modified the GHS-System by the regulation 1272/2008/EC with special hazard statements (EUH statements, art. 25), so-called Precautionary and Hazard Statements. Thus, occurring hazards and safety statements elicited by chemical substances and preparations will be covered for a better working place condition.

Under the Regulation of REACH, especially covered are old and new substances as well as polymers. Biocide agents need to be registered and regulated according to the law of Chemicals in a special section (§ 12a). In the European Community Classification, the Biocidal Products Directive 98/8/EC (BPD), the classification of biocides, divides them into four main groups: "Disinfectants (and general biocidal products), preservatives, pest control" and "other biocidal products." These in turn are broken down into 23 product types (i.e., rodenticides, insecticides, repellents).

In 2013, this regulation is expected to be completely replaced by the new Guideline EU No. 528/2012, comprising the materials as cited above. In addition, the regulation will be updated by nanomaterials and "treated articles," i.e., materials that intentionally incorporate or have been treated with biocidal products. Generally, under the REACH regulation (CA/59/2008), nanomaterials get an official interest, the "Second Regulatory Review on Nanomaterials" currently

adjusts the scientific knowledge about this topic on the European level (SWD (2012) 288). The European Commission focuses its basic interest on key enabling technologies (KET), which include mainly nanotechnologies with all their biological implications. Especially the Scientific Committee on Consumer Safety (SCCS) and the EMA are authorized to work continuously on the risk assessment of nanomaterials concerning consumers and patients.

Starting in 2006 (REACH – 1907/2006 EC), when the quantity of produced or imported chemicals is basically adjusted to quantitative limits of 1 t/annum, the registration at the European Chemicals Association in Helsinki (ECHA) is obligatory. In the case of substances of very high concern (SVHC), the industry and the importers are obligated to supply information on all those substances exceeding a concentration of 0.1 % of weight in the mixture (cf. REACH – Art. 31–33). Every 2 years, new findings on persistence, bioaccumulation, and toxicity of substances listed as "SVHC" have to be published by the ECHA agency. The Candidate List of December 2012 included all 138 SVHC substances, of which 74 are carcinogenic, and 15 mutagenic, while a further 71 substances cause toxic effects on reproduction (cf. ECHA).

According to Article 33 (2), the consumer can request information from any supplier of an article containing a substance at a concentration above 0.1 % (w/w). The manufacturer is in turn obligated to provide the consumer with all necessary information, to allow safe use of the article in daily use. In Germany, electronic request may be directed toward UBA (reach-info.de) with the legal guarantee of response within 45 days.

Endocrine disrupters are defined as exogenous substances which affect the endocrine system. This can happen in three different modes of action: (i) The action of a naturally occurring hormone can be imitated, such as testosterone or estrogen. (ii) Hormone receptors in cells can be occupied, i.e., the normal hormone action is blocked. (iii) Synthesis, transport, metabolism, and kinetics of hormones are impaired, which consequently disturbs the physiological level of a hormone. Medical treatment with natural or synthetic hormones as well as various edible plant ingredients in addition to the synthetically produced endocrine disrupting agents can play such disturbing roles.

From a toxicological aspect, the man-made chemicals appearing in industry, agriculture, or in consumer goods have a very high significance. The extreme persistence and ubiquitous distribution of some compounds in our environment is exceptionally dangerous (cf. POPs – Persistent Organic Pollutants, see above). Substances interfering with the endocrine system belong to the following classes of chemicals: (i) PCBs – polychlorinated biphenyls, dioxin, and combustion products (i.g. B(a)P); (ii) products generated during manufacture of or contained in plastics: phthalates (in PVC) and bisphenol A; (iii) pesticides (insecticides such as DDT, herbicides such as atrazine and nitrofen, fungizides); (iv) household products such as alkylphenols (nonylphenol) as well as octylphenol (in rubber vulcanization); and finally, some heavy metals, such as lead and cadmium, are of relevance

(mercury : see below – Contaminants). These organic pollutants enter the human body via water, food, or, in some cases, cigarette smoke or ambient air. This demonstrates the importance to keep drinking water, food, and the ambient air as clean as possible for future regulations.

The insecticide endosulfan is another important example for the exposure of ground water sources. It has explicitly been banned in the Stockholm Reach List in 2011 as POP No. 22. As a biological indicator for the quality of our environment, the distribution of health honey-bee swarms is decisive for the worldwide supply of the human population with fruit products – as an important part of our nutrition. The new class of neonicotinoids such like clothianidin, imidaclopramid, and thiamethoxan –applied as insecticide spraying of seeds – has been proved to be toxic for the bee population (EFSA – Neonicotinoides, 2013). As a consequence of a report from EFSA, this group of herbicides is recommended to be forbidden for 2 years from July 2013 by the European Commission, which represents a quasi-moratorium.

In the Appendices VII up to X of the REACH regulation (Art. 12), the registration in a data record describing physicochemical, toxicological, and ecotoxicological information is classified depending on tonnage: VII (amount of substance $\geq$1 t/a), VIII ($\geq$10 t/a), IX ($\geq$100 t/a), X ($\geq$1,000 t/a). As listed in Appendix No. VIII, every substance produced or imported at a quantity $>$10 t per year has to be registered in the CSR (Chemical Safety Report, acc. to Art. 10 b, REACH).

Concerning carcinogens, the International Agency for Research on Cancer (IARC) in Lyon is responsible for the evaluation and publishing of monographs. IARC Monographs are the leading documents with detailed descriptions on carcinogens and mutagens. The Agency follows the general rules of WHO and assembles remarkable scientific competence in cancer research and allied fields.

## Pesticides

Under the term "pesticides," the following substances are listed: insecticides, herbicides, fungicides, plant growth regulators, rodenticides, biocides, and veterinary medicines. These chemical compounds are applied to prevent or control pests and parasites of plants or animals causing harm during the production, processing, storage, transport, or marketing of food and agricultural goods.

EU-Regulation 2002/79/EC appoints the maximum levels for "certain pesticide residues in and on cereals, foodstuffs of animal origin and certain products of plant origin." In Germany, this regulation has been transformed into national law and is listed as "Maximum permissible amount of residue" (germ.: RHmV) expressing a technical zero-tolerance of 0.01 mg/kg of raw material of eatable cereals, fruits, and vegetables and of processed forms of food. By all means, this zero-tolerance may be exceeded by a factor of 100, under the prerequisite that the defined substance is not able to provoke any detectable damage to the consumer.

## Occupational Health and Safety

The OECD ("Organisation for Economic Cooperation and Development") is the supervisor of the regulation and coordination for biocides employed in the EU and important industrial nations. To put these substances into the market of the European community, it is necessary in Germany to involve the "Federal Institute for Occupational Safety and Health" (in german: BAUA). For the registration of new chemical compounds, it is appropriate to apply the Commission Directive 93/67 EEC, which determines the principles of risk evaluation to man and the environment. This is in agreement with the Council Directive 67/548/EEC. Herein, the classification, packaging, and labeling of dangerous substances are controlled to provide protection for public and occupational health as well as the environment on the whole. Dangerous substances are classified according to the degree of hazard and the nature of the risk.

The protection of people at their workplace situations is of vital importance because of lifelong contacts in daily routine iteration. In this background, the production, transport, and application of various chemical preparations have to be considered in terms of Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) to protect workers against health and safety hazards. The corresponding Directives in force are 2004/10/EC and 2004/9/EC (see also Tweedale 2011).

## Poisoning by Chemicals for General Use: Especially in Children

Children compared to adults are even more perceptive to damages caused by hazardous substances. The Guideline 2009/48/EU (put into force in 2011) is responsible for a general safety-standard and chemical safety of toys and other products inside the EU. The 2. GPSGV (published in Germany as "Geräte- und Produkt-Sicherheits-Gesetz") includes a special 'Regulation on Safety of Toys'. Many possible intoxication limits are weaker than before and importers – in accordance with REACH – are not restricted by these limitations as they are valid in Germany at present ("Karzinogene, mutagene, reproduktionstoxische (CMR)... Stoffe..." www.umweltdaten.de/publikationen). The allowed maximum concentrations of CMR substances are apparently too high and endanger the safety of our children.

Consequently, in the Annex XVII of REACH, an appropriate restriction has to be admitted. In tires, mouse pads and plastic clothing and articles for children and infants (for example, feeding bottles and soothers) are softeners from plastic production containing concentrations harmful to health. For example, bisphenol A is contained in a wide range of end-user applications as diverse as DVDs, spectacles, optical lenses, and reusable water bottles as well as in various medical equipments. This antioxidant component of softeners plays a crucial role in articles of daily use for children. In addition to its toxic effects on reproduction, there is an enormous accumulation in the biosystem, which therefore demands strict

evaluation in the recent EU risk regulation (EU Risk Assessment Report 2008). In children's feeding bottles, bisphenol A is apparently eliminated, but hazardous carcinogenic compounds such as benzophenone can still be detected in the plastic material of items used by infants. A dramatic example of a 20-fold exceeding above the allowed concentration (cf. Reach, Annex XVII; cf. CMR, 1 page before) has been found in toys. Some derivatives of phthalates obviously have been synthesized to circumvent the existing regulation under REACH.

The industrial countries and the developing countries of the Third World are in a diverse manner confronted with the problem of accidental poisoning in children. While in the Third World Countries, kerosin, caustic agents, insecticides, and herbicides are predominant sources of poisoning, in the developed world unintentional household poisoning as well as pharmaceutical products rank in the first place (Abbas 2012; Meyer et al. 2007).

Acute poisoning in children and adults is a specific problem. The diagnosis and treatment must be very fast and efficient. In order to prevent poisoning and to promote better care for intoxicated patients, the "Association of Poison Centres and Clinical Toxicologists" (EAPCCT), with its head office in Brussels, was founded. Thus, the EAPCCT brings together the activities of 56 countries in all continents – particularly through poisons information centers and poisons treatment centers in both the European Community and also the WHO organizations. In a global comparison, only about 46 % of the WHO member states agree to be part of the "World directory of poisons centers". Particularly the EAPCCT Working Group on Poison Centre activities plays an increasing role in regulatory processes in the European Community (e.g., REACH) as well worldwide. Furthermore, the EAPCCT is now editing an official journal, namely, *the Clinical Toxicology*. EAPCCT members are engaged in the scientific organizations IUTOX and EUROTOX, the International and European Union of Toxicology, with the intention of monitoring the progress of toxicological knowledge.

The national implementation is reflected in many countries, including Germany, Austria, and Switzerland in the form of selected poisons centers (www.vergiftungszentrale.de), which take in hand the risk evaluation, therapeutic, and preventive measures following urgent requests by call.

The hazardous accumulation in the human environment requires attention to the implications on the genetic material. That also includes the importance to study possible effects on successive generations. Such transgenerational phenomena have recently been described, and have provoked effects on the development of physiological, neural, metabolic, and behavioral phenotypes in adult individuals (cf. Crews et al. 2012; Skinner et al. 2012). In experiments on animals, it could be definitely demonstrated: How descendants of exposed progenitor individuals perceive and respond to a chemical restraint stress challenge.

Epigenetic transgenerational inheritance evoked by environmental hazardous substances has been determined by common epigenetic control regions in the genome and specific transcriptomes on the tissue-level. In case of the unwanted pesticide by-product TCDD, implications were reported in human populations that are exposed to dioxin. They are not only experiencing declines in fertility and

increases in adult onset disease, but most importantly, it could be realized that there is a risk of transmission to later generations. Experiments with similar results in the parents and the F3-generation animals have been performed using the fungicide vinclozolin. Such toxic substances show remarkable permanent presence also in drinking water – as detected in long-term biomonitoring. This is also valid for the herbicide atrazin and its main metabolite desethylatrazin. In 1986, about 400 l of atrazin reached the Rhine River via waste water (cp. Comp. Ciba-Geigy/Basel). The legitimate indignation in the public resulted in the further prohibition (in 1991), to apply such hazardous pesticides and herbicides to the environment.

2,3,7,8-TCDD, the toxicant from the Seveso disaster (1976), is an unwanted by-product, that never had an application itself. It is the most toxic congener of the 75 dibenzodioxines and 135 dibenzofurans (PCDDs and PCDFs, cp.: ipsc.jrc.ec.europa.eu; "Substances covered by Seveso II Directive") and is used as standard No. 1 for the evaluation of all congeners, as listed by the ECHA Agency (s. above). The persistence half-life of TCDD on soil surfaces may vary from less than 1 year to 3 years, but half-lives in soil interiors may be as long as 12 years. Screening studies have shown that TCDD is generally resistant to biodegradation (U.S. Environmental Protection Agency; epa.gov/. . ./factsheets. . .dioxin). Consequently, biomonitoring appears to be obligatory, because of such extreme half-life times of TCDD and other dioxines. From a thorough epidemiological analysis focused on approximately 3,500 male chemical workers, it was found that there was a significant correlation between the serum TCDD concentration and cancer mortality.

From an ethical point of view, infants and children are of outstanding significance for our common future. A suitable discipline focusing this forward-looking thinking is "Developmental Toxicology." This novel discipline considers the peculiar physiological and pathophysiological conditions during the development of the human organism and its susceptibility for toxic influences. This includes the creative establishment of sensitive analytical methods. To educate the responsible personal and to find contributions to this evolving field of activity, the following scientific sources can be recommended: EPA – U.S. Environmental Protection Agency, cf. Children's Health; Journals as EPH – Environmental Health Perspect, cf. Perera et al. (2012), and Shrader-Frechette (2012).

To include all citizens and customers in Europe for the protection of health and environment, the national implementation in Germany has been achieved by means of the former "Pollutant Release and Transfer Register." Now the access is realized via the electronic platform "thru.de," publicly available in Germany (12/2012, Environment Agency: UBA).

Corresponding with the global structure, institutions like WHO and EU execute their role for health protection of citizens and environmental safety regarding the risk evaluation of chemicals. Herein the European Environment Agency (EEA) and the Organisation for Economic Cooperation and Development (OECD) play an outstanding role for an intact environment and occupational health strategies. In this spirit, the German Action Programme Environment and Health (APUG – apug.de) is implementing the international activities, in order to set preventive impulses for the children's health.

## Food and Feed

The German food and feed law LFGB ("Lebensmittel- und Futtermittel-Gesetzbuch") came into force in September 2005. Thereby, the European basic regulations were implemented in the effective German food law. Along the line of food-production, all manufacturing and processing steps are included. Beside of food the law is valid for articles for daily use, feed, and cosmetics. According to priority, the safety of food is high ranking. Manufacturers, distributors, and traders have to guarantee perfect quality in each case. In addition, product identification and traceability must be assured during all steps of processing.

In various scandals, it has been obviously observed that many times the contents and the labeling of food preparations have been illegally changed. However, the safety and reliance of the consumers have to be guaranteed against fallacy and falsification of the declared components in food products. Just recently, in Europe, horse meat has been passed off as beef. In this connection § 11 (1), 1 of the amended LFGB (composition, description, and identification) from July 2009 to 2011 offers a reference point for effective prosecution by the appropriate control authorities.

## Food Additives, Sweeteners, and Contaminants

Food additives are used for the preservation or enhancement of taste and appearance of food supply and may be divided into numerous categories (ca. 20). A few selected examples are antioxidants, food colors, flavors, and sweeteners. This scheme is under continuous development by the Codex Alimentarius Commission, established by FAO and WHO to harmonize international food standards (online: codexalimentarius.org).

In three different Directives of the EU, the requirements are laid down with regard to the safety and purity criteria of: Sweeteners (Directive 2008/60/EU); additives other than sweeteners and colors (Directive 2008/84/EC); and Directive 2008/128/EC for colors. These substances may only be brought to the market, if there is a technological need for their use, if they do not mislead the consumer, and if they do not present any health hazard. The daily use of sweeteners (e.g., saccharin, cyclamate, aspartame, and recently also sucralose and stevia) has to be considered with critical awareness.

In the EU, the food additives are categorized in well-known lists according to E-numbers (E100 up to E1525 (expandable); cf. Regulation No. 1129/2011/EU).

During the past few years, in incidents which dealt with contaminated food, the problems were related to dioxin, nitrofuran, pesticides, etc. Cultivation and production processes as well as humid storage may contribute to enrichment of microorganisms and of mycotoxins such as aflatoxin. The Council Regulation 315/93/EEC defines the level of toxic contaminants which are based on data collections from the EFSA agency. Nowadays, maximum levels for certain contaminants in food are set in Commission Regulation 1881/2006/EC. Detailed determinations are scored for: nitrate, mycotoxins (among others aflatoxins, ochratoxin A, patulin), metals

(lead, cadmium, mercury, tin), 3-MCPD, dioxins,and dioxin-like PCBs as well as polycyclic aromatic hydrocarbons B(a)P. Human exposure to PCBs is primarily caused by consumption of contaminated food. As a consequence of 60-fold differences in maternal hepatic cytochrome (CYP1A2) levels and 12-fold variability in AH-Receptor affinity in humans the neurotoxic effects on children can considerably diversify. This depends not only on the exposition, but especially on their genetic precondition (mechanistic study in k.o.-mice in Curran et al. 2011). The International Convention of 140 states on mercury has been adopted in Geneva (UNEP – United Nations Environment Programme, 2013). The worldwide emission of 3,900 t of highly toxic mercury needs to be reduced and these are particularly related to washingout during gold mining and emissions from coal power stations. Amalgam fillings of caries cavities also constitute an individual risk.

The Rapid Alert System for Food and Feed (RASFF) of the European Commission is responsible for the continual observation of risks, which are identified in food, feed, and food contact material in a member state or imported from nonmember states into the EU. All hazard reports have to be sent immediately to the responsible national administration (in Germany: BVL – bvl.bund.de/rasffmeldung).

## Food Supplements and Health Claims

The most common food supplements consist of vitamins and/or minerals. Food supplements must contain any of these ingredients in quantities that are sufficient to have a verifiable effect on the body. Moreover, it is required that food supplements are sold in small quantities, for example, as pills or fluids, and that they are labeled with information about the recommended daily dose (ADT-value; cf. Directive 002/46/EC and amended in Commission Regulation 1170/2009).

The regulations lay stress on the absolute prohibition of unproven health claim declarations in labels and descriptions and must comply with the rules laid down for food supplements in Regulation (EC) No 1924/2006 of the European Parliament and of the Council. Therefore, there has to be a strict differentiation between supplements and approved medicinal products and the appropriate regulations by the EU or the national legislation (cf. in Germany: NemV – Nahrungsergänzungsmittel-Verordnung 2004 (Amendment 2011)).

## Cosmetics

A growing field in the modern consumer world is cosmetics where ingredients are continually changing. In Germany, cosmetics are under LFGB – (Lebensmittel- und Futtermittel-gesetzbuch) control. Cosmetics comprise products for hair and skin care, as well as for mouth care and dental hygiene. The relevant substances may enter the body and biosystem through the body surface and reach the other tissues via blood stream.

Allergic reactions are the most frequent side-effects caused by various mixtures containing colors, synthetic or natural fragrances as well as diverse additives. Routinely, cosmetics have to be screened for possible effects on fertility, genetics, and particularly on mutagenic and carcinogenic potency. For example, the use of permanent aromatic-amine hair dye seems to be related to an increased risk of bladder cancer in individuals who possess certain drug-metabolizing enzyme polymorphisms (Koutros et al. 2011).

For producers, the EU opened the Cosmetic Products Notification Portal (CPNP) that regulates the registration and notification procedure for new products. Concurrently, the producers and importers are obliged to disclose their frame recipes, so that the applied toxic constituents are available urgently for the poison centers in an emergency situation.

Recently, the new cosmetics Directive 1223/2009/EU is now in force while the former Council Directive 76/768 will be canceled in July 2013. Animal experiments should be substituted by alternative in vitro methods or cell culture systems (Animal protection law; EU Directive 1223/2009). For selective toxicological endpoints like reproduction toxicity and toxicokinetics, restricted implementation of animal studies appears to be inevitable.

## Drugs: Approval, Certification, and Safety

Approved drugs are characterized by efficacy, safety, and quality (AMG – §1) of its active pharmaceutical ingredient(s) as well as of the additives. In case of drugs, the protection of consumers requires a highly qualified safety system regarding approval, manufacturing, and delivering of a prescribed drug by the pharmacist and even more in life-saving clinical situations. In Germany, the competent authority for human medicinal products is the BfArM. On the other hand, the Paul-Ehrlich-Institute (PEI) is responsible for sera, vaccines, test allergens, antigens, and blood preparations. The governmental competence is attributed to the Ministry of Health (BMG).

All biotechnological drugs as well as the innovative chemical drugs have to be authorized through a Centralised Procedure (CP) at the European Medicines Agency (EMA). In this connection, gene and (stem) cell therapy are intrinsic components of modern drug therapy. Apart from the national authorization procedures in Germany, new authorization procedures have been created on the basis of regulations and directives of the European Commission. The Centralised Procedure (CP) effects a license throughout the EU. In order to obtain a license within more than one EU country simultaneously, the applicant has to initiate a so-called Decentralised Procedure (DCP) or to apply for a “Mutual Recognition Procedure” (MRP).

### Narcotic and Psychotropic Substances

The strictest drug regulations are established for anesthetic and narcotic substances because of their extreme potential to develop addiction and dependency. By way of

the distinct rules and regulations of the medical prescription and the release by the pharmacist, limited portions of narcotic substances have been accurately defined. These may be maximally two active substances combined, which are released within 30 days for an individual patient. The tightened control of all prescribed narcotic drugs is realized by means of identical printed form copies in triplicate. These are confidently delivered by the physician, the pharmacist, and in the end, the supervising "Federal Opium Agency" is implicated. A statistical evaluation of all data supplies the material for national as well as regular reports to the International Narcotics Control Board (INCB) in Vienna. Accordingly, the total turnover of narcotic drugs and psychotropic substances used for medical and scientific purposes is documented and verifiable.

In correspondence with the so-called BtMVV * ("Ordinance on the Prescriptions of Narcotic Drugs" BGBl, last amended in July 2009), the maximum permissible dose is listed in detail for each narcotic substance in §2. Equivalent to the steps of importing the raw substances and their subsequent manufacturing, the "Ordinance concerning the Foreign Trade in Narcotic Drugs" (BtMAHV – amended June 2001) and the "Ordinance concerning the Domestic Trade in Narcotic Drugs" (BtMBinHV – dito) are the relevant legislative regulations in Germany. The administrative control of the consumption of narcotic substances in Germany is under the responsibility of the Federal Institute BfArM, especially the "Federal Opium Agency" (BOPST). Its tasks are established comprehensively in the German Narcotics Act (BtMG). The problem of currently modified psychoactive substances as derivatives of the classic narcotics – such as amphetamines, cocaine, etc. – are considered in the updated version of the BtMÄndV (cf. 26. Amendment of the Narcotic Drug Act, July 2012).

Import, export, manufacturing, and marketing of narcotic and psychotropic substances require a harmonized international cooperation between the EU member states and the countries involved in the cultivation and preparation of precursor substances (e.g., EC Regulations 273/2004 and 1277/2005; Precursors Monitoring Act: cp. EMCDDA in Lisbon (European Monitoring Centre for Drugs and Drug Addiction) – March 2008). A complete "List of Narcotic Drugs under International Control" is published in form of the 'Yellow List' by the International Narcotics Control Board.

## Doping

Various supplements could also be applied to improve performance or even for performance enhancement (doping) in competitive sport disciplines (cf. List of Prohibited Substances (2013) according to **WADA** and **NADA**, resp.). Each year, the substances and methods, that lead to improved performance, are updated, and also health risks or violation of the international "fair-play spirit" in sports are revised. Scandalous regulatory violations are generally well known in the public and are associated with substance classes such as erythropoietin, testosterone (anabolic analogs), or ß2-agonists like clenbuterol. Sports physicians in Germany

responsible for the optimal performance of their athletes find helpful support in the "Beispielliste zulässiger Medikamente 2013" (NADA), for their efforts to prescribe permitted drugs only. The application of certain drugs for enhancement in high-performance sports will be prosecuted according to the recent amendment of the drug law (16. Novelle AMG, § 6a : 'Prohibition of Drugs for Use in Doping').

### CHMP

The Committee for Medicinal Products for Human Use (CHMP) is a scientific board within the EMA responsible for precise examination of the substantial dossiers in the process of drug approval. During the preclinical investigations and the different phases of essential clinical trials, a vast quantity of molecular, physiological, and pharmacological data represents the basis for comprehensive decision about the risk-benefit ratio. Likewise, it is important that there should be a collection of monitoring data during therapeutic use of a drug since threatening side-effects lead to a negative risk-benefit evaluation and consequently withdrawal from the market.

From CHMP, the EPAR as an European Evaluation Report of highest quality is published for the respective active ingredient in all official EU languages, which reports precisely the relevant criteria to judge the risk-benefit decision. The equivalent medical compilation in Germany is a "Summary of Product Characteristics/Fachinformation" which is issued under the professional supervision of the Federal Agency, BfArM.

### CVMP

To comply with the conditions of drug approval in the case of medicinal products for veterinary use, the EMA (Committee for Medicinal Products for Veterinary Use) is responsible. If the relevant Committee concludes that the quality, safety, and efficacy of the concerning product are sufficiently proven, it adopts a positive opinion. This is the prerequisite for the legal implementation by the European Commission. Veterinary and medicinal products for human use may give reason for regulatory measures. Both kinds of drugs excreted and metabolized accumulate via sewage plants in the ground water and drinking water and thus pose a risk to human health.

Consequently, environmental persistent pharmaceutical pollutants (EPPPs) may have a considerable environmental impact on the human environment, but there was insufficient documentation available. As a result of the diffuse dissemination of EPPPs such as estrogens in the environment, vulnerable populations like children and adolescents, men and women in reproductive age and embryos/fetuses as well as elderly or weak persons are under hazardous exposure.

Pharmaceuticals and their metabolic products present an emerging global issue. The special attention should be focused on a qualified equipment of sewage plants for the elimination of as many as possible of the PPCPs (pharmaceuticals and personal care products; cf. Kümmerer (2010)).

To consider the safety aspects in patients younger than 18 years, the paediatric-use marketing authorisations (PUMA) are granted by the European Medicines Agency for medical products that are intended exclusively for pediatric use.

The main aim of the Paediatric Regulation is to improve the children's health in Europe. The Paediatric Regulation came into force in the European Union from January 2007 (Regulation 1901/2006/EC). Its principal impact was the establishment of the Paediatric Committee (PDCO), which is responsible for coordinating the Agency's work on medicines for children.

In Germany, "The Drug Act" (AMG – 16. Novelle (BGBl. Okt. 2012) includes all legislative regulations related to the approval, marketing, and supply of drugs, the European directives are implemented continuously. In cases of serious events of drug side-effects, the manufacturers have to inform all physicians and medical experts by an urgent letter ("Rote Hand Brief," corresponding with "Dear Doctor Letter"). Its content is coordinated following concerted consultations with the physicians organizations (AKdÄ – Drug Commission of the German Medical Association) and governmental institutions (BMG, BfArM, PEI, federal state authorities) to assure the highest level of safety for patients and consumers.

The Pharmacovigilance Risk Assessment Committee (PRAC) ensures the inclusion of the European Medicines Agency (EMA) in the assessing and monitoring of safety issues for human medicines.

The International Conference on Harmonisation (ICH) is a project that brings together the regulatory authorities of Europe, Japan, and the United States, representing the top-selling drug markets worldwide. Experts from the regulatory agencies and from the pharmaceutical industry in the three regions discuss scientific and safety aspects of pharmaceutical products for human use. ICH guidelines are used as guidance for the U.S. Food and Drug Administration (FDA) and have been adopted as law in several countries as well.

## Self-commitments of the Chemical Industry

Initiatives of the chemical industry and their international associations in their traditional perception should be accepted as a quite progressive contribution for the sustainment of environment and human health. In the form of self-commitments, specific agreements have been arranged following intensive discussions and negotiations with the public administration. "Responsible Care" and "Corporate Social Responsibility" are representative agreements for responsible management of chemicals, products, and processes. The GRI (Global Reporting Initiative, in Europe: CEFIC) Guideline G3.1 (2006) has been elaborated, evaluating companies by a vast number of indicators (>100). This GRI Indicator Protocol Set includes criteria of biodiversity and emissions and 30 of them enable conclusions on total environmental expenditures. The GRI – organizing Global Conferences on Sustainability and Transparency (e.g., Amsterdam 2013) – cooperates worldwide with the governmental organizations OECD, UNEP, and UNCTAD. The raising awareness of sustainable developments of products and industrial processes presupposes partnership with governments as well as public organizations in the

effective implementation of environmentally responsible behavior. The bigger part of responsibility has to be maintained by the public institutions and international organizations. The globalization process gives fresh impetus to safeguard landscapes, underground, and the atmosphere against exposure to risky industrial products, as documented in Greenhouse Gas Emissions (most important are $CO_2$, $CH_4$, and $N_2O$).

The ecological footprint and consequent natural resource balancing are main criteria for the intergenerational equity. By means of reducing the overconsumption of irreplaceable natural resources – as ethically esteemed – the oceans, underground resources as well as clean air and water must be preserved in favor of the successive generations.

## References and Recommended Reading

### Chemicals

Abbas SK, Tikmani SS, Siddiqui NT (2012) Accidental poisoning in children. J Pak Med Assoc 62(4):331–334
CLP – Regulation – EC No. 1272/2008
Commission Directive 93/67/EEC. cp. www.eur-lex.europa.eu/
Chemicals: "Risks to man and the environment"
Crews D, Gillette R et al (2012) Epigenetic transgenerational inheritance of altered stress responses. PNAS 109:9143–9148
EU Regulation No.s 756/7 of 24 Aug. 2010, amending EC Regulation No. 850/2004
EU Risk Assessment Report (2008) EINECS No. 201–245–8, Hum Health Add
Meyer S, Eddleston M et al (2007) Unintentional household poisoning in children. Klin Pädiatrie 219(5):254–270
Perera FP, Tang D et al (2012) Prenatal Polycyclic Aromatic Hydrocarbon (PAH) exposure and child behavior at age 6–7 years. Environ Health Perspect 120(6): 921–926
REACH – Registration, Evaluation, Authorisation and Restriction of Chemicals.www.reach-info.de (LINK: EU – > VO-Text REACH) – EC 1907/2006
EUR – Lex – 32006R1907
Shrader-Frechette K (2012) Taking action on developmental toxicity: scientist's duties to protect children. Environ Health 11:61–73
Skinner MK, Manikkam M et al (2012) Epigenetic transgenerational inheritance of somatic transcriptomes and epigenetic control regions. http://genomebiology.com/2012/13/R91
Tweedale AC (2011) Uses of 'Good Laboratory Practices' by regulated industry and agencies, and the safety of bisphenol A. J Epidemiol Community Health 65(6):475–476
UNECE/GHS – United Nations Economic Commission. www.unece.org/trans/danger/publi/ghs/ghs_rev03

### Chemicals/Biocides

BAuA. www.baua.de
IARC. www.iarc.fr
UBA. www.umweltbundesamt.de

## Food and Feed

BMELV. www.bmelv.de
BVL. www.bmelv.de (national)
BfR. www.bmelv.de
Curran CP, Nebert DW et al (2011) In utero and lactational exposure to PCBs in mice: adult offspring show altered learning and memory depending on Cyp 1a2 and Ahr genotypes. Environ Health Perspect 119:1286–1292
EFSA. www.efsa.europa.eu. (Europe)
Koutros S, Silverman DT et al (2011) Hair dye use and risk of bladder cancer in the New England bladder cancer study. Int J Cancer 129:2894–2904
LFGB – Lebensmittel- und Futtermittel-Gesetzbuch (22. Aug. 2011)
www.umwelt-online.de

## Drugs

AMG Arzneimittel-Gesetz (Germany)
BfArM. www.bfarm.de
EC No. 726/2004 – Centralized Procedure (amended 31.03.2004)
Guideline 2001/83/EC
EMA. www.ema.europa.eu/ema
Kümmerer K (2010) Pharmaceuticals in the environment. Annual Rev Environ Res 35:57–75
PEI. www.pei.de

## Narcotic and Psychotropic Substances

BtMAHV – Ordinance concerning the Foreign Trade in Narcotic Drugs, last amended June 2001 (Fed. Law Gaz. I, p 1180)
BtMBinHV – Ordinance concerning the Domestic Trade in Narcotic Drugs, dito (Fed. Law Gaz. I, p 1416)
BtMÄndV–(cf. 26. Amendment of the Narcotic Drug Act, July 2012)

# Observance of Susceptible Population Groups in Regulatory Toxicology

Ursula Gundert-Remy

## Contents

General Considerations ..... 720
Are Susceptible Subgroups Protected by the Default Safety Factors? ..... 720
Specific Groups and Regulations ..... 720
Specific Sensitivity in Different Life Periods ..... 721
Prenatal Period ..... 721
Age up to Six Months ..... 721
Child and Youth ..... 722
Elderly (>70 Years of Life) ..... 723
Pregnancy ..... 723
Influences of Diseases ..... 723
Impairment of Excretory Organs ..... 723
Other Organs ..... 724
Gender-Specific Aspects ..... 724
Recommended Reading ..... 725

**Abstract**

When setting health-based threshold values or assessing MOS values, awareness has increased that subgroups in the population may exist which respond with a higher susceptibility than the majority of the population. It is not an easy task to take increased susceptibility into consideration. Even if we know or at least have some indications that, for example, newborn might be more susceptible than adults, it is hard to account for the difference in terms of numeric figures because of lack of data.

U. Gundert-Remy
Institute for Clinical Pharmacology and Toxicology/Institut für Klinische Pharmakologie und Toxikologie, Charité Universitätsmedizin Berlin, Berlin, Germany
e-mail: ursula.gundert-remy@charite.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_86, 

## General Considerations

When assessing substances or exposure situations, e.g., accidental release of dangerous chemicals, it has to be considered whether in the remit of the assessment, susceptible subpopulations may exist. If so their specific situation has to be reflected in the assessment and the measures taken. Potential sensible subgroups are the unborn child, the neonates, the elderly, and the subjects with allergic conditions.

## Are Susceptible Subgroups Protected by the Default Safety Factors?

Intraspecies variability and susceptibility has to be taken into consideration when setting health-based threshold levels like ADI values for pesticides. The conventional default value of 10 has been introduced by JECFA (Joint Food and Agriculture Organization – World Health Organization Expert Committee on Food Additives) in the 1950s and was taken up by Joint Food and Agriculture Organization – World Health Organization Meetings on Pesticide Residues (JMPR). The default factor of 10 was subdivided in a factor covering variability in toxicokinetics and a subfactor covering variability in toxicodynamics. It has been proposed to allocate equal factors of 3.14 for both parts of the total factors. Other scientists proposed to use a subfactor of 4 for the toxicokinetic and a subfactor of 2.5 to cover variability in toxicodynamics. Retrospective analysis of data from clinical studies shows that the factor of 10 is sufficient to cover intraindividual variability with the exception of cases in which the drug is metabolized by a polymorphically expressed enzyme (e.g., CYP 2D6 or CYP 2C18). As the studies were mainly from phase-I studies in which health young adults with predefined body weight and body height take part, the variability of the general population may be greater than in the population of young healthy adults.

## Specific Groups and Regulations

The need to consider special subpopulations is laid down in several guidances for risk assessment and for the use of the TTC concept (EFSA 2012). However, there is no general factor to be used and a case-by-case consideration is recommended.

In Germany, the protection of children is explicitly expressed in several regulations. Table 1 gives some examples.

A second group which is often mentioned as being specifically sensitive is the group of pregnant women. It should be born in mind that it is not the pregnant women who is the sensitive subject but the unborn child who's development when exposed to environmental may be impaired.

Other groups are the elderly which are mentioned when discussing about specific sensitivity towards exposure against chemicals, the female population in general, and asthmatics. The drug legislation foresees specific trials for children. Elderly and

**Table 1** Examples for specific regulation for children

| Regulation | Specific parameter |
|---|---|
| Ordinance on toys for children | Heavy metals |
| Order on dietetic foodstuff | Organochloro- compounds |
| Protection of minors | Alcohol |
| Drug law | Specific doses for children, contraindications |

women have to be included into the trial population and their data may be requested to be analyzed separately. In the following part, we will discuss the state of the knowledge including also subjects with impaired excretory organ function.

## Specific Sensitivity in Different Life Periods

### Prenatal Period

Birth defects due to prenatal exposure towards chemical substances, drugs, infections, and other environmental influences are the main causes for mortality in early life. Drugs which are known to cause birth defects are among the groups of cytostatic drugs, sex hormones, anticonvulsives (such as valproic acid), and antipsychotics. Intrauterine infections which can cause birth defects are rubella, cytomegalovirus infections, syphilis, and toxoplasmosis. Radiation at higher doses may cause structural abnormalities of the brain and the eye. Exposure against some chemicals and environmental agents during pregnancy has been described as causing birth defects. Organic solvent sniffing may cause craniofacial abnormalities, similar to the syndrome after alcohol abuse in pregnancy. Further defects are associated with glycolether and alcohol abuse such as microcephalus and intellectual impairment. Some other agents have been imputed. The data are however inconclusive including pesticides exposure and arsine exposure. Also, the exposure of the father has been imputed to be causally related to birth defects without clear results (tobacco, grass, pesticides, anesthetics, and lead).

It is however beyond doubt that smoking, even secondary smoke during pregnancy, is related with low birth weight.

The influence of intrauterine exposure towards chemicals on the development of cancer is controversial in most of the cases. Diethylstilbestrol is known as causing vaginal carcinoma in young adults, twenty years after intrauterine exposure.

### Age up to Six Months

In this period starting at birth, toxicokinetic differences are well documented. The activity of most of the CYP enzymes is lower at birth, maturating during the first 6 months and reaching the level of the adult within the first year. The enzyme proteins for phase-II conjugation reactions are expressed whereby for some glucuronyl transferases the levels at birth are at the adult level, for others a reduced capacity is

known (e.g., UTG 1A1 or UTG1B15). As far as known today, sulfation is fully expressed at birth, and activity of acetyltransferase may reach adult levels only at the age of 2. Absorption through skin is enhanced due to the reduced thickness. Distribution of substances is different because of the lower relation between fat and body weight and the higher relation between water and body weight. In the first weeks the blood/brain barrier is not anatomically developed which plays a role in the development of "kernicterus" in the newborn where physiologically a high turnover of erythrocytes containing the fetal hemoglobin in newborns leads to high bilirubin levels. In cases of different blood groups between mother and child, the incompatibility leads to lysis of erythrocytes. The high bilirubin levels in conjunction with the impaired ability to conjugate bilirubin to bilirubin glucuronide and the impaired blood/brain barrier are components of the enrichment of bilirubin in cerebral structures such as basal ganglia and brainstem nuclei. The status "kernicterus" may lead to severe neurological deficit and even death.

The renal function as measured by glomerular filtration rate is reduced to an extent of up to 50 % in early life and is gradually increasing to the normal level within the first 6 months. The physiological changes are all in the direction that the elimination and excretion of xenobiotics is prolonged which at the same level of exposure leads to higher internal exposure when compared with the adult. Depending on whether the parent compound or the metabolite is the toxic agent, this may result in a high (parent compound is the toxic agent) or lower (metabolite is the toxic agent) sensitivity. Brain, bones, immune system, and endocrine and reproductive organs are developing in the postnatal period over years. Thus, specific susceptibility can be present also after the first months in life. In particular the development of the brain is a highly susceptible physiological process which might be critical. In this respect, lead and also PCBs may play a role in negatively influencing intellectual capacity.

## Child and Youth

The kinetics in these age groups is not much different from the kinetics in the adults. In school kids the metabolizing enzymes are rather highly expressed so that the clearance is somewhat higher than in adults. It is to be noted that windows of development exist during which exposure towards chemicals may have negative influences. Continuously developing organs are the brain (influence of marihuana?) and bones. Closure of epiphyses can be influenced by chinolones. The immune system is developing until the age of young adults. Exposure towards some chemicals might negatively influence the development of the immune system. Among the chemicals imputed to cause immunotoxicity are organotin compounds, pesticides (methoxychlor, heptachlor), and polyhalogenated aromatic hydrocarbons (2,3,7,8-tetrachlordibenzo-p-dioxine). A special awareness has been raised for the exposure towards compounds exhibiting sexual hormonal activity, so-called endocrine active substances. There is no doubt that high exposure levels can influence the sexual development in animals; the effect of much lower exposure levels in the human population is not yet finally assessed.

### Elderly (>70 Years of Life)

Physiological functions may be caning with increasing age whereby the variability of these processes is extremely high. Generally, the excretory functions are declining with age. Liver mass as well as liver blood flow are slightly reduced in high age (mass, reduction of 17 % between the age of 20 and the age of 80 years; liver blood flow, reduction of 0.3–1.5 %/year). Reduced liver mass and liver blood flow may cause a reduced metabolic clearance of chemical compounds. Depending on whether the parent compound or the metabolite is the toxic agent, this may result in a high (parent compound is the toxic agent) or lower (metabolite is the toxic agent) sensitivity. The renal function measured by glomerular filtration rate is also a function of age. The physiological change is a reduced renal blood flow (2 %/year) and a reduced renal organ mass (up to 30 % in the very elderly). Substances which are excreted by the kidneys (e.g., PFOA) are much slower excreted. It should be noted that at the same external exposure level, a twofold higher internal exposure will result if the renal function is reduced to 50 %.

On the toxicodynamic field, there are some observations showing a reduced number of receptors. This concerns ß receptors, alpha-2 receptors, and insulin, glucagon, steroid, dopamine, and prolactin receptors. The results if transferred to the in vivo situation should result in a lower susceptibility. Whereas for some effects (effect of beta-blockers, effect of insulin), data in humans and animals are available, no generalization can be made, and for chemicals this field is not yet studied in detail.

### Pregnancy

It is well known that the body of the pregnant woman undergoes physiological changes with an increased blood volume, body weight, and hormonal changes. Nevertheless, the difference between pregnant women and nonpregnant women for three substances (caffeine, midazolam, and metoprolol) is not more than a factor of two. Hence, major and important changes are not present.

## Influences of Diseases

### Impairment of Excretory Organs

Impairments of excretory function in liver or renal diseases are the same as described for the very elderly. At the same level of external exposure, the level of internal exposure is several times higher as compared with healthy subjects, potentially causing an increased effect. Theoretically, in patients with reduced lung surface such as in emphysema patients, the reduced surface should lead to a reduced internal exposure. On the other hand, substances which are excreted by exhalation should accumulate in these patients. However, there are no data

confirming the theoretical considerations. It is known that in emphysema patients and in patients with asthma, chemicals acting on the airways have an increased effect ("higher sensibility" of these patients).

### Other Organs

Systematic studies are not found in the literature. It is to be inferred from biology that patients with impaired bone marrow due to pretreatment with cytostatic drugs may have an increased effect from chemicals acting on the blood and bone marrow such as benzene. Likewise, patients with impaired immune function either inborn or due to treatment with immunosuppressive drugs may be at a greater risk than healthy subjects exposed towards immunotoxicants. In the current risk assessment, no special considerations apply for subgroups with preexisting conditions.

## Gender-Specific Aspects

Physiological differences are obvious between males and females. Different chromosomal status and hormonal levels determine the phenotypic body appearance, in particular in the adult life. Men do have higher mean body weights and higher mean height when compared with the mean values for women. The body composition differs in such that men have a greater muscle mass in terms of proportion of their body weight, whereas women do have a greater proportion as fat mass.

The hormonal situation in women is characterized by cyclic changes in the estrogens and gestagens in the lifetime between menarche until menopause; in a great number of women using hormonal contraception, the situation is changed. During pregnancy the level of hormones increases from about 50 pg/ml (ovulation) to 25 ng/ml in week 40 of pregnancy; this is a factor of 500. In the menopause the sex hormonal levels are declining. In males extreme changes in sex hormonal levels are not known.

Gender-related differences of metabolizing enzymes are known; they are, however, not impressive. The activity of CYP3A4 is some 20 % higher in women as compared to men. There are subtle changes of hormonal influences on CYP 1A2 activity as the half-life of theophylline and caffeine may be different within the cycle, and men do have a bit higher activity of CYP1A2. As the changes and differences are rather small, they are covered in the intraspecies factor of 10.

In animal studies, we know some sex-specific reaction and the results of studies have to be assessed cautiously. Renal tubular damage related to $\alpha 2-$microglobulin excretion is found in male rats only, and the finding is without relevance for the human, males as well as females. On the other hand, breast tumors in female rats are difficult to assess with respect to the relevance for humans as it is the leading tumor in females. It may also occur in men, however, only in rare cases. Carcinoma of the

prostate and mesothelioma of the testes are clearly only occurring in males. Thus, findings in animals are difficult to interpret.

Although physiological differences and sex differences in susceptibility and findings in animals exist, we do not know similar differences towards chemicals, with the notable difference of sex hormones. At present, the effect of endocrine active substances is hotly debated. The question remains unresolved until now whether these substances in environmental concentration may negatively impact male fertility. Also the increase in breast cancer in females has been discussed in the context of exposure towards chemicals with estrogenic activity present in the environment.

## Recommended Reading

Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H (2012) Anatomical, physiological and metabolic changes with gestational age during normal pregnancy: a database for parameters required in physiologically based pharmacokinetic modelling. Clin Pharmacokinet 51(6):365–396

ACUTEX (2006) http://www.ineris.fr/centredoc/TGD_06DR055.pdf

Bruckner JV (2000) Differences in sensitivity of children and adults to chemical toxicity: the NAS panel report. Regul Toxicol Pharmacol 31:280–285

EPA (2003) Overview of the special vulnerability and health problems of children. Paper series on children's health and the environment

EFSA (2012) www.efsa.europa.en/de/efsajournal/pub/2750.htm

Ginsberg G, Hattis D, Sonawane B (2004) Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. Toxicol Appl Pharmacol 198:164–183

Gleiter CH, Gundert-Remy U (1996) Gender differences in pharmacokinetics. Eur J Drug Metab Pharmacokinet 21(2):123–128

Goodman JE, Witorsch RJ, McConnell EE, Sipes IG, Slayton TM, Yu CJ, Franz AM, Rhomberg LR (2009) Weight-of-evidence evaluation of reproductive and developmental effects of low doses of bisphenol A. Crit Rev Toxicol 39(1):1–75

Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leede JS, Kauffman RE (2003) Developmental pharmacology – drug disposition, action, and therapy in infants and children. N Engl J Med 349:1157–1167

Meibohm B, Beierle I, Derendorf H (2002) How important are gender differences in pharmacokinetics? Clin Pharmacokinet 41(5):329–342

Shi S, Klotz U (2011) Age-related changes in pharmacokinetics. Curr Drug Metab 12(7):601–610

Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, Woodruff TJ, Vom Saal FS (2012) Endocrine-disrupting chemicals and public health protection: a statement of principles from the endocrine society. Endocrinology 153(9):4097–4110

# Toxicological Report

Thomas Gudermann, Harald Mückter, John H. Duffus, and Hermann M. Bolt

## Contents

Key Points ..... 728
Expert Requirements ..... 728
Report Essentials ..... 728
Required Basic Information for Experts ..... 729
Cause and Effect Relationships ..... 730
Occupational Disease and Workplace Issues ..... 731
Adverse Drug Effects and Drug-Related Disease ..... 736
Reduction in Earning Capacity ..... 736
Cross-Links to Regulatory Affairs ..... 737
Recommended Reading ..... 737
Resources ..... 737

**Abstract**

The toxicological report is the cornerstone in court and business when it comes to the weighing of arguments in scientifically based trials and investigations dealing with human and environmental health concerns. The toxicological expert who is

T. Gudermann (✉) • H. Mückter
Walther-Straub-Institute of Pharmacology and Toxicology, Ludwig-Maximilians-Universität München, Munich, Germany
e-mail: thomas.gudermann@lrz.uni-muenchen.de; mueckter@lrz.uni-muenchen.de

J.H. Duffus
The Edinburgh Centre of Toxicology, Edinburgh, Scotland, UK
e-mail: j.h.duffus@blueyonder.co.uk

H.M. Bolt
Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany
e-mail: Hankinson@ifado.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_98, 

responsible for its preparation is usually regarded as an unbiased and independent expert in the field, and her/his statements should be as concise and reasonable as possible. Depending on the case of interest, the toxicological report may eventually become a piece of evidence, sometimes with far-reaching consequences.

## Key Points

*Toxicologists may be asked for their expertise in criminal, labor, social, and civil affairs.*

*The starting point for expert reports is the court direction for evidence, which is mandatory and specific to the expert.*

*An expert report has to meet typical requirements in form and content as outlined below.*

## Expert Requirements

Among the various experts a toxicologist is no exception. Toxicological experts must be independent, neutral, and honest. After receiving a formal legal request for a statement, toxicologists have to check whether the issue falls within their scientific and (or) technical competence. If this is not the case, a prompt informative reply to the client is mandatory. Once the toxicologist has accepted a request for a statement, he (she) is responsible for the unbiased fulfillment of the job and is solely obliged to provide sound scientific reasoning based on the state of the art.

An important expert requirement is a fundamental knowledge of the specific field of law. The expert should approach the subject and its related terms from the point of view of a lawyer, in order to be able to explain any investigations, methods, and results in court. Another requirement is clear and unambiguous presentation – in written and oral form – of the details and facts pertaining to the case. Expert witnesses may be challenged by critical questions in court. It is not the expert's duty to come up with new hypotheses or suspicions outside the realm of toxicology, and it is the duty of judges, attorneys, and lawyers to find out the truth. Finally, an expert's mandate is not negotiable.

## Report Essentials

Traditionally, expert reports consist of two parts. Part I is the prologue in which the expert details all the facts that were known to him when arriving at the final statement. These facts include (i) the relevant parts of the disclosed file as received from the court or client, (ii) known facts of the medical history if individuals are involved, and (iii) obtained laboratory results with analytical

data and/or specimens, clinical findings, testimonies, and any other conclusive evidence.

Part II is the expert's assessment, which is based solely on evidence and findings that were elaborated on in Part I. It is a good habit to begin Part II with the question (s) that were asked to the expert. Any assessment should not come as a plain repeat of evidence and findings presented in Part I. It should also not reiterate the contents of the filed information. Instead, it should contain the detailed reasoning – step by step – as to how the expert arrived at the presented conclusions. These conclusions may be given as a statement followed by the relevant arguments or may follow the logical and scientific reasoning.

An optional third part that is highly recommended for complicated or large reports is a short summary in which the essential arguments and conclusions are presented as a concise fact sheet, preferably by repeating each interrogatory issue in front of the expert's answers.

Toxicological reports should be written in plain language that educated laymen are able to understand. The text should be comprehensible without sacrificing precision. Any special medical or scientific terms should be avoided or should be explained and referenced. This may be accomplished in parentheses or footnotes. For large reports a glossary may be appropriate, and references should be made to it in the text.

## Required Basic Information for Experts

The toxicologist will be asked as an expert about relationships between exposure of one or more individuals to an identified or suspected health hazard or environmental situation. That is why any information about the exposure is of utmost importance (see chapter "► Exposure Scenarios in Toxicology"). It may already be included in the file of the court or client, or it will be determined by the toxicologist, based on his (her) own investigations that are clearly presented in the report.

Of similar importance is the determination and assessment of any suspected toxic effects. These may be symptoms, diseases, epidemiologic findings, or environmental abnormalities. If the filed data include observations, statements, expert statements of medical specialists, environmental scientists, etc., these may be included in the toxicologist's reasoning. It is common practice to rank any relationship or association of identified causes and observed effects according to probability. Unfortunately, the English language uses different terms to measure certainty, and toxicologists face a dilemma in putting scientific data into the context of the official language used in court. This is reflected in the three terms "likeliness," "likelihood," and "probability." Table 1 lists the various expressions that are being used to describe any causative or plausible link between an exposure and its outcome.

**Table 1** Degrees of likelihood

| | |
|---|---|
| Impossible | 0 % |
| Possible | ≤50 % |
| Somewhat probable | 60 % |
| Probable | 70 % |
| Very probable | 80 % |
| With high probability | 90 % |
| With utmost probability | 99 % |
| With certainty | 100 % |

## Cause and Effect Relationships

There are different concepts of cause in various areas of jurisprudence. Essentially, jurisdiction and the theory of law differentiate between the theory of equivalence (in criminal law), the theory of adequate causation (in general civil law), and the theory of essential conditions (social and insurance law). The theory of equivalence describes an action as causal with respect to its success, i.e., the intended action or observed effect, if and only if it cannot be omitted without obviating the specific success. In contrast, general civil law defines an action as causal with respect to its success if the specific circumstances would normally lead to this success and not some rare, odd, or unlikely peculiarities that are not generally adequate and would have been ignored given the normal (natural) course of things or events. Additionally, the theory of essential conditions requires that a specific cause or event has substantially contributed to the observed "success," e.g., a specific disability.

An example of forensic toxicology may clarify this issue. On a workbench, in his shed, a 60-year-old gardener has kept an aqueous solution of paraquat, a herbicide, in a glass bottle that would normally contain sparkling water. The original labels were removed, and there is a handwritten warning sign, but no other hint of the toxic content. In the absence of the gardener, a 16-year-old neighbor who by chance is the son-in-law of the gardener's sister broke into the secured shed and by mistake took a great mouthful of the toxic water to satisfy his thirst. The ensuing lung edema, followed by a permanent pulmonary damage, eventually caused the death of the boy. The criminal trial is now to investigate the accident in order to determine if a case of familial homicide or a bodily harm with fatal consequences has occurred. The judge may decide that the gardener's action was grossly negligent, because the paraquat solution caused the fatality and the gardener ignored necessary protective measures. Any civil court will approach the case in terms of due compensation, e.g., when asked whether the boy's parents should compensate the gardener for the broken shed door, but may arrive at a similar decision since the break-in is a minor delict that would not normally lead to death, while the toxic drink does. Had the boy survived with a permanent disability, any social court would have attested a reduction in earning capacity. Again, the toxic drink was directly causal and not the illegal break-in.

## Occupational Disease and Workplace Issues

Within the forensic frame the court will define the precise questions that the toxicology report must deal with. Therefore, it is absolutely mandatory that the expert strictly adheres to these specific questions. A rather complicated issue is the elaboration of a causal medical assessment in terms of the social law. Quite often this question arises if the underlying cause is an occupational illness. In such cases insurance companies, e.g., the Accident Prevention & Insurance Association, may be obliged to provide fair compensation for health care and rehabilitation. Recognized occupational diseases are listed below under the specific causative agent or substance. An occupational disease is accepted if the medical diagnosis has been confirmed, the exposure conditions have been unequivocally verified, and the medical evidence confirms cause and effect. The latter is usually agreed if the causal relationship between exposure and disease has been assessed as probable. That is, there is more reason to argue in favor than against the assumed relation of cause and effect.

ILO List of Occupational Diseases (rev 2010)

1. Occupational diseases caused by exposure to agents arising from work activities
   - 1.1. Diseases caused by chemical agents
     - 1.1.1. Diseases caused by beryllium or its compounds
     - 1.1.2. Diseases caused by cadmium or its compounds
     - 1.1.3. Diseases caused by phosphorus or its compounds
     - 1.1.4. Diseases caused by chromium or its compounds
     - 1.1.5. Diseases caused by manganese or its compounds
     - 1.1.6. Diseases caused by arsenic or its compounds
     - 1.1.7. Diseases caused by mercury or its compounds
     - 1.1.8. Diseases caused by lead or its compounds
     - 1.1.9. Diseases caused by fluorine or its compounds
     - 1.1.10. Diseases caused by carbon disulfide
     - 1.1.11. Diseases caused by halogen derivatives of aliphatic or aromatic hydrocarbons
     - 1.1.12. Diseases caused by benzene or its homologues
     - 1.1.13. Diseases caused by nitro- and amino- derivatives of benzene or its homologues
     - 1.1.14. Diseases caused by nitroglycerine or other nitric acid esters
     - 1.1.15. Diseases caused by alcohols, glycols or ketones
     - 1.1.16. Diseases caused by asphyxiants like carbon monoxide, hydrogen sulfide, hydrogen cyanide or its derivatives
     - 1.1.17. Diseases caused by acrylonitrile
     - 1.1.18. Diseases caused by oxides of nitrogen
     - 1.1.19. Diseases caused by vanadium or its compounds
     - 1.1.20. Diseases caused by antimony or its compounds
     - 1.1.21. Diseases caused by hexane
     - 1.1.22. Diseases caused by mineral acids
     - 1.1.23. Diseases caused by pharmaceutical agents

1.1.24. Diseases caused by nickel or its compounds
1.1.25. Diseases caused by thallium or its compounds
1.1.26. Diseases caused by osmium or its compounds
1.1.27. Diseases caused by selenium or its compounds
1.1.28. Diseases caused by copper or its compounds
1.1.29. Diseases caused by platinum or its compounds
1.1.30. Diseases caused by tin or its compounds
1.1.31. Diseases caused by zinc or its compounds
1.1.32. Diseases caused by phosgene
1.1.33. Diseases caused by corneal irritants like benzoquinone
1.1.34. Diseases caused by ammonia
1.1.35. Diseases caused by isocyanates
1.1.36. Diseases caused by pesticides
1.1.37. Diseases caused by sulphur oxides
1.1.38. Diseases caused by organic solvents
1.1.39. Diseases caused by latex or latex-containing products
1.1.40. Diseases caused by chlorine
1.1.41. Diseases caused by other chemical agents at work not mentioned in the preceding items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to these chemical agents arising from work activities and the disease(s) contracted by the worker

1.2. Diseases caused by physical agents
1.2.1. Hearing impairment caused by noise
1.2.2. Diseases caused by vibration (disorders of muscles, tendons, bones, joints, peripheral blood vessels or peripheral nerves)
1.2.3. Diseases caused by compressed or decompressed air
1.2.4. Diseases caused by ionizing radiations
1.2.5. Diseases caused by optical (ultraviolet, visible light, infrared) radiations including laser
1.2.6. Diseases caused by exposure to extreme temperatures
1.2.7. Diseases caused by other physical agents at work not mentioned in the preceding items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to these physical agents arising from work activities and the disease(s) contracted by the worker

1.3. Biological agents and infectious or parasitic diseases
1.3.1. Brucellosis
1.3.2. Hepatitis viruses
1.3.3. Human immunodeficiency virus (HIV)
1.3.4. Tetanus
1.3.5. Tuberculosis
1.3.6. Toxic or inflammatory syndromes associated with bacterial or fungal contaminants
1.3.7. Anthrax

1.3.8. Leptospirosis
1.3.9. Diseases caused by other biological agents at work not mentioned in the preceding items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to these biological agents arising from work activities and the disease(s) contracted by the worker

2. Occupational diseases by target organ systems
   2.1. Respiratory diseases
      2.1.1. Pneumoconioses caused by fibrogenic mineral dust (silicosis, anthraco-silicosis, asbestosis)
      2.1.2. Silicotuberculosis
      2.1.3. Pneumoconioses caused by non-fibrogenic mineral dust
      2.1.4. Siderosis
      2.1.5. Bronchopulmonary diseases caused by hard- metal dust
      2.1.6. Bronchopulmonary diseases caused by dust of cotton (byssinosis), flax, hemp, sisal or sugar cane (bagassosis)
      2.1.7. Asthma caused by recognized sensitizing agents or irritants inherent to the work process
      2.1.8. Extrinsic allergic alveolitis caused by the inhalation of organic dusts or microbially contaminated aerosols, arising from work activities
      2.1.9. Chronic obstructive pulmonary diseases caused by inhalation of coal dust, dust from stone quarries, wood dust, dust from cereals and agricultural work, dust in animal stables, dust from textiles, and paper dust, arising from work activities
      2.1.10. Diseases of the lung caused by aluminium
      2.1.11. Upper airways disorders caused by recognized sensitizing agents or irritants inherent to the work process
      2.1.12. Other respiratory diseases not mentioned in the preceding items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to risk factors arising from work activities and the disease(s) contracted by the worker
   2.2. Skin diseases
      2.2.1. Allergic contact dermatoses and contact urticaria caused by other recognized allergy- provoking agents arising from work activities not included in other items
      2.2.2. Irritant contact dermatoses caused by other recognized irritant agents arising from work activities not included in other items
      2.2.3. Vitiligo caused by other recognized agents arising from work activities not included in other items
      2.2.4. Other skin diseases caused by physical, chemical or biological agents at work not included under other items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to risk factors arising from work activities and the skin disease(s) contracted by the worker

2.3. Musculoskeletal disorders
  2.3.1. Radial styloid tenosynovitis due to repetitive movements, forceful exertions and extreme postures of the wrist
  2.3.2. Chronic tenosynovitis of hand and wrist due to repetitive movements, forceful exertions and extreme postures of the wrist
  2.3.3. Olecranon bursitis due to prolonged pressure of the elbow region
  2.3.4. Prepatellar bursitis due to prolonged stay in kneeling position
  2.3.5. Epicondylitis due to repetitive forceful work
  2.3.6. Meniscus lesions following extended periods of work in a kneeling or squatting position
  2.3.7. Carpal tunnel syndrome due to extended periods of repetitive forceful work, work involving vibration, extreme postures of the wrist, or a combination of the three (from: http://www.ilo.org/wcmsp5/groups/public/@dgreports/@dcomm/@publ/documents/publication/wcms_150323.pdf)
  2.3.8. Other musculoskeletal disorders not mentioned in the preceding items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to risk factors arising from work activities and the musculoskeletal disorder(s) contracted by the worker
2.4. Mental and behavioural disorders
  2.4.1. Post-traumatic stress disorder
  2.4.2. Other mental or behavioural disorders not mentioned in the preceding item where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to risk factors arising from work activities and the mental and behavioural disorder(s) contracted by the worker

3. Occupational cancer
3.1. Cancer caused by the following agents
  3.1.1. Asbestos
  3.1.2. Benzidine and its salts
  3.1.3. Bis-chloromethyl ether (BCME)
  3.1.4. Chromium VI compounds
  3.1.5. Coal tars, coal tar pitches or soots
  3.1.6. Beta-naphthylamine
  3.1.7. Vinyl chloride
  3.1.8. Benzene
  3.1.9. Toxic nitro- and amino-derivatives of benzene or its homologues
  3.1.10. Ionizing radiations
  3.1.11. Tar, pitch, bitumen, mineral oil, anthracene, or the compounds, products or residues of these substances
  3.1.12. Coke oven emissions
  3.1.13. Nickel compounds
  3.1.14. Wood dust
  3.1.15. Arsenic and its compounds

3.1.16. Beryllium and its compounds
3.1.17. Cadmium and its compounds
3.1.18. Erionite
3.1.19. Ethylene oxide
3.1.20. Hepatitis B virus (HBV) and hepatitis C virus (HCV)
3.1.21. Cancers caused by other agents at work not mentioned in the preceding items where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure to these agents arising from work activities and the cancer(s) contracted by the worker

4. Other diseases
   4.1 Miners' nystagmus
   4.2 Other specific diseases caused by occupations or processes not mentioned in this list where a direct link is established scientifically, or determined by methods appropriate to national conditions and practice, between the exposure arising from work activities and the disease(s) contracted by the worker

With regard to liability, law systems are quite different among countries of the European Union and may even be more different between the so-called G8 nations and other countries of the world. Employees may be protected against health injuries from workplace exposure, but to varying degrees. In the United States the Division of Federal Employees' Compensation (DEFC) at the US Department of Labor will oversee compliance with the Federal Employees' Compensation Act (FECA) through its district offices, which are located throughout the country. In principle, the act treats permanent and temporary employees alike. However, the employee must provide medical and factual evidence to establish the essential elements of the claim, i.e., that the claim was filed within the statutory time requirements of the FECA, the injured or deceased person was an employee within the scope of the FECA, the employee suffered from an injury or disease, the employee was in the performance of duty when the injury occurred, and the condition in question resulted from the injury. If the injury has not been reported, no benefits will be paid. The restrictions on these reports are rather strict: If medical treatment is required, a special form has to be submitted – usually within 4 h of the request. Retroactive issuance is not permitted under any circumstances. If the damaging event was a toxic substance, the toxicologist will have to fill in the gaps and answer the questions put forward by the district officers. In Germany, for example, the social courts have to decide if an employee will receive compensation for an acquired disease that has been linked to workplace exposure. If the disease is on the list of known occupational health problems, the Accident Prevention & Insurance Association ("Berufsgenossenschaft" in Germany) will cover any illness-related expenses (for therapy and rehabilitation, if appropriate). If the disease is not on the list, the worker's health insurance would have to pay the costs to restore the worker's health. Once a causal relationship between a toxic exposure and an observed disease has been established, but the disease is not (yet) listed above, there is an exemption clause that will allow the worker's condition being treated as an occupational disease. In order to do so, four criteria have to be met: (i) a special

group of workers or employees has to be identified that will be exposed to certain influences at the workplace more seriously than the general population and the affected person, i.e., the patient, must be a member of that group; (ii) the claimed influences must be able – according to the state of the art and medical science – to cause the observed health injury; (iii) scientific knowledge about the newly suspected influences was not available or insufficient, when the last revision of the list of known occupational disease was made, or has not been thoroughly checked; and (iv) a causal relationship between worker's or employee's duties and the ensuing illness must be a deemed-to-satisfy provision. Once again, the toxicological expert will have to provide evidence or deny such a link, before the patient will be promoted to an applicant for compensation. The expert has to deliver his (her) scientific opinion for each of the four points and the statements must respect the available literature in the fields of occupational and social medicine. If the expert fails at any point, chances are that the whole report with all its arguments will be dismissed in court. Conversely, a carefully written report with convincing data and evidence may provide enough reason to resume hearings and discussions that may eventually lead to the filing of the newly discovered condition as an occupational disease.

## Adverse Drug Effects and Drug-Related Disease

For a long time adverse drug effects have been recognized as inevitable sequelae of certain medical treatments. Modern drug safety requirements and consumer protection regulations require that such information be provided to the patient on a case-by-case basis. The physician who prescribes a certain drug is usually held responsible for informing the patient. It should be emphasized that despite potentially severe adverse effects, drug treatment is warranted if the benefits for an individual outweigh the risks. Recent trends appear to enforce the patients' rights of clear and understandable information about the most frequent and (or) most serious adverse drug effects. Failure to provide this clarification at the beginning of the treatment may be sued as medical malpractice. Nowadays, toxicologists may be asked for their scientific opinion in court. In such cases it is mandatory that the expert is absolutely unbiased and independent, scientifically sound, and has no personal history that might be deemed a conflict of interest.

## Reduction in Earning Capacity

One of the issues most often dealt with in Labor Courts is the reduction of earning capacity. Even minor shifts in the granted percentage will have great repercussions on the patient's compensation and benefits. However, the toxicological expert should be aware that clinicians and specialized physicians generally provide such an assessment. Only in exceptional cases will the toxicological expert be asked for some scientific statement. The most notable exception is occupational cancer and

carcinogenesis when the toxicologist should provide his/her expertise and give an estimate of likely sequelae and prognosis, based on the most recent research and study results in the field of interest.

## Cross-Links to Regulatory Affairs

The above-given examples should have made clear that the toxicological report may have a strong impact on later amendments of jurisdiction. For example, a new entity may be added to the list of occupational diseases if an increasing number of expert reports emphasize its relevance and the experts communicate the need for such a revision. In Germany, the Contergan Trial (1968–1970) was the most prominent example of how toxicological experts might take part in the decision-making processes in politics and public opinion and thus trigger a fundamental revision of the country's law. The German Medicines Law, which became effective in 1971, was significantly influenced by the science of toxicology and by toxicologists and other experts in the field and reciprocally stimulated toxicological research in Germany and elsewhere.

## Recommended Reading

Brodsky SL (2009) Principles and practice of trial consultation. Guilford, New York

Wall WJ (2009) Forensic science in court: the role of the expert witness. Wiley-Blackwell, Chichester/West Sussex/Hoboken

Stephens T, Brynner R (2001) Dark remedy: the impact of thalidomide and its revival as a vital medicine. Basic Books, New York

## Resources

ECHA: Practical Guide 14: How to prepare toxicological summaries in IUCLID and how to derive DNELs. http://echa.europa.eu/documents/10162/13655/pg_14_on_hazard_endpoint_en.pdf. Last seen on 11 March 2013

O'Neal JT (2010) Causation determination in workers' compensation and toxic tort cases. http://www.health.state.mn.us/divs/eh/hazardous/topics/eegrandrounds/causation.pdf. Last seen on 11 March 2013

# Risk Management in Toxicological Disasters

Kai Kehe and John H. Duffus

## Contents

History ..... 740
Characteristics of Toxicological Disasters ..... 740
Management of Toxicological Disasters ..... 742
The Local Regulatory Framework ..... 742
Preparedness ..... 743
Training, Drill, and Exercises ..... 743
Identification of Potential Hazard Sources ..... 743
Identification of Hazards Through Emergency Forces ..... 743
Detection ..... 744
Information on Toxic Substances ..... 744
Limits of Exposure ..... 744
Physical Protection ..... 745
Decontamination ..... 745
Drugs ..... 745
Centers of Expertise ..... 745
Research and Development ..... 746
Risk Management ..... 746
References ..... 746

**Abstract**

Toxicological disasters are rare events with high impact to health and environment. Medical preparedness in these disasters requires policy, planning, and a preorganized response system. This chapter briefly describes the main parts of the planning process and the needed legal framework to support this

K. Kehe (✉)
Bundeswehr Medical Academy, Military Medical Research and Development, Munich, Germany
e-mail: kai.kehe@lrz.uni-muenchen.de

J.H. Duffus
The Edinburgh Centre of Toxicology, Edinburgh, Scotland, UK
e-mail: J.H.Duffus@blueyonder.co.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_87, 

process. Precautions (production, storage, transport, use) have to be taken to minimize the chance of hazardous substances to be released. Toxicological profiles and medical countermeasures should be readily available in databases. The response system has to plan and exercise the management of toxicological disasters on a regularly basis. Financial support is needed to support continuous toxicological research and to build up specialized medical response teams. These efforts taken together should form an efficient risk management system.

## History

The release of poisonous substances can lead to disaster. For example, ergot alkaloids caused mass poisoning during the middle ages. More recently, the extensive use of chemical warfare agents during World War I and the Iraq–Iran war (1980–1988) caused hundreds of thousands of soldiers to be injured. Terrorists from the Aum Shinrikyo Cult released sarin in Matsumoto and Tokyo in 1994 and 1995. The Tokyo subway attack resulted in 12 fatalities and 980 injured persons. More than 5,000 people thought they might have been poisoned and consequently sought medical help. The most serious recent toxicological disaster took place in Bhopal in 1984 when 42 t of methyl isocyanate leaked into the air from reservoirs of a local chemical plant. 5,000 fatalities and more than 200,000 long-term injuries were counted within an area of 20 $km^2$. These examples clearly demonstrate the need to plan, organize, and prepare appropriate medical countermeasures.

Toxicological disasters may be of natural or of human origin (Table 1). Disasters which produce toxic gases in combination with fire (Table 2) or those resulting from chemical warfare agents (Table 3) are the most relevant to this chapter.

The onset of clinical symptoms after release of toxic substances may vary between seconds and days depending on the nature of the poison. An **accident** is typified by its being manageable by local response teams, which are sufficient to handle the incident without outside aid. Medical **disasters** are defined by great damage or loss of life in numbers or time frames that overwhelm the local community's medical services. This means that the relationship between the capacity of local response teams and their need for external aid is more important to qualify an incident as a disaster than is the total number of intoxicated persons or fatalities. An unintended small-scale release of toxic substances is a toxicological accident; an intended release may occur during a terrorist or military attack and may lead to a disaster.

## Characteristics of Toxicological Disasters

A substantial release of toxic substances will cause mass casualties over the affected area. Amount and identity of the released hazardous materials will be unknown during the first phase of response but must be determined for risk

**Table 1** Causes of disasters

| Natural disasters | Disasters of human origin |
|---|---|
| *Physical* | *Industrial* |
| Flooding, earthquake, storm, fire, volcanos | Fire, explosion, release of toxic industrial chemicals or liquids |
| *Biological* | *Transportation* |
| Epidemic, toxins | Release of toxic substances during air, ground, or sea transport |
| *Chemical* | *Large fire* |
| Toxic contamination of water, toxic gases (e.g., volcanos) | Schools, universities, hospitals, high-rise buildings |
| | *Wars* |
| | Conventional war, weapons of mass destruction |
| | *Polluted areas* |
| | Old ammunition, stockpiled, or dumped weapons of mass destruction |
| | *Terrorism* |
| | Explosion, hijacking airplanes, dirty bombs, use of weapons of mass destruction |

**Table 2** Toxic gases and fire

| Gases | Sources |
|---|---|
| *Systemic effects* | |
| Carbon monoxide | Hydrocarbons |
| Cyanide | Polyurethane, acrylonitrile |
| *Local irritant, lung-damaging gases* | |
| Hydrogen chloride | Polyvinyl chloride |
| Formaldehyde | Paper, cellulose |
| Hydrogen fluoride | Teflon |
| Isocyanate | Polyurethane |
| Nitrous gases | Celluloid, cellulose nitrate, artificial fertilizer |
| Sulfur dioxide | Sulfur-containing chemicals |
| Acrolein | Polyolefins, cellulose |
| Phosgene | Organochlorine compounds |

estimation and for the initiation of appropriate countermeasures. Complex mixtures of toxic gases characterize fire accidents, necessitating rapidly available analytical methods close at hand. Air may be heavily polluted as well as soil, buildings, and persons. For this reason, personal protective equipment is essential to enable rescue operations within the contaminated area. However, personal protective equipment is heavy and uncomfortable. Thus, medical treatment of injured and (or) intoxicated patients is severely impaired.

**Table 3** Weapons of mass destruction (chemical warfare agents, toxins)

| *Chemical warfare agents* | *Examples* |
|---|---|
| Nerve agents | VX, sarin, cyclosarin, tabun, soman |
| Vesicants | Sulfur and nitrogen mustards, lewisite |
| Blood agents | Hydrogen cyanide, arsine |
| Choking agents | Chlorine, phosgene, diphosgene |
| Incapacitating agents | Quinuclidinyl benzilate |
| Toxins | |
| bacterial toxins | Botulinum toxin[a] |
| | Staphylococcal enterotoxin B[a] |
| Mycotoxins | T-2 mycotoxin[a] |
| | Aflatoxins |
| | Phallotoxins, amatoxins |
| Plant toxins | Saxitoxin |
| | Ricin[a] |

[a]These toxins are listed as biological warfare agents

Spreading of solid or liquid toxic substances may be accelerated by the evacuation of contaminated patients, animals, or traffic through the hot zone. Thus, all contaminated persons and materials have to be thoroughly decontaminated before leaving the hot zone. As it is very likely that some persons will escape the situation before the hot zone is completely controlled, it is crucial to set up a decontamination line in front of hospitals to protect these medical facilities.

## Management of Toxicological Disasters

### The Local Regulatory Framework

Massive release of toxic substances affects both environment and infrastructure. Thus, a wide range of capabilities is necessary to deal with a toxicological disaster. Planning the initial response is a responsibility of local authorities and communities. There are legal requirements to ensure proper planning and training of local public health, emergency response, and other authorities (fire and ambulance services, police force, and civil defense) to respond to and to manage the incident scene in its early phases. In later phases, more specialized help is needed, and special civilian and military response units are necessary to provide more sophisticated expertise. The legal framework to manage this kind of disaster differs from nation to nation and even within a nation. Because of this, delays may occur and the delays may complicate the situation. Continuous political leadership is needed to overcome these complications. In times where civil defense is reduced due to tight budgets, it is important to enhance risk perception so that the highest-risk areas are given priority for appropriate control measures to be taken (Health Aspects of Biological and Chemical Weapons 2013).

## Preparedness

***Legal Framework.*** A consistent legal framework of responsibilities, tasks, proceedings, alarm plans, and communication lines is crucial for the successful management of disasters or catastrophes.

The availability of points of contact and key personnel 24 h and 7 days a week is essential.

## Training, Drill, and Exercises

Efficient collaboration between different agencies and organizations depends on their current knowledge and practical skills. Regular exercises including the highest political levels are necessary to achieve an appropriate level of preparedness. All participants have to be prepared for their tasks before they can assume full collective responsibility. Such preparation necessitates a profound understanding of toxicological risks and possible countermeasures. Necessary protective equipment should be available in sufficient quantity to deal with any possibility of a major disaster. Trainees should be instructed on a regular basis to ensure a safe handling of protective equipment.

## Identification of Potential Hazard Sources

To counteract potential terrorism, there must be integrated intelligence systems supporting interagency activities against organized terrorist organizations that may plan to use very toxic substances. It is particularly important to discover their capacity to produce and release such substances. It is also important to compile a database identifying industrial facilities that store and produce substances that terrorists might use. Additionally, transport routes should be known and analyzed for potential risks. Regulatory approaches that may be applied include the UN Recommendations on the Transport of Dangerous Goods, Dangerous Goods Emergency Action Code List 2011, and Canadian Transportation of Dangerous Goods Regulation.

To supplement the above activities, an epidemiological surveillance system should be introduced for early detection of the effects of any hazardous substances that may be released into the community.

## Identification of Hazards Through Emergency Forces

Toxic chemicals (solids, liquids, or gases) that can harm people, other living organisms, property, or the environment are classified as dangerous goods. Regulation of such chemicals should be enforced by local regulatory agencies. Proper labeling of chemicals is needed for the safety of emergency forces and to ensure proper countermeasures. The Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)

is a European Union Regulation to enforce safe use of chemicals (see chapter "▶ Reach (and CLP). Its Role in Regulatory Toxicology"). A comparable law is the US Toxic Substances Control Act.

As described in the previous section, transport of dangerous goods is strictly regulated at both international and national level. This requires properly defined labeling of dangerous goods to speed up the identification of risks for emergency personnel. First responders especially must be familiar with the labeling system. In addition, an analytical task force should be available to identify unknown chemicals and to ensure that the correct measures are taken to minimize their potential to cause harm.

## Detection

Various devices are available for the rapid detection of unknown chemicals, and detection systems have been optimized for a number of civil or military scenarios. Unfortunately, there are always new and unforeseen events occurring. Thus, it is essential to have available first class analytical chemists and a well-equipped laboratory in order to devise and apply new methods which may be necessitated as new problems are presented. To ensure reliable results, sample collection and preservation (e.g., with refrigeration and in lightproof containers) must be properly carried out, and transportation to the analytical laboratory must be as quick as possible.

In addition to the addresses of the laboratories that are involved in normal emergency response, emergency response units should also keep a list of addresses of laboratories with special knowledge and skills (e.g., universities, industry, and the armed forces).

## Information on Toxic Substances

Companies that use highly toxic substances should develop a risk management program (RMP). Information, including material safety data sheets, should be available for protective, diagnostic, and medical countermeasures. Poison information centers should have this information available because they have a central role in the initial planning of medical countermeasures. Specialized knowledge about chemical warfare agents will be available from military sources. Specialized Internet databases may also provide detailed information. Regulatory laws under the European REACH regulation should eventually ensure the availability of all relevant data regarding industrial chemicals.

## Limits of Exposure

A number of exposure limits have been defined for the safety of human beings, workplace, buildings, and environment. These limits are of minor importance during the emergency management of toxic catastrophes. However, they are helpful to estimate limits of short-time exposure for emergency personnel during the initial phase of disaster

management. Additionally, they are useful for information of exposed population about possible hazards. These limits become highly relevant in the aftermath of the accident in ensuring that harmful consequences are kept to a minimum.

## Physical Protection

Toxic contamination of air and body surfaces requires physical protection of airways, eyes, and skin. Without certain knowledge of the nature of the released toxic substance, it is necessary to wear whole body protection and a self-contained breathable air supply. Airway protection may be sufficient in some cases if the potentially toxic substance involved has been identified. Masks, suits, gloves, and overshoes should be made of appropriate material with a high protection factor. In order to choose the right equipment, it is necessary to have data readily available defining relevant protective properties.

## Decontamination

Contaminated persons have to be decontaminated either before leaving the hot zone or before entering a medical facility. It is crucial that intoxicated persons do not contaminate hospitals. The preparedness of hospitals and the public medical service is a legal requirement in some nations. Sufficient amounts of clean water and decontamination equipment should be available. Wastewater must be collected and decontaminated as well. Hospital emergency plans should contain information about the traffic routes to and from the medical facility, and hospitals should have sufficient stockpiles of antidotes, beds, and blankets.

## Drugs

Life-threatening poisoning is a rare event. Usually, stockpiles of specific drugs and antidotes to treat intoxicated patients are small and not sufficient to cope with catastrophes, but sufficient amounts of lifesaving antidotes should always be kept available for emergency personnel contaminated after damage to their protective equipment. Further stockpiles of antidotes and other drugs must be available at short notice. Distribution depots should be located to ensure short transportation times. Pharmaceutical companies should participate in planning for rapid supply of necessary drugs.

## Centers of Expertise

Centers of expertise for the management of toxicological catastrophes are rare. They may be part of a ministry, university, company, or even of the military. Laws

should ensure that a sufficient quantity and quality of toxicological expertise is available on a national level. This expertise is especially needed after a toxicological incident and should be responsible for human biomonitoring to follow up exposed patients.

## Research and Development

National authorities have to make their own assessment as to what may be a possible threat for their nation. However, basic scientific expertise is needed as well as capability to respond to an incident. In order to sustain toxicological research, sufficient financial support has to be secured as well as research institutes at the medical faculties of the main universities. Medical students and doctors should be supported to participate in toxicological research programs. These programs are needed to support the education of new scientists with a medical background. This future scientific expertise is necessary to support ongoing research. Currently a small number of scientists have to deal with a plethora of urgent toxicological problems:

- Development of new antidotes
- Development of a system to decontaminate injured patients
- Development of a decontamination treatment which can be applied to eyes and mucous membranes
- Development of new methods to analyze and monitor toxicological exposures

## Risk Management

The WHO suggests implementation of a structured planning process to meet the needs of effective risk management. The following is the step-by-step approach suggested (Health aspects of chemical accidents 1994):

1. Identify the hazards.
2. Evaluate the hazards to determine the probability and severity of the initial risk.
3. Introduce risk-reduction strategies.
4. Quantify the residual risk, and decide what risk is acceptable.
5. Monitor the risk management program, and repeat the process as required.

## References

Health Aspects of Biological and Chemical Weapons (WHO) http://www.who.int/csr/delibepidemics/biochem1stenglish/en/index.html. Accessed 3 May 2013

Health aspects of chemical accidents (OCDE/GD (94)1) (1994) OECD, Paris

http://www.epa.gov/osweroe1/lawsregs.htm#accident. Accessed 3 May 2013

# Institutionalized Participation in Regulatory Toxicology

Andreas D. Kappos

## Contents

Introduction ..... 748
Why Participation? ..... 748
Participatory Risk Management ..... 749
Legal Background of Participation ..... 749
Rationale and Benefits of Public Participation in Risk Decisions ..... 750
Who Should Participate? ..... 751
Models of Participation ..... 752
  Publication of Decision with a Set Period to Submit Objections ..... 752
  Hearing ..... 752
  Round Table ..... 752
  Advisory Board ..... 753
  Mediation ..... 753
  Cooperative Discourse ..... 753
Evaluation of Participation ..... 754
Conclusions ..... 755
References ..... 755
  Resources ..... 756

**Abstract**

Public participation in risk management decisions has a sound legal base in Europe. In democracy it replaces the authoritarian top-down risk management. Participation of the public changes the role of scientific toxicology. Toxicologists should state clearly their models, assumptions, and resulting uncertainties and strictly separate scientific analysis from extrapolation and opinion.

A.D. Kappos
Former Head of Department of Health and Environment, Hamburg Health Authority, Frankfurt, Germany
e-mail: adkappos@t-online.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_88, 

Participatory risk management is based on the notion of the emancipated citizens, who are capable of informed risk appraisal and enable them to contribute their interests, beliefs, and values. The rationale and supposed benefits of public participation in decisions about environmental health risks are discussed, and different models applied so far are presented.

## Introduction

The *Aarhus Convention* of 1998 (UNECE 1998), signed by 40 countries and the European Union, guarantees the rights of the public to participate in decision-making in environmental matters. Substantial evidence shows clearly that an adequate involvement of *stakeholders* and persons concerned in environmental health decisions achieves more effectively better and enduring results. Goals of participation should be to de-emotionalize conflicts, to reconcile different points of view, and to develop a common basis of assessment and consensual strategies for solutions, which do justice to the distinct interests of the different stakeholders. Participation enables better acceptance of decisions. The unsolvable points of conflict may be unearthed, and last not least the transparency of political decisions increased.

## Why Participation?

In the classic concept of *"top-down" risk management*, public administration regulates risks for the public or for single individuals using adequate measures, which in their discretion best suits the problem. The background of the decision, such as the scientific facts and their *uncertainties*, the *conflicts of interest*, the accepted compromises and weighing processes, and the remaining residual risk, remains hidden to the public. The role of the toxicologists as a scientific expert is to propose suiting treatment policies to the administration. Their expert statements serve to justify decisions that are founded on scientific facts, but depend as well on *normative values*. The underlying assumption of this risk management model is as follows: The administration well intentioned represents public welfare whereas stakeholders want only to enforce their special interests.

In the 1970s and 1980s, sensational incidents and in hindsight obviously wrong risk assessments by governmental policy makers scandalized by the media put public confidence in the ability of the administration to regulate risks for the benefit of the general public seriously in question. People's trust in the impartiality and objectivity of scientific risk statements was lost. Affected parties demanded loudly a participation in risk decisions. The political implementation of controversial techniques, such as genetic engineering or waste incineration, got almost impossible if they were felt threatening, justified or not. Small risks were overregulated, large neglected. A rethink was strongly indicated.

## Participatory Risk Management

Social science risk research indicates that the *rating of environmental risks* on human health depends not only on its height but at least equally on the uncertainty of the scientific facts and on the values the rating persons have. In a society, in which pluralism of values prevails, risk assessment and management must be democratically legitimized. The paradigm of risk management as a participatory process in mutual faith replaces the "top-down" model of risk management. It is characterized by a broad participation of all people concerned, the so-called stakeholders.

In this counter-model of a *participatory risk management*, the authorities provide basically the framework for process-oriented regulations. Decision processes take place as locally as possible, if possible at the local level where the risks occur. Science is not presented anymore as the single factor determining the decisions about risks, but as one factor among others, such as economic criteria and value-led trade-offs. Science is accessible to all people involved.

Participatory risk management changes the role of scientific toxicology. Toxicology is required to adhere strictly to scientific data and to refer only to facts that are proven according to sound scientific methodology. On the other side, scientists have to be explicit about the extent and limits of their knowledge. They should state clearly their models, assumptions, and resulting uncertainties and strictly separate scientific analysis from extrapolation and opinion. For the public it is critical to know not only what definite knowledge is but also what is still ambiguous.

Participatory risk management is based on the notion of the emancipated citizen, who is capable of informed risk appraisal. The concept means being in a position to make an informed risk appraisal on the basis of knowing the objectively demonstrable consequences of risk-generating events or activities, the residual uncertainties, and other risk-relevant factors and to rate the risks according to the individual's values for shaping his own life and in correspondence to his personal criteria for assessing the acceptability of these risks for society as a whole. Once this capacity for *informed risk appraisal* on the part of the citizen is acknowledged, it is the task of the authorities to build up and maintain the communication base necessary for this purpose. In the context of risk communication, there is a need for all forms of communication, from simple documentation of results, through targeted information offerings, to forms of dialog and of participation in the decision-making process.

## Legal Background of Participation

The participation of each person in decisions that affect them in democracy is viewed as a fundamental right. In 1989 all the Ministers of the Environment and of Health of the European region of WHO signed the *European Charter on Environment and Health* (WHO-Euro 1989), which states: Every individual is

entitled to . . .information and consultation on the state of the environment, and on plans, decisions and activities likely to affect both the environment and health (and to) participation in the decision-making process.

*Principle No. 10 of the Rio Declaration* (UNEP 1992) declares that environmental issues are best handled with participation of all concerned citizens, at the relevant level. At the national level, each individual shall have appropriate access to information concerning the environment that is held by public authorities, including information on hazardous materials and activities in their communities and the opportunity to participate in decision-making processes.

These fundamental rights were materialized in the UN/ECE Convention on Access to Information, Public Participation in Decision-Making and Access to Justice in Environmental Matters (UNECE 1998), signed 1989 in Aarhus, Denmark, by more than 40 states and the European Union, the so-called *Aarhus Convention*. In the framework of EU legislation, rights of the public to be informed and to participate in decisions are guaranteed in the *EU Strategic Environmental Assessment Directive* 2001/42/EC (EU 2001) and the *EU Public Participation Directive* 2003/35/EC (EU 2003). Both directives are binding on the Member States and accordingly have been implemented into national law.

## Rationale and Benefits of Public Participation in Risk Decisions

The benefits of *stakeholder participation* in risk decisions are obvious (Table 1). The participation of people concerned by risks or generally interested in risk issues should eliminate the widespread suspicion of the public towards authorities and established science. It should ensure the transparency of the foundation, framework, and underlying assumptions of the decision and finally promote their acceptance. For the authorities participation of the public provides opportunity to become acquainted with the fears and worries of the citizens and their specific concerns, which they can take into account. Local experience and knowledge can be utilized for risk management. Finally, public participation encourages the participants to focus on arguments rather than on ideological contradictions. Timely involvement of the persons concerned may possibly avoid time-consuming legal disputes and heavy conflicts fought out in the media or in the political arena. However,

**Table 1** Benefits of stakeholder participation in risk management

| |
|---|
| Democratization of the decision processes |
| Inclusion of different values in society |
| Promotion of a better understanding of administrative decisions by the public |
| Improvement of the knowledge base |
| Saving of time and costs |
| Trust building |
| Supporting acceptance of decisions by the public |

participation is no panacea. Objective clash of interests cannot always be settled, and unsolvable conflicts must ultimately be decided at the political level.

Participation adjusts to the three key challenges of rational risk management. What issue dominates a specific process and defines what type of participation is most useful. If the complexity of scientific data as to the cause-effect relationship prevails, then the main issue will be clarification and explanation of difficult-to-understand scientific facts to lay people. Main conflicts arise at interprofessional level. The objective of public participation in this case is to inform about the scientific facts and make the scientific debates transparent to the public. Thus, it serves the understanding between experts and laymen.

If predominantly the *uncertainty* about the level of risk is under debate on account of methodological uncertainties, statistical variability or limitations, and uncertainty of scientific knowledge, then it will be important to find the narrow path between excessive caution and irresponsible negligence. Risk benefit considerations may be the remedy of choice (see chapter ▶ Risk-Benefit Considerations in Toxicology). A balance must be struck between the burdens of those who have to bear the risk and the benefit of those who create the risk.

If *ambiguity of risk*, which means different interpretation and evaluation of scientific facts according to differences in values, is the issue, then the acceptability of risks has to be negotiated and finally decided. Cultural, social, and ethical values have to be taken into account. In this case participation serves to improve understanding of different positions and to guarantee a fair and equitable procedure.

## Who Should Participate?

The process should include credible representatives of the full spectrum of parties, who are interested in or will be affected by a decision (Table 2). It should be structured to encourage their voluntary commitment. Basically, anyone who feels affected by a specific risk has the right to participate. In practice only few people will have such an immediate interest to sacrifice time and money required to fully

**Table 2** Stakeholders in participatory risk management

| |
|---|
| Local initiatives concerned with the risk issue |
| Representatives of cultural, ethnic, or economic groups and associations |
| Local authorities |
| Public health service |
| Industry and chambers of commerce, business associations |
| Local practitioners and their association |
| Trade unions |
| Environmental associations |
| Relevant research institutions |
| Institutions responsible for standard setting |

participate in the decision process. Such people feel personally affected in their lifestyles, their health, their economic interests, or their values and organize themselves in grassroots initiatives. But this must not lead to the fallacy that grassroot initiatives represent only the interests of a small minority. Experience has shown that risk managers often be wrecked, if they doubt whether these initiatives represent the general public and if they try to play the so-called silent majority against them.

In order to decide, who has to be involved, authorities have to ask the questions: Who is affected by the risks (but also of the measures to eliminate the risks)? Who has additional information or expertise? Who was affected by similar risks in the past? Who could be upset if not invited?

## Models of Participation

In the past various *models of a public participation* have been applied.

### Publication of Decision with a Set Period to Submit Objections

The decisions of the authority are made accessible to the public. Everyone can raise written objections within a prescribed period. The authority must deal with them. Participation is aimed mainly at professionals and associations.

### Hearing

Similar to the previous model arguments from interested parties could be raised and publicly discussed with the authority. In this and the previous model, the objective is to bring arguments to the authority's notice, which was not considered in the initial decision. According to all experience, the effect on the final decision is low, because the authorities are not bound to take additional arguments into account. Usually the participation takes place at a time, at which the cause has largely decided and authorities hate to revise once taken decisions. Hearings are legally prescribed in a number of European environmental laws. Participation is typically restricted to persons or institutions with a "legitimate" interest on the issue.

### Round Table

The experience that hearings in practice contribute little to a de-escalation of risk-related conflicts leads to the establishment of so-called round tables in particular settings. The objective of this exercise is to negotiate with as many opponents, as possible, at an early stage of the decision process with the hope that the final decision will be accepted by most of the stakeholders. Critical for the success of a round table is the inclusion of all people concerned, a collaborative formulation

**Table 3** Five key principles for effectively melding scientific analysis and public participation

| |
|---|
| 1. Ensuring transparency of decision-relevant information and analysis |
| 2. Paying explicit attention to both facts and values |
| 3. Promoting explicitness about assumptions and uncertainties |
| 4. Including independent review of official analyzes and/or engage in a process of collaborative inquiry with interested and affected parties |
| 5. Allowing for iteration to reconsider past conclusions on the basis of new information |

From (NRC 2008)

of the problem, a good faith communication, and last not least the transparency of all decision-relevant information. Specifically the role of scientific expertise has to be considered carefully and accessible to all participants. As put by the Panel on Public Participation in Environmental Assessment and Decision Making of the US National Research Council generally, it should be wise to follow five key principles for effectively melding scientific analysis and public participation (Table 3).

## Advisory Board

An Advisory Board is made up of representatives of the interested parties and experts and accompanies a planned project from the beginning. The Board must be clearly predetermined competences to interfere with the decision process. In practice the delegated members of the advisory board are endangered to decouple themselves increasingly over time from the interest of their base.

## Mediation

In addition to the participating interest groups of the "round table," an impartial arbitrator (mediator) is appointed to guarantee a fair deliberation. Its role is to promote the integration of diverging positions. So to speak, the mediator should act as a catalyst for consensus. Mediation is indicated, where conflicts between stakeholders are evident in the run-up of risky projects.

## Cooperative Discourse

This model, proposed by (Renn 1999), consists of three steps: identification and selection of concerns and values of the stakeholders, identification of impacts and consequences of different policy options by experts, and finally evaluation of potential solutions by a panel of randomly selected citizens. In the last step, the stakeholders and the experts contribute only as witnesses. They provide their arguments and scientific evidence to the panel, which ultimately decides on the

**Table 4** Models of public participation in risk management: pros and cons

| Participatory models | Pros | Cons |
|---|---|---|
| **Publication with objections** | Transparency | Minor influence on decision |
| **Hearing** | Transparency, platform for diverging arguments | Minor influence on decision, no settlement of conflicts |
| **Round table** | Fairness | Time-consuming questionable legitimacy |
| **Advisory board** | Anticipated settlement of conflicts, expertise, competence to decide | Limited participation, questionable legitimacy alienation from public |
| **Mediation** | High potential to settle conflicts, fairness | Time consuming, low efficiency |
| **Cooperative discourse** | Adequate to the problem, effective, efficient | Costly |

various options. In this model the phases of elicitation of scientific facts, elaboration of deviant values of people concerned, and weighing of facts and values are clearly separated into three panels with varying participants.

Pros and cons of the different participatory models are summarized in Table 4.

## Evaluation of Participation

In spite of the legal establishment of public participation, its application in practice has been criticized repeatedly (SRU 2002; NRC 2008; Pohjola and Tuomisto 2011). But the evaluation of public participation has been focused on process and access rather than on outcomes, but what is important from the point of view of participation in risk assessments and management is the influence allowed for the stakeholders in the different settings. The framing of a risk assessment approach can be a significant constraining factor for potential effectiveness of participation. For example, the commonly applied approach to environmental health assessment treats stakeholder involvement and public participation rather as an add-on brought about by legal requirements than as an essential aspect of risk assessment or decision-making. The common current practices of participation are not necessarily always in line with the latest discourses in the literature, and the law seldom requires very high degrees of openness to public participation. It is usually built on the conventional frameworks of administrative decision-making. Professional risk assessors and policy makers fear losing their power, although they should see themselves as feeding an open collaborative process with their expertise. Pohjola et al. addressing the issue of effective participation developed a concept of five dimensions of openness of risk management, which include the scope of participation, access to information, timing of involvement of the public, aspects of the issue the participants are allowed to contribute, and the weight given to the contributions in the final decision. The framework of openness provides a context for evaluation and

constructive criticism of contemporary institutions and practice of public participation in risk assessment, and policy making, and a basis for developing new models. Openness should not, however, be considered as an end in itself, but rather a means for advancing societal development through creation and use of broadly distributed collective knowledge upon issues of great societal relevance (Pohjola and Tuomisto 2011).

## Conclusions

Participation in risk assessment and policy making upon issues of environment and environmental health has become a commonplace. There are numerous good examples of public participation to manage risks at the local level. At the national or international level, specifically for the management of toxic substances in consumer-relevant products, they are scanty or completely missing. Existing experience with participatory risk management shows that participation can be an invaluable part of risk assessment and decision-making. There are no simple "best practices" that provide universal guidance (NRC 2008). Therfore the creativity of risk managing authorities is challenged.

The strength of participation is that aided by discursive methodology, it is possible to weigh arguments by rational and political legitimized criteria before deciding. A formally structured and organized deliberate procedure takes notice of scientific expertise, laws, norms, social interest, and people's values. If it is conducted in a fair and representative manner, it will integrate rational, emotional, and normative statements and opens a perspective to solve conflicts of interest. Prerequisite is the willingness to debate, to learn, and to compromise.

It is not possible to predict in advance how effective a particular participation process will be to avoid or lessen conflicts. Participation has limitations. It is reasonable only if there is still something to decide and if willingness to compromise exists on the side of all parties involved and last not least if the public is supported by trustworthy scientific experts. Participation is not effective, if it depraves to unlimited debating. Besides well-intended openness, the majority of concerned citizens will always remain bystanders.

## References

EU (2001) Strategic environmental assessment directive (2001/42/EC)

EU (2003) Public participation directive (2003/35/EC)

NRC (2008) National Research Council: Public participation in environmental assessment and decision making. In: Dietz T, Stern PC (eds) Panel on public participation in environmental assessment and decision making, National Academic Press. http://www.nap.edu/catalog/12434.html. Accessed 30 Oct 2012

Pohjola MV, Tuomisto JT (2011) Openness in participation, assessment, and policy making upon issues of environment and environmental health: a review of literature and recent project results. Environ Health 10:58–71

Renn O (1999) A model for an analytic-deliberative process in risk management. Environ Sci Technol 33:3049–3055

SRU (2002) The German advisory council on the environment: environmental report 2002. Towards a new leading role. Ch.2.3 Citizens and the enabling state. http://www.umweltrat.de/SharedDocs/Downloads/EN/01_Environmental_Reports/2002_Environmental_Report_summary.pdf?__blob=publicationFile. Accessed 30 Oct 2012

UNECE (1998) Aarhus convention on access to information, public participation in decision-making and access to justice in environmental matters. http://www.unece.org/env/pp/documents/cep43e.pdf. Accessed 30 Oct 2012

UNEP (1992) Rio declaration on environment and development. http://www.unep.org/Documents.Multilingual/Default.asp?documentid=78&articleid=1163. Accessed 30 Oct 2012

WHO-EURO (1989) European charter on environment and health. http://www.euro.who.int/__data/assets/pdf_file/0019/114085/ICP_RUD_113.pdf. Accessed 30 Oct 2012

## Resources

Risk Commission (2003) Ad hoc commission on revision of risk analysis procedures and structures as well as of standard setting in the field of environmental health in the federal republic of Germany. Final report. www.apug.de/archiv/pdf/Risk_Commission_Final_Report.pdf. Accessed 30 Oct 2012

http://www.unece.org/fileadmin/DAM/env/documents/2006/pp/ece%20mp%20pp%205_E.pdf

http://www.riskworld.com/

http://www.eeb.org/activities/transparency/main.htm

# Risk Communication

Werner Lilienblum and Marianne Lilienblum

## Contents

Objectives and Skills of Good Risk Communication ..... 758
Elements of Effective Risk Communication ..... 759
Basics and Basic Rules of Good Communication ..... 760
Important Requirements for Effective Risk Communication ..... 761
Preventive Risk Communication ..... 762
Public Hearings for Preparing Decisions by Authorities or in Politics ..... 762
Risk Communication in Case of Hazardous Incidents and Transport Accidents with Release of Dangerous Substances ..... 763
Contact with *Media* (Press, Television, Broadcast) ..... 764
Risk or Hazard Information on Chemical Substances and Products ..... 765
References ..... 765
Recommended Reading ..... 766
Resources ..... 766

**Abstract**

This chapter is primarily written for scientists concerned with risk assessment for human health and environmental protection and involved in communicating and explaining scientific risk issues to the public. Besides following basic rules of good communication, risk experts have to find solutions to overcome specific barriers in the dialogue with laymen, well-informed citizens, or other experts and also with public media. This includes adapting the dialogue to the audience, to explain complex scientific facts and their legal context perspicuously, and to achieve trust and a truthful dialogue atmosphere when discussing with citizens

W. Lilienblum (✉)
Formerly: Governmental Institute of Public Health, Hannover, Germany
e-mail: werner.lilienblum@t-online.de

M. Lilienblum
Faculty of Social and Behavioural Sciences, University of Amsterdam, Amsterdam, Netherlands
e-mail: m.lilienblum@hotmail.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_119, 

or stakeholders. Eventually, the risk expert should contribute to reach agreements or other options (including disagreements) when assessing risks. For the interaction with public media, the risk expert should keep particular rules in mind. *In conclusion*, awareness of obstacles in communication and acquiring special communication skills and training are mandatory for risk scientists before being confronted with a discussion about risks with the public and public media.

## Objectives and Skills of Good Risk Communication

*Risk communication* was defined as "an interactive process of exchange of information and opinions on risk among risk assessors, risk managers, and other interested parties" (WHO 1998). Consequently, basic objectives of good risk communication are on the one hand the *exchange of information* on facts, in particular features, extent, and probabilities of risks discussed and on the other hand *exchange of opinions* about appraisals, concerns, fears, and anxieties about these risks. Risks perceived by individuals or society are also *perceived risks associated with emotions*. Ignoring individual emotions or personal values connected to the issue would be like disregarding the major part of an iceberg below the water surface while talking only about the visible part. With regard to the exchange of opinions, it is often difficult for scientists to deal with the expectations beyond the factual level. An important reason is that scientists in the role of risk experts usually evaluate risks in a different manner than individuals or public media do (see Table 1). However, risk experts may evaluate risks differently in areas where they are laymen.

Important aims for the *risk management* are mutual understanding in the communication and agreement, e.g., about the implementation of methods for the reduction of risks, insofar as tolerated by the different opinions and interests of the involved parties. Whether such objectives can be achieved, strongly depends on the opinions and interests of the protagonists and stakeholders concerned. The role of the risk expert in such a situation may become difficult in case his opinion is attributed to particular interests; consequently, his objectiveness and credibility may be doubted by individuals or parties with different interests or opinions.

The preparation of an official decision, e.g., a public hearing is a typical case where risk communication is necessary. Due to the legal frame, particular rules should be considered for risk communication.

Involved parties in risk communication are as follows:

- Risk experts from different institutions (engineers' offices, industry, authorities, scientific institutions)
- Citizens with diverse levels of knowledge and education
- Public media (television, broadcast, print media)
- Action groups (citizens), consumer councils
- Companies and associations of industry
- Other administrative authorities, bodies, agencies

**Table 1** Risk perception and risk appraisal by risk experts and citizens

| Risk experts | Citizens |
|---|---|
| High degree of abstraction | Real and subjective observations |
| Strict application of scientific methods/procedures | Preference of intuitive approaches |
| Application of statistical or probabilistic methods | Expectation of inevitable (deterministic) developments |
| Determination of acceptable risk values/limit values as basis for measures by risk management | Striving for 100 % safety |
| Comparison of different risk scenarios by mathematical/abstract figures | Consideration of single/separate incidents; refusal of the comparison of different risk scenarios |
| Statistical average person as a reference, e.g., 70 kg average bodyweight | Personal or social relationship to (potential) victims, sympathy, dismay/shock |

Ref.: German Advisory Council on the Environment (SRU), 1999 (modified)

– Political lobby groups
– Other protagonists or stakeholders

Some of these different parties have in common that they have been confronted with warning messages about hazards or risks while having diverse and often little previous knowledge about risks. Hence it is important for risk assessing institutions to integrate risk communication as a permanent discourse in their external communication in order to reduce information deficits and to promote a realistic perception of risks in the general population (see chapter "▶ Risk Comparison in Toxicology" in the book). Especially government agencies and industrial companies and associations should feel their responsibility to this invitation (see References).

The larger the differences between groups, the more difficult will it be to manage the risk communication process and the higher will be the potential for conflicts:

– Previous knowledge of risks and level of information about particular risks
– Risk perception
– Acceptance of certain risks
– Distribution of benefits and expenses of a risk-reduction measure
– Extent and kind of the exposure to a risk
– Particular interests

Particular interests may be of economic nature including suspected decrease in value (e.g., lower costs for the industry, gain or loss of employment, residential sites close to a planned industrial plant) or political, moral, or ideological interests, just to mention some examples.

## Elements of Effective Risk Communication

*Every message is only as credible as the source that tells it!* When important decisions have to be taken, the success of the parties depends on their credibility

and the trust that other parties have in them. When an obvious charlatan appears more credible than a risk expert, something went wrong in the debate.

Criteria for the trust of citizens in scientific statements are as follows:

- Trust in a message
- Trust in the person that states it (personal reputation)
- Credibility and trust in the information source
- Credibility and trust into the scientific institution
- Social climate which may ease or constrain the confidence building

## Basics and Basic Rules of Good Communication

Being proficient in the basics and rules of communication is a requirement for effective risk communication. There is much information easily available in guidebooks and on the Internet about how to achieve and to perform good and efficient communication. It is recommended to join advanced training courses additional to the study of appropriate literature. Taking courses of rhetoric may also be useful but will cover only a subarea.

A message is a basic element of communication. In the following, some theoretical background is provided on the components of a message: *Every message is only as good as the part of it which is reaching the recipient!* This does not only apply to the factual content but also to three other layers of a message. Based on the psychological work of Paul Watzlawick on communication, ("We talk even when we're not saying anything" is just one of Watzlawick's five axioms on communication), Schulz von Thun (1998) developed the *four-sides model of communication* (also known as *communication square* or *four-ears model)*. The four sides have the function to clarify the four layers of a message:

- *Layer of matters and facts* of a message
- *Self-revealing* and *self-disclosure* by the sender
- Layer of *relationship* between sender and recipient of the message
- Layer of *appeal* from sender to recipient

The simple example of the four sides of the communication square is used to illustrate that communication is multilayered. Consequently, risk experts would be well advised to look more closely at the basics of communication in order to avoid blunders in communication, to practice one's own abilities, and (similarly important) to detect attempts of manipulation.

On the *layer of matter and facts*, the recipient gets information about the communicated issues. Clear structure, logical order of factual arguments, and comprehensible wording (spoken or written, inclusive of an explanation of technical terms) can help the recipient to encode the message the way it is intended. The senders' request for feedback about how the message has been perceived can help to detect any misunderstanding and provides the opportunity for correction.

On the *layer of self-revealing and self-disclosure*, the sender of a message gives information about themselves, e.g., about their role in the process of communication, competence, points of view, ideals, other parts of their personality, or about

their actual mental state. This partly happens as an intentional self-revealing but also as an unconscious disclosure.

On the *layer of relationship*, the sender communicates openly or subliminally what they think about the recipient or about the way they define the relationship. The way of talking (wording, body language, intonation, etc.) may express, e.g., respect, friendliness, disinterest, or contempt toward the recipient.

On the *layer of appeal*, the sender openly or subliminally attempts to influence the recipient in order to think, feel, or act in a way intended by the sender.

On each of the four levels of a message, each perceived signal triggers a sensation or a reaction of the recipient. On the level of self-disclosure and on the level of relationship, effects of subliminal or unconscious signals of the sender might be particularly tenuous.

Risk experts in particular have to take into account that protagonists of other parties, as being recipients, critically compare the factual information with their sensations of the other three levels for incongruities. A message that is consistent on all of the four levels of communication is one of several important requirements for credibility and trust; another term, which means the same, is authenticity. If all of these four levels of a message reach the receiver and are encoded in the intended way, the risk expert has successfully resolved one of several obstacles with regard to credibility and trust.

## Important Requirements for Effective Risk Communication

Additionally to being proficient of the basics of good communication, the risk expert is expected to translate technical terms into a language that is comprehensible for everybody (including unversed citizens) and to arrange mutual understanding.

Important tools and techniques for this challenging task are as follows:

- Translating technical language into everyday language
- Simplifying circumstances and line of thought to the essential, without omitting relevant information
- Illustrating complex matters with examples and comparisons of everyday life

Additional useful strategies to gain trust and credibility are as follows:

- Disclosure of all relevant factual information and transparency with regard to their personal role/function
- Dynamic communication policy and rapid and comprehensive information, e.g., after accidents happened (important for institutions involved)
- Reliable adjustment of information
- Acknowledging ambiguities and uncertainties
- Responding to emotions of the public
- Showing presence and leadership skills

Also, the risk expert has to consider that abstract determinism and reductionism often inherent to a scientific approach can be rarely reconciled with the thinking of the citizen in social relations. Furthermore, good risk communication is not

a one-way communication: Sending a message does not mean that the receiver passively absorbs the message like a sponge. It is a two-way communication with both an active sender and active receivers with own opinions and own perceptions.

And finally, all parties have to be conscious about the fact that good risk communication is an important requirement, but not a guarantee for comprehension or agreement.

## Preventive Risk Communication

### Public Hearings for Preparing Decisions by Authorities or in Politics

It is necessary to distinguish between public hearings that are based on existing law, e.g., in connection with the approval/authorization of hazardous industrial plants and public or expert hearings for preparing changes of legislation (laws, ordinances, etc.). It is inherent for legal procedures such as approval/authorization that the competent authority is bound by law to approve any petition if the applicant fulfills the legal requirements. In such a case, the authority often has only a small margin of decision, contrary to expectations of citizens concerned. Therefore, it is important for a successful progress of the procedure to provide detailed and clear explanations of the frame of legislation to the public or citizens involved, already at the beginning of the hearing. This may help to restrain unrealistic expectations of citizens with regard to the realization, prevention, or substantial limitations of the project.

Similarly important is the detailed explanation of undetermined legal terms by the management of the hearing such as the *principle of adequate means*. (In Germany derived by Article 20, No. 3 of the German Constitution (Basic Law)). This principle has the function to limit the extent of restrictions the authority can impose, e.g., because technical measures reducing risks may be expensive. In addition, other important legal terms should be explained to the audience such as the *generally recognized codes of practice* or the *current state of scientific knowledge and practice*, if applicable. This kind of hearings is normally managed by a leading official of the competent authority, who is often a lawyer specialized in administrative law. The risk expert should be familiar with such legal terms.

The risk expert in such hearings usually has the role of an appraiser. Often the applicant proposes an accredited or well-respected expert to be appointed by the authority.

The following criteria are essential for generating credibility and acceptance of the appraiser by citizens or stakeholders involved:

- *Perceived* independence
- *Perceived* competence
- Consistency of scientific reasoning
- Fairness
- Willingness and the competence of responding to different opinions or contradictory arguments

Perception of independence is as important as independence itself but it is subjective. Independence and zero tolerance of conflicts of interest (COL) are driven by societal organizations. An important criterion of independence is the affiliation of the risk expert. Risk experts from industry are normally not regarded as independent although they often strive to be. A consultant or appraiser, dependent on mandates from industry because of a high degree of specialization, may be generally regarded as independent; however, he should preferably demonstrate his independence and credibility by consistency of reasoning and convincibility. Competent authorities and other public scientific institutions often have introduced a passage in their rules of internal procedure which assures that the scientist is independent from instructions with regard to scientific appraisal issues he is responsible for. Usually this does not apply to risk managing authorities such as ministries where often hierarchy and political considerations predominate. In case the risk scientist acts the role of an appraiser in public hearings or at other public occasions, he should explain in detail his role in the procedure as far as necessary and also comment on the issue of his scientific independence, if applicable.

Public hearings intended for the preparation of regulatory changes may leave more scope for discursive procedures and external moderation. Also for this kind of hearings, the above-described role of the risk expert applies.

## Risk Communication in Case of Hazardous Incidents and Transport Accidents with Release of Dangerous Substances

All organizational and communicative actions are primarily focused on warding off any damage or on limiting the extent of damage. However, good risk communication starts far in advance to hazardous incidents, namely, by specifying procedures for approval/authorization of industrial plants with intrinsic dangerous potentials. Required information and communication includes scenarios of accidents, emergency (management) plans, and appropriate practice and exercises on a regular basis (SFK 1997; OECD 2002).

By information and training in advance, the emergency staff is usually familiar with the local conditions and also with possible accidental releases, and will arrive immediately in case of an incident.

With regard to the transport of hazardous goods on roads, rails, or shipping lanes, the focal point is concentrated on safety-related provisions on the means of transportation in order to prevent accidents. This applies also to the transport company's reliability, aiming to reduce the probability of technical or human failure and to limit the impact of an accident on human health or the environment, respectively.

In case of hazardous incidents, a smooth and rapid interaction with adequate communication lines between the responsible institutions and authorities is crucial for the conduct of emergency plans, in particular when substance release exceeded the plant boundary. This requires careful planning and coordination in order to prevent or to limit damage to a minimum.

In addition to the elucidation of the cause(s) of an accident, extensive information of the public on hazards or risks, which may have existed or may still exist, has to be carried out. The necessity of medical diagnostic examination or treatment of exposed individuals has to be decided case by case. Such decisions require adequate scientific justification and knowledge of the adequate and available chemical and medical investigation methods but also some (political) flair in order to avoid or to minimize public criticism.

## Contact with *Media* (Press, Television, Broadcast)

Risk experts are in great demand, especially when accidents in the field of chemistry, scandals around food, etc., have happened because of the public attention focused on these issues. However, they are also consulted for less big issues.

There are different reasons why risk experts are getting consulted by the media. It might be pure interest in information, but journalists could also already have a certain idea of how to present a particular issue and which statements (or headlines) ought to be supported by means of an expert. In the latter case, the risk expert would provide a scientific disguise for the story the journalist has in mind; only certain parts of the interview would appear in the article/interview, utilized to support the journalist's idea of the story. Before agreeing to the interview, catch up on the background of the request! Clarify in advance incase that you want to have a look at the final material, before it gets published.

Furthermore, the following is recommended:

Preparation

Think about and prepare *in advance* how you can formulate in a few words what you want to say. It is important to keep it simple so that laypeople can follow it. Also illustrate your statements with examples to make abstract things comprehensible. Besides, illustrations are often better understood, maintained and reproduced than abstract explanations.

Comprehensibility

Journalists are busy people and therefore are often only superficially informed. If you leave the translation of your complicated technical wording to the journalist (everyday language is necessary for a broad public), the risk of an incorrect reproduction increases. Keep in mind and take care about your statements being clear and comprehensible to laypeople. Also avoid jargon; the meaning of terms has to be (made) clear to non-insiders.

Statements

The time on air or the space in the article is restricted: be short and concise. Be aware of the fact that journalists often omit details when editing the material in order to create a clearer picture or to enhance comprehension. Also, the public's ability to maintain information is limited (and reproduction of information is even more).

While listening to you, journalists often already listen and think in an editing mode: Which sentences are short and concise; what is an expedient quotation in the context?

Provide the journalist with appropriate (short and clear) statements which are "ready for use"; this increases the probability that the statement will get published correctly (because the journalist can easily use it), and it decreases the risk of an incorrect report due to subsequent need for editing (shortening or simplification).

In certain cases, you can consider a media training session to practice how to appear in front of a camera, how to react on critical or delicate questions, or how to communicate your message effectively in a convincing and intelligible way (*perceived* competence).

## Risk or Hazard Information on Chemical Substances and Products

Last, but not the least, an important area of preventive risk communication is the design of written product information for consumers and for workers handling the hazardous material. Form and extent of such product information is partly stipulated by laws, regulations, or by non-legislative technical rules and standards intended as technical minimum requirements.

Some typical examples of important product information are (see also chapter "▶ Health Hazards Classification and Labeling", Author Desel of this book) as follows:

- Labeling of dangerous substances, preparations, articles with regard to their dangerous properties by hazard pictograms, hazard statements (risk phrases), precautionary statements, and material safety data sheets (MSDS) according to the Globally Harmonizing System (GHS)
- Labeling of ingredients, e.g., additives in food, often listed as codes (E numbers) or the list of ingredients in cosmetic products
- Instructions for use and warning notes of medicinal products or articles of daily use

Beyond the official legally provided requirements, the written information has to be short but complete and comprehensible. Editorial review by laymen is recommended. Especially in case of complex information, graphic images may be more comprehensible than pure text. Readability (the fine print!) is often more important than the handiness of the package insert; not everybody can be expected to have as good eyes as the designer of the leaflet.

## References

German Advisory Council on the Environment (Sachverständigenrat für Umweltfragen, SRU) (1999): Umwelt und Gesundheit- Risiken richtig einschätzen. Sondergutachten (in German) http://www.umweltrat.de/SharedDocs/Downloads/DE/02_Sondergutachten/1999_SG_UmweltundGesundheit.html

OECD (2002) Guidance Document on Risk Communication for Chemical Risk Management. OECD Environment, Health and Safety Publications, Series on Risk Management No. 16. ENV/JM/MONO 18. 25-Jul-2002, Paris. http://search.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO%282002%2918&docLanguage=En

Schulz von Thun F (1998) Miteinander Reden, Teil 1, Störungen und Klärungen; Teil 2, Stile, Werte und Persönlichkeitsentwicklung, Sachbuch 60606. Teil 3, Das "Innere Team"und situationsgerechte Kommunikation, Sachbuch 60545. Rowohlt, Hamburg (in German)
SFK (1997) Accident Commission/Störfall-Kommission (SFK) Guide for Plant Safety. SFK-GS-06. http://www.kas-bmu.de/publikationen/sfk_gb/sfkgs06engl.pdf

## Recommended Reading

Renn O (2008) Risk governance: coping with uncertainty in a complex world. Earthscan, London. ISBN 978-1-84407-291-0; 978-1-84407-292-7
Wiedemann PM, Clauberg M, Börner F (2011) Risk communication for companies. Fischer Verlag. http://www.wiedemannonline.com/blog/wp-content/materialien/downloads/Risk%20communication%20for%20companies.pdf

## Resources

Centers for Disease Control and Prevention, CDC http://www.cdc.gov/healthcommunication/risks/index.html
European Food Safety Agency, EFSA http://www.efsa.europa.eu/en/efsawhat/riskcommunication.htm
Four-sides model of communication http://en.wikipedia.org/wiki/Four-sides_model
World Health Organization, WHO (1998) http://www.who.int/foodsafety/publications/micro/feb1998/en/index.html

# Dealing with Diseases That Have Been Attributed to Chemical Exposures

Thomas Zilker

## Contents

The Disease and Its Regulatory Aspects ..... 768
Environmental Health Investigation ..... 768
Medical History ..... 768
Uncommon Somatic Disorders ..... 769
Disorders Not Able To Be Diagnosed by Conventional Medicine ..... 770
Social History ..... 770
Previous Findings ..... 770
Findings ..... 771
Blood Sampling ..... 771
Monitoring in Environmental Medicine ..... 771
Specimen Collection and Processing Procedure ..... 772
Evaluation of Results of Human Biomonitoring ..... 773
Reference Values for Human Biomonitoring ..... 773
Toxicologically Derived Human Biomonitoring Values ..... 773
Limitation of Biomonitoring ..... 773
Dealing with Environmental Patients from a Psychological and Psychiatric Perspective ..... 774
Psychiatric Diagnostics ..... 774
Therapy ..... 775
Recommended Reading ..... 776

**Abstract**

The cause behind the so-called MCS or IEI (Idiopathic Environmental Intolerance) is a mental or psychogenic or psychosomatic disorder.

Although the regulated threshold values in environmental media are aimed at virtually eliminating adverse health effects due to toxic substances, there are

T. Zilker
Formerly head of the Department for Clinical Toxicology, Medizinische Klinik Klinikum rechts der Isar Technical University München, Munich, Germany
e-mail: T.Zilker@LRZ.TUM.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_89, 

patients who attribute their illnesses to pollution. Only rarely is it possible to construct causality between the symptoms complained of and an exposure to toxicants. The communication is often disrupted. Only close cooperation between the family doctor and the doctors of environmental medicine can alleviate the suffering of these patients.

## The Disease and Its Regulatory Aspects

Patients presenting themselves to the doctor as being environmentally ill often complain of a strong feeling of being unwell, the cause of which had not been medically explained so far. The symptoms can include listlessness, fatigue, disturbed concentration, muscle pain, and irritation of mucous membranes. Patients attribute the cause to pollution and/or an increased sensitivity to ubiquitous pollutants. They hope for identification of the contaminant, either by having the specific symptoms pertaining to that contaminant, or by means of pollutant analysis. This vague clinical picture is also referred to as *multiple chemical sensitivity* (MCS). This concept is based on the notion that sensitization to various chemicals is triggered by previous exposure to pollutants, as a rule usually accompanied by odor intolerance.

*Regulatory aspects* are present in two ways. Firstly, patients demand more stringent threshold values so that they, with their hypersensitivity, are protected. Secondly, with its human biomonitoring, environmental medicine has a method of investigation, the results of which can be assessed on the basis of scientifically derived values (reference values, HBM values).

## Environmental Health Investigation

The examination of the environmental health patient consists, as is common, of taking a medical history, a physical exam, and – if necessary and useful – apparatus-based diagnostics and laboratory tests. Consultations with specialists should be requested if the symptoms are outside the specific area of expertise of the examining doctor. The following disciplines are required: internal medicine, clinical toxicologist, dermatologist, allergist, neurologist, occupational medicine, laboratory medicine, and – last but not least – the psychosomatic specialist and/or psychiatrist. When it concerns prevention and counseling, Public Health specialists and doctors from the Public Health Service are involved.

## Medical History

While this statement reportedly made by a wise physician certainly still holds true: “Whoever does not have a diagnosis after taking the medical history,

is badly off," apparatus or laboratory-based examinations are often given precedence. But as experience has shown, patients with ailments associated with environmental health issues are often not helped by these kinds of diagnostic investigations, and so taking the medical history is of utmost importance. This serves not so much the purpose of inquisitorial questioning of the patient, but rather of creating a ***basis of trust*** from the very beginning. Although time consuming, the patient should be allowed to express himself and should be listened to.

However, in order not to miss or forget important symptoms or associations, giving patients a ***questionnaire*** to answer beforehand has proved invaluable. As these questionnaires cover all possible symptoms and associations imaginable connected to living and working areas and are therefore very extensive, it makes sense to let the patient have a questionnaire well in advance of the consultation and to give oneself enough time to study it before seeing the patient.

The following categories are covered in the questionnaire:

1. Symptoms, with information about the time of onset, the duration, intensity, and the frequency
2. The disposition of the subject regarding familial clustering of diseases, hypersensitivities, allergies, and certain diseases
3. Potential exposure due to lifestyle factors such as natural stimulants, smoking, drugs, medication, sport, and leisure activities

Furthermore, ***exposure possibilities*** within the living accommodation, the surroundings, the household furniture, the use of domestic chemicals, and potential exposure in the workplace, by means of traffic, food, animal contact, and travel have all to be elicited.

Should suspicion fall and harden on a particular exposure, the search for possible vectors must be carried out. To this end, air, water, dust, food, utensils, products, and clothing are called into question. Of course, the maximum concentration that can be present in these vectors also plays a significant role. It is important to ask about first-time exposure and a one-off acute event. The doctor taking the history should not get too set in any one direction, but should remain open. He has to accept the patient's explanations, but must not overlook any symptom complex that might indicate a non-environment-related disease. That is why a doctor well trained in internal medicine with a broad subject knowledge and who has possibly come across many, even rare, diseases is predestined to be especially suitable for taking patient case histories.

## Uncommon Somatic Disorders

In our department, for example, some uncommon diseases have been diagnosed, such as acromegaly, hyperthyroidism, hypothyroidism, Wilson's disease, Mediterranean fever, cardiomyopathy, diabetes mellitus, insulinoma, pheochromocytoma, chronic lymphocytic leukemia, sarcoidosis, and various connective tissue diseases. In the

neurological department, we came across Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis and meningioma with disturbance of the sense of smell. Chronic carbon monoxide poisoning accompanied by nausea and headache, usually due to faulty flues and chimneys, are among these misattributions. Uncovering medical or neurological disorders despite the aforementioned diagnoses is still somewhat rare.

## Disorders Not Able To Be Diagnosed by Conventional Medicine

As a rule, none of the somatic disorders defined by conventional medicine are diagnosed. In the case of somatic disorders, when the medical history is being taken, very often quite specific indicators are mentioned, for example pain at very typical locations such as the stomach, gall bladder, kidneys, lower abdomen or thorax, after meals, during exercise, climbing stairs, in the night, or a loss of appetite with weight loss, bloody stools, blood in the urine; however, patients with environmental disorders do not present in the same way. Rather, they complain of pain that cannot be exactly localized, and their presenting signs are vague but manifold. Such patients specify muscular pain that migrates and is not precisely localized. Reports of occurrences of feelings of numbness without exact localization and constancy arise repeatedly. Problems such as indigestion with nausea but without vomiting or increased stool frequency are also often specified. Nervousness, fatigue, dizziness, a burning feeling on the skin or mucous membranes, palpitations, dyspnea without cyanosis, or spasticity are also complained of frequently. Disturbed sleep patterns are also included, but without specifying whether the problem lies in falling asleep or sleeping through the night.

## Social History

Besides taking the medical history and asking about all the presenting signs and symptoms, care should be taken to enquire after the social environment. It is to be noted that most of the patients appear dissatisfied with their social environment, but not because money is short, or the employer is putting on the pressure, or the partner is being difficult, but rather because they are experiencing problems due to their symptoms that were there in the first place.

## Previous Findings

Before patients visit a clinic for environmental medicine, as a rule they have already been seen by doctors from various disciplines. Many of these patients keep a special file folder that they bring with them to the consultation, usually containing files of

numerous previous examinations. Mostly, the previous examiners could not decide on a common diagnosis. Such patients lay great importance on receiving original copies of the findings as quickly as possible in order to store them in this folder. Included in these investigations, besides laboratory screening and human biomonitoring tests, there are investigations of their surroundings. These had usually been carried out by institutions specialized in measuring minute concentrations of chemicals in the air, in dust, and in materials to be found in the home environment. Upon exceeding the background values or reference values of one method, a whole host of complaints are then specified that could match the chemical that has exceeded the reference value.

## Findings

A complete physical examination, which should have an internal medicine focus as well as a neurological focus, is essential! One works from top to bottom. In addition to the reflex test, the neurological exam incorporates the extended-hand test, Romberg's test, Babinski, Hackengang, evaluation of superficial and deep sensibility, and testing the diadochokinesis.

## Blood Sampling

At a time of cost constraints within health care, some restrictions on laboratory tests should be in place. They should be selective and targeted at searching out any organ pathologies that may already have been indicated in the examination. As a rule, electrolytes, serum liver values, serum kidney values, blood sugar, and blood count with leukocytes, erythrocytes, and platelets are determined, along with an inflammatory parameter, preferably CRP. In respect of the biomonitoring, according to laboratory guidelines either blood or urine should be collected, depending on the pollutant. The urine should be checked at the same time for bacteria and erythrocytes.

## Monitoring in Environmental Medicine

The patients' hypothesis is that a pollutant has caused their suffering. Case history and chronological sequence of the complaints should indicate the direction the search has to take to find the source. Basically, one can distinguish between ***environmental monitoring*** (ambient (bio)monitoring) and human biomonitoring. Environmental monitoring, in connection with patients, only makes sense if a suspected source is known. Often, this is the dwelling place. A possible increase in pollution in the dwelling place can be ascertained by measuring the ambient air, preferably preceded by a specialized tour of the dwelling and

history taking with respect to any potential sources of contamination. Nowadays, only rarely and usually only temporarily following any new fittings or renovations, can an increased concentration of pollutants be found within the dwelling that are likely to induce ailments. In this connection, odor nuisance can be a relevant factor.

*Human biomonitoring* can be divided into exposure biomonitoring, effect biomonitoring, and susceptibility biomonitoring. With ***exposure biomonitoring***, the substances absorbed from the environment are registered in body materials, mostly in blood/plasma/serum and/or urine. Biomonitoring of hair, saliva, breast milk, or fatty tissue rarely makes sense. It is not correct to determine formaldehyde contamination on the basis of the identification of formic acid in urine, because formic acid accumulates during metabolism even without exposure to formaldehyde. One speaks of ***effect biomonitoring*** if the poison itself can be measured less well than one of its effects, which thereby do not have to be toxic yet. A classic example of effect biomonitoring is determining plasma cholinesterase (PchE) to assess a contamination with organophosphates (also see chapter "► Background Exposure Versus Additional Exposure in Human Biomonitoring"). ***Susceptibility monitoring*** is served by measuring biomarkers that indicate the individual susceptibility to toxic influences. An example of this is determining the expression of detoxification enzymes, or, in the field of allergology, the determination of immunoglobulin E.

## Specimen Collection and Processing Procedure

The first thing to consider is what the appropriate materials to use for exposure biomonitoring are. As a rule, blood is a suitable medium to determine an exposure occurring within the near past, while urine covers a somewhat longer time period and especially because most substances are found in higher concentrations in urine than in blood. For some substances, hair can provide evidence of an exposure that took place up to several months previously. However, in the case of hair it is difficult to distinguish between internal and external exposure. Blood fat provides a suitable medium to determine lipophilic substances. For this purpose, larger amounts of blood are needed. Teeth or bones allow for gauging exposures lying further in the past, even over the course of years.

In order to choose the most suitable material for biomonitoring, it is important to know about the metabolism of a particular substance. So for material with low renal excretion and high metabolism in the liver, blood/plasma/serum are better and more suitable for analysis than urine. Lindane is a classic example of this. Unaltered lindane appears in urine in only the slightest amounts, making plasma the more suitable material for ascertaining lindane.

As a rule, 10 ml of EDTA blood and/or 50 ml of urine are required. It is important to ensure that no contamination occurs. As a matter of principle, venous blood, for example, should not be drawn via metal cannulae when ascertaining levels of metals, but rather by means of polyethylene cannulae in

situ. This is particularly critical when drawing blood for aluminum, chromium, and nickel analysis, because for these metals there is a high risk of contamination from the surroundings. Urine samples are collected as a random specimen or timed collection specimen. In turn, the most suitable collection vessels for this purpose are made of polyethylene or polystyrene. For determining organochlorine biocides (e.g., DDE, HCB, PCB) in blood, glass tubes should be used throughout the procedure.

## Evaluation of Results of Human Biomonitoring

The assessment of the results can be done in two ways. On the one hand, in comparison to the reference value that reflects the background level to be found nowadays in the population, and on the other hand by means of toxicologically derived values such as the BAT values for the workplace or the HBM values for the environmental area (see chapters "► Background Exposure Versus Additional Exposure in Human Biomonitoring," "► Importance of Exposure Level for Risk Toxicological Assessment," and "► Limit Values and Guideline Values in Regulatory Toxicology").

## Reference Values for Human Biomonitoring

The comparison of the measured level of contamination of the patient with the reference value provides information about whether a greater than average concentration is present. Excess above the reference value has per se no toxicological relevance.

## Toxicologically Derived Human Biomonitoring Values

A comparison of the measured level of contamination of the patient with the toxicologically derived HBM value provides information about the degree of health risk (see chapter "► Importance of Exposure Level for Risk Toxicological Assessment"). Unfortunately, HBM values are only available for a few substances.

## Limitation of Biomonitoring

Many patients who visit an environmental medical clinic are not aware of which pollutant is causing their ailments. This can lead to an ineffectual, very broadly based biomonitoring. From this, one might conclude that biomonitoring does not have a good cost-benefit ratio at a time when savings are called for in health care. This contrasts with legal concerns: If a patient feels that a certain substance is poisoning him, he has the right to demand clarity. A common problem lies in the

interpretation of results. Doctors often certify that, when a reference value is exceeded, then a patient has a health-related risk. This is pure nonsense, because the reference value is not derived toxicologically. Announcing such erroneous findings results in patients being frequently misdiagnosed as "poisoned." If everything is unclear, further help can be obtained via ambient monitoring. If under ambient monitoring increased exposure to a specific toxicant is established, under certain circumstances this can be specifically searched for by human biomonitoring. Ambient biomonitoring is, however, not a medical service and, as a rule, must be paid for by the patients themselves.

## Dealing with Environmental Patients from a Psychological and Psychiatric Perspective

With the present state of knowledge, one must assume that a psychological component (toxicophobia, phantom risk) plays a role with many patients. In order to meet patients' needs, in addition to the somatic-orientated and well-trained doctors such as general practitioners or internists, there should be doctors trained in psychosomatic medicine or psychiatry.

## Psychiatric Diagnostics

Before the therapy, the "gods" have made the diagnosis. As already pointed out above, all known serious somatic disorders must be excluded. Once this is done, the patient has to be examined by a psychiatrist/psychosomatic doctor. As a large-scale study done on 308 patients in cooperation with the toxicology and psychiatry departments has shown, in 35.3 % of them the presenting symptoms can be completely explained as belonging to a mental disorder. In 21.6 %, there was an underlying physical condition that could explain the symptoms adequately. In 22.2 %, the simultaneous presence of a mental disorder and a somatic disorder could justify the symptoms. In 1.6 %, the patients' ailments could be explained by the impact of a pollutant. This leaves a group of 14.1 % remaining where the ailments could neither be explained somatically nor mentally. These patients were suffering from an impairment of well-being that was neither mental nor psychosomatic.

For an experienced psychiatrist or doctor of psychosomatic medicine, it should not normally be difficult to narrow down the type of mental dysfunction, so long as the patient is prepared to undergo one or more verbal consultations. However, if one wants as scientific and comprehensible a diagnosis as possible, a battery of psychological and/or psychiatric tests is essential. In our study of over 300 patients, of course, not all environmental patients were found to have the same personality or psychiatric disorder, but rather virtually any kind of distinctive personality type or known psychiatric diagnose could be found (Fig. 1).

What they all have in common is simply the belief that the perceived ailments are the result of environmental pollutants. Under somatoform disorders, we

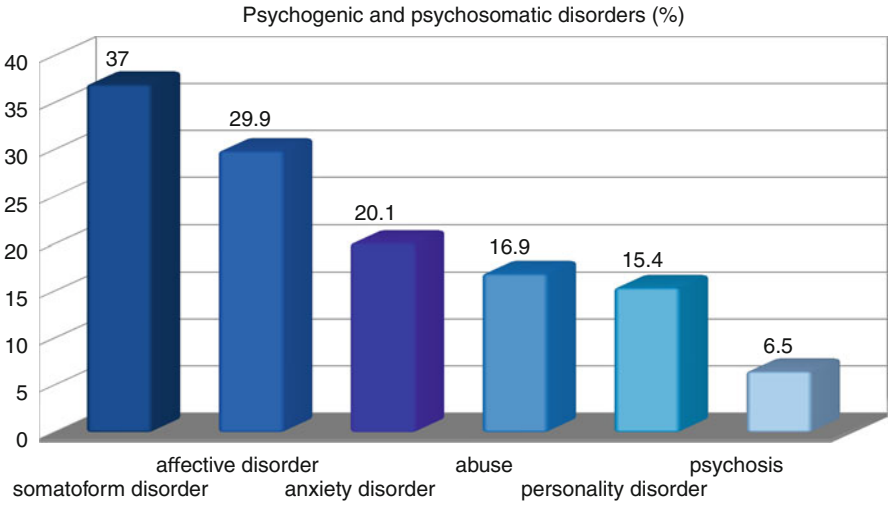



**Fig. 1** Psychiatric diagnoses in patients with environmental disorders ($n = 308$) (some of the patients have multiple diagnoses)

understand psychosomatic diseases; under affective disorder, depression and/or manic depression; under anxiety disorders, the phobias; and under psychotic disorders, the schizophrenias.

## Therapy

Treatment is determined by the diagnosis, of course. If indeed a relevant pollutant exposure can be proved, then the avoidance of this exposure is the decisive therapy. However, if a pollutant exposure is only suspected on the basis of a psychiatric disorder, then psychotherapy comes into play. In the case of ***anxiety disorders***, behavior therapy brings fast and good results. In a few of these anxiety disorders, treatment can also be supported by use of psychotropic drugs. The ***depressive*** disorders usually require a combination of psychotherapy and antidepressant pharmacotherapy. Depending on the severity of the depression, treatment with antidepressants can stand in the foreground (major depression), or a balanced combination therapy consisting of medication and psychotherapy as in the case of milder forms of depression (dysthymia).

***Substance abuse*** necessitates detoxification and then withdrawal treatment in an appropriate, specialized clinic that works particularly with group therapy and sociotherapy. Following on from this, self-help groups are particularly meaningful. Schizophrenic disorders require treatment with predominantly high-potency neuroleptic drugs and social therapeutic measures after resolution of the productive symptoms. ***Personality disorders*** are unfortunately difficult to treat. Sociotherapy, psychotherapy, and psychotropic drug therapy can lead to improvement.

There is repeated emphasis on not taking patients with environmentally associated disorders seriously, and that they should not be unnecessarily ***psychiatrically treated***. Which normal-thinking, emphatic-feeling doctor would not take such a patient seriously? On the other hand, psychiatry is part of conventional medicine; "psychiatrization" is not done by doctors. Rather, this is more a phenomenon of society, and it is actually time to overcome stigmatization by means of education. To this end, long years of positive public relations are needed, as has long been the case for other kinds of impairment. Of course, it is useless to try to talk the patient out of his notion that his symptoms are related to environmental toxicants. Letting go of these symptoms is only possible at the end of treatment. Especially in the case of somatic disorders, symptom-orientated psychotherapeutic measures seem sensible. One begins with relaxation training, such as autogenic training, progressive muscle relaxation, or hypnosis. This is combined with deep psychologically orientated focal therapy and cognitive behavioral therapy. Symptom relief is not sought after. The aim is to reduce social isolation.

Mechanisms need to be found to help other things become more meaningful rather than just ***dealing with the toxicant*** (coping strategies). This way, quality of life is improved. Forces, that is, healthy aspects of the psyche, are reactivated. In individual cases, there are reports of success in the aforementioned therapy forms with these kinds of environmentally related somatic disorders. Patients that exclude any psychogenesis – and they are many – cannot be reached at this point in time. Patients who are ambivalent about accepting a psychosomatic illness are treatable with patience. Patients who accept psychotherapy are to a large extent capable of improvement and in about 40 % (personal experience) of cases can be cured.

It is interesting that even though patients seek help from alternative medicine therapy forms, they mostly do not break off contact with conventional medicine and carry on expecting help from that quarter, too. This may be an indication that alternative therapies such as chelation agent therapy, antioxidant therapy, electro-acupuncture, or cleansing methods by means of hydrotherapy or Ayurveda therapy do not always lead to success. The psyche needs a valve in order to stabilize itself. Only if changes are possible can the symptoms be minimized. Suppressed conflicts or guilt is often the reason for externalizing, which means that the blame (the toxicant) for a particular plight is sought for in the outside world, because otherwise the burden of internal tensions would be unbearable. It is my wish that such patients may be helped, but this will only work with interdisciplinary cooperation and patience.

## Recommended Reading

Bornschein S, Hausteiner C, Konrad F, Förstl H, Zilker T (2006) Psychiatric morbidity and toxic burden in patients with environmental illness: a controlled study. Psychosom Mod 68(1):104–109

Bornschein S, Hausteiner C, Zilker T, Bickel H, Förstl H (2000) Psychiatric and somatic morbidity of patients with suspected multiple chemical sensitivity syndrome (MCS). Nervenarzt 71(9):737–744

Hausteiner C, Bornschein S, Bickel H, Zilker T, Förstl H (2003) Psychiatric morbidity and low self-attentiveness in patients with environmental illness. J Nerv Ment Dis 191(1):50–55

Hausteiner C, Bornschein S, Zilker T, Henningsen P, Förstl H (2007) Dysfunctional congnitions in idiopathic environmental intolerances (IEI)- an integrative psychiatric perspective. Toxicol Lett 171(1–2):1–9

Hausteiner C, Mergeay A, Bornschein S, Zilker T, Förstl H (2006) New aspects of psychiatric morbidity in idiopathic environmental intolerances. J Occup Environ Med 48(1):76–82

Staudenmayer H, Binkley KE, Leznoff A, Phillips S (2003a) Idiopathic environmental intolerance: part 1: a causation analysis applying Bradfoord Hill's criteria to the toxicogenic theory. Toxicol Rev 22(4):235–246

Staudenmayer H, Binkley KE, Leznoff A, Phillips S (2003b) Idiopathic environmental intolerance: part 2: a causation analysis applying Bradford Hill's criteria to the psychogenic theory. Toxicol Rev 22(4):247–261

# Reach (and CLP). Its Role in Regulatory Toxicology

Walter Aulmann and Nathan Pechacek

## Contents

Aim ..... 780
"No Data – No Market": Registration of Chemical Substances ..... 781
Information Gathering and Closing of Knowledge Gaps ..... 782
Quality Check of Gathered Information ..... 785
Hazard Identification and Assessment ..... 787
Chemical Safety Assessment (CSA) ..... 790
Evaluation ..... 793
Authorization and Restriction ..... 794
Outlook ..... 794
References ..... 795

**Abstract**

This chapter intends to illustrate the general framework of REACH and will specifically focus on the aspects which are important for a regulatory toxicologist. The principles of its individual elements Registration, Evaluation, Authorization, and Restriction are explained. The current progress is described and an outlook to the final stage of the transition period is given.

In its 1999 "White paper" the European Union published the result of an analysis of the existing chemical legislation identifying a number of problems. The paper challenged the existing allocation of resources applying different approaches for new and existing substances. For new substances, even at low tonnage levels, a burdensome and expensive notification was required. On the

W. Aulmann (✉)
Ecolab Deutschland GmbH, Monheim, Germany
e-mail: walter.aulmann@ecolab.com

N. Pechacek
Ecolab Inc, St. Paul, MN, USA
e-mail: nathan.pechacek@ecolab.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_92, 

other hand, producers were not obliged to submit similar data packages for existing substances. Moreover, the systematic evaluation of existing chemicals had turned out inefficient. After an intensive dialogue with all stakeholders, the REACH regulation was adopted in 2006. REACH stands for Registration, Evaluation, Authorization and Restriction of Chemicals. Since entering into force REACH is gradually replacing several directives such as the former Dangerous Substances Directive (DSD) (Council Directive 67/548/EEC), the Dangerous Preparations Directive (DPD) (Council Directive 1999/45/EC), the Restriction Directive, (Council Directive 76/769/EEC) and the Regulation on Existing Substances. (Council Regulation No 793/93 EEC)

It is impossible to give a comprehensive overview in this chapter of all the aspects of REACH, because the regulation and the accompanying guidance documents cover over 20,000 pages. This chapter intends to illustrate the general framework of REACH and will specifically focus on the aspects which are important for a regulatory toxicologist.

**List of Abbreviations**

| | |
|---|---|
| C&L | Classification and labelling |
| CMR | Carcinogenic, mutagenic, reproductive toxicant |
| CSR | Chemical safety report |
| DNEL | Derived no-effect level |
| DU | Downstream user |
| ECHA | European chemicals agency |
| ES | Exposure scenario |
| eSDS | Extended safety data sheet |
| GHS | Globally harmonized system of classification and labelling of chemicals |
| GLP | Good laboratory practice |
| IUCLID | International uniform chemical information database |
| NACE | Nomenclature générale des activités économiques dans les communautés européennes |
| NOAEL | No observed adverse effect level |
| PBT | Persistent, bioaccumulative, toxic |
| RMM | Risk management measures |
| SIEF | Substance information exchange forum |
| SME | Small or medium enterprise |
| SVHC | Substances of very high concern |
| vPvB | Very persistent very bioaccumulative |

## Aim

REACH aims to ensure a high level of protection for human health and the environment while at the same time enhancing competitiveness and innovation

with a focus on less hazardous materials. The regulation applies to substances on their own, substances in mixtures, and in articles and is directly applicable in all member states of the European Union.

For the management of the technical, scientific, and administrative aspects of REACH at the community level, a new institution was founded. This is the European Chemicals Agency (ECHA) in Helsinki, Finland. In order to support industry and authorities to meet their obligations under REACH, technical guidance documents were developed involving experts from various stakeholders (e.g., industry, member states, and nongovernmental organizations). These documents aim to facilitate the implementation of REACH. They are not prescriptive but summarize generally acknowledged good practice (ECHA 2009). It should be stated that these guidance documents are not legally binding.

In order to maintain the workability of the system, reduced requirements from the obligation to register exist for intermediates under strictly controlled conditions as well as some exemptions for selected groups of substances (e.g., polymers). Compared to the previous system, an important change is the shift in responsibility for the risk management of substances, which now resides with the industry. Depending on its role as a manufacturer, importer, or downstream user (e.g., formulators), industry has to comply with specific duties and obligations. A major task for industry is to provide data by filing registrations to ECHA.

One of the goals of the United Nations Conference on Environment and Development in 1992 was the development of globally harmonized criteria for classification and labeling with a view to facilitating worldwide trade while protecting human health and the environment. Over a period of 12 years the Globally Harmonized System of Classification and Labeling of Chemicals ('GHS') had been developed within the United Nations (UN). The new Classification, Labeling and Packaging (CLP) regulation in 2008 implemented the GHS in the European Union.

REACH and CLP do not stand isolated from each other. Instead, there are many close links between the CLP regulation and the REACH regulation. Both regulations make use of a uniform terminology and definitions. This ensures maximum consistency in the application of chemical legislation within the European Union in the context of global trade. The infrastructure used is basically the same. Both regulations make use of the same legislative bodies like the European Chemicals Agency (ECHA) as listed under title X of the REACH regulation. National helpdesks have been established to provide advice to suppliers and any other interested parties, in particular small- and medium-sized enterprises (SMEs), on their respective responsibilities and obligations under both regulations. The same applies to the technical guidance notes for the application of the CLP criteria. They are included in the compendium of supporting documents for REACH on the ECHA website.

## "No Data – No Market": Registration of Chemical Substances

The review programs on existing chemicals had indicated that safety data were often lacking even for high production volume chemicals. To overcome this

issue REACH obliges producers and importers of chemicals to systematically compile safety information in a registration dossier.

Tonnage per year manufactured or imported serves as a crude indicator for exposure of man and the environment, which triggers the extent of information that has to be filed in the registration.

## Information Gathering and Closing of Knowledge Gaps

A registrant who manufactures or imports a substance has to collect all available and relevant information for hazard identification and assessment. In many cases, the information gathered may consist of actual test data. However, other types of information may also be sufficient, especially when used in a weight of evidence (WoE) approach (see below). This means that information on intrinsic properties of substances may be obtained by means other than tests. REACH provides the options to refrain from further studies when adequate evidence is available from other sources. In such cases the regulation speaks of "information." When crisp facts are presented based on studies, this is denominated as "data."

The registrant is obliged to incorporate all relevant available information in the registration dossier, using the IUCLID-software format. To that end the existing physicochemical, toxicological, and ecotoxicological information are gathered.

Sharing of information on substances among the registrants is strongly encouraged by REACH to reduce testing on vertebrate animals and agree on a harmonized classification. Whenever possible, information shall be shared with other registrants in a Substance Information Exchange Forum (SIEF). SIEFs are formed on the basis of the preregistration (described below).

In the tonnage driven test program REACH follows a tiered approach to allocate resources appropriately. REACH offers special transition periods for the so-called phase-in substances. They apply to substances already manufactured or imported. To make use of the transition period actors had to preregister their candidate phase-in substances in 2008. This phase is over now and the option to preregister can only be used in some exceptional cases. On the basis of the preregistration files, ECHA built up a database bringing together all potential registrants for the same substance in a SIEF. Registration on phase-in substances follows certain tonnage levels and hazard potential. By December 2010, all phase-in substances exceeding a volume of 1,000 t/year and all substances ($>$ 1 t/year) classified as carcinogenic, mutagenic, or toxic to reproduction or toxic to the environment ($>$ 100 t/year) required registration. The deadlines for the lower tonnage levels will be 2013 and 2018 (see Fig. 1).

For non-phase-in substances, a so-called inquiry has to be sent to ECHA to make sure that the substance intended for a new registration was not filed in parallel by another actor. ECHA has to approve the inquiry confirming that to their knowledge no duplicate registration efforts are undertaken for the described chemical. Without this confirmation testing on vertebrates in order to meet the information requirements is prohibited.

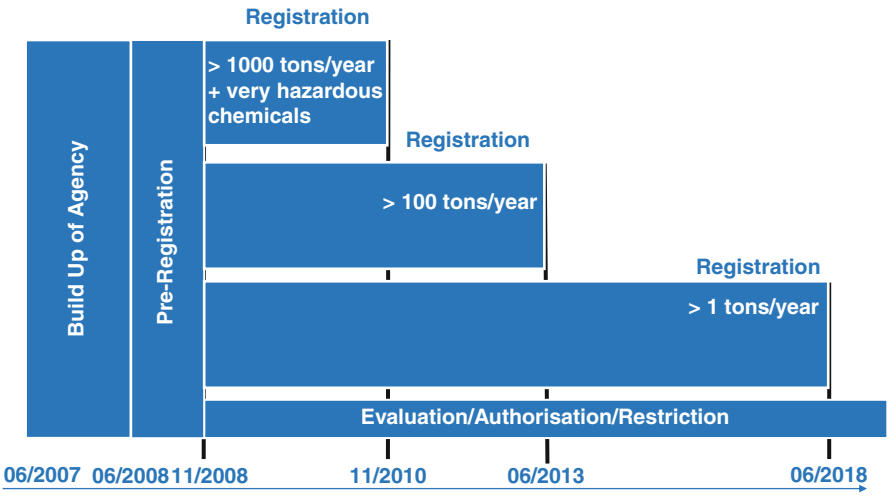



**Fig. 1** Timeline for the registration of phase-in substances

In case of data gaps, the registrants have to generate new data for all substances to be registered or provide sufficient justification on why the data is not needed. For example, certain tests can be waived based on physicochemical properties or on exposure. Animal tests may always be taken into account as the last resort. Also, information on exposure, use, and risk management measures has to be collected.

For the tonnage up to 100 t/year the information requirements are laid down in annexes VII and VIII (see Table 1). If relevant information for more end points is available, this needs to be submitted as well, regardless of whether information on this given end point is required at this tonnage level or not.

Compared to the requirements for the tonnage band up to 100 t, the test program for *higher* tonnage levels needs to be tailored in a substance-specific manner. Substance characteristics and information already gathered will trigger the data requirements for the next stage (i.e., under annex IX and X). The following additional toxicological information may be required under the regime of annex IX and X:

- Repeated-dose toxicity – subchronic
- Prenatal developmental toxicity study
- Two-generation study
- Carcinogenicity study

In the field of ecotoxicity and environmental fate, further investigations may be needed on:

- Effects on aquatic organisms after long-term exposure
- Toxicity on terrestrial organisms
- Bioaccumulation
- Degradation

**Table 1** Information requirements for substances below 100 t/year

| Annex no. | End point |
|---|---|
| VII | Melting point |
| VII | Boiling point |
| VII | Relative density |
| VII | Vapor pressure |
| VII | Surface tension |
| VII | Water solubility |
| VII | Partition coefficient |
| VII | Flash point |
| VII | Auto flammability |
| VII | Flammability |
| VII | Explosive properties |
| VII | Self-ignition temperature for solids |
| VII | Oxidizing properties |
| VII | Skin corrosion/irritation |
| VII | Eye irritation |
| VII | Skin sensitization |
| VII | Bacterial gene mutation (Ames) |
| VIII | Chromosome mutation in vitro |
| VIII | Gene mutation in mammalian cells |
| VIII | In vitro gene mutation |
| VII | Acute toxicity oral |
| VIII | Acute toxicity inhalation |
| VIII | Acute toxicity dermal |
| VIII | Subacute toxicity test |
| VIII | Reproductive toxicity screen |
| VIII | Developmental toxicity |
| VII | *Daphnia* acute |
| VII | Algae test |
| VIII | Fish acute |
| VIII | Activated sludge |
| VII | Biodegradation in water |
| VIII | Hydrolysis in water |
| VIII | Adsorption/desorption |

VII – requirements of annex VII: 1 to $<$ 10 t/year
VIII – requirements of annex VIII: 10 to $<$ 100 t/year

At this tonnage level the registrant has to file a test proposal to ECHA upfront. The proposal needs to be endorsed before the registrant can launch any test.

In order to generate information on intrinsic properties of substances for a registration under REACH, the test method regulation 440/2008/EC has to be consulted. It contains a compendium of methods for the determination of physico-chemical properties, of toxicity and other health effects, and of ecotoxicity. The regulation is regularly updated, especially taking into account progress that is being

made in the OECD program for the development of test methods and when appropriate validated methods become available to replace, reduce, or refine animal testing. Due to different processing speeds in incorporating new methods into the regulation, the OECD and the EU compendium do not always match each other. In some cases the OECD had been ahead, like for tests on eye damage (OECD 437 and 438). In other cases it was the EU, like in the test method to identify skin irritation (test method No. B46). Interestingly, some of the tests in the REACH standard test program are not contained in the test method regulation, such as the "repeated-dose reproductive screening" tests, which were adopted in 1992 as OECD test guidelines No. 422 and 421. Tests to identify intrinsic adverse properties for human health in general have to follow the principles of Good Laboratory Practices (GLP). Strong prescriptions for record keeping and quality assurance procedures are a prerequisite for acceptance of data submissions to ECHA.

In an ideal world the test of first choice would be a definitive test. This type of test provides sufficient stand-alone information on dose response and adverse effects to make a final decision with regard to hazard assessment. However, in many cases, data from screening tests go first in a tiered testing strategy. Screening tests are generally used for preliminary decision making and to set priorities for more definitive tests. In special cases additional investigations are needed which do not follow a standard approach but are tailor-made for the hazard assessment of a specific substance. Those adjunct tests help to interpret the results of other tests and provide information useful for the hazard and risk assessment process.

Generation of in vitro data is explicitly encouraged by REACH. Often in vitro methods are mechanistically based; they provide a direct relationship between the biological effects observed and the biological effects of interest. REACH annex XI stipulates that positive findings from in vitro screening tests can be used for an assessment (e.g., hazard classification). However, negative findings cannot be readily used to exonerate a substance from having a specific toxic effect. To that aim the method must have undergone validation and must be a definitive test for classification. The latter indirectly implies that it has gained regulatory acceptance.

Physicochemical data are often used for initial considerations of a specific hazard potential. Knowledge on selected physicochemical properties (e.g., water solubility, acidity, alkalinity, hydrophilicity/lipophilicity) can foster an understanding whether a substance is bioavailable and to which extent it may be metabolized or be locally toxic.

## Quality Check of Gathered Information

All information has to be assessed for its reliability, relevance, and adequacy.

Reliability is the inherent quality of the information as related to preferably standardized methodology and the way experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. In general,

**Table 2** Klimisch codes/category of reliability

| |
|---|
| ***Reliability 1*** **(reliable without restriction)** |
| Test data generated according to an internally accepted test guideline study (OECD, EU, ICH) |
| Comparable to guideline study |
| Test procedure according to national standards (DIN, etc.) |
| ***Reliability 2*** **(reliable with restrictions)** |
| Acceptable, well-documented publication/study report which meets basic scientific principles |
| Basic data given: comparable to guidelines/standards |
| Comparable to guideline study with acceptable restrictions |
| ***Reliability 3*** **(not reliable)** |
| Method not validated |
| Documentation insufficient for assessment |
| Does not meet important criteria of today standard methods |
| Relevant methodological deficiencies |
| Unsuitable test system |
| ***Reliability 4*** **(not assignable)** |
| Only short abstract available |
| Only secondary literature (review, tables, books, etc.) |

the Klimisch code system allows ranking of the information for further review. The system consists of four categories (Klimisch et al. 1997) (Table 2):

1. Reliable without restrictions
2. Reliable with restrictions
3. Not reliable
4. Not assignable

In general only Klimisch category 1 and 2 data are suitable for a registration dossier.

Relevance is the extent to which data and tests are appropriate for a particular hazard assessment. Adequacy is the usefulness of the data for hazard and risk assessment purposes.

Available human data are taken into account for the identification of intrinsic hazardous properties as it concerns human health. However, in cases other than pharmaceuticals, human data generally are scarce. For industrial chemicals the main types of human data are case reports and epidemiological studies. Clinical studies are rarely available for industrial chemicals since clinical trials with humans are not allowed for hazard identification. They generally serve as confirmatory tests for the assumption on safety made previously on the basis of nonhuman data.

In case reports and epidemiological studies the actual human exposure may be poorly characterized. Diagnosis confirmed by expert physicians may also be missing. Confounding factors may not have been accounted for such as confounding chemical exposures. Small group sizes may compromise the statistical strength of evidence. Many other factors may compromise the validity of human data. In clinical studies the selection of individuals for the test and the control groups

must be handled with care. Therefore, the assumption of low uncertainty that is generally attributed with human data is often associated with an overall low data quality and poor documentation.

Poison control centers may provide evidence if there is an issue with a product on the market. If the statistics from poison centers do not give any alerts, this does not constitute proof that a chemical does not have adverse effects.

## Hazard Identification and Assessment

For the purpose of the hazard assessment, basically the rules of the CLP regulation apply. These rules are described comprehensively and in detail in the chapter "► Health Hazards Classification and Labeling."

In this respect the WoE approach is a guiding principle of REACH and CLP. A legally binding definition of "weight of evidence" has been adopted in the European CLP regulation in annex I:

> A weight of evidence determination means that all available information bearing on the determination of hazard is considered together, such as the results of suitable in vitro tests, relevant animal data, information from the application of the category approach (grouping, read-across), (Q)SAR results, human experience such as occupational data and data from accident databases, epidemiological and clinical studies and well documented case reports and observations. The quality and consistency of the data shall be given appropriate weight. Information on substances or mixtures related to the substance or mixture being classified shall be considered as appropriate, as well as site of action and mechanism or mode of action study results. Both positive and negative results shall be assembled together in a single weight of evidence determination.

Additional provisions are laid down in REACH annex XI, which provides options for meeting the information requirements in annex VII–X by other means than testing.

In a weight of evidence approach different pieces of available information are weighted. This may be necessary when several reliable studies are available with conflicting results. Moreover, evidence from less reliable information is weighted for the hazard assessment. WoE is end point specific and in general needs expert judgment.

Among the end points for which the WoE approach is fairly progressed is the hazard identification of local skin and eye effects which is demonstrated in the flow chart (ECHA 2012). For these end points all available information is pooled in an initial phase. This reflects read-across from similar substances, QSAR predictions, pH and alkaline/acidic buffer reserve, in vitro tests, and human experience. As an example for a WoE approach, Table 3 illustrates the evaluation and testing strategy for local skin effects.

The WoE approach grants flexibility and may reduce the costs and avoid animal tests. A major pitfall is that it is associated with lower legal certainty.

**Table 3** Decision tree for classification of skin effects

| | | |
|---|---|---|
| **Part 1 Retrieving existing information** | | |
| ***Existing data on physicochemical properties*** | | |
| 1a | Is the substance an organic hydro peroxide or an organic peroxide? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify as:<br>Corrosive (Skin Corr. 1B) if the substance is a hydro peroxide or<br>Irritating (Skin Irrit. 2) if the substance is a peroxide<br>or<br>Provide evidence for the contrary<br>Proceed to step 1b |
| 1b | Is the pH of the substance $\leq 2$ or $\geq 11.5$? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify as corrosive. Where classification is based upon consideration of pH alone (see step 7), Skin Corr. 1A should be applied<br>Proceed to step 1c |
| 1c | Are there other physical or chemical properties that indicate that the substance is irritating/corrosive? →<br>↓<br>No<br>↓ | Yes:<br>Use this information for WoE analysis (step 7)<br>Proceed to step 2 |
| ***Existing human data*** | | |
| 2 | Are there adequate existing human experience data which provide evidence that the substance is corrosive, irritant, or non-irritant? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify accordingly<br>Proceed to step 3 |
| ***Existing animal data from irritation/corrosivity studies*** | | |
| 3 | Are there data from existing studies *on irritation and corrosion* in laboratory animals, which provide sound conclusive evidence that the substance is a corrosive, irritant, or non-irritant? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify accordingly (either Skin Corr. 1A or Skin Corr. 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification)<br>Proceed to step 4a |
| ***Existing data from general toxicity studies via the dermal route and from sensitization studies*** | | |
| 4a | Has the substance proven to be a corrosive, irritant, or non-irritant in a suitable acute dermal toxicity test? →<br>↓ | Yes:<br>If test conditions are consistent with OECD 404, consider to classify accordingly (Skin Corr. 1A or Skin Corr. |

(*continued*)

**Table 3** (continued)

| | | |
|---|---|---|
| | No<br>↓ | 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification)<br>Proceed to step 4b |
| 4b | Has the substance proven to be a corrosive or an irritant in sensitization studies or after repeated exposure? →<br>↓<br>No<br>↓ | Yes:<br>This information cannot be used for considering a concrete classification conclusion but must be used exclusively within the integrated WoE judgement (step 7)<br>Proceed to step 5a |
| ***Existing (Q)SAR data and read-across*** | | |
| 5a | Are there structurally related substances (suitable "read-across" or grouping), which are classified as corrosive (Skin Cat. 1) on the skin, or do suitable QSAR methods indicate the presence/absence of corrosive potential of the substance? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify or not<br>Proceed to step 5b |
| 5b | Are there structurally related substances (suitable "read-across" or grouping), which are classified as irritant on the skin (Skin Cat. 2), or do suitable (Q)SAR methods indicate the presence/absence of irritating potential of the substance? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify or not<br>Proceed to step 6a |
| ***Existing* in vitro *data*** | | |
| 6a | Has the substance demonstrated corrosive properties in an OECD adopted in vitro test? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify as corrosive. If discrimination between Skin Corr. 1A/1B/1C is not possible, Skin Corr. 1 must be chosen<br>Proceed to step 6b |
| 6b | Are there acceptable data from a validated in vitro test (adopted by OECD or not), which provide evidence that the substance is an irritant or non-irritant? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify accordingly (Skin Irrit. 2 or no classification)<br>Proceed to step 6c |
| 6c | Are there data from a suitable in vitro test, which provide sound conclusive evidence that the substance is an irritant ? →<br>↓<br>No<br>↓ | Yes:<br>Consider to classify as Skin Irrit. 2<br>Proceed to step 7 |

(*continued*)

**Table 3** (continued)

| **Part 2 Weight of evidence analysis** | |
|---|---|
| 7 Taking all existing and relevant data (steps 1–6) into account, is there sufficient information to make a decision of whether classification/labeling is necessary, and – if so – how to classify and label? →<br>↓<br>No<br>↓ | Yes:<br>**Classify accordingly (Skin Corr. 1A or Skin Corr. 1B or Skin Corr. 1C or Skin Irrit. 2 or no classification)** |
| **Unable to classify substance for skin corrosion/irritation** | Decision to carry out new tests data should be made in compliance with REACH requirements |

## Chemical Safety Assessment (CSA)

If a substance to be registered requires classification as dangerous or turns out to be persistent, bioaccumulative or toxic (PBT), or very persistent and very bioaccumulative (vPvB), the risk has to be characterized in a chemical safety assessment. The exposure assessment and the risk characterization has to be targeted at the specific hazard that had been identified, be it either for human health, physicochemical hazard assessment, environmental hazard assessment, or PBT or vPvB assessment.

The chemical safety assessment is required for all substances subject to registration under REACH in quantities of 10 t or more per year per registrant. Its aim is to ensure that all risks are identified and under control (see Fig. 2) by relating exposure to threshold levels for hazards (ECHA, 2009).

Exposure assessment is a tiered approach for which three levels are available. Entry into the process is possible at any of the three tiers. This *basic exposure assessment* can be used for all target groups (i.e., workers, consumers, humans via the environment). The tool which is mostly used for the basic assessment is the ECETOC targeted risk assessment tool (ECETOC 2004, 2009). With relatively limited entry data, results can be quickly obtained.

The next higher assessment tiers can be for example *sector-specific generic exposure scenarios*. They are based on sets of 'use scenarios' agreed along the supply chain between manufacturers and importers on one hand and downstream users on the other hand. Risk management measures may have to be considered in order to ensure a use is safe to human health and the environment. Exposure scenarios are the communication tool to the user describing how to use a substance in a safe way. They are filed in the CSR together with other information and communicated to DUs via an annex to the safety data sheet (SDS), resulting in an extended safety data sheet (eSDS).

Whenever needed, *tier 3-specific assessments* may be needed by modeling cases of specific applications as an outcome of joint efforts between supplier and

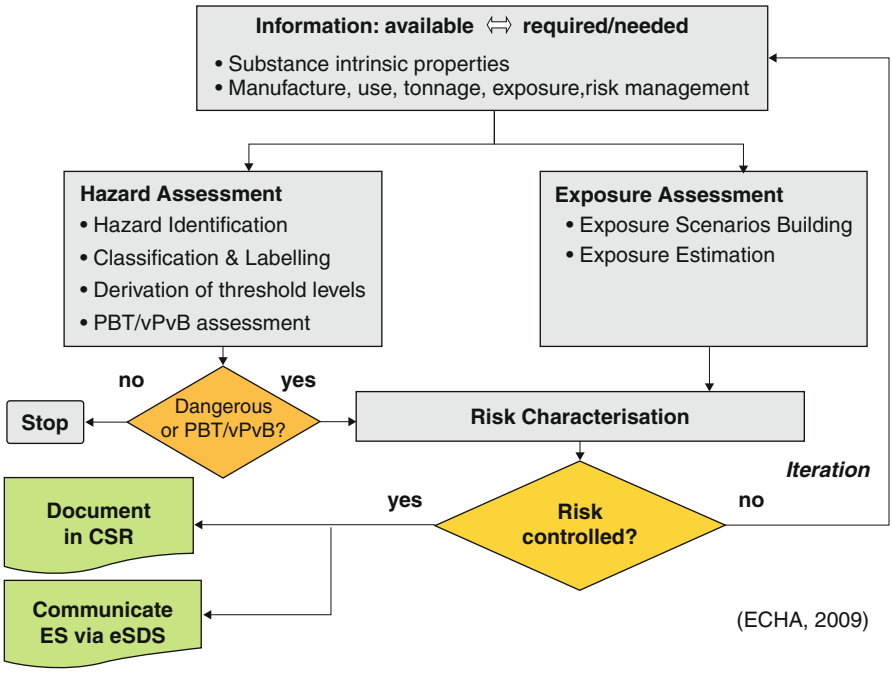



**Fig. 2** Flow scheme for the chemical safety assessment

downstream user. Exposure assessment may then be based on actual measurements from downstream use.

(For more details see the chapter on "▶ Importance of Exposure Level for Risk Toxicological Assessment").

The last phase of the chemical safety assessment is risk characterization. Exposure levels have to be related to threshold doses or concentrations for which no adverse effects are expected. They are denominated as "derived no-effect levels" (DNELs).

DNEL setting must account for several aspects. All conditions of manufacturing or use must be addressed. Different factors have to be applied depending on the target (workers, general population [consumers, humans via the environment]). Specific target organ toxicity data after acute and repeated exposure needs to be evaluated with special regard to the character of effects (systemic versus local).

The first step in the derivation of a DNEL is the decision on the initial point-of-departure (POD) value. Most frequently, the lowest reliable, relevant, and adequate no observed adverse effect level (NOAEL) from the registration data set is used as the POD. At this dose or concentration no adverse, treatment-related findings are observed. The NOAEL can be considered as the initial dose descriptor forming the basis of a risk assessment. The initial dose descriptor may require modification to address specific needs of the risk characterization. Assessment factors (AF, commonly referred by other regulatory jurisdictions as

| | | ECETOC | ECHA TGD |
|---|---|---|---|
| Interspecies | rat –human | 4 | 4 |
| | 'Remaining differences' | | 2.5 |
| Intraspecies | Worker | 3 | 5 |
| | General population | 5 | 10 |
| Exposure Duration | 28 days → 90 days | 3 | 3 |
| | 90 days → 2 years | 2 | 2 |
| | 28 days → 2 years | 6 | 6 |
| Route-to-route | Oral → inhalation | | 2 |

**Fig. 3** Assessment factors for DNEL setting

"modifying" or "uncertainty" factors) are then applied to the POD or modified POD. Such factors address interspecies and intraspecies uncertainty and variability, exposure duration differences, dose–response considerations, and the overall quality of the dataset. The various AFs are multiplied, and the resulting product is used to divide the POD (or modified POD) to derive a DNEL. The DNEL implicates that humans should not be exposed to doses or concentrations above this level. Exposures below the DNEL are considered acceptable and are considered to not pose an unacceptable risk.

The ECHA technical guidance provides standard default assessment factors for DNEL setting. Several reviews showed that the ECHA factors are significantly more conservative than any other currently existing health benchmarks. Consequently the DNELs derived, especially for the worker population, are far lower than existing occupational exposure levels (OELs) utilized in Europe like the MAK and SCOEL values (Kreider and Spencer Williams 2010). Figure 3 provides a comparison of assessment factors as used, e.g., by ECETOC, and as described by the ECHA guidance (ECETOC 2010; ECHA 2009).

Risks are regarded as adequately controlled under REACH when the exposure levels to the substance are below the DNELs. This renders a risk calculation ratio (RCR) below 1. If risks are not under control, an iterative process starts. The chemical safety assessment has to be refined, either by obtaining more data on the properties of the substance or by refining the exposure estimations to achieve more realistic estimates or implementing risk management measures.

As a result of the risk characterization, the registrant defines the appropriate risk management measures to adequately control the risks. The measures are communicated down the supply chain by extended safety data sheets (eSDS). If for a specific use of a substance the risks are not under control, the supplier may designate and communicate a use which he does not support. To this end chapter 1.2 of the safety data sheet ('uses advised against') is the right place for communication.

Exposure scenarios make use of the "use descriptor system" and were derived from the European NACE system for economic activities (ECHA 2010). They describe the conditions under which a substance is manufactured and used. In some cases, certain risk management measures (e.g., protective gloves, respiratory protection) may help render the use under control. All in all exposure scenarios facilitate communication along the supply chain (see Table 4).

**Table 4** Use descriptor system under REACH

| Descriptor types | | (Examples) |
|---|---|---|
| SU | 23 sectors of use | SU 11 : manufacture of rubber products |
| PROC | 25 process categories | PROC 15: use as a laboratory reagent |
| PC | 40 product categories | PC17: hydraulic fluids |
| AC | 39 article categories | AC3-2: electrical batteries and accumulators |
| ERC | 22 environmental release categories | ERC6c: production of plastics |

**Table 5** Evaluation processes under REACH

| Item | By whom? |
|---|---|
| Technical completeness check | ECHA (automatically, under IUCLID) |
| Dossier evaluation (ECHA) | ECHA |
| Substance evaluation (CoRAP) | Competent authorities of the member states |
| Evaluation of test proposals (annex IX und X) | ECHA |

Exposure scenarios are a set of conditions [usually based on a Process Category (PROC code) for workers, Product Category (PC Code) or Article Category (AC) for consumers, or Environmental Release Category (ERC) for the environment] that describe how a substance can be safely used throughout its life cycle. Such scenarios include the necessary operational conditions (OCs) and risk management measures (RMMs). For each exposure scenario, the exposure levels of humans and the environment need to be determined. The exposure scenarios will cover all supported uses and life stages of the substance.

If a downstream user notes that the own uses are not covered in the safety data sheet of the supplier, he can either contact its supplier in order to get the own uses covered or prepare an own CSA. To that end he may make use of the available information in the safety data sheet of the supplier.

The chemical safety assessment is documented in the Chemical Safety Report (CSR). A chemical safety report must always be up-to-date.

A good documentation system is required to ensure that duplication of efforts (assessments, tests) is avoided.

## Evaluation

Registrations dossiers are initially subjected to a technical completeness check (TCC), which has to be distinguished from the phase of dossier evaluation (see Table 5). A completeness check is conducted automatically for each registration dossier before a registration number is allocated, whereas the subsequent dossier evaluation is done on a spot-check basis by ECHA, in general aiming to cover 5 % of the registration files. Additionally, a substance evaluation is being performed for

some specific substances of high concern, being selected in the "Community Rolling Action Plan" (CoRAP). The responsibility for this evaluation is with the different member states which scrutinize whether a substance constitutes a risk to human health or the environment.

## Authorization and Restriction

The REACH regulation intends to filter out substances of very high concern (SVHC). High concern is raised by substances that are CMRs, PBTs, or vPvBs. Apart from these SVHCs which are explicitly mentioned in the regulation, Article 57 contains an opening clause that enables competent authorities to carry out individual case-by-case examinations for further substances. In cases of "scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern," these substances may also be proposed for authorization. Probable candidates for this procedure are endocrine disruptors.

Authorizations of uses for the placing on the market are granted by the Commission if the risks arising from their use are adequately controlled, in principle when derivation of limit values is possible. In those cases where a substance causes an unacceptable risk to human health or to the environment, its substitution has to be considered. The considerations have to take into account whether suitable alternatives are available which are economically and technically feasible. In cases where no adequate control can be ensured (e.g., non-threshold substances), a socioeconomic analysis (SEA) has to be prepared, demonstrating that the benefit of further use of the substance outweighs societal risk.

*Authorized* substances are generally banned except for some specific uses, which have been authorized by the EU Commission after application. In contrast, the use of *restricted* substances is permitted with the exception of some specific uses being prohibited. Apart from a total ban, "Risk Management Options" (RMO) can be applied, by prohibiting certain uses, where risks are NOT adequately controlled.

## Outlook

In the first REACH registration phase, approximately 27,000 dossiers were filed for about 4,600 phase-in substances with a total cost of € 2.1billion. Dossiers are steadily being updated based on new information. In parallel, experience on evaluation is beginning to grow. About 5 % of the dossiers are subjected to a dossier evaluation performed by ECHA. For substance evaluation, the dialogue between the evaluating competent authorities and the registrants has just started (CoRAP). Five years after the REACH regulation came into force, no knowledge is available yet for authorizations and restrictions.

From a global perspective REACH is an unprecedented chemical legislation, which continuously increases the safety information on chemical substances

in the EU. Many new threshold values have been and will be generated, and communication along the supply chain is intense. REACH is a highly complex regulation which requires huge endeavors from all impacted stakeholders.

However, a final conclusion on the overall scientific, societal, and economic impact of REACH cannot yet be drawn. The comprehensive picture will only be available after 2018, when the last phase-in registration phase has ended. Regardless, it is fair to already conclude that REACH, though it is a European-specific regulation, is having global implications on industry. Additionally, many regulatory jurisdictions outside of Europe are closely monitoring the impact of REACH and are considering updating existing regulations or adopting new regulations that model after REACH.

**Acknowledgment** The authors would like to thank Dr. Volker Soballa for helpful discussions and competent feedback.

## References

ECETOC (2004) Technical report no 93 targeted risk assessment. Brussels. ISSN-0773-8072-93

ECETOC (2009) Technical report no 107. Brussels - addendum to targeted risk assessment report no 93. ISSN-0773-8072-107

ECETOC (2010) Technical report no 110. Guidance on assessment factors to derive a DNEL. ISSN-0773-8072-110. Brussels

ECHA (2009) Guidance in a nutshell on chemical safety assessment. http://echa.europa.eu/documents/10162/13632/nutshell_guidance_csa_en.pdf

ECHA (2010) Guidance on information requirements and chemical safety assessment chapter R12: use descriptor system. ECHA 2010-G05—EN. http://echa.europa.eu/documents/10162/13632/information_requirements_r12_en.pdf

ECHA (2012) Guidance to Regulation (EC) no 1272/2008 on classification, labeling and packaging (CLP) of substances and mixtures. http://guidance.echa.europa.eu/docs/guidance_document/clp_en.pdf

European Union (2008) Regulation (EC) no 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures (CLP), amending and repealing Directives 67/548/EEC (DSD) and 1999/45/EC (DPD), and amending Regulation (EC) No 1907/2006

Klimisch HJ, Andreae M, Tillmann U (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5

Kreider ML, Spencer Williams E (2010) Interpreting REACH guidance in the determination of the derived no effect level (DNEL). Regul Toxicol Pharmacol 58:323–329

# Bridging. The Regulation of Toxic Mixtures

Walter Aulmann and Nathan Pechacek

## Contents

Evaluation of the Degree of Mixture Modification:
The Concept of "Minor Modifications" ..... 798
Assessment of Mixtures on the Basis of Existing Toxicological
Information on Similar Mixtures (i.e., "Bridging") ..... 798
Use of Weight of Evidence (WOE) and Expert Judgment ..... 800
References ..... 801

**Abstract**

Although they have been in use in certain sector-specific applications already in the past (e.g., in the detergents industry under the so-called trustee-expert model) (AISE 1993), bridging principles are increasingly gaining a broad regulatory acceptance for the hazard assessment of mixtures under the new globally harmonized system for classification and labelling of chemicals (GHS). This chapter explains the basic concepts and highlights their specific relevance for classification purposes.

To ensure accurate hazard classification without resorting to unnecessary testing, existing toxicity data of a relevant reference mixture should be applied to a similar mixture when technically justifiable. At a practical level, a reference mixture is defined as a mixture of known composition which (1) has been tested for a toxicological or ecotoxicological endpoint of interest (e.g., skin or eye irritation/corrosion) or (2) for which reliable information is available allowing the determination of its classification and labelling in compliance with the

W. Aulmann (✉)
Ecolab Deutschland GmbH, Monheim, Germany
e-mail: walter.aulmann@ecolab.com

N. Pechacek
Ecolab Inc, St. Paul, MN, USA
e-mail: nathan.pechacek@ecolab.com

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_114, 

existing regulatory framework. This should also include data on applicable physical-chemical properties (e.g., mixture pH and reserve alkalinity/acidity in the case of pH extreme formulations), as well as information on the toxicological properties and classification(s) of individual substances.

EU Regulation (EC) No 1272/2008 on the classification, labelling, and packaging of substances and mixtures ("CLP Regulation") incorporates the concept of using reference mixtures by suggesting the following steps in the process:

- Evaluation of the degree of mixture modification which introduces the concept of "Minor Modification"
- Assessment of mixtures on the basis of existing toxicological information on similar mixtures (i.e., bridging principles)
- Use of weight of evidence and expert judgment

## Evaluation of the Degree of Mixture Modification: The Concept of "Minor Modifications"

A separate and complete toxicity assessment of a modified mixture does not need to be carried out if the modification, in comparison to a classified reference mixture for which toxicity data are available, is only minor. In this context, "minor" means that the modification lies within the permitted variations in initial concentrations of the hazardous constituents, as shown in Table 1.

Constituents contained in mixtures at low levels are assumed to not generally influence the toxicity profile of a mixture. Such constituents present in a mixture below regulatory-specified concentration cutoff limits (i.e., generic or chemical specific) for a toxicity endpoint of consideration generally do not have to be considered for calculation of classification according to the GHS additivity approach (United Nations 2005). If the composition variations introduced in the modified mixture exceed those that are considered "minor," then the mixture should be evaluated with additional bridging principles to determine the acceptability of applying existing toxicological information from a potentially similar mixture.

## Assessment of Mixtures on the Basis of Existing Toxicological Information on Similar Mixtures (i.e., "Bridging")

In the absence of adequate toxicity information for a mixture, the potential applicability of available toxicity information for similar mixtures should be considered. The underlying concept encompasses the comparison (i.e., "bridging") of the available technical information pertinent to the assessment of the toxicological endpoint of interest (e.g., skin corrosion/irritation profile) of well-defined reference mixtures to similar mixtures.

A prerequisite for bridging data from a reference mixture to another mixture are the availability of sufficient compositional and physical-chemical information on both mixtures. This generally requires the following information of relevant

**Table 1** Permitted composition variations for consideration as a minor modification

| Initial concentration range of the constituent[a] | Permitted variation in initial concentration of the constituent |
|---|---|
| <2.5 % | ±30 % |
| 2.5 % < C < 10 % | ±20 % |
| 10 % < C < 25 % | ±10 % |
| 25 % < C < 100 % | ±5 % |

[a]Constituents do not necessarily have to be aligned from a CASRN perspective. Constituents with *different* CASRNs with similar modes of action and the same hazard category can be clustered into the same constituent group

mixture constituents: identification at the CASRN level, concentration(s) in the relevant mixture(s), toxicological profile, and classification by any applicable regulatory authority(ies).

The following "bridging principles" are considered:

- *Concentration of highly hazardous mixtures* – Where a mixture is already classified in the highest hazard category, then it can be assumed that a more concentrated mixture will also be in the highest hazard category.
- *Dilution* – Where the test mixture is diluted with a substance (diluent) that has an equivalent or lower hazard category than the least hazardous original substance, then it can be assumed that the respective hazard of the new mixture is equivalent to that of the original tested mixture.
- *Interpolation within one toxicity category* – Defines how much the concentration of a hazardous mixture may vary without changing the classification. Usually two reference mixtures are needed which are broadly similar and in the same hazard category for a given endpoint. Typically, a third mixture has the same hazardous constituents in concentrations that are between those of the two reference mixtures and can be classified the same as the reference mixtures. It is noted that this approach may allow greater variation for individual constituents than those permitted in the "Minor" Modification method highlighted in Table 1.
- *Substantially similar mixtures* – Notes that if constituents with the same hazard category and the same potency are exchanged in a mixture, the hazard category of the mixture does not change. Potency may be expressed by specific concentration limits.
- *Batching* – If a batch of a mixture is produced under a controlled process, then it is assumed that the hazards of each new batch are equivalent to those of previous batches. This method cannot be used where there is significant variation between batches which may affect hazard classification.

The bridging principles "Interpolation within one toxicity category" and "Substantially similar mixtures" are considered to be the most relevant for comparison of a reference mixture to another mixture. Detailed rules for the use of bridging principles are given in the CLP Regulation and further illustrated in the Guidance on the application of Regulation (EC) No 1272/2008.

## Use of Weight of Evidence (WOE) and Expert Judgment

Generally, the criteria for using the bridging principles for the classification of mixtures on the basis of a reference mixture are narrow and have limited use. Subsequently, the use of expert judgment to support a classification or non-classification of a previously unassessed mixture is critical.

Bridging test data require knowledge of the chemistry and toxicological profile of the product categories in question, chemical factors impacting the toxicity profile of the mixture, as well as the appropriate expertise to weigh the relevance of the evidence of different types of test systems and information. The latter is particularly important when the classification of the reference mixture is based on heterogeneous data sets including data from scientifically valid but not fully validated methodologies or in cases where conflicting information is available.

Given the complexity of these issues, a WOE determination based on expert judgment is advocated. Classification of a mixture based on WOE requires the consideration of *all* available information bearing on the determination of a given health endpoint. This includes results of suitable in vitro tests, relevant animal data, chemical category information, quantitative structure activity relationship (QSAR) results, and human experience taken from occupational, epidemiological, clinical, and well-documented case report studies. The quality and consistency of the information has to be given appropriate consideration. Positive and negative results should be assembled together in a single WOE determination.

The priority given to different pieces of information is generally determined on a case-by-case basis using expert judgment. Some guidance is provided by the CLP Regulation to address conflicting findings (European Union 2008). From this guidance, some basic qualitative rules can be established for the classification and labelling of mixtures:

1. When several studies with conflicting results are available for one reference mixture, the quality and reliability of the studies, as well as their relevance for classification and labelling, have to be taken into account. Toxicity data derived from in vitro studies (animal or clinical) are generally viewed as acceptable if they receive a reliability score of 1 or 2 according to the Klimisch criteria (Klimisch et al. 1997).

   When the reliability of studies is comparable, studies considered to be most relevant for the particular hazard in humans are given more weight in the assessment. A general relevance hierarchy of the following is observed: human studies > animal studies > in vitro studies. However, deviation from this hierarchy may occur on a case-by-case basis when study specifics are considered. Hence, the final assessment of which studies are most relevant to the assessment of the human hazard is subject to expert judgment.
2. In case the study results of two or more reference mixtures are in conflict, the degree of similarity of the reference mixture to the other mixture, in addition to the factors mentioned above, will need consideration. The data from the reference mixture that is judged to be the closest to the comparison mixture under assessment should be given the greatest weight.

3. In cases of no appreciable differences in study reliability or ranking, the study giving rise to the highest concern should be taken as the key study for the classification of the comparison mixture.

## References

European Detergents Association (AISE) (1993) AISE/IKW trustee expert model, http://www.ikw.org/haushaltspflege/themen/informationen-fuer-hersteller/trustee-expert-model-treuhaender-gutachter-modelltgm/f5127bd59cb449c96e3bb30c34ebe2d3/

European Union (2008) Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006

Klimisch HJ et al (1997) A systematic approach to evaluating the quality of experimental toxicological and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5

United Nations (2005) Globally harmonized system of classification and labelling of chemicals (GHS) first revised edition. United Nations, New York/Geneva, ISBN 92-1-116927-5

# Risk Assessment of Food Additives

Rainer Gürtler

## Contents

Introduction and Legal Background ..... 804
Data required for the Risk Assessment ..... 805
Toxicokinetics ..... 806
Genotoxicity ..... 806
Subchronic and Chronic Toxicity and Carcinogenicity ..... 807
Reproductive and Developmental Toxicity ..... 808
Other Studies ..... 808
Derivation of an Acceptable Daily Intake ..... 809
Perspective ..... 811
References ..... 811
Resources ..... 812

**Abstract**

Food additives are substances used for technological purposes, such as preservation, sweetening, or coloring, during the production of food. The requirements for the risk assessment of food additives are described in a recent guidance document of the European Food Safety Authority (EFSA). According to this guidance, a tiered approach which balances toxicity data requirements against the risk is applicable for the risk assessment of food additives. The approach was established to evaluate the following core areas: toxicokinetics, genotoxicity, toxicity (encompassing subchronic toxicity, chronic toxicity, and carcinogenicity), and reproductive and developmental toxicity. Additional studies on other toxicological endpoints may be required on a case-by-case basis. The approach consists of three tiers. It provides guidance to applicants in designing their toxicity testing strategy in which the decision on the requirement for further

R. Gürtler
Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany
e-mail: Rainer.Guertler@bfr.bund.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_116, 

testing can be based on the results of certain initial studies. While maintaining the high level of consumer safety, the application of this tiered approach could result in a smaller number of animal tests or more refined animal studies, compared to the requirements applied before, and thus benefit animal welfare. This chapter provides the legal background and delineates the principles and requirements for the risk assessment of food additives based on the EFSA guidance for submission for food additive evaluations.

## Introduction and Legal Background

The use of chemical substances in order to maintain the quality of food has been a common procedure for a very long time. Accordingly, questions about the safety of these substances were addressed already many years ago. In 1955, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations established the *Joint FAO/WHO Expert Committee on Food Additives* (*JECFA*). In its first report, JECFA provided general principles for the use of food additives and pointed out that "critically designed animal tests of the physiological, pharmacological and biochemical behavior of a proposed additive can provide a reasonable basis for evaluating the safety of use of a food additive at a specified level of intake" (WHO 1957). Procedures for the testing of food additives were published by JECFA in 1958 (WHO 1958). At the European level, the Scientific Committee for Food (SCF) was established by the Commission of the European Communities in 1974. The SCF evaluated food additives and advised the European Commission. In the course of reorganization of scientific committees, the SCF was renamed in 1997 as *Scientific Committee on Food* (*SCF*). The SCF was active until the European Food Safety Authority (EFSA) was established in 2003. Since then, food additives have originally been evaluated by the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) which was, however, split into the Panel on Food Additives and Nutrient Sources Added to Food (ANS Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) in 2008. Thus, currently the EFSA ANS Panel is responsible for the risk assessment of food additives in the European Union.

Any substances added intentionally to food including flavoring substances and processing aids might be considered as food additives; however, according to European legislation, the term *food additive* is restricted to those substances which are used for technological purposes only. According to Article 3 of Regulation (EC) No. 1333/2008, a food additive "shall mean any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of

such foods." According to Article 6 of Regulation (EC) No. 1333/2008, a permission for the use of food additives can only be given for a substance provided that "(a) it does not, on the basis of the scientific evidence available, pose a safety concern to the health of the consumer at the level of use proposed; (b) there is a reasonable technological need that cannot be achieved by other economically and technologically practicable means; and (c) its use does not mislead the consumer." Accordingly, the safety of food additives is to be assessed by the *EFSA ANS Panel* prior consideration by the European Commission for market authorization. Applicants seeking such an authorization are required to provide all the relevant data.

## Data required for the Risk Assessment

The requirements for the risk assessment of food additives are described in a guidance document adopted by the ANS Panel in 2012 (EFSA 2012a). This guidance replaces the guidance established in 2001 by the European Commission's former SCF (SCF 2001) on which EFSA's AFC and ANS Panels based their risk assessments previously. According to the new guidance, a *tiered approach* which balances toxicity data requirements against the risk is applicable for the risk assessment of food additives. The approach was established to evaluate the following core areas: toxicokinetics, genotoxicity, toxicity (encompassing subchronic toxicity, chronic toxicity and carcinogenicity), and reproductive and developmental toxicity. Additional studies on other toxicological endpoints may be required on a case-by-case basis. The approach consists of three tiers. It provides guidance to applicants in designing their toxicity testing strategy in which the decision on the requirement for further testing can be based on the results of certain initial studies. While maintaining the high level of consumer safety, the application of this tiered approach could result in a smaller number of animal tests or more refined animal studies, compared to the requirements applied before, and thus benefit animal welfare.

According to EFSA's *guidance for submission of food additive applications*, a minimum set of data is required for all food additives at Tier 1. It covers data on absorption (i.e., systemic availability), genotoxicity in vitro, and subchronic toxicity. Depending on the results, further toxicity studies are required at Tier 2 and Tier 3. Tier 2 studies will be required in order to generate more extensive data for substances which are absorbed, demonstrate toxicity or genotoxicity in Tier 1 tests. If higher tier testing is required based on results in one of the core areas, such testing would only be required in this core area but not in the others, e.g., where results from the subchronic toxicity study require further Tier 2 studies but Tier 1 in vitro genotoxicity is clearly negative, there would be no need for Tier 2 follow-up of genotoxicity. Tier 3 testing should be performed on a case-by-case basis. Results at higher tiers will in principle supersede results observed at lower tiers.

## Toxicokinetics

The aim of investigations on systemic availability is to establish whether the substance or its breakdown products are absorbed from the gastrointestinal tract. In this respect, the physicochemical properties (e.g., molecular weight, hydro- and lipophilicity) of the substance should be considered as well as models for bioavailability from in vitro and in vivo studies. Demonstration of negligible absorption, either through experimental studies or based on theoretical considerations, may be considered as a scientific justification for not undertaking higher tiered toxicological studies, provided that the results of the genotoxicity tests are clearly negative and no toxicity in the subchronic toxicity study is observed at Tier 1. Absorption data available for structurally related substances may contribute useful information. However, the required sensitivity to determine negligible absorption levels will generally necessitate in vivo studies using labeled compounds. If the absorption cannot be considered negligible at Tier 1, further data on absorption, distribution, metabolism, and excretion (*ADME*) including identification and quantification of metabolites are required at Tier 2. Basic single dose toxicokinetic parameters, e.g., area under the curve of plasma concentration of the compound against time after oral administration, maximum concentration, time to reach maximum concentration, elimination half-life, and bioavailability, should be determined based on in vivo studies according to the Organisation for Economic Cooperation and Development (OECD) Technical Guidance (TG) No. 417. A range of dose levels should be applied in order to examine the linearity of kinetic parameters and possible saturation. The trigger for Tier 3 studies would be limited or slow excretion or any other mechanism resulting in bioaccumulation. In such a case, studies with repeated doses in experimental animals or human kinetic data from volunteer studies may be required.

## Genotoxicity

The in vitro investigations on *genotoxicity* at Tier 1 should cover gene mutations, and structural and numerical chromosomal alterations, as recommended by the EFSA Scientific Committee (EFSA 2011). In line with this recommendation, a bacterial reverse mutation assay (OECD TG 471) and an in vitro mammalian cell micronucleus test (OECD TG 487) are required for all food additives. Any inconclusive, equivocal, or positive results observed with in vitro tests at Tier 1 should be further investigated. According to EFSA's guidance document on *genotoxicity testing strategies* (EFSA 2011), inconclusive and equivocal test results may be clarified by further in vitro testing, but in vivo studies might also become necessary. A positive result in a Tier 1 study requires follow-up at Tier 2 in order to investigate whether the hazard is expressed in vivo. In case of negative in vivo genotoxicity tests, it is crucial to demonstrate, based on cytotoxicity or kinetic data, that the target tissue was exposed. Suitable tests for a follow-up of results from

Tier 1 studies are an in vivo micronucleus test (OECD TG 474), a transgenic rodent somatic and germ cell gene mutation assay (OECD TG 488), and an in vivo Comet assay (an OECD TG is still pending; however, an EFSA report on minimum criteria for the acceptance of in vivo alkaline Comet assay reports (EFSA 2012b) could be consulted until an OECD guideline is available). Detailed advice on strategies for genotoxicity testing is given in an opinion of the EFSA Scientific Committee (EFSA 2011). Genotoxicity in vivo is to be considered as an adverse effect per se, even in cases where cancer bioassays are negative, since genotoxicity is also implicated in diseases other than cancer and one of the aims for genotoxicity testing is to identify substances which could cause heritable damage in humans (EFSA 2011). There is no Tier 3 for genotoxicity testing. If a substance is positive at Tier 2, it is usually assumed that it is a somatic cell genotoxin and will be potentially carcinogenic and also mutagenic in germ cells. Such substances are not considered acceptable as food additives. Hence, careful consideration should be given to animal welfare before conducting any further in vivo studies (EFSA 2012a). It should, however, be noted that the assessment of genotoxicity is generally based on all available data and that the quality and reliability of data is taken into consideration (EFSA 2011). Accordingly, it is important to differentiate between indication and clear evidence for genotoxicity in vivo. An indication for in vivo genotoxicity would require further clarification.

## Subchronic and Chronic Toxicity and Carcinogenicity

A *subchronic toxicity* study should be performed for a period of at least 90 days in rodents (OECD TG 408) at Tier 1. The design of this study should be modified to include the assessment of some additional parameters described in the more recent guideline on repeated-dose 28-day oral toxicity studies in rodents (OECD TG 407). Toxicity observed in the subchronic toxicity study would trigger investigation of chronic toxicity at Tier 2. A chronic toxicity study may reveal effects which were not observed in subchronic studies, or it may confirm effects at the same or even lower doses than those applied in the subchronic study. *Chronic toxicity* and *carcinogenicity* are to be investigated at Tier 2 either separately (OECD TG 452 and 451) or in a combined study (OECD TG 453). The EFSA ANS Panel noted in its guidance document that there was a considerable debate in recent years in the area of risk assessment of pharmaceuticals about the value of the two rodent species approach for the evaluation of carcinogenicity and that this debate has led to the suggestion that there may be no need to continue investigating carcinogenicity routinely in two species (EFSA 2012a). The Panel supported this position and recommended, deviating from former requirements, to perform the studies with a single species only, generally the rat. However, under certain circumstances, e.g., indications for species specific effects, a study in a second species may become necessary. At Tier 3, studies on the *mode of action* may be performed if required.

## Reproductive and Developmental Toxicity

The subchronic toxicity study performed at Tier 1 provides only limited information on reproductive toxicity and no information on developmental toxicity. It provides information on potential effects on the reproductive organs and, if assessed, the estrous cycle, but it does not cover fertility and the whole reproductive cycle. However, the decision on whether studies on reproductive and developmental toxicity are required could be based on the outcome of the subchronic toxicity study, provided that the absorption of the substance is negligible. Studies on *reproductive and developmental toxicity* will generally be required for substances which are systemically available. In addition, any indication for effects on reproductive organs or parameters in the subchronic toxicity study will trigger testing for reproductive and developmental toxicities at Tier 2. This comprises a prenatal developmental toxicity study (OECD TG 414) in rabbits and an Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443). According to the OECD Guideline 443, the EOGRTS covers parameters on reproductive endpoints, pre- and postnatal developmental endpoints, and specific endpoints on developmental neurotoxicity, immunotoxicity, and *endocrine disruption*. The EOGRTS protocol includes the assessment of parameters which can be used for the decision on whether assessment of a second generation is required. The risk assessment may be based on the results of these studies; however, the effects observed might trigger additional studies at Tier 3, e.g., on endocrine effects, developmental neurotoxicity (OECD TG 426), and mode of action (EFSA 2012a).

## Other Studies

In addition to the core areas for evaluation, other studies may be required for the risk assessment of food additives, e.g., studies on *immunotoxicity*, hypersensitivity, and food intolerance. Likewise, human studies, e.g., ADME studies and tolerance studies, could provide useful information (EFSA 2012a). However, the quality and reliability of tolerance studies is mainly dependent on the study design. The prevalence of intolerances against food additives which are already on the market could reliably only be determined with placebo-controlled double-blind oral challenge tests, a condition which is met only by a few studies (Simon 2003; Wilson and Bahna 2005).

Several symptoms have been attributed to food additive exposure, but the cause-and-effect relationship has not been well demonstrated in all (Wilson and Bahna 2005). *Allergenicity* may result from the consumption of food additives which are proteins or peptides, e.g., lysozyme (E 1105) and invertase (E 1103), while *pseudoallergenicity* could be due to other (nonprotein) food additives. The EFSA ANS panel noted in its guidance document (EFSA 2012a) that there are no validated studies in laboratory animals which would allow assessment of the potential of a substance to cause allergic reactions in susceptible individuals

following oral exposure. The panel recommended to consult the EFSA guidance on the allergenicity of genetically modified organisms (EFSA 2010) if the additive is a potential allergen, e.g., a protein or a peptide, or contains residues of proteins or other known allergenic molecules (EFSA 2012a). However, the panel pointed out that defining a threshold or a No Observed Adverse Effect Level (NOAEL) is difficult and that, accordingly, an adverse effect would be taken into account on a case-by-case basis (EFSA 2012a).

For new food additives, an indication for immunotoxicity may be obtained from the studies performed at Tier 1 and Tier 2. The subchronic toxicity study in rats (OECD TG 408) performed at Tier 1 involves investigation of a number of parameters that may be indicative of an immunotoxic or immunomodulatory effect, e.g., changes in spleen and thymus weights relative to body weight in the absence of overt toxicity, histopathological changes in these and other organs of the immune system, as well as changes in total serum protein, albumin:globulin ratio, and in the hematological profile of the animals. Such effects may be confirmed or, alternatively, observed for the first time in Tier 2 studies, notably the EOGRTS (OECD TG 443), but also in chronic toxicity/carcinogenicity studies conducted according to OECD TGs 452, 451 or 453. In the EOGRTS, a cohort of animals is specifically dedicated to assess the potential impact of exposure on the developing immune system. If the results from these studies provide indication for immunotoxicity, additional studies may be performed at Tier 3 in order to investigate the underlying mechanisms of the effects seen and to assess their relevance for the risk assessment (EFSA 2012a). The EFSA ANS panel noted in its guidance document that there are no OECD guidelines for such extended specialized studies and recommended to consult a WHO guidance for immunotoxicity risk assessment for chemicals (WHO 2012).

## Derivation of an Acceptable Daily Intake

A main purpose of the risk assessment of food additives is the derivation of an *Acceptable Daily Intake* (*ADI*) as a health-based guidance value. An ADI is the estimated maximum amount to which individuals may be exposed daily over their lifetimes without appreciable health risk. Based on the most sensitive endpoint from a range of toxicological hazards and their dose–response relationships, a No Observed Adverse Effect Level (NOAEL) or a benchmark dose lower confidence limit (BMDL) is established and used as point of departure for deriving an ADI (EFSA 2009, 2012a). This point of departure is divided by an uncertainty factor which covers uncertainties due to the extrapolation of data from animal studies to the human situation as well as individual variabilities. Generally, a factor of 100 is applied as a default value. A smaller factor could be applied if human data are available, e.g., toxicokinetic data, which allow for comparison of internal doses in experimental animals and humans. A factor larger than 100 would be applicable if additional uncertainties were to be covered. The ADI is expressed in mg per kg body weight and is established for compounds for which a threshold mechanism of

toxicity can be demonstrated. The ADI is applicable to the general population except infants below 12 weeks (WHO 1978; SCF 1998).

Based on a numerical ADI and an exposure assessment, conditions of use, e.g., maximum level for certain food categories, can be derived for food additives by risk managers. The current conditions of use applicable in the European Union are defined in Commission Regulation (EU) No. 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No. 1333/2008. For some food additives, there are no numerical ADIs. For substances of very low toxicity which, on the basis of the available data on toxicity and intake, do not represent a hazard to health, e.g., citric acid and citrates (E 330–E 333), the outcome of the evaluation by JECFA or SCF was "ADI not specified." Such substances may be used at "*quantum satis*" level. According to Regulation (EC) No. 1333/2008, "'*quantum satis*' shall mean that no maximum numerical level is specified and substances shall be used in accordance with good manufacturing practice, at a level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled."

An occasional exceedance of the ADI might be tolerated provided that (i) there is still a tolerable *margin of safety* between the NOAEL and the exposure, (ii) the effect on which the ADI was derived is not observed after acute exposure and (iii) the exceedance does not occur frequently so that the long-term exposure is not significantly affected (Gürtler 2010).

For compounds for which no safe level of exposure can be anticipated, for example, genotoxic carcinogens, an ADI would not be established. Such substances would not be acceptable as food additives. For the assessment of the risk resulting from levels of unavoidable contaminants or residuals in the additive which are genotoxic and carcinogenic, the ANS Panel generally uses the *Margin of Exposure* (MOE) approach described in an EFSA Scientific Committee opinion (EFSA 2005, 2012c). The *Threshold of Toxicological Concern* (TTC) approach would be considered for the evaluation of unavoidable genotoxic residuals, for which carcinogenicity data are not available. In such cases, exposures for high level consumers at the proposed maximum use levels would be expected to be below the TTC for genotoxic compounds of 0.15 μg/person/day (EFSA 2012d). The TTC approach could also be applied to low-exposure substances such as impurities, metabolites, and degradation products of deliberately added substances for which genotoxicity data may be unavailable (EFSA 2012a).

In 2010, a re-evaluation program was established in the European Union for all existing food additives. According to Regulation (EU) No. 257/2010, food additives which were permitted before 20 January 2009 shall be subject to a new risk assessment carried out by EFSA. In order to prioritize the evaluation of more than 300 food additives, priority criteria such as time since the last evaluation by SCF or EFSA, the availability of new scientific evidence, and exposure were applied. The highest priority was assigned to *food colors* for which the evaluations were to be completed until the end of 2010, while the lowest priority was applied to *sweetener*, except aspartame, which should be evaluated until the end of 2020.

## Perspective

The current EFSA guidance document for food additive evaluations provides a flexible approach which acknowledges the use of integrated testing strategies and alternative methods in order to complement the data required in this guidance. In some cases, e.g., for the evaluation of aspartame, physiologically based pharmacokinetic modeling was applied and it may be expected that such modeling will become more relevant for the evaluation of additives in future. "In silico/(quantitative) structure activity relationships (QSAR)" methods may contribute to evaluate impurities and metabolites. Special studies may be used to investigate the mode of action and "*Omics*" *methods* might contribute in this respect. However, "Omics" methods do not have any relevance for the risk assessment of food additives as yet. In the future, "Omics" and "*In silico*/(*Q*)*SAR*" *methods* might also be used for screening purposes before embarking on any Tier 1 testing.

## References

COMMISSION REGULATION (EU) (2011) No. 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No. 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Off J Eur Union L 295/1

EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA J 282:1–31

EFSA (2009) Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. EFSA J 1150:1–72

EFSA (2010) EFSA panel on genetically modified organisms (GMO); scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA J 8(7):1700

EFSA (2011) EFSA Scientific Committee; scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA J 9(9):2379

EFSA (2012a) EFSA panel on food additives and nutrient sources added to food (ANS); guidance for submission for food additive evaluations. EFSA J 10(7):2760

EFSA (2012b) European Food Safety Authority; minimum criteria for the acceptance of in vivo alkaline comet assay reports. EFSA J 10(11):2977

EFSA (2012c) Statement on the applicability of the Margin of Exposure approach for the safety assessment of impurities which are both genotoxic and carcinogenic in substances added to food/feed. EFSA J 10(3):2578

EFSA (2012d) Exploring options for providing preliminary advice about possible human health risks based on the concept of threshold of toxicological concern (TTC). EFSA J 10(7):2750

Gürtler R (2010) Safety of food additives from a German and European point of view. Bundesgesundheitsblatt Gesundh Gesundh 53(6):554–560 (Article in German)

REGULATION (EC) (2008) No. 1333/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on food additives. Off J Eur Union L 354/16

SCF (1998) Opinion of the Scientific Committee of Food on the applicability of the ADI (acceptable daily intake) for food additives to infants. 17 Sept 1998. http://ec.europa.eu/food/fs/sc/scf/out13_en.html

SCF (2001) Guidance on submissions for food additive evaluations by the Scientific Committee on Food. Opinion expressed on 11 July 2001. http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

Simon RA (2003) Adverse reactions to food additives. Curr Allergy Asthma Rep 3(1):62–66

WHO (1957) General principles governing the use of food additives. First report of the Joint FAO/WHO Expert Committee on Food Additives. FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129. http://whqlibdoc.who.int/trs/WHO_TRS_129.pdf
WHO (1958) Procedures for the testing of intentional food additives to establish their safety for use (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958. http://whqlibdoc.who.int/trs/WHO_TRS_144.pdf
WHO (1978) Evaluation of certain food additives. Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 617. World Health Organization, Geneva. http://whqlibdoc.who.int/trs/WHO_TRS_617.pdf
WHO (2012) Guidance for immunotoxicity risk assessment for chemicals. IPCS Harmonization Project Document; No. 10. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf
Wilson BG, Bahna SL (2005) Adverse reactions to food additives. Ann Allergy Asthma Immunol 95(6):499–507

## Resources

European Food Safety Authority (EFSA) publications. http://www.efsa.europa.eu/en/publications.htm. Accessed 27 July 2013
Joint FAO/WHO Expert Committee on Food Additives (JECFA) publications and database of evaluation summaries. http://www.who.int/foodsafety/chem/jecfa/publications/en/index.html. Accessed 27 July 2013
Organisation for Economic Cooperation and Development (OECD) Guidelines for the testing of chemicals, Section 4, Health effects. http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788. Accessed 27 July 2013
Scientific Committee on Food (SCF) outcome of discussions. http://ec.europa.eu/food/fs/sc/scf/outcome_en.html. Accessed 27 July 2013

# Risk Assessment of Food Components with Botanical Origin

Birgit Dusemund

## Contents

Botanicals in Food – an Introduction ..... 813
Specifics in the Risk Assessment of Botanicals: Some Examples ..... 814
Guidance for Safety Evaluation: An Approach by EFSA ..... 817
Legal Background ..... 819
Future Perspectives ..... 822
References ..... 823

**Abstract**

In view of a persisting trend to use botanicals and botanical preparations especially in food supplements but also in replacing synthetic additives or flavorings in food, aspects of the safety evaluations of plants and plant-derived components in food are discussed. In addition risk assessment regarding unintentional intake of botanical ingredients via contamination of food with whole plants, plant parts, or their components is addressed. Examples are presented taking the complexity in composition of botanicals and their matrix effects into account. Requirements and principles of present guidelines for the safety evaluation of botanicals and their components for food use, including a presumption of safety approach based on existing knowledge, are outlined. The essentials of relevant regulatory frameworks are summarized, and an outlook on possible future developments is given.

## Botanicals in Food – an Introduction

*Plant*-derived food forms an intrinsic part of traditional diets all over the world. Furthermore, diets rich of vegetables and fruits are associated with health benefits.

B. Dusemund
Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany
e-mail: Birgit.Dusemund@bfr.bund.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_117, 

As part of a continuing trend to a preference for natural, "organic," *herbal*, and plant-based products, especially *food supplements* (synonym: *dietary supplements*) based on *botanicals*[1] and their preparations[2] became widely available on the European and North American market. This was accompanied by the tendency to replace synthetic food additives or flavorings in food products by those of botanical origin. Novel foods or genetically modified foods represent another source of intentional dietary intake of botanicals and are not dealt with here (see chapter "▶ Risk Assessment of Novel Food and Genetically Modified Food and Feed"). Unintentional exposure of consumers to botanicals and their components is occurring via *contamination* of food with whole plants, plant parts, or their ingredients, e.g., as a consequence of inadequate harvesting or cleaning methods, adulteration or due to carry over from animal feed through meat or animal products, or via mistaking edible and nonedible plants (Speijers et al. 2010; Koleva et al. 2012; Dusemund et al. 2012). The use and occurrence of plants and their components in feed are not a subject of this chapter.

The use of a large variety of botanicals in food supplements and related products includes that of certain medicinal plants. In particular plants used for therapeutical purposes may contain a diversity of biologically active substances such as alkaloids; cardiac glycosides; mono-, di-, tri-, and sesquiterpenes; and anthraquinones for which the induction of desired or adverse effects depends on dosage. As known from many lawsuits the borderline between plant-based food supplements (not subjected to an authorization procedure in the European Union (EU)) and plant-based medicinal products (subjected to an authorization procedure in the EU) is often difficult to define. In relevant literature the need of an EU-wide harmonized approach based on appropriate systematical safety evaluations of botanicals and botanical preparations used in food supplements is described to assure that intake doses of biological active plant components in these products are within safe limits (EFSA 2009a, b; Silano et al. 2011).

## Specifics in the Risk Assessment of Botanicals: Some Examples

Independent from the role of the botanical, being either added for sensory, technological, or health purposes to a food product or occurring as a natural typical component or an impurity in food, risk assessments of botanicals and their preparations differ from that of clearly defined single substances as a component of food. Thus, it has to be considered that botanicals generally consist of a *complex mixture of phytochemicals*. Regarding the active principle of interest for technological or health reasons, the purity is often rather low, and the remaining substances are not characterized sufficiently. A botanical preparation of a specific species may be

[1]This term refers to plants, including algae, fungi, and lichens, and parts of plants as a whole or cut.

[2]This term refers to preparations obtained by all kind of processing, e.g., pressing, extraction, fractionation, concentration, drying, and/or fermentation.

represented by different extracts varying in composition, due to the use of different source materials (e.g., different botanical subspecies, different geographical origin and conditions of growth and harvesting, different parts of a plant) and different extraction methods and solvents. In case of selective extractions enrichments of natural ingredients of concern or contaminants such as heavy metals or pesticides may occur. Pharmacological and toxicological effects of botanical products are usually associated with their contents of secondary plant metabolites which can vary among plants belonging to the same species or variety as a consequence of multiple biotic and abiotic factors. These parameters have to be taken into account by defining the *identity and specifications* of botanical preparations which of cause is also basically important to exclude adulteration and misidentification of plants. Furthermore it has to be ascertained in the risk assessment that specifications of the products of trade and those investigated in the toxicity testing are the same or allow read across.

These reasonings may be illustrated by an example. For the natural botanical food color lutein (E 161b), belonging to the category of food additives, the content of total carotenoids/saponified carotenoids as coloring matter may be as low as 5 % when extracted from grass, nettle, lucerne, or spinach but amounts to $\geq$ 60 % when *Tagetes erecta* flowers are used as a source. While the EFSA (European Food Safety Authority) Panel on Food Additives and Nutrient Sources Added to Food (ANS) in the process of reevaluation of food additives could establish an ADI of 1 mg/kg body weight (bw)/day for the more concentrated extracts (total carotenoids $\geq$ 60 %) from *Tagetes erecta* flowers, it decided that the database available is too limited to conclude that the ADI also applies to lutein preparations of lower purity or from other sources (EFSA 2010, 2011a).

Furthermore, in a botanical biologically active substances usually occur accompanied by chemically related compounds formed, e.g., as precursors or byproducts of biosynthesis pathways. Due to similar structures a variety of components of a botanical or botanical preparation may have the same structural alerts for specific toxicity endpoints, may react as agonists or antagonists regarding receptor-mediated reactions, or may compete for binding sites in metabolizing enzymes responsible for activation, deactivation, or elimination. In consequence *interactions with accompanying ingredients* which may weaken or enhance the toxic effects of a known substance of concern have to be considered in the risk assessment of plant materials (*matrix effects*). Thus, in general, basing the risk assessment of botanicals, botanical preparations, or botanical contaminants on the exposure and toxicity data of only one active ingredient and ignoring the accompanying ones may be inappropriate. It also becomes evident that breeding methods, influencing biosynthesis pathways, may change the pattern in which bioactive substances occur in a botanical and thus affect its toxicity profile. If botanical preparations of different plants are used in combinations, which is frequently observed, the possibilities of interactions increase.

Possible interactions including synergistic effects have been addressed, e.g., in the risk assessments of botanical contaminants in food and feed recently performed by national authorities and EFSA. Evaluating the contamination of poppy seeds, derived from *Papaver somniferum*, by opium alkaloids, in addition to the presence

of morphine that of codeine as a precursor in the biosynthesis of morphine, has been considered, both alkaloids acting as agonists binding to the μ-opioid receptors (BfR 2005; EFSA 2011b). An altered pattern of alkaloids was, e.g., observed in Australian poppy seeds originating from poppy cultivars developed by genetic regulation of certain enzymatic biosynthesis processes to give a high yield of thebaine and oripavine, two other intermediates in the biosynthesis of morphine, which are used as precursors in drug synthesis (EFSA 2011b). In the risk assessment of ergot alkaloids occurring in the sclerotia of *Claviceps purpurea*, which are contaminating grain, reference is made to a sum of ergot alkaloids including, i.a., ergometrine, ergotamine, ergosine, ergocristine, ergocryptine, and ergocornine. They have in common the tetracyclic ergoline ring system which is associated with their activity as ligands for adrenergic, serotonergic, and dopaminergic receptors (EFSA 2012a). Additive toxic effects have also been taken into account in the risk assessments of pyrrolizidine alkaloids, which have been performed for the contamination of salad with parts of *Senecio vulgaris* (BfR 2007) and for the occurrence of pyrrolizidine alkaloids in honey (BfR 2011a; EFSA 2011c). A cumulative assessment approach is recommended for all 1,2-unsaturated pyrrolizidine alkaloids, the double bound being a prerequisite for metabolic activation to genotoxic carcinogens (COT 2008; BfR 2011a; EFSA 2011c). Interactions may also play a role regarding the toxicokinetic and toxicodynamic effects of polyphenols contained in green tea extracts derived from the leaves of *Camellia sinensis* which are inter alia used in food supplements. It has been hypothesized that the principal ingredient (−)-epigallocatechin-3-*O*-gallate (EGCG), taken as part of a green tea extract, shows slower elimination than when used as an isolated compound due to competition for binding sites in metabolizing enzymes with other accompanying polyphenols in the extract (EFSA 2009a).

The risk evaluation of botanicals which have been traditionally used for years as food or *herbal* medicine is often based on the *experiences and data from human exposure*, covering potentially epidemiological and clinical studies, reports of adverse health effects, and case reports of intoxications, while data of validated toxicological studies in experimental animals are often sparse.

Furthermore, botanical ingredients that commonly occur at low levels in botanical components of the diet may be extracted and reintroduced in concentrated form in certain specific food supplements or related health products resulting *in comparatively high exposure levels* (EFSA 2004). There are some examples where partly even serious adverse effects have been assigned to this type of products. Thus, capsules containing high-dosed dried green tea extracts taken for weight-loss purposes have been associated with severe liver toxicity, while these adverse effects are not reported for traditional consumption of green tea infusions (EFSA 2009a; Speijers et al. 2010). Certain case reports on adverse cardiovascular effects are suspected of being associated with intake of food supplements advertised for weight loss or improvement of physical fitness containing high doses of (−)-synephrine as part of bitter orange (*Citrus aurantium* ssp. *aurantium*) extracts partly in combination with caffeine. No adverse effects are known from (−)-synephrine exposure via traditional foods such as orange juice or bitter orange marmalades (NTP/NIEHS 2004; EFSA 2009a; Health Canada 2011; BfR 2012a).

## Guidance for Safety Evaluation: An Approach by EFSA

In view of the described specific aspects in the safety evaluation of plant-based food supplements and their expanding market volume and increasing variety with numerous claims, EFSA saw a need for a better characterization of the botanicals in use and for a harmonization of their risk assessments (EFSA 2004). To identify the data needed to assess the safety of botanicals and to suggest a science-based approach for the safety assessment, EFSA developed the "*Guidance on safety assessment of botanicals* and botanical preparations intended for use as ingredients in food supplements" (EFSA 2009b). The guidance is intended to assist risk assessors and food manufacturers considering the safety of a given botanical. Even though the guidance is focussed on the use of botanicals in food supplements, EFSA emphasizes that the principles of the approach chosen are applicable also to other uses of botanicals and botanical preparations in the food and feed areas. This guidance does not refer to the use of botanicals or botanical preparations for use as a novel food or botanicals representing genetically modified food, since for both categories special guidances do exist (see chapter "▶ Risk Assessment of Novel Food and Genetically Modified Food and Feed").

The information considered as necessary for a botanical or botanical preparation is *technical*, *exposure*, and *toxicological* data. The *technical* data comprise details on (i) the identity and nature of the source material, (ii) the manufacturing process of the botanical (preparation), (iii) its chemical composition, (iv) its specifications, (v) its stability in food (supplements), (vi) the proposed uses and use levels, and (vii) the information on existing assessments. Regarding *exposure* data information is required on (i) the anticipated exposure via the food supplement, (ii) the cumulative exposure via different categories of food, including food supplements, and medicinal products, (iii) the modality of use, and (iv) the information on historical use of different categories of food, including food supplements, and medicinal products. Regarding the *toxicological* data, studies on toxicity and toxicokinetics including metabolism of botanicals and botanical preparations should be conducted using internationally agreed protocols.

A two-level tiered approach for the safety evaluation of the botanical (preparation) is proposed depending on the available knowledge. It consists primarily on "*level A*" of a risk assessment in which, based on all available data, a decision is derived if there is (i) a safety concern, (ii) no safety concern, or (iii) a need for additional data. In the latter case, the requirement for further testing is specified on a subsequent level ("*level B*").

On "*level A*" the decision "no safety concern" may be based on the principle of a "presumption of safety." The guidance describes that a "*presumption of safety*" could be applied when available data would allow to conclude that exposure to known levels of the botanical (preparation) has occurred in large population groups for many years without reported adverse effects and that thus no additional data are judged necessary for the safety evaluation. Requirements for a "presumption of safety" are that not only use levels but also chemotypes of the botanicals and the chemical composition of the botanical preparations should be in line with

historically used ones and intakes due to the intended levels of use are within the range of intake levels derived from the European Member States' average diets. The approach relies mainly on the objective of not significantly increasing exposures beyond the levels linked to the safe history of use.

If specific compounds of concern can be well defined on "*level A*," the evaluations can focus on them. For a botanical (preparation) with a potential to contain toxic, addictive, psychotropic, or other substances that may be of concern, "presumption of safety" can be applied only if there is convincing evidence that these undesirable substances are either absent or significantly reduced or inactivated during processing. In these cases a "presumption of safety" of the botanical (preparation) is only justified when the overall exposure to the substances of concern is not too high compared to existing health-based guidance values such as the acceptable/ tolerable daily intake (ADI/TDI). Consideration of exposure to the substance of concern in relation to the Threshold of Toxicological Concern (TTC) values may also be helpful. When the botanical (preparation) contains substances that are both genotoxic and carcinogenic, the "Margin of Exposure" (MOE) approach (EFSA 2005) could be applied. Furthermore, the EFSA guidance (EFSA 2009b) addresses the possibility that the kinetic and toxicodynamic of a naturally occurring substance could be modified by the matrix in which it is present which may result in reduced or increased toxicity. Advice is also given regarding read across between two different preparations of a botanical or between different botanicals for which equivalence of composition data and consumption patterns regarding the substances of concern are a precondition.

On "*level B*" decision is taken which additional studies are needed for those botanicals or botanical preparations for which a "presumption of safety" was not justified on "*level A*" because, e.g., the anticipated intake is significantly higher than the estimated historical intake level or the historical intake level cannot be assessed. According to the EFSA guidance (EFSA 2009b), the study requirements on "*level B*" can be deduced from the "Guidance on submissions for food additive evaluations by the Scientific Committee on Food (SCF 2001)."[3] The spectrum of toxicological data asked for comprises primarily studies on toxicokinetics including metabolism, genotoxicity, and subchronic toxicity. Depending on the outcome of these studies and other specific relevant information, further studies, e.g., on reproductive toxicity, developmental toxicity, neurotoxicity, immunotoxicity, and chronic toxicity/carcinogenicity, may be required. The specifications and identity criteria for the botanical preparation(s) used for the toxicity studies and their relationship to the final product to be used in the food supplement should be described in detail.

The adequacy of the two-level tiered approach described in the guidance document was tested with a selected number of examples including botanicals known to contain

[3]This guidance was replaced in 2012 by the "Guidance for submission for food additive evaluations by the EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS)" (EFSA 2012c).

acute or subchronic toxic substances or potentially genotoxic carcinogens and botanicals with an established history of food use. The results of this study were published in a report illustrating how to apply the guidance document (EFSA 2009a).

As a further tool for risk evaluation of botanicals, EFSA listed more than 1,200 plant genus, species, and varieties in a "Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements" (EFSA 2012b; Silano et al. 2011). Its purpose is to draw the attention of manufacturers and food safety authorities to possible safety issues when these botanicals are used in food. There is no final judgment as to whether botanicals listed in the Compendium are safe or not safe for food use.

The new "Guidance for submission for food additive evaluations" (EFSA 2012c) by the EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) is relevant for the safety evaluation of botanical preparations used as food additives. It reflects widely the principles of the "Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements" presented above.

The existing guidance for the use of botanical preparations as flavorings is dealt with in chapter "► Risk Assessment of Food Additives."

## Legal Background

The use and the occurrence of plants in food are heterogeneous and therefore subject to different national and international regulatory frameworks, which in general are based on the outcome of the scientific risk assessments of competent national or international authorities, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or the EFSA.

The Codex Alimentarius Commission, established by FAO and WHO in 1963, provides a global framework. It develops harmonized international food standards, guidelines, and codes of practice to protect the health of the consumers which are also relevant for botanical components in food. For food additives of botanical origin, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) publishes specifications and safety evaluations including health-based guidance values (e.g., Curcumin (E 100): JECFA 2004, 2006). On the basis of these monographs, the Codex Committee on Food Additives may endorse permitted maximum levels for individual food additives. Another Codex panel, the Codex Committee on Contaminants in Foods, establishes or endorses permitted maximum levels or guideline levels for contaminants including naturally occurring toxicants in food and feed. For grains and pulses, the Codex Alimentarius standards state, e.g., that they shall be free from the following toxic or noxious seeds in amounts which may represent a hazard to human health: crotalaria (*Crotalaria* spp.), corn cockle (*Agrostemma githago* L.), castor bean (*Ricinus communis* L.), and Jimson weed (*Datura* ssp.) (CODEX STAN 153-1985; CODEX STAN 171-1989; CODEX STAN 172-1989; CODEX STAN 199-1995; CODEX STAN 201-1995).

In Europe the use of botanicals and botanical preparations in food is regulated by national and EU legislations, where mainly the latter will be addressed in the following.

Overall botanicals and botanical preparations found in food fall under the basic regulations of the General Food Law (Regulation (EC) No. 178/2002/EC), in which Article 14 protects against unsafe food and which attributes responsibility for the safety of the products on the market to the food business operators.

It is known that conventional plant-derived food, e.g., certain fruits, vegetables, herbs, and spices may contain a variety of naturally occurring substances of concern, such as α-solanine, furocoumarins, cyanogenic glycosides, capsaicin, coumarin, estragole, and thujone. With a normal diversified diet the intake of these substances generally only amounts to low levels not known to cause any health damage in humans. However, for preventive action, national authorities are informing the public by communicating nutritional recommendations to avoid potential risks of high or excessive individual consumption of a certain foodstuff; e.g., to consume cassia cinnamon, containing relatively high levels of the hepatotoxic coumarin only in moderate amounts (BfR 2012b), or advising against excessive intake of extremely hot chili sauces with high concentrations of capsaicin, which may cause irritation of mucous membranes, nausea, vomiting, and hypertension (BfR 2011b). Specific regulations exist for some of the abovementioned naturally occurring food ingredients, but they refer only to flavoring purposes (Regulation (EC) No. 1334/2008). They aim at avoiding an increase of exposure not allowing to add the isolated substances to food (e.g., capsaicin, coumarin, estragole, thujone) and restricting their concentration in compound food containing flavorings or flavoring food ingredients (coumarin, estragole, thujone). Further details on the regulations of flavorings in general are given in chapter "▶ Risk Assessment of Food Additives."

Regarding plant-based food additives, present uncertainties regarding the differentiation between natural food colors, being food additives (e.g., beetroot red (E 162), an extract of beetroots), and coloring foods (e.g., concentrated cherry juice), considered to be normal characteristic natural food ingredients, are noteworthy. In contrast to the latter, food additive colors undergo an approval procedure (Regulation (EC) No. 1331/2008), need labeling as an additive (Directive 2000/13/EC), and have to meet the purity criteria set out in Commission Regulation (EU) No. 231/2012. A criterion to determine whether or not a food color is an additive is that of "selective extraction of the pigments" which is per definition associated with the manufacturing of a food color considered as an additive (Annex I of Regulation (EC) No. 1333/2008). Further details on regulations of food additives in general are outlined in chapter "▶ Risk Assessment of Food Additives."

Regulations for novel foods and genetically modified foods of botanical origin are not presented here but in chapter "▶ Risk Assessment of Novel Food and Genetically Modified Food and Feed."

With respect to food supplements the use of botanicals and botanical preparations has not yet been harmonized within the EU but is regulated in the Member

States by differing national rules. Partly, (positive) lists of safe botanicals and/or (negative) lists of botanicals which are banned or restricted for food use have been established. In some EU Member States, plant-based food supplements are subject to a notification procedure before being introduced to the market (Bast et al. 2002). In the EU Directive 2002/46/EC gives general provisions for food supplements and specifically addresses modalities for use of vitamins and minerals listed in the annexes to this directive. It also allows the use of "other substances with a nutritional or physiological effect," for which no further definition or regulation is given. However, it is generally understood that this term could include botanicals and botanical extracts besides substances such as amino acids, enzymes, pre- and probiotics, and essential fatty acids (Silano et al. 2011). Furthermore, there is an announcement in the 8th recital of this directive that specific rules concerning these other substances as ingredients of food supplements should be laid down at a later stage, provided that adequate and appropriate scientific data become available.

If a harmful effect on health is suspected for a botanical or botanical preparation, a procedure based on Article 8 of the food fortification legislation (Regulation (EC) 1925/2006) may be initiated by the European Commission which may result in a placement of the botanical (preparation) in Annex III of this regulation. On the basis of a risk assessment by EFSA, it can be banned (Annex III, Part A), restricted in use (Annex III, Part B), or in case of uncertainties reevaluated on the basis of additional safety data (Annex III, Part C). So far, no botanical (preparation) has been regulated by this means.

Nutrition and health claims regarding botanical food supplements are regulated by Regulation (EC) 1924/2006. This regulation does not foresee an assessment of the safety of the product carrying the claim.

As far as botanical contaminants are concerned, Article 2 of the Council Regulation (EEC) No. 315/93 stipulates that, where necessary, maximum tolerances for specific contaminants shall be established in order to protect public health. Thus, Commission Regulation (EC) No. 1881/2006 lays down maximum levels for certain contaminants in foodstuffs, but regarding natural toxicants at present, it only includes various mycotoxins.

In the USA the use of botanicals in food supplements (dietary supplements) is regulated under the Dietary Supplement Health and Education Act of 1994 (DSHEA), which places the burden on the Food and Drug Administration (FDA) to prove that a dietary supplement presents a significant or unreasonable risk of illness or injury under the labeled conditions of use. Under certain conditions a notification process is required for new dietary ingredients that were not marketed before October 15, 1994. The law requires the collection of all adverse event reports by manufacturers, distributors, and retailers of dietary supplements and the reporting of serious adverse event reports to the FDA. Due to case reports on adverse effects, FDA published a final rule on February 11, 2004, declaring dietary supplements that contain ephedrine alkaloids adulterated on the basis that these products present an unreasonable risk of illness or injury (FDA 2010).

## Future Perspectives

Since plant-based preparations are widely present in different categories of food commodities with expanding market volume and increasing exposures of consumers, the need for a better chemical and toxicological characterization and a harmonized systematic approach of scientific risk assessment of botanicals and botanical preparations is growing.

The establishment of the EFSA guidance document for the safety assessment of botanicals and botanical preparations (EFSA 2009b) was a first step to achieve similar standards in safety evaluations performed by the national competent authorities of Member States. The next step would be to determine which botanicals should be evaluated with priority considering significant levels of substances of concern, reports on adverse effects, increases of intake rates, and negative lists of member states (EFSA 2009b).

While the EU legislation is covering the use of botanical additives and flavorings, an EU-wide approach is lacking for plant-based food supplements. Especially for botanicals or botanical preparations used in products, where the borderline between food supplements (not subjected to an authorization procedure in the EU) and medicinal products (subjected to an authorization procedure in the EU) is difficult to define, the need for a harmonized approach based on validated safety evaluations becomes evident. This should guarantee that intakes of toxicologically relevant botanical components are within safe limits. Factual two options for legal measures may be considered as already mentioned above. Thus, either the framework of Directive 2002/46/EC may be extended, taking for botanicals and botanical preparations the same approach already used for harmonizing vitamins and minerals, or, as foreseen under Article 8 of Regulation (EC) 1925/2006, lists in Annex III may be established.

In addition activities are expected with respect to the definition of coloring foodstuffs. A catalogue of clear criteria will presumably soon be established on how to distinguish coloring foods, which are not requiring a safety evaluation and an approval, from additive food colors which need an authorization. With respect to consumer safety, unambiguous definitions of coloring foods and their uses are considered to be necessary to ensure that their application does not lead to toxicologically unacceptable higher exposures with coloring components, accompanying substances, and impurities of these sources than would result from their normal dietary intake. Legal definitions and specific provisions regarding the use of coloring foods might be necessary.

Furthermore, recent EFSA risk assessments of botanical contaminants, namely, of opium alkaloids in poppy seeds and of ergot alkaloids as well as of pyrrolizidine alkaloids in food and feed (EFSA 2011b, c, 2012a), may result in legal consequences. A decision by the European Commission may be taken if in these cases there is a need for legal measures in the frame of Council Regulation (EEC) No. 315/93 laying down community procedures for contaminants in food to protect public health. Other measures may also be taken, such as the development of codes of practice. This has been especially recommended in a discussion paper by the

Codex Committee on Contaminants in Foods to prevent and reduce pyrrolizidine alkaloid contamination of food products (FAO/WHO 2011).

## References


Bast A, Chandler RF, Choy PC, Delmulle LM, Gruenwald J, Halkes SB, Keller K, Koeman JH, Peters P, Przyrembel H, de Ree EM, Renwick AG, Vermeer ITM (2002) Botanical health products, positioning and requirements for effective and safe use. Environ Toxicol Pharmacol 12:195–211

BfR (2005) BfR recommends provisional daily upper intake level and a guidance value for morphine in poppy seeds. BfR health assessment No 012/2006, 27 December 2005. http://www.bfr.bund.de/cm/349/bfr_recommends_provisional_daily_upper_intake_level_and_a_guidance_value_for_morphine_in_poppy_seeds.pdf

BfR (2007) Salad mix contaminated with groundsel containing pyrrolizidine alkaloids. BfR opinion No 028/2007, 10 January 2007. http://www.bfr.bund.de/cm/349/salad_mix_contaminated_with_groundsel_containing_pyrrolizidine_alkaloids.pdf

BfR (2011a) Chemical analysis and toxicity of pyrrolizidine alkaloids and assessment of the health risks posed by their occurrence in honey. BfR opinion No 038/2011, 11 August 2011. http://www.bfr.bund.de/cm/349/chemical-analysis-and-toxicity-of-pyrrolizidine-alkaloids-and-assessment-of-the-health-risks-posed-by-their-occurence-in-honey.pdf

BfR (2011b) Too hot isn't healthy – foods with very high capsaicin concentrations can damage health. BfR opinion No 053/2011 of 18 October 2011. http://www.bfr.bund.de/cm/349/too-hot-isnt-healthy-foods-with-very-high-capsaicin-concentrations-can-damage-health.pdf

BfR (2012a) Gesundheitliche Bewertung von synephrin- und koffeinhaltigen Sportlerprodukten und Schlankheitsmitteln. Stellungnahme Nr 004/2013 des BfR vom 16. November 2012. http://www.bfr.bund.de/cm/343/gesundheitliche-bewertung-von-synephrin-und-koffeinhaltigen-sportlerprodukten-und-schlankheitsmitteln.pdf

BfR (2012b) New insights into coumarin contained in cinnamon BfR opinion No 036/2012, 27 September 2012. http://www.bfr.bund.de/cm/349/new-insights-into-coumarin-contained-in-cinnamon.pdf

CODEX STAN 153-1985. www.codexalimentarius.net/download/standards/51/CXS_153e.pdf

CODEX STAN 171-1989. www.codexalimentarius.net/download/standards/56/CXS_171e.pdf

CODEX STAN 172-1989. www.codexalimentarius.net/download/standards/57/CXS_172e.pdf

CODEX STAN 199-1995. www.codexalimentarius.net/download/standards/62/CXS_199e.pdf

CODEX STAN 201-1995. www.codexalimentarius.net/download/standards/64/CXS_201e.pdf

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs 20.12.2006, OJ (EC)2006 No L 364/5

Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council, OJ (EC) 2012 No L 83/1

COT (Committee on Toxicity of Chemicals in Food, Consumer, Products and the Environment) (2008) Statement on pyrrolizidine alkaloids in food. http://cot.food.gov.uk/pdfs/cotstatementpa200806.pdf

Council Regulation (EEC) No 315/93 of 8 February 1993 laying down community procedures for contaminants in food, OJ (EC) 1993 No L 37, 1–3

Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs, OJ (EC) 2000 No L 109/29

Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ (EC) 2002 No L 183/51

Dusemund B, Soffers AEMF, Rietjens IMCM (2012) Plant-derived contaminants in food. In: D Schrenk (ed) Chemical contaminants and residues in food. Woodhead Publishing series

in food science, technology and nutrition: No 235. Woodhead Publishing Limited, Oxford, pp 394–417
EFSA (2004) Discussion paper on botanicals and botanical preparations widely used as food supplements and related products: coherent and comprehensive risk assessment and consumer information approaches, Brussels, 25 June 2004. EFSA/SC/26 final
EFSA (2005) Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. EFSA J 282:1–31. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620763354.htm
EFSA (2009a) Advice on the EFSA guidance document for the safety assessment of botanicals and botanical preparations intended for use as food supplements, based on real case studies. EFSA J 7(9):280. http://www.efsa.europa.eu/en/supporting/doc/280r.pdf
EFSA (2009b) Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements. EFSA J 1249:1–19. http://www.efsa.europa.eu/en/efsajournal/pub/1249.htm
EFSA (2010) Scientific opinion on the reevaluation of lutein (E 161b) as a food additive on request of the European Commission. EFSA J 8(7):1678. www.efsa.europa.eu
EFSA (2011a) Scientific opinion on the re-evaluation of lutein preparations other than lutein with high concentrations of total saponified carotenoids at levels of at least 80 %. EFSA J 9(5):2144. http://www.efsa.europa.eu/de/efsajournal/doc/2144.pdf
EFSA (2011b) Scientific opinion on the risks for public health related to the presence of opium alkaloids in poppy seeds. EFSA J 9(11):2405. http://www.efsa.europa.eu/de/efsajournal/doc/2405.pdf
EFSA (2011c) Scientific opinion on pyrrolizidine alkaloids in food and feed. EFSA J 9(11):2406. http://www.efsa.europa.eu/de/efsajournal/doc/2406.pdf
EFSA (2012a) Scientific opinion on ergot alkaloids in food and feed. EFSA J 10(7):2798. http://www.efsa.europa.eu/en/efsajournal/doc/2798.pdf
EFSA (2012b) Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements. EFSA J 10(5):2663. www.efsa.europa.eu/efsajournal
EFSA (2012c) Guidance for submission for food additive evaluations. EFSA panel on food additives and nutrient sources added to food (ANS). EFSA J 10(7):2760. http://www.efsa.europa.eu/en/efsajournal/doc/2760.pdf
FAO/WHO (Food and Agriculture Organization/World Health Organization) (2011) Discussion paper on pyrrolizidine alkaloids, Joint FAO/WHO food standards programme, CODEX Committee on Contaminants in Foods, 5th session, The Hague, The Netherlands, 21–25 March 2011
FDA (2010) Dietary supplements-import and domestic (implementation date: 03/26/2010) includes pen & ink changes as of 07/08/10 to parts I and II, Food and Drug Administration. http://www.fda.gov/Food/ComplianceEnforcement/FoodCompliancePrograms/ucm071547.htm
Health Canada (2011) Synephrine, octopamine and caffeine health risk assessment (HRA) report, Robin Marles, file number: 172091, date review approved 2011-05-16. http://www.nutratechinc.com/advz/Studies2011/Safety/S1%20Health%20Canada%200511.pdf
JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2004) Evaluation of certain food additives and contaminants. Sixty-first report of the Joint FAO/WHO expert committee on food additives. WHO technical report series 922. Geneva
JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2006) Combined compendium of food additive specifications - all specifications monographs from the 1st to the 65th meeting (1956–2005). FAO JECFA monographs series, No 1, Vols 1–3. Food and Agricultural Organization of the United Nations, Rome
Koleva II, van Beek TA, Soffers AEMF, Dusemund B, Rietjens IMCM (2012) Alkaloids in the human food chain – natural occurrence and possible adverse effects. Mol Nutr Food Res 56:30–52
NTP/NIEHS (2004) Bitter orange (Citrus aurantium var. amara) extracts and constituents (±)-p-synephrine [CAS No. 94-07-5] and (±)-p-octopamine [CAS No. 104-14-3]. Review of

toxicological literature. National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, North Carolina, VIII + 73 p. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Bitterorange.pdf

Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings, OJ (EC) 2008 No L 354/1

Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ (EC) 2008 No L 354/16

Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ (EC) 2008 No L 354/34.

Regulation (EC) No 1924/2006. Corrigendum to regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ (EC) L 12/3

Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ (EC) L 404/26

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ (EC) 2002 No L 31/1

SCF (2001) Guidance on submissions for food additive evaluations by the Scientific Committee on Food. 12 July 2001. http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

Silano V, Coppens P, Larranaga-Guetaria A, Minghetti P, Roth-Ehrang R (2011) Regulations applicable to plant food supplements and related products in the European Union. Food Funct 2:710

Speijers G, Bottex B, Dusemund D, Lugasi A, Toth J, Amberg-Müller J, Galli C, Silano V, Rietjens IMCM (2010) Safety assessment of botanicals and botanical preparations used as food supplements; emerging issues when testing a proposed tiered approach. Mol Nutr Food Res 54:175–185

# Risk Assessment of Novel Food and Genetically Modified Food and Feed

Annette Pöting, Marianna Schauzu, Birgit Niemann, and Regina Schumann

## Contents

Legislation ..... 828
Novel Foods and Food Ingredients ..... 828
Food and Feed Derived from Genetically Modified (GM) Plants ..... 830
Risk Assessment of Novel Foods and Food Ingredients ..... 831
Characterization and Specification ..... 832
Production Process ..... 833
Previous Exposure and Experience Gained ..... 833
Intended Consumption and Extent of Use ..... 834
Microbiological Aspects ..... 835
Nutritional Evaluation ..... 835
Toxicological Evaluation ..... 836
Risk Assessment of Food Derived from Genetically Modified Plants ..... 838
Risk Assessment Strategy ..... 838
Molecular Characterization ..... 839
Comparative Analyses ..... 839
Toxicological Assessment ..... 840
Allergenicity ..... 842
Nutritional Assessment ..... 844
Future Directions ..... 844
Novel Foods and Food Ingredients ..... 844
Food and Feed Derived from GM Plants ..... 845
References ..... 846

**Abstract**

Since 1997, novel foods and food ingredients are subjected to Regulation (EC) No 258/97, which requires an authorization or notification before novel food products can be placed on the EU market. Food and feed derived from

A. Pöting (✉) • M. Schauzu • B. Niemann • R. Schumann
Department of Food Safety, Federal Institute for Risk Assessment (BfR), Berlin, Germany
e-mail: Annette.Poeting@bfr.bund.de; Maria-Anna.Schauzu@bfr.bund.de; Birgit.Niemann@bfr.bund.de; Regina.Schumann@bfr.bund.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_118, 

genetically modified plants now fall within the scope of Regulation (EC) No 1892/2003. This chapter provides an overview of the respective legislation as well as the strategies and scientific criteria for the risk assessment, which forms an essential part of the authorization procedures and is to be performed according to specific guidance of the European Food Safety Authority.

## Legislation

### Novel Foods and Food Ingredients

With the coming into force of Regulation (EC) No 258/97 concerning novel foods and novel food ingredients (EC 1997a) (Novel Foods Regulation), an authorization procedure for placing food and food ingredients on the market was established for the first time in Member States of the European Community (since 1993 European Union – EU). The Novel Foods Regulation applies to food and food ingredients, which have not been used for human consumption to a significant degree within the Community before the reference date 15 May 1997 and which fall under the categories as specified in Table 1.

As laid down in Article 3 (1) of the Regulation, novel foods and food ingredients must not:

- Present a danger for the consumer
- Mislead the consumer
- Differ from foods or food ingredients, which they are intended to replace, to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer

According to Article 4 (1) of the Regulation, applicants have to submit a request to the competent food assessment body (Competent Authority) of the Member State in which the novel food product is to be marketed for the first time (Fig. 1). The request should contain the necessary information for the risk assessment including the studies carried out and any other information, which is available to demonstrate that the food or food ingredient complies with the criteria laid down in Article 3 (1). Within a period of 3 months from receipt of a request, the competent food assessment body of the Member State has to carry out an initial assessment. The Member State needs to forward this report, indicating whether or not the novel food product requires an additional assessment, to the Commission. Within 60 days from the date of circulation of the report, Member States or the Commission may make comments or present reasoned objections to the marketing of the product concerned. If an additional assessment is considered necessary or an objection is raised, the European Food Safety Authority (EFSA) has to be consulted on any matter considered likely to have an effect on public health. Based on the EFSA opinion, the Commission asks the Standing Committee on the Food Chain and Animal Health (SCFCAH), which consists of Member States' representatives, for an opinion on a draft decision for authorization. The decision is taken with

**Table 1** Categories of novel foods and food ingredients as specified in regulation (EC) No 258/97 (according to the revised version of 18 April 2004)

| Food and food ingredients |
|---|
| With a new or intentionally modified primary molecular structure |
| Consisting of or isolated from microorganisms, fungi or algae |
| Consisting of or isolated from plants and food ingredients isolated from animals, except for those obtained by traditional propagating or breeding practices and having a history of safe food use |
| To which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism, or level of undesirable substances |

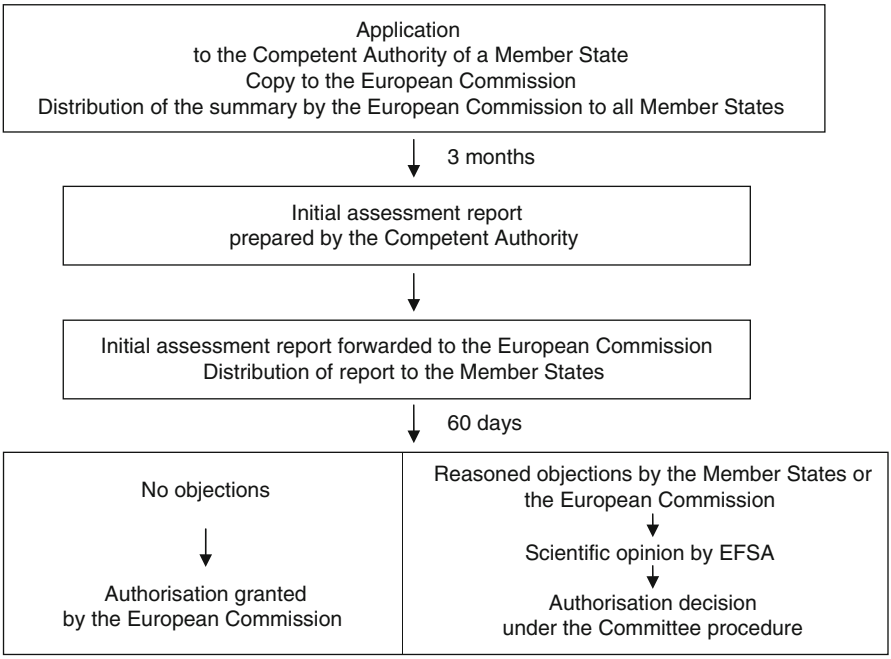



**Fig. 1** Authorization procedure for novel foods and food ingredients according to Article 4 (1) of Regulation (EC) No 258/97

qualified majority. Commission Decisions are published in the *Official Journal of the European Union*.

According to Article 3 (4) of the Regulation, a simplified procedure (notification) may apply to specific foods or food ingredients which, on the basis of the scientific evidence available and generally recognized, or on the basis of an opinion delivered by one of the competent food assessment bodies, are substantially equivalent to existing foods or food ingredients with regard to their composition, nutritional value, metabolism, intended use, and the level of undesirable substances contained therein.

According to Article 5, the applicant may notify the Commission of the placing on the market when he does so (accompanied by the relevant information), while in practice a scientific opinion of one of the Member States' Competent Authorities generally forms the basis of a notification. Each year, the Commission publishes a summary of these notifications in the *Official Journal of the European Union*.

## Food and Feed Derived from Genetically Modified (GM) Plants

The governing of the placing on the market of genetically modified organisms (GMOs) began in 1990 when Directive 90/220/EEC (EEC 1990) entered into force. Since EU Directive 90/220/EEC focused mainly on environmental aspects, the Novel Foods Regulation (EC 1997a) was established to provide for a specific safety assessment of, among other categories, (a) foods and food ingredients containing or consisting of GMOs, and (b) foods and food ingredients produced from but not containing GMOs, with an authorization procedure obligatory for the first category and a notification procedure for the latter (see section on "Novel Foods and Food Ingredients"). Only the notification procedure was used in 1997 and 1998 by certain Member States for placing on the market specific food products such as refined oils derived from GM rape or cotton seeds or processed maize products. Criticism from some EU Member States concerning the procedures for the placing on the market as well as the requirements for the safety assessment led to a de facto moratorium in the EU, and no further permits for cultivation of GM plants were issued in accordance with Directive 90/220/EEC after October 1998.

With Directive 2001/18/EC (EC 2001a) replacing Directive 90/220/EEC, a first step was taken to overcome the so-called moratorium by introducing traceability and post-market monitoring plans as well as mandatory labeling provisions for GMOs. Most importantly, the optional consultation of the Commission's Scientific Committees, e.g., if objections were raised by Member States, was made obligatory. A second step followed in April 2004 when Regulation (EC) No 1829/2003 on genetically modified food and feed (EC 2003) became effective, which replaced the GM food related part of the Novel Foods Regulation. It dismissed the simplified notification procedure and instead requires an authorization for all GMO-derived products. The old system has been replaced by a "one door–one key" procedure for the scientific assessment and the authorization of GMOs and derived food and feed. A single risk assessment is conducted and a single authorization is granted for a GMO and its possible uses. However, cultivation of a GMO still needs an additional authorization in accordance with Directive 2001/18/EC. Authorizations are limited to a 10-year period but are renewable. GMO-derived foods and feeds which have been lawfully placed on the EU market before Regulation (EC) No 1829/2003 (EC 2003) entered into force can be further marketed, provided that they had been notified to the Commission by 17 April 2004. Applications for renewal of these authorizations are required within 9 years from the date of which the products were first placed on the market.

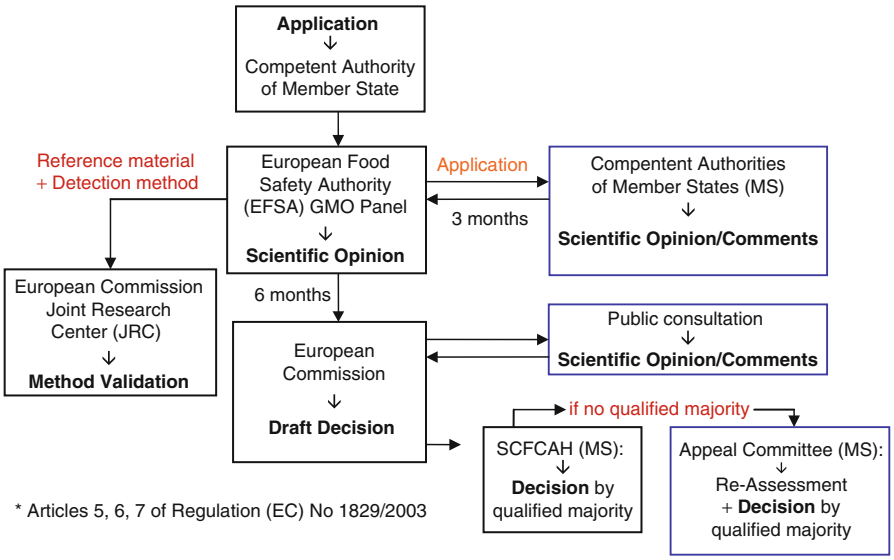



**Fig. 2** Authorization procedure for food and feed derived from GMOs according to Regulation (EC) No 1829/2003

The safety assessment of food and feed derived from GMOs as well as the assessment of environmental risks is the responsibility of EFSA, but the Competent Authorities of Member States are invited to provide comments. Based on the EFSA opinion, the European Commission drafts a proposal for granting or refusing authorization. The SCFCAH then decides whether to accept the Commission's proposal through a weighted voting system. If the proposal is neither accepted nor rejected by a qualified majority of Member States, it is referred to an Appeal Committee. If this Committee takes no decision within 3 months, or does not reach a qualified majority indicating that it opposes the proposal, the European Commission can adopt its decision (Fig. 2).

## Risk Assessment of Novel Foods and Food Ingredients

Guidance for the risk assessment of novel foods and food ingredients elaborated by the Scientific Committee on Foods (SCF), the expert committee advising the Commission on questions of food safety before EFSA was established in 2002, is provided in Commission Recommendation 97/618/EC (EC 1997b). This guidance is intended to support the responsible person in preparing an application and to facilitate the evaluation by the national competent food assessment bodies, thus contributing to a harmonized evaluation. As a general principle, the risk assessment should be performed on a case-by-case basis. Considering the wide variety of potential novel food products (see Table 1), no specified requirements but more

**Table 2** Information requirements for the risk assessment of novel foods and food ingredients

| |
|---|
| Specification |
| Effects of the production process |
| Previous exposure and experience gained |
| Intended consumption and extent of use |
| Microbiological information |
| Nutritional information |
| Toxicological information |

general recommendations have been intentionally laid down, thus allowing for sufficient flexibility in the evaluation process. Table 2 lists the essential information requirements.

EFSA and the SCF have issued a large number of scientific opinions on specific applications since the publication of Commission Recommendation 97/618/EC. The experience thus gained as well as the outcome of a Scientific Colloquium discussing the information needed for the evaluation of applications (EFSA 2010a), and further developments related to other fields of EFSA's risk assessment activities, in particular on food additives (EFSA 2012a; see chapter "► Risk Assessment of Food Additives") and botanicals (EFSA 2009a; see chapter "► Risk Assessment of Food Components with Botanical Origin"), have influenced the application of the original guidance. The following sections provide more comprehensive information related to the specific aspects of the risk assessment.

## Characterization and Specification

Chemical characterization and specification of the novel food or food ingredient is required to ensure that the product intended to be marketed complies with the material tested and evaluated. In the case of single substances, the information should include the chemical name, CAS number, molecular and structural formulae, molecular weight, physicochemical properties as well as other data considered relevant to support the identity of the substance. For simple chemical mixtures, the identity and proportion of the substances present in the mixture should be indicated in addition to the parameters listed above for the single substances. In the case of complex mixtures, for example, a food of plant origin, the constituents contained therein cannot be always fully characterized. Data on chemical composition should include relevant macro- and micronutrients and any constituents that may give rise to concern, e.g., due to their chemical, physiological, toxicological, anti-nutritive, or allergenic properties. Taxonomic classification of the source as well as information on the geographical origin, growth, and harvesting conditions and the plant parts to be used is also required.

The specifications of a novel food product should define the requirements concerning its identity, composition, purity, limits of impurities including chemical

and microbial contaminants, residues from the production process, and – in the case of plant-derived novel food products – limits of specific naturally occurring substances raising safety concerns.

## Production Process

A detailed description of the production process is required, comprising information on the raw materials and/or chemical substances used, the process(es) by which the starting materials are converted to the product including relevant process parameters, as well as methods of purification and preparation of the final product. These data serve to identify substances in the final product, which may pose a health risk to consumers and thus need further evaluation.

Foods and food ingredients produced using a new production process constitute a specific category of novel products (see Table 1). According to the present legislation, they only fall within the scope of Regulation (EC) No 258/97 if the new process "gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances." Whether such changes occur can only be assessed after a thorough evaluation considering the available scientific information on possible effects of the new technology as well as analytical data on the nature and extent of effects induced in the specific food product treated under the conditions intended to be applied. A food or food ingredient produced by a related traditional method and/or the respective untreated product may serve as an appropriate comparator. If physical, chemical, and/or biological changes are identified, the potential impact on relevant nutritional, toxicological, and microbiological parameters of the final product has to be assessed. The first submitted application in this category concerned fruit-based preparations pasteurized using high-pressure treatment, which were intended to replace thermally pasteurized fruit preparations. Based on the initial assessment report, which concluded that high-pressure treatment does not cause relevant changes to the major constituents when compared with heat pasteurization, the marketing was authorized in 2001 (EC 2001b). The evaluations of recent applications concerning UV light-treated yeast, milk, and bread have not yet been completed (date July 2013).

## Previous Exposure and Experience Gained

Information on the extent of previous and/or present food use in non-EU countries of a product considered novel in the EU and the experience gained from such use is highly relevant for the risk assessment. Data on intake levels and duration of consumption as well as a description of the traditional methods applied to obtain and prepare the food are – besides other information – required to establish a "history of safe use" (Constable et al. 2007). Information on other uses can also

contribute to the evaluation: For example, an application of a plant-derived product as a traditional medicine may indicate the presence of pharmacologically active substances.

Dried fruit pulp derived from the fruit of the Baobab tree (*Adansonia digitata*) and Chia seed (*Salvia hispanica*) are novel food ingredients, which were accepted for marketing in the EU largely on the basis of a documented history of traditional consumption without indications of adverse health effects (FSA 2007; EFSA 2009b).

## Intended Consumption and Extent of Use

Assessment of exposure constitutes an essential part of the risk assessment. If a novel food ingredient is intended to be added to conventional foodstuffs, information on the consumption of these foodstuffs (or food categories) and on the use levels of the novel ingredient are required for an assessment of its intake. Consumption data should preferably be obtained from the EFSA Comprehensive European Food Consumption Database, and a guidance document for its use has been elaborated (EFSA 2011a). Population groups with high intake should be identified, especially if particularly sensitive groups are concerned, namely, children, pregnant, and lactating women, the elderly, or individuals with specific health risks.

On a case-by-case basis, a Post-Launch Monitoring (PLM) may be required to confirm whether the outcome of the intake assessment, which is generally based on a number of assumptions, reflects the actual situation after market introduction. Such a PLM was requested when yellow fat spreads with added phytosterolesters, intended to lower blood cholesterol levels, were for the first time authorized in the EU (EC 2000). The Commission Decision obliged the applicant to estimate the extent to which the novel food reached the target group, i.e., people trying to control their elevated blood cholesterol levels, and to estimate consumption of phytosterols from this source in other population groups. The outcome of this study (SCF 2002a) and the experience gained in other fields were taken into account in a review article on the purpose, aims, methodologies, and limitations of PLM applied to novel foods (Hepburn et al. 2008). Also the authorization decision related to synthetic lycopene (EC 2009) included an obligation to perform a PLM. Due to concerns that the wide use in a number of different foods might lead to a lycopene consumption higher than the Acceptable Daily Intake (ADI) (see section on "Toxicological Evaluation"), the company marketing synthetic lycopene, as well as those marketing other lycopene preparations authorized for the same uses, was requested to provide information on the quantities provided to their customers for the production of food products as well as data on product launches of foods with added lycopene. In the light of the new information and an updated intake assessment, the use of lycopene as a novel food ingredient is expected to be reviewed in 2014.

## Microbiological Aspects

As a matter of principle, novel foods and food ingredients must not pose a microbiological risk to consumers. According to Regulation (EC) No 852/2004 on the hygiene of foodstuffs (EC 2004a), which applies to all stages of production, processing, and distribution of food intended for human consumption, food business operators have the primary responsibility to ensure food safety. The establishment of and compliance with microbiological criteria as well as the implementation of production procedures based on the Hazard Assessment and Critical Control Points (HACCP) principles together with the application of good hygiene practice should ensure the microbiological safety of a food. With respect to the evaluation of novel foods and food ingredients, identification of the microorganisms present and analysis of relevant metabolites is required.

Novel foods containing or consisting of live microorganisms with claimed health benefits related to gut function or the immune system (so-called probiotics) would require special attention with regard to potential microbiological risks. However, the strains presently used, mostly *Lactobacilli* or *Bifidobacteria*, are not covered by Regulation (EC) No 258/97 since they have been used in the EU before the reference date 15 May 1997. If a microorganism falls into the scope of the Novel Foods Regulation, a comprehensive characterization is required, including taxonomic identification, information on the source, previous use in food or feed, pathogenicity, toxigenicity, virulence, genetic stability, and the capability to exchange genetic material, for example, genes conferring antimicrobial resistance. Microbial metabolic activities, which might lead to the production of toxicologically relevant substances, and possible effects on the composition and functions of the normal intestinal microflora, should also be considered. Additional aspects have to be considered if the microorganism has been genetically modified (EFSA 2011b).

## Nutritional Evaluation

Comprehensive compositional information with respect to the contents of macro- and micronutrients forms the basis of each nutritional evaluation. The effects of other food constituents, which might impact on the biological efficiency of nutrients (antinutrients), should be assessed, taking into account the influence of processing, storage, and further preparation of the novel food. Considering also the intended use and consumption levels, these data should allow a conclusion on whether the novel food product is nutritionally equivalent to a comparable traditional food or food ingredient, which it might replace. If this is the case, further nutritional testing is generally not required.

If nutritional equivalence cannot be established, the potential implications for the nutrient supply of consumers have to be assessed. This may particularly apply to novel products with claimed nutrition and/or health benefits, which have the

potential to replace traditional foods or food ingredients in the diet and thus induce relevant changes in consumption patterns. For a comprehensive evaluation, human data are generally required. Studies should be based on well-defined hypotheses and provide information on nutritional and metabolic aspects relevant to the novel food product, target group(s), anticipated consumption levels, and duration of use. Specific attention should be paid to the particular physiological characteristics and metabolic requirements of population groups like infants, children, pregnant and lactating women, and the elderly, if these are included in the target group(s). It is emphasized that the assessment of potential benefits does not fall within the scope of the Novel Foods Regulation; nutrition and health claims need an authorization according to Regulation 1924/2006 (EC 2006).

Foods with added plant sterols, comprising of sterols, their hydrogenated forms (stanols), and the respective esters, required a specific nutritional risk assessment. Plant sterols interfere with intestinal cholesterol absorption and reduce blood concentrations of low density lipoprotein (LDL) cholesterol by 5–15 % without affecting high density lipoprotein (HDL) cholesterol. Some human studies also indicated interference with the absorption of carotenoids, mainly β-carotene, and fat-soluble vitamins, such as vitamin E. Although no serious concern was deduced regarding the role of β-carotene as a vitamin A precursor, except in situations where vitamin A requirements are greater than normal as in pregnancy, lactation, or infancy, consumption of carotenoid-rich fruits and vegetables was recommended to counterbalance the reduction of β-carotene and fat-soluble vitamin levels in blood. Furthermore, risk-managers were advised to take appropriate measures in order to prevent excessive consumption (SCF 2002b). On the basis of this opinion, several foods with added plant sterols were authorized for marketing in the EU under specified conditions of use. The products have to be clearly labeled with regard to portion sizes and the amount of sterols contained therein in order to allow consumers to easily restrict their intake to a maximum of 3 g per day (EC 2004b).

## Toxicological Evaluation

The toxicological requirements depend on the category (see Table 1) and nature of the novel food product, which may range from single substances via simple chemically defined mixtures to complex foods containing a wide variety of unidentified constituents. In principle, novel food ingredients should be evaluated like food additives, which are also intentionally added to foods. Some of the ingredients, have been the subject of an application under the Novel Foods Regulation, for example, synthetic lycopene to be used as antioxidant, and have already been authorized as food additives for specific technological purposes (EC 2008a). Synthetic lycopene was evaluated in accordance with the Guidance of the SCF on submissions for food additive evaluations (SCF 2001), which was applicable at the time of submission. Genotoxicity testing using a battery of in vitro and in vivo tests showed that properly stored and formulated synthetic lycopene protected from

oxidative damage was not mutagenic. Based on a No-Observed Adverse Effect Level (NOAEL) of 50 mg/kg bw per day in a chronic toxicity study in the rat and applying an uncertainty factor of 100, an ADI for humans of 0.5 mg/kg bw per day was derived, referring to lycopene from all sources (EFSA 2008a). Considering this ADI and the evaluation of a specific application under the Novel Foods Regulation (EFSA 2008b), the use of synthetic lycopene in a number of food categories was authorized (EC 2009). For other substances, e.g., synthetic dihydrocapsiate and zeaxanthin, the database was not considered sufficient to derive an ADI, but the safety margins between the respective NOAEL in a subchronic rat toxicity study and the anticipated intake were considered sufficient to accept the novel ingredients under the conditions of use, as proposed by the applicants (EFSA 2012b; EFSA 2012c). Also for "Glavonoid," an extract derived from the root of *Glycyrrhiza glabra* L. (liquorice root), the main conclusions were based on the results of a subchronic rat toxicity study. Using a Benchmark Dose (BMD) modeling approach for analysis of the critical effects, a specified intake level of "Glavonoid" per day was considered safe (EFSA 2011c). An updated guidance for food additive evaluations was published in 2012 (EFSA 2012a), and specific guidance on genotoxicity testing strategies applicable to food and feed safety assessment is also available (EFSA 2011d). For detailed information, it is referred to the chapter "► Risk Assessment of Food Additives."

Considering complex novel products, for example, foods of plant origin, the need for testing depends on the concerns raised with respect to the possible presence of toxicologically relevant constituents. Information from the scientific literature, data on composition, and the experience gained from previous or present use for food and/or other purposes may provide relevant information in this respect. If – based on the available information – the safety cannot be established, the testing program should include a subchronic (90-day) toxicity study in rodents (OECD 1). Since the testing of whole foods raises difficulties, in particular, with respect to dose selection and the formulation of nutritionally balanced diets, specific advice related to the study design, data reporting, and interpretation of results has been provided (EFSA 2011e). Depending on the study outcome, as well as compositional data and/or previous experience, additional specific studies may be required, e.g., to address possible effects on the gastrointestinal tract; the immune, nervous, or endocrine system; and reproductive functions and development. Genotoxicity testing covering the usual endpoints should be performed if there are indications for the presence of mutagenic constituents. When testing whole foods using in vitro systems, special technical problems may be encountered due to possible effects of food constituents on the growth medium or the test cells, which are unrelated to mutagenicity; appropriate assays should thus be carefully selected (EFSA 2011d).

Noni juice obtained from the fruits of the Noni tree (*Morinda citrifolia*) is an example of a complex food, that was extensively tested. No indications for genotoxicity were found using several in vitro and in vivo test systems, and two subchronic rat toxicity studies showed no adverse effects. The SCF did not consider it appropriate to use the NOAEL determined in feeding studies with a whole food to

derive an ADI, but consumption of Noni juice was accepted based on the safety margin between the NOAEL and anticipated intake levels, and taking into account the experience gained in non-EU countries (SCF 2002c).

The potential occurrence of allergic reactions should also be considered in the risk assessment of novel foods and food ingredients. While validated test methods to predict de novo sensitization after oral intake are not available to date, potential cross-reactivity of immunoglobulin E (IgE) antibodies present in sera from allergic individuals can be studied applying in vitro methods, such as Radio or Enzyme Allergosorbent Assay (RAST or EAST) and Enzyme Linked Immunosorbent Assay (ELISA). This approach was applied, for example, in the case of Chia seed. Using sera from a number of food allergic patients, sera from peanut allergic individuals showed specific IgE-binding to proteins from Chia seed. Furthermore, individuals sensitive to sesame reacted positively in skin prick tests with Chia seed protein (EFSA 2009b). Concerns related to potential allergenicity need not lead to a rejection of authorization but require the implementation of risk management measures in order to protect potentially affected consumers. The use of Chia seed was thus restricted to a limited number of foods and requires clear labeling of this novel ingredient (EC 2013a).

## Risk Assessment of Food Derived from Genetically Modified Plants

### Risk Assessment Strategy

Genetic engineering allows insertion into the plant genome of specific genes encoding new traits such as herbicide tolerance, insect resistance, or modified nutritional characteristics of GM crops. The strategy and criteria for the risk assessment have been outlined in the Guidance for risk assessment of food and feed from genetically modified plants (EFSA 2011f), which is based on the internationally accepted recommendations of the Codex Alimentarius (WHO/FAO 2009). The strategy is based on the concept of substantial equivalence, which makes use of the idea that the non-modified plant with a history of safe use for consumers and/or animals can serve as comparator when assessing the safety and nutritional value of the modified plant and derived food or feed. Application of this comparative approach is considered a pragmatic tool for identifying differences between the GM plant and its non-GM comparator including intended as well as unintended changes. Similar to traditional breeding techniques, unintended effects could potentially be caused by genetic rearrangements or metabolic perturbations.

Each assessment starts with a comprehensive molecular characterization of the genetic modification followed by a comparative analysis of compositional, phenotypic, and agronomic characteristics of the GM plant in relation to its comparator grown under the same environmental conditions. Identified differences are then subject to further evaluation with regard to their potential impacts on human

and animal health. The objective of the risk assessment is to determine whether the GM plant and derived food and feed are as safe and nutritious as the respective products from traditionally bred crops. In the following sections, relevant aspects of this evaluation are described.

## Molecular Characterization

The molecular characterization aims at the identification of intended and potential unintended changes caused by the genetic modification, in particular those that might raise safety concerns. Information on the source of the gene(s) to be introduced and on the history of consumption of the newly expressed protein(s) by humans and/or animals as well as data on a possible relationship with proteins known to cause adverse effects is of particular importance (see sections on "Toxicological Evaluation" and "Allergenicity").

The methods applied need to be described in detail including a characterization of functional elements and other components of the vector(s) used for transformation and the complete sequence of the nucleic acid intended to be inserted. Information related to the GM plant should include a general description of the introduced trait(s), its mode of action, and the resulting changes in the plant's phenotype and/or metabolism. In order to determine whether rearrangements have occurred within the transgene construct or at the insertion site(s) and whether endogenous genes might have been disrupted or new open reading frames have been created through the insertion, the sequence of the inserted DNA and of both genomic flanking regions needs to be known. The amino acid sequences deduced from the open reading frames present in the insert and spanning the junction sites are to be compared to sequences of known allergens and toxins using bioinformatic tools and up-to-date databases. If the modification leads to the expression of new proteins, data on protein levels are required. In other cases, e.g., gene silencing approaches or where biochemical pathways have been intentionally modified, analysis of specific RNA(s) or metabolite(s) is relevant. Information on the stability of the new traits is also needed.

For GM plants containing stacked events ("stacks"), which can be obtained, for example, by conventional crossing of GM plants containing the respective single events, the main objective of the analysis is to assess the potential for any interactions between the events which may raise safety concerns. For this purpose, it needs to be analyzed whether the structure of the inserts as present in the respective single events has been conserved in the stack. Comparative data on gene expression are also required.

## Comparative Analyses

Genetically modified plants are subjected to thorough comparative analyses of their compositional, phenotypic, and agronomic characteristics, which aim to

identify differences between the GM plant and derived food and feed and its comparator. This requires the application of two complementary tests: The test of difference is used to verify, whether the GM plant, apart from the intended modification, is different from its comparator. The test of equivalence is used to verify whether the characteristics of the GM plant fall within the normal range of natural variation, which is estimated from a set on non-GM reference varieties with a history of safe use. In the case of herbicide-tolerant GM plants, it also needs to be examined whether the intended herbicide has an impact on the plant's characteristics. Detailed guidance for the selection of comparators, the design of field trials, and the statistical analysis of the data generated in the comparative analyses have been provided (EFSA 2010b; EFSA 2011g). Those characteristics that show differences between the GM plant and the comparator and/or lack of equivalence with non-GM varieties taking into account natural variation need to be considered further in the safety assessment (see section on "Toxicological Assessment").

Alterations in the phenotype and agronomic characteristics are identified through a comparison of parameters such as yield, plant morphology, flowering time, growing degree days to maturity, duration of pollen viability, response to plant pathogens and insect pests, and sensitivity to abiotic stress. With respect to the comparison of composition, an OECD Task Force is continuing to elaborate Consensus Documents on compositional considerations for new varieties of crops identifying key components that should be analyzed as well as their ranges of variation (OECD 2). The parameters should comprise key macronutrients (protein, fat, and carbohydrates), micronutrients (vitamins and minerals), antinutrients, natural toxins, and allergens as well as other plant metabolites characteristic for the plant species. A fatty acid profile should be provided for oil-rich plants and an amino acid profile for plants used as an important protein source. Depending on the introduced trait, analysis of metabolites of potentially modified metabolic pathways is also required. Considering that the comparative analyses can only detect differences in known characteristics or parameters, EFSA recommends that non-targeted profiling technologies, such as genomics, transcriptomics, proteomics, and metabolomics, should be further explored for use in the comparative analyses (EFSA 2011a).

## Toxicological Assessment

Any biologically relevant changes in the GM plant and/or derived food and feed, comprising intended as well as unintended effects of the genetic modification, are to be evaluated with regard to a potential impact on human and animal health. More specifically, this assessment needs to consider the presence of newly expressed proteins and/or other new constituents as well as changes in the levels of endogenous constituents beyond normal variation.

### New Proteins

Proteins are nutritionally relevant food constituents, which are consumed daily from different sources and degraded in the gastrointestinal tract. Although most proteins do not induce adverse effects after oral uptake, the risk assessment should consider potential toxicity and allergenicity. Proteins introduced into plants by genetic modification thus need to be thoroughly characterized, including the amino acid sequence, molecular weight, and information on posttranslational modification, such as glycosylation. A description of the protein's function and, in the case of enzymes, information on the substrate specificity and possible reaction products is required. Bioinformatics-supported (in silico) searches for homology to proteins known to cause adverse effects may provide indications of potential toxicity or anti-nutritive effects. In addition, the stability of the protein under relevant processing and storage conditions for the food derived from the GM plant and its stability toward digestive enzymes, such as pepsin, should be analyzed (see the section on "Allergenicity"). The evaluation should also consider potential interactions between the newly expressed proteins as well as interactions with other plant constituents.

The available data and in particular information on a history of safe consumption determine whether toxicological studies have to be performed. If the protein is derived from a traditional food plant and already consumed as part of the normal diet without indications of adverse effects, specific toxicity testing is not required. However, if the database is insufficient or the available information, for example, the results of the bioinformatics-supported studies, raises safety concerns, a repeated dose 28-day oral toxicity study using rodents should be performed according to the OECD Guideline 407 (OECD 1). Depending on the outcome of this study, further targeted examinations may be required. Protein produced using a microbial expression system is generally used in the safety studies instead of the protein expressed in the plant: in this case, the structural and functional equivalence of the proteins should be demonstrated by appropriate methods, such as comparison of the amino acid sequence, molecular mass, peptide mapping, physical-chemical properties, posttranslational modification and, if applicable, enzyme activity.

### New Constituents Other than Proteins and/or Changed Levels in Endogenous Constituents

The genetic modification can lead to the formation of new constituents and/or changes in the levels of constituents, which occur naturally in the unmodified plant, beyond normal variation. Typical examples for the latter are food crops used for oil production like soybeans or maize with modified fatty acid patterns in seed and rice varieties (*Oryza sativa*) with a higher content of β-carotene (pro-vitamin A) intended to be grown and consumed to reduce vitamin A deficiency in specific countries. Of agronomic interest is also the generation of GM crops resistant to plant pathogens due to the synthesis of specific secondary metabolites.

In addition to these intended changes, unintended alterations in the levels of endogenous constituents may result, which also need further evaluation.

Considering the wide variety of possible changes, a case-by-case evaluation taking into account the knowledge of the physiological function and potential toxicity of the respective constituent is generally required. Depending on the available information, additional examinations including toxicological studies may be needed. The specific testing strategy should be selected according to the EFSA guidance for food additive evaluations (EFSA 2012a), as described in the chapter "▶ Risk Assessment of Food Additives." Toxicological testing is not required if there is a documented history of safe consumption of the respective constituent.

### Testing of Whole Foods

According to the EFSA guidance, testing of the whole food and/or feed derived from a GM plant is only required if the composition has been substantially modified or if there are indications for the potential occurrence of unintended effects. Feeding studies should also be considered for GM plants containing stacked events if there are indications of possible interactions between the events stacked within the plant. Such indications may be obtained from the molecular characterization, the comparative analyses, and knowledge of the mode of action of the newly expressed proteins.

If considered necessary, a subchronic (90 days) feeding study in rodents should be conducted applying a protocol, which is based on OECD Guideline 408 for the testing of chemicals (OECD 1). This study was assessed to have sufficient specificity and sensitivity to act as a sentinel study in order to detect toxicologically relevant differences as well as nutritional deficiencies which may be due to the expression of new constituents and alterations in the levels of naturally occurring constituents, including those resulting from unintended effects of the genetic modification (EFSA 2008c). Since the testing of whole foods/feeds raises particular difficulties, a specific guidance document provides advice how to design, perform, and evaluate these studies (EFSA 2011e). Preparation of appropriate test diets is a key element since nutritional imbalance, which may complicate the interpretation of findings, has to be avoided. Furthermore, the guidance makes recommendations with respect to dose selection, animal housing, determination of sample size and power, statistical evaluation of data as well as the interpretation of study findings. Depending on the outcome of this 90-day feeding study, further toxicological testing may be needed, for example, studies on reproductive and/or developmental effects or chronic toxicity. Studies with young rapidly growing animal species, such as broiler chickens as an animal model for non-ruminants, may provide additional information on the possible occurrence of unintended effects.

## Allergenicity

Immunoglobulin E (IgE)-mediated reactions represent the main form of food allergy, and most of the constituents responsible for the allergenicity are proteins.

Therefore, all newly expressed proteins in GM plants have to be assessed for their potential to cause allergic reactions. For this purpose and in line with the recommendations of EFSA (EFSA 2010c) and the Codex Alimentarius (WHO/FAO 2009), an integrated case-by-case approach, also called weight-of-evidence approach, is applied. Given the lack of complete predictability, it is necessary to consider several aspects to obtain a cumulative body of evidence, which minimizes the uncertainty with regard to the protein in question.

In each case, it should be verified whether the source of the introduced gene encoding the protein is allergenic. If the genetic material was derived from wheat, rye, barley, oats, or related cereal crops, the newly expressed protein should also be assessed for a possible role in the elicitation of gluten-sensitive enteropathy or other enteropathies, which are not IgE-mediated. In addition, a search for amino acid sequence homology and/or structural similarities between the protein and known allergens should be performed to identify potential IgE cross-reactivity. Sequence identity of 35 % and higher over a window of at least 80 amino acids is considered as a threshold for further examinations.

If there are indications of sequence homology or structural similarities, in vitro tests measuring the capacity of specific IgE present in serum of allergic patients to bind the test protein should be performed. This specific serum screening should also be applied if the source of the introduced gene is considered allergenic, even if no sequence homology to known allergens was identified. Individual sera from well-characterized allergic individuals and immunochemical tests, such as Radio or Enzyme Allergosorbent Assay (RAST or EAST), Enzyme Linked Immunosorbent Assay (ELISA), or electrophoresis followed by immunoblotting, should be used for this purpose. Stability to digestion by proteolytic enzymes has been considered a characteristic of allergenic proteins. Although the correlation is not absolute, the pepsin resistance test performed under standardized conditions is still regarded as relevant additional information. It is also acknowledged that this in vitro test does not reflect the physiological conditions of digestion with respect to pH conditions and the presence of food matrix constituents. In vitro cell-based tests and in vivo tests using animal models may provide relevant information but have not yet been validated for use in the allergenicity assessment.

If the recipient of the introduced gene is known to be allergenic, for example, soybeans (*Glycine max*), it cannot be excluded that unintended effects of the genetic modification might have resulted in higher levels of naturally occurring endogenous allergens and thus increased the allergenicity of the whole food. In order to assess this possibility – as specifically required by the EFSA guidance – the levels of relevant naturally occurring endogenous allergens in the modified plant are to be compared with those in the non-GM comparator. Proteomics or immunochemical in vitro tests like RAST or ELISA with sera from allergic individuals are regarded as appropriate analytical methods. Post-market monitoring (PMM) may be required on a case-by-case basis to confirm the absence of an increased allergenic risk after market introduction. However, in the scientific opinions on GM plants published by EFSA to date (EFSA GMO Opinions up to June 2013), no specific concern requiring a follow-up by PMM was identified.

### Nutritional Assessment

The nutritional assessment should consider the composition of the food with respect to the levels of nutrients and antinutrients, the bioavailability and biological efficacy of nutrients as well as the anticipated dietary intake of the foods and resulting nutritional impact. If the comparative compositional analysis (see section on "Comparative Analysis") has not shown biologically relevant differences in relation to the non-GM comparator except for the introduced trait(s), no further studies to demonstrate nutritional equivalence are required. If compositional characteristics are different, they have to be evaluated for their nutritional relevance. Taking account of current dietary recommendations and nutritional reference values, it may in some cases be sufficient to base the evaluation on an assessment of the anticipated changes in intake levels of the relevant nutrient(s), which may result from replacement of the respective traditional food product. In other cases, for example, if an altered bioavailability raises nutritional concerns, specifically designed animal studies may be required.

A soybean variety showing a modified fatty acid profile in seed was the first GM plant with an intended change in nutrient levels evaluated by EFSA. Main changes in relation to commercial non-GM soybean varieties were an increased proportion of oleic acid (C18:1) and decreased proportions of linoleic acid (C18:2), α-linolenic acid (C18:3), and palmitic acid (C16:0). The nutritional assessment focused on soybean oil, the main product for human consumption. Based on consumption data from the United Kingdom, the resulting changes in intake levels of relevant fatty acids were calculated considering different scenarios, which included total replacement of conventional vegetable oils by oil derived from the GM soybean. The replacement model generally reflects a theoretical extreme case, which may overestimate the actual intake. As expected, the replacement would increase oleic acid intake and decrease palmitic acid intake, which is in line with current dietary recommendations (EFSA 2010d). Also with regard to the anticipated decreased intake of α-linolenic acid and linoleic acid, no nutritional concerns were identified (EFSA 2012d).

## Future Directions

### Novel Foods and Food Ingredients

Stakeholder consultations on a Commission discussion paper and on an impact assessment of a possible revision of the Novel Foods Regulation have revealed the need to update the existing Regulation. In consequence, the Commission submitted a proposal for revision to the Council and the European Parliament intending to simplify legislation and administrative procedures (EC 2007). This proposal contained several definitions of terms (including "novel foods," "traditional foods from a third country," and "history of safe food use in a third country"), and

clarified that foods modified by new production processes, such as nanotechnology, should be covered by the Regulation. Most relevant intended changes were the move from a decentralized to a centralized authorization procedure comprising an evaluation of all categories of novel products by EFSA according to harmonized and transparent risk assessment criteria. It was also proposed to replace the now applicant-linked authorization by a general authorization, provided that the novel food product complies with an appropriate specification. In consequence, the notification procedure according to Article 3 (4) for foods and food ingredients assessed as substantially equivalent to existing products would have been abolished. For traditional foods from third countries, a simplified procedure was envisaged: If a history of safe food use is demonstrated in the country of origin and the Member States and EFSA do not present reasoned safety objections, the novel food product could be marketed in the EU on the basis of a notification. All authorized novel products as well as those notified from third countries should be included in respective Community lists, comprising a specification of the product concerned and, where appropriate, the conditions of use, additional specific labeling requirements to inform the consumer and/or post-market monitoring (PMM) requirements. After a period of negotiations between the Commission, the Council and the European Parliament, which were unable to agree on specific issues, the revision of Regulation (EC) No 258/97 finally failed in March 2011, and to date (June 2013), no new proposal has been submitted by the European Commission.

## Food and Feed Derived from GM Plants

Recently, a Commission Implementing Regulation on applications for authorization of GM food and feed in the EU, which specifies the requirements for the presentation and preparation of applications submitted under Regulation (EC) No 1829/2003, was published (EC 2013b). With respect to the risk assessment, this Implementing Regulation follows for the greater part the guidance of EFSA. However, it requires a 90-day feeding study in rodents for each single transformation event, while EFSA requires this study only in specific cases (see section on "Testing of Whole Foods") (EFSA 2011f). Although numerous feeding studies of 90 days and longer duration have been performed with materials derived from GM plants of different species without indications of adverse effects (EFSA GMO Opinions; EFSA 2008c; Snell et al. 2012), there are still diverging views between the food and feed assessment bodies of EU Member States on the relevance and necessity of feeding studies with whole food/feed. This has led to the situation that the 90-day feeding study is now mandatory. The Commission will review this requirement on the basis of new scientific information, particularly the outcome of the research project "GMO Risk Assessment and Communication of Evidence (GRACE)" under the 2012 work programme of the seventh Framework Programme for Research (FP7) expected to be available by the end of 2015.

## References

Constable A, Jonas D, Cockburn A, Davi A, Edwards G, Hepburn P, Herouet-Guicheney C, Knowles M, Moseley B, Oberdörfer R, Samuels F (2007) History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food Chem Toxicol 45:2513–2525

EC (1997a) Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Off J Eur Commun L 43:1–6

EC (1997b) Commission recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under regulation (EC) No 258/97 of the European Parliament and of the Council (97/618/EC). Off J Eur Commun L 253:1–36

EC (2000) Commission decision of 24 July 2000 on authorising the placing on the market of 'yellow fat spreads with added phytosterol esters' as a novel food or novel food ingredient under regulation (EC) No 258/97 of the European Parliament and of the Council (2000/500/EC). Off J Eur Commun L 200:59–60

EC (2001a) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing council directive 90/220/EEC. Off J Eur Commun L 106:1–38

EC (2001b) Commission decision of 23 May 2001 authorising the placing on the market of pasteurised fruit-based preparations produced using high-pressure pasteurisation under Regulation (EC) No 258/97 of the European Parliament and of the Council (2001/424/EC). Off J Eur Commun L 151:42–43

EC (2003) Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Off J Eur Union L 268:1–23

EC (2004a) Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Off J Eur Union L 139:1–54

EC (2004b) Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters. Off J Eur Union L 97:44–45

EC (2006) Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Off J Eur Union L 404:9–25

EC (2007) Proposal for a regulation of the European Parliament and of the Council on novel foods and amending Regulation (EC) No XXX/XXXX [common procedure] (presented by the Commission). Brussels, 14.Jan 2008. COM (2007) 872 final. 2008/0002 (COD) http://ec.europa.eu/food/food/biotechnology/novelfood/COM872_novel_food_proposal_en.pdf

EC (2008a) Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Off J Eur Commun L 354:16–33

EC (2009) Commission Decision of 30 April 2009 authorising the placing on the market of lycopene as novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2009/362/EC). Off J Eur Union L 110:54–57

EC (2013a) Commission Implementing Decision of 22 January 2013 authorising an extension of use of Chia (Salvia hispanica) seed as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (2013/50/EU). Off J Eur Union L 21:34–35

EC (2013b) Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Off J Eur Union L 157:1–48

EEC (1990) Council Directive of 23 April 1990 on the deliberate release into the environment of genetically modified organisms (90/220/EEC). Off J Eur Commun L 117:15–27

EFSA GMO opinions. http://www.efsa.europa.eu/en/panels/gmo.htm

EFSA (2008a) Scientific opinion of the panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission on the safety in use of lycopene as a food colour. EFSA J 674:1–66
EFSA (2008b) Scientific opinion of the panel on panel on dietetic products, nutrition and allergies on a request from the European commission on the safety of synthetic lycopene. EFSA J 676:1–25
EFSA (2008c) Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Report of the EFSA GMO panel working group on animal feeding trials. Food Chem Toxicol 46:S2–S70
EFSA (2009a) EFSA scientific committee. Guidance on safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements. EFSA J 7(9):1249
EFSA (2009b) Scientific opinion of the panel on dietetic products nutrition and allergies on a request from the European commission on the safety of 'Chia seed (*Salvia hispanica*) and ground whole Chia seed' as a food ingredient. EFSA J 996:1–26
EFSA (2010a) 13th EFSA scientific colloquium: what's new on novel foods. Amsterdam, The Netherlands. EFSA 19–20 Nov 2009
EFSA (2010b) EFSA panel on genetically modified organisms (GMO). Scientific opinion on statistical considerations for the safety evaluation of GMOs. EFSA J 8(1):1250
EFSA (2010c) EFSA panel on genetically modified organisms (GMO). Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA J 8(7):1700
EFSA (2010d) EFSA panel on dietetic products, nutrition and allergies (NDA). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 8(3):1461
EFSA (2011a) EFSA. Use of the EFSA comprehensive European food consumption database in exposure assessment. EFSA J 9(3):2097
EFSA (2011b) EFSA panel on genetically modified organisms (GMO). Guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA J 9(6):2193
EFSA (2011c) EFSA panel on dietetic products, nutrition and allergies (NDA). Scientific opinion on the safety of "Glavonoid®", an extract derived from the roots or rootstock of *Glycyrrhiza glabra* L., as a novel food ingredient on request from the European Commission. EFSA J 9(7):2287
EFSA (2011d) EFSA scientific committee. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA J 9(9):2379
EFSA (2011e) EFSA scientific committee. EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA J 9(12):2438
EFSA (2011f) EFSA panel on genetically modified organisms (GMO). Scientific opinion on guidance for risk assessment of food and feed from genetically modified plants. EFSA J 9(5):2150
EFSA (2011g) EFSA panel on genetically modified organisms (GMO). Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. EFSA J 9(5):2149
EFSA (2012a) Panel on food additives and nutrient sources added to food (ANS). Guidance for submission for food additive evaluations. EFSA J 10(7):2760
EFSA (2012b) EFSA panel on dietetic products, nutrition and allergies (NDA). Scientific opinion on dihydrocapsiate. EFSA J 10(7):2812
EFSA (2012c) EFSA panel on dietetic products, nutrition and allergies (NDA). Statement on the safety of synthetic zeaxanthin as an ingredient in food supplements. EFSA J 10(10):2891
EFSA (2012d) EFSA panel on genetically modified organisms (GMO). Scientific opinion on application (EFSA-GMO-NL-2010-78) for the placing on the market of herbicide-tolerant, increased oleic acid genetically modified soybean MON 87705 for food and feed uses, import and processing under regulation (EC) No 1829/2003 from Monsanto. EFSA J 10(10):2909

FSA (2007) Advisory Committee on Novel Foods and Processes (ACNFP). Initial opinion on an application under the novel foods regulation for baobab dried fruit pulp as a food ingredient. http://www.food.gov.uk/multimedia/pdfs/baobabinitialopinion.pdf

Hepburn P, Howlett J, Boeing H, Cockburn A, Constable A, Davi A, de Jong N, Moseley B, Oberdörfer R, Robertson C, Wal JM, Samuels F (2008) The application of post-market monitoring to novel foods. Food Chem Toxicol 4:9–33

OECD 1 OECD guidelines for the testing of chemicals. http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

OECD 2 consensus documents for the work on the safety of novel foods and feeds: plants. http://www.oecd.org/env/ehs/biotrack/consensusdocumentsfortheworkonthesafetyofnovelfoodsandfeedsplants.htm

SCF (2001) Guidance on submissions for food additive evaluations by the Scientific Committee on Food (opinion expressed on 11 July 2001). http://ec.europa.eu/food/fs/sc/scf/out98_en.pdf

SCF (2002a) Opinion of the Scientific Committee on Food on a report on post launch monitoring of "yellow fat spreads with added phytosterol esters". (expressed 26 Sep 2002) http://ec.europa.eu/food/fs/sc/scf/out144_en.pdf

SCF (2002b) General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects on β-carotene. (expressed 26 Sep 2002) http://ec.europa.eu/food/fs/sc/scf/out143_en.pdf

SCF (2002c) Opinion of the Scientific Committee on Food on Tahitian Noni® Juice (expressed on 4 December 2002). http://ec.europa.eu/food/fs/sc/scf/out151_en.pdf

Snell C, Bernheim A, Bergé J-B, Kuntz M, Pascal G, Paris A, Ricroch AE (2012) Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: a literature review. Food Chem Toxicol 50:1134–1148

WHO/FAO (2009) Foods derived from modern biotechnology, 2nd edn. WHO, Rome

# Notification of Cosmetic Products and Dangerous Mixtures in Regulatory Toxicology

Herbert Desel, Pieter Brekelmans, and Ronald de Groot

## Contents

Poisons Centers Have an Important Role in Clinical and Regulatory Toxicology ..... 850
Notification of Product Information for Poisons Centers Facilitates Rapid Toxicological Risk Assessment ..... 850
The Cosmetic Products Notification Portal ..... 851
Harmonized Notification of Hazardous Products According to CLP Article 45 (4) ..... 851
References ..... 852
Resources ..... 853

**Abstract**

Poisons centers (PC) support medical management of poisoning cases in all parts of the health system, in particular in emergency cases, by supplying specific information on the toxicity of agents involved and by advising on medical management of the poisoning case. Detailed product information on cosmetic products and hazardous mixtures has to be notified by industry to facilitate the PC services. Harmonized notification formats and procedures and Europe-wide product databases as the Cosmetic Products Notification Portal help to increase data quality and to reduce notification workload.

The authors are active participants in the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) Working Group on Poisons Centres' Activities/European Regulatory Issues, and as poisons center experts they take part in discussions on harmonization of product notification for Poisons Centres with the European Commission, industry, and other stakeholders.

H. Desel (✉)
GIZ-Nord Poisons Center, University Medical Center Göttingen - Georg-August-Universität, Göttingen, Lower Saxony, Germany
e-mail: hdesel@med.uni-goettingen.de; hdesel@giz-nord.de

P. Brekelmans • R. de Groot
National Poisons Information Center (NVIC), University Medical Center Utrecht, Utrecht, GA, The Netherlands
e-mail: P.Brekelmans@umcutrecht.nl; R.degroot-5@umcutrecht.nl

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_95, 

## Poisons Centers Have an Important Role in Clinical and Regulatory Toxicology

Today, about 80 *poisons centers* (poison control centers, poison information centers, PC) play an important role in toxicological risk assessment and management of human (and animal) poisoning cases in Europe, especially in emergency medicine. Worldwide, about 200 PC are registered in a directory of the World Health Organization. PC either are independent public institutions or are affiliated with a (university) hospital or a governmental authority. Traditionally, PC are fully funded by the public health system, but since recently other ways of financing the centers are used, like cooperation with industry on emergency number service for the Safety Data Sheet and continuous reporting on exposure cases with products of the contracting company.

In PC, toxicologically trained medical doctors and other experts (specialists in poison information, *SPIs*, e.g., pharmacists or nurses) give information to general practitioners, medical doctors at the hospital, or other professional healthcare personnel on poisoning severity, clinical symptoms, and medical treatment of patients exposed to a toxic agent. If a physician is calling the PC service, toxicological risk assessment, the medical diagnosis, and the individual poisoning management plan can be worked out. As an intoxication progresses and/or if a patient is admitted to the hospital, often several calls are needed to complete risk assessment, advice, and case recording. In most countries, PC also give advice directly to the exposed patient or to family members, especially if children are exposed. An "all-clear" can be given in the majority of exposures and thus saves thousands of unnecessary presentations to the emergency medical system every day.

Poisons centers are often contacted by local, national, and/or European authorities and by industry to report on their experiences with specific poisonings. To answer these questions – and to analyze cases for medical studies – PC register all exposure cases in local or national case databases. Thus, PC case databases are recurrently analyzed to describe poisoning frequencies and to identify poisoning trends, e.g., novel poisoning risk arising from new agents and products (*toxicovigilance*).

## Notification of Product Information for Poisons Centers Facilitates Rapid Toxicological Risk Assessment

The most important part of the PC service is to perform or to support toxicological risk assessment. Together with findings of the physical examination of the patient and in ambiguous cases the results of toxicological lab investigations, the risk assessment constitutes the basis for correct medical diagnosis and subsequent medical decisions on patient treatment and monitoring.

To facilitate toxicological risk assessment after exposure to a potentially hazardous commercial product, access to toxicity-related product information, especially detailed information on the product formula, is needed. European Union (EU) regulations, directed to a harmonization and centralization of product notification in EU Member States, are described below.

## The Cosmetic Products Notification Portal

According to Article 13 of the Cosmetics Regulation (EC No 1223/2009, European Parliament and Council 2009), there is an obligation for all manufacturers and importers (into the EU) of cosmetic products to notify product information to a central European database, the *Cosmetic Products Notification Portal* (*CPNP*). The CPNP is located at the European Commission Services in Brussels. Poisons centers of all EU Member States have access to the complete CPNP dataset; competent national authorities have access to a data subset needed for market surveillance. PC can either access the CPNP online or download all or a subset of data for import into local product databases. Local product data download enables reliable and easy linkage between poisoning case documentation and product formula in the PC databases to facilitate (toxicovigilance) reporting (see above).

Notification to the CPNP is performed online through a secured website. One of three different formats can be used: (1) frame formulations for products with ingredients of no or low toxicological concern under most exposure conditions to be anticipated (indicating maximum concentrations for ingredients of low toxicity; a set of frame formulations is predefined by a working group of the European Commission, poisons centers, and cosmetic industry), (2) frame formulation and notification of exact concentrations for ingredients with moderate or high toxicity, or (3) the full formula with exact concentration for all ingredients. Besides the formula, further data have to be notified, e.g., exact and complete product name(s) in all relevant languages, pH value, and image of the packaging. Product *renotification* is needed (1) if the product dataset has changed (e.g., additional product name used in another country of marketing), (2) if the product formula changes, or (3) in case of error in the dataset.

## Harmonized Notification of Hazardous Products According to CLP Article 45 (4)

Article 45 of the CLP (Classification, Labelling and Packaging of Substances and Mixtures) Regulation (EC No 1272/2008, European Parliament and Council 2008) states that poisons centers shall have at their disposal all information needed to carry out *the tasks for which they are responsible*, i.e., mainly for toxicological risk assessment in emergency cases.

For this purpose, all EU Member States had to appoint a body or bodies responsible for receiving this information including *the chemical composition of mixtures placed on the market and classified as hazardous on the basis of their health or physical effects*. The appointed body, i.e., PC or governmental authority in some Member States, continuously collects product information notified by companies, i.e., *importers and downstream users*. Product data have to be kept confidential and must not be used for any other purpose than (1) medical management of poisoning cases or (2) poisoning prevention measures (including statistical data analysis).

However, as in the preceding legislation, Article 45 does not yet exactly describe what information is required and how it should be notified (de Groot et al. 2007). And thus, varying product notification requirements in different EU Member States (leading to additional workload for industry) could still remain. This shortcoming was recognized at a late stage in the development of the *CLP Regulation* and corrected by adding paragraph 4 to Article 45. According to paragraph 4, the European Commission Services have carried out a review process to see whether harmonization of product notification is feasible, including consultations with relevant stakeholders such as the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT), industry, and national authorities from 2009 to 2011. A review report was published in 2012 (European Commission – Enterprise and Industry Directorate-General 2012) with positive result: On the basis of this review, the European Commission will develop a regulation adding an annex to the CLP Regulation that will define the format (and procedures) for an EU-wide harmonized electronic notification of product information for all EU poisons centers in the future. According to the Commission's review the main features of the harmonized format will be the following: (1) in contrast to the requirements for the Safety Data Sheets, also nonclassified ingredients have to be notified if present above a threshold concentration, (2) concentration of ingredients in a mixture can be notified using well-defined ranges, (3) a Unique Product Identifier (UPI), which includes a company-identifier component that will have to be printed on labels, will be part of the notification in order to facilitate identification of products and formulas involved in poisoning incidents, (4) the notification contains a product category for each mixture notified (a European Product Category System shall be developed for this purpose), and (5) the format is described as eXtended Markup Language (XML) Scheme.

## References

De Groot et al (2007) Article 17 of the Preparations Directive 1999/45/EC is differently implemented in EU member states. RIVM report 233900001/2007. http://www.rivm.nl/Bibliotheek/Wetenschappelijk/Rapporten/2007/juli/Article_17_of_the_Preparations_Directive_1999_45_EC_is_differently_implemented_in_EU_Member_States_A_survey_on_how_Poisons_Information_Centres_become_informed_on_dangerous_preparations. Accessed 28 Oct 2012

European Commission – Enterprise and Industry Directorate-General (2012) Harmonisation of information for poison centres. Review according to Article 45(4) of Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures, Brussels. http://ec.europa.eu/enterprise/sectors/chemicals/classification/poison-centres/index_en.htm#h2-1. Accessed 21 Oct 2012

European Parliament and Council (2008) Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures . . .. Off J Eur Union (EN) L 353:1–1355

European Parliament and Council (2009) Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Off J Eur Union (EN) L 342:59–209

## Resources

European Commission Services (2012) Cosmetic products notification portal (CPNP) – cosmetics – consumer affairs (Consumer affairs policy website). http://ec.europa.eu/consumers/sectors/cosmetics/cpnp. Accessed 28 Oct 2012

European Commission Services (2012) Cosmetic products notification portal (CPNP) – User Manual, Brussels. http://ec.europa.eu/consumers/sectors/cosmetics/files/pdf/cpnp_user_manual_en.pdf. Accessed 28 Oct 2012, also available in all other official EU languages

European Commission Services (2012) Poison centres – chemical – enterprize and industry. http://ec.europa.eu/enterprise/sectors/chemicals/classification/poison-centres. Accessed 28 Oct 2012

# Chemical and Biological Weapons and Their Regulation

Horst Thiermann, Kai Kehe, Julia Riehm, and Lothar Zöller

## Contents

Introduction and History ..... 856
Chemical Weapons ..... 857
Definitions ..... 857
Characteristics of Chemical Weapon Agents (CWA) ..... 857
Biological Weapons ..... 861
Definition ..... 861
Characteristics of Biological Toxins ..... 862
Risk Assessment Aspects ..... 862
Risk Management ..... 865
Biological Weapons Convention (BWC) ..... 865
Chemical Weapons Convention (CWC) ..... 865
National Regulations: Installation of Preparedness Standards ..... 866
References ..... 868
Recommended Reading ..... 868

**Abstract**

Chemical and biological agents have been used as weapons since ancient times. But it was only after the disastrous use of this type of agents in World War I that international efforts were made to prohibit them. These efforts were very

H. Thiermann (✉)
Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany
e-mail: HorstThiermann@bundeswehr.org

K. Kehe
Bundeswehr Medical Academy, Military Medical Research and Development, Munich, Germany
e-mail: KaiKehe@bundeswehr.org

J. Riehm • L. Zöller
Bundeswehr Institute of Microbiology, Munich, Germany
e-mail: JuliaRiehm@bundeswehr.org; LotharZoeller@bundeswehr.org

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_121, 

successful and continue until today. But nevertheless, it was not possible to forestall their use completely. This chapter gives a short introduction in the field. It also characterizes some important agents and outlines, what is necessary to be prepared against a possible attack.

## Introduction and History

Chemical warfare agents are chemicals, which have a very high toxicity and may therefore be misused as weapons to cause death or disease among the target population. For historical reasons, the term "chemical warfare" agent includes synthetic chemicals (toxicants), but usually does not include the toxins, which are poisons produced by living organisms. Toxin agents are often taken as a subgroup of biological agents (see below). However, for the toxicological risk assessment, there is no basic difference between toxicants and toxins. The disabling effect of such weapons on target persons is horrific. It is in the nature of such agents that they will without differentiation affect the exposed population.

It is probably a result of the widespread use of chemical weapons during World War I that international efforts were made, to restrict and ban such agents. In 1925, the use of asphyxiating, poisonous, or other gases and of bacteriological methods of warfare were prohibited and included in the Geneva Protocol. Mandatory regulations regarding the possession and development of warfare agents followed in 1968 (Chemical Weapons Convention) and 1972 (Biological Weapons Convention). Despite these regulations, several offenses occurred. The exile Bulgarian Markov died after an attack with ricin toxin in London in 1978. About 10 years later, members of the Japanese Aum Shinrikyo cult tried to poison attendants of a royal wedding party spraying medium supernatant from cultures of neurotoxin-producing *Clostridium botulinum* strains. According to the American "Working Group on Civilian Biodefense," 19,000 l of botulinum neurotoxin were produced during the 1990s in Iraq. Officially, there are no existent biological warfare programs nowadays. However, their presence cannot be completely denied as there are no legal control mechanisms. In 1995, the sarin subway attack was of terrorist origin. Such an attack is able to scare a whole nation and has high impact on politics and decision making. In 2013, ricin toxin was used in a bioterror attack in the United States when three series of letters containing the substance were sent to officials and even the President. Although nobody was injured, the news attracted public attention and intensive media coverage worldwide. Chemical warfare agents are likely to be used in terrorist attacks as they are relatively easy to produce and designed to have a high lethality.

The use of poison in military conflicts is very old. One of the first attempts to use toxic substances in military operations was during the Cirraean war [595–585 BC]. The city of Kirrah was attacked by the Amphictyonic League of Delphi. A secret water supply of the city was poisoned with *Helleborus* roots . Helleborin caused severe diarrhea and weakened the defenders of the city. This is believed to be the first report of chemical warfare. Later in history, more toxic substances have been stockpiled and used as chemical weapons.

For example, historical documents claim the Assyrians to consciously poison their enemies by the application of *Claviceps purpurea*'s ergot in the sixth century BC. Later in time, one of Hannibal's warfare strategies aimed at throwing poisonous snakes on Pergamenes' ships.

Chemical warfare agents are still stockpiled and available for military use. After the last chemical war between Iran and Iraq 30 years ago, there was a long lag-period, in which there was no proof for the use of chemical or biological weapons in war. However, the situation changed dramatically, when in August 2013, news on a possible use of chemical weapons in a populated area in Syria made the headlines.

## Chemical Weapons

### Definitions

Article II of the Chemical Weapons Convention (CWC) defines a *toxic chemical* as "any chemical which through its chemical action on life processes can cause death, temporary incapacitation or permanent harm to humans or animals." *Toxic chemicals* and/or devices (munitions) to disperse toxic chemicals are regarded as *chemical weapons*. Toxic chemicals, synthesized for military purposes, used in this context, are also called chemical weapon agents (CWAs). *Old chemical weapons* are produced before 1925.

CWAs are commonly classified as blood, blister, nerve, psychological, and pulmonary agents. This classification is commonly used but scientifically not correct, e.g., blood agents do not solely react with blood constituents. Blister agents may cause (more severe) systemic poisoning.

The CWC Annex of Chemicals distinguishes so-called Schedule 1–3 chemicals, which are regarded as CWAs.

Schedule 1 substances are toxic chemicals which have been used as chemical weapons or may be used for manufacturing chemical weapons (Table 1). Their civil use is limited. Some of the Schedule 1 chemicals have limited use in medicine or research. Saxitoxin and ricin are also Schedule 1 substances.

Toxic chemicals with possible use as chemical weapons or in their manufacturing process and which have legal use as well are listed in Schedule 2 (small-scale applications) and Schedule 3 (large-scale applications).

### Characteristics of Chemical Weapon Agents (CWA)

#### Nerve Agents

Organophosphorus (OP) compounds are widely used pesticides in agriculture. More than 200,000 deaths after OP poisoning occur worldwide. The main causes are of suicidal nature or accidents. A subgroup of OP compounds has highly toxic properties and was stockpiled as chemical weapons. OP nerve agents are divided

**Table 1** Examples of chemical warfare agents listed in Schedule 1 and their physicochemical properties

| Substance [NATO code] | Chemical name | CAS | MW | Boiling point [°C] | Freezing point [°C] | Vapor pressure [mmHg at 20 °C] | Vapor density [air = 1.0] | Solubility in water [g/100 g $H_2O$, 20 °C] |
|---|---|---|---|---|---|---|---|---|
| Schedule 1 | | | | | | | | |
| Sarin [GB] | Isopropyl methylphosphonofluoridate | 107-44-8 | 140.1 | 158 | −56 | 2.1 | 4.9 | Miscible |
| Soman [GD] | Pinacolyl methylphosphonofluoridate | 96-64-0 | 182.2 | 167–200 | −42 | 0.4 | 6.3 | 2.1 |
| Tabun [GA] | Dimethylamidocyanoethylphosphate | 77-81-6 | 162.1 | 220–246 | −50 | 0.037 | 5.6 | 9.8 |
| Cyclosarin [GF] | *O*-cyclohexyl-methylfluorophosphonate | 329-99-7 | 180.2 | 239 | −30 | 0.044 | 6.2 | 0.37 |
| VX | S-(2-diisopropyl aminoethyl) *O*-ethyl methyl phosphonothiolate | 50782-69-9 | 267.4 | 298 | −51 | 0.0007 | 9.2 | 3 |
| HD | Bis(2-chlorethyl) sulfide | 505-60-2 | 159.1 | 227.8 | −50 | 0.072 | 5.6 | <1 |

into two groups: G agents and V agents. G agents contain a fluorine or cyanine as leaving group, whereas V agents contain a sulfur substituent leaving group.

### Clinical Picture

OP poisoning shows typically the signs and symptoms of cholinergic crisis. Respiration is the most critical affected system. Severe poisoning causes respiratory depression, bronchosecretion, bronchospasm, and paralysis of respiratory muscles. Additional effects are miosis, increased secretions from glands, increased peristaltic activity, vomiting, general muscle weakness and twitching, hypothermia, bradycardia and hypotension, convulsions followed by unconsciousness.

### Toxicodynamic

Acetylcholinesterase (AChE) is one of the fastest acting enzymes of the human body, which hydrolyzes the cholinergic transmitter acetylcholine (ACh), thereby inactivating its action on muscarinic or nicotinic receptors. Membrane-bound AChE is located at cholinergic synapses, and neuromuscular junctions. Soluble AChE is present in the cerebrospinal fluid and in cholinergic nerve terminals. Nerve agents phosphorylate AChE at the active enzyme site, thereby inhibiting activity. As a consequence, ACh accumulates and overstimulates cholinergic receptors, leading to a cholinergic crisis. Antidotal therapy is directed either to competitively displace acetylcholine from the receptor (atropine) or to remove causally the nerve agent from its binding site (reactivation). To the later end, "reactivators" so-called oximes (e.g., obidoxime, pralidoxime) were introduced in causal therapy. This therapeutic strategy appears suitable in case of poisoning with several nerve agents (Sarin, VX). Unfortunately, however, AChE inhibited by several nerve agents can hardly be reactivated, e.g., tabun. Moreover, bound nerve agents undergo an "aging" process, where an alkyl or alkoxy group leaves the nerve agent AChE complex. The velocity of aging is dependent on the nerve agent and is extremely rapid in case of soman (aging half time about 2 min in humans). The "aged" complexes can no longer be reactivated. As a consequence, AChE reactivators as well as atropine should be given within minutes after exposure. Nevertheless, symptomatic treatment, e.g., artificial ventilation, may be necessary.

### Biomonitoring, Bioanalytic, and Verification

To confirm clinical diagnosis based on typical signs and symptoms of cholinergic crisis, determination of red blood cell, AChE activity appears appropriate. This parameter can be determined even under field conditions or bedside within few minutes by the ChE check mobile that is commercially available as certified as medical products in Europe or in the United States by the Testmate®. Under several circumstances, however, ongoing treatment may be necessary, especially when active poison remains longer in the body than early administered antidotes. In such cases, aside from atropine, oxime treatment may be necessary for a longer period. To enable optimized patient-oriented application of oximes as long as needed, a laboratory test system, the so-called cholinesterase status, was established and is commercially available since early 2013. Apart from these clinically most

relevant parameters, the analysis of intact nerve agent, its metabolites as well as protein adducts in body fluids are possible in special laboratories. However, for such analytical tasks, advanced techniques are necessary that are available only in a few laboratories.

### Long-Term Effects

After exposure of organophosphate insecticides, an organophosphate-induced delayed neuropathy (OPIN) has been described. This clinical picture has not been observed in survivors of nerve agent poisoning. No reports about mutagenic, cancerogenic, or teratogenic effects after sarin, tabun, or VX poisoning have been published.

## Vesicants

Sulfur mustard (bis(2-chloroethyl)sulfide, HD) was first synthesized in 1822 by Despretz. In World War I, it has been extensively used as chemical weapon and was called the "king of war gases." During World War II, nitrogen analogues such as ethylbis(2-chloroethyl)amine (HN-1), bis(2-chloroethyl)methylamine (mechlorethamine, HN-2), and tris(2-chloroethyl)amine (trichlormethine, HN-3) were synthesized in the United States. All these agents share their ability to induce skin blistering and were classified as "vesicants." Sulfur mustard is by far the most produced and stockpiled vesicant until today.

### Clinical Picture (Short and Long Term)

Skin contact with sulfur mustard liquid or gas will produce blisters after a symptomless interval of several hours. Gaseous exposure affects more moist and hairy regions of the body as the genito-anal region, the chest, and axillae. The eyes are very susceptible. Even low vapor exposure results in ocular injury with severe blepharospasm. Inhalation of sulfur mustard vapor damages mainly the upper part of the respiratory tract. The trachea and bronchial epithelia become necrotic and detach from the wall (pseudomembranes). Besides this local effects, absorption of sulfur mustard results in systemic poisoning. Reproductive and developmental toxicity, gastrointestinal effects (vomiting, diarrhea), hematological effects (pancytopenia), and immunosuppression have been reported.

### Toxicodynamic

Sulfur mustard is a lipophilic, alkylating substance with two reactive moieties. Sulfur mustard can easily penetrate the skin or other body surfaces and reacts with a huge variety of molecules. It can alkylate macromolecules and cross-link them. The most important reaction is with the DNA. Sulfur mustard reacts predominantly with guanine at the $N_7$ position, which accounted for 61 % of total DNA alkylation. Less likely are cross-links, 17 % of alkylations involve two guanines (G-alkyl-G). However, cross-linked DNA strands are difficult to repair and cell division may result in DNA strand breaks, which are lethal lesions of the cell. Apoptotic cell death occurs with a delay of several hours.

Despite a century of research and deeper insight in the pathophysiology of sulfur mustard poisoning, no causal treatment has been identified so far.

### Late Effects

Sulfur mustard poisoning results in a variety of late effects. The most common late effects were found in the respiratory tract (42.5 %), eyes (39 %), and skin (24.5 %).

The most disabling late effects after sulfur mustard inhalation are respiratory disorders, e.g., bronchiolitis obliterans, chronic obstructive pulmonary disease, asthmatoid bronchitis, and bronchial stenosis.

Late effects at the eyes are chronic keratoconjunctivitis. Only a few of exposed soldiers (0.5 %) complain of a delayed type of ulcerative keratitis, which occurs several years after exposure and results in opafication of the cornea.

Balali-Mood et al. (2005) published a study on soldiers heavily exposed to sulfur mustard. The most important dermatological late effects are hyperpigmentation (55 %), hypopigmentation (25 %), erythematous papular rash (42.5 %), dry skin (40 %), multiple cherry angiomas (37.5 %), and skin atrophy (27.5 %).

As a DNA-damaging agent, it has been linked to several forms of cancer observed in workers or soldiers. Lung cancer (e.g., adenocarcinoma) has been reported in workers of sulfur mustard production facilities. Skin cancer (e.g., basalioma) may occur at exposed sites.

## Biological Weapons

### Definition

Biological weapons may be used for strategic or tactical reasons to intimidate, incapacitate, or kill an opponent, single individuals or entire groups. The highest risk of a deliberate release of a biothreat agent currently arises from bioterrorism. Numerous species of highly infectious bacteria or viruses and various biological toxins have been misused as biological warfare agents in the past or are associated with an inherent risk to be misused due to their specific properties. Moreover, some species of fungi and parasites are listed as potential biothreat agents by some authors. Listing and current ranking of biothreat agents can be accessed at the websites of the American CDC, in the Chemical Weapons Convention, in the textbook of military medicine, or in the NATO handbook on the medical aspects of NBC defensive operations (AMedP-6(B)).

Among the biological warfare agents, biological toxins in contrast to live bacteria and viruses represent a group of noninfectious substances. Only toxins that can be utilized independently of their producer organisms are considered as autonomous biothreat agents and must be differentiated from toxins that are produced by the microorganisms during the course of infection and act as pathogenicity factors, such as the toxins of *Bacillus anthracis*. Biothreat toxins may cause incapacitating, severe intoxication, or even death in exposed humans or animals. Early in history, various poisonous substances used to be employed not only for man's own survival but also to attack enemies. For the toxicologist, the risk assessment of toxin-derived "biological warfare agents" is principally the same as that of chemical warfare agents.

## Characteristics of Biological Toxins

Toxins represent a subset of biothreat agents, which are also called mid-spectrum agents. They are noninfectious and do not reproduce in the host. The clinical picture usually appears after a shorter latency period as compared to infectious agents. Naturally occurring biological toxins are synthesized by plants (curare, ricin), fungi (aflatoxins), amphibians (dart frog's batrachotoxin), bacteria (botulinum neurotoxin), or algae (paralytic shellfish poison) and are mostly part of the self-protection strategies of the producing organism. The structures of biological toxins range from high-assembly biotoxins to simple bioregulator molecules: Complex AB toxins are produced by bacteria or plants. They consist of a binding (B) and an active (A) domain and interfere with internal cell functions. The binding subunit (A) binds to a cell surface receptor and enables the transport of the cytotoxic B-subunit into the cell. Their size ranges from 25kD to 200kD (Table 2). Other toxins are non-peptide substances and rather bioregulator molecules. Their onset of action is immediate in contrast to AB toxins with a latency period of hours, sometimes days. Their molar mass is smaller ranging from 300 g/mol to 3,000 g/mol (Table 2). They are also markedly stable under different environmental conditions, versus heat and pH alterations. They can even be synthesized in vitro (SXT), which is not possible for the proteinaceous toxins. The trichothecene mycotoxins belong to the non-peptide substances and, moreover, are contact poisons. They gained notoriety as the "yellow rain" agent during the 1970s and 1980s in Cambodia and Laos, Southeast Asia, which is – for lack of unambiguous evidence – not without controversy.

Toxicological effects of biological toxins were studied mostly after alimentary uptake. However, more severe physiological consequences may result from exposure through a non-enteric route. Intentional exposure to toxins in aerosol and droplet clouds and after subcutaneous injection has occurred. Yet only few and inconsistent data is available with regard to the associated health effects. A variety of nonspecific clinical symptoms and multiorgan effects may develop depending on the way of exposure, ranging from acute emesis and diarrhea, nervous disorders, cardiovascular alterations, hemostatic derangements, skin toxicity, and multiorgan failure to chronic syndromes such as immunosuppression, weight loss, decreased reproductive capacity, and bone marrow damage.

## Risk Assessment Aspects

Due to their relative ease of production and immense toxicity, some biological toxins are considered as potential biological warfare agents. The Centers for Disease Control (CDC, Atlanta, United States) provide the most widely used priority categorization of bioterrorism agents according to the risk to national security associated with them. Features determining the categorization are the ease of transmission/dissemination, the mortality rates, and the public health impact. The botulinum neurotoxins are classified as category A (highest priority). Ricin, staphylococcal enterotoxins, further clostridial

**Table 2** Characteristics of a selection of biological toxins without subtype differentiation

| Origin | Name | Short | Listing | Main effect | Main pathophysiology | $LD_{50}$ | Specific prophylaxis/ treatment | Size (kD) | Gold standard detection |
|---|---|---|---|---|---|---|---|---|---|
| Bacteria | | | | | | | | | |
| *Clostridium botulinum* | Botulinum neurotoxins | BoNT | AMedP-6(B)<br>Cat A (CDC) | AB toxin, neurotoxic | Flaccid paralysis, botulism | 0.002 | Antiserum, vaccine (limited) | 160 | Mouse bioassay |
| *Clostridium perfringens* | Epsilon toxin | – | AMedP-6(B)<br>Cat B (CDC) | Pore-forming toxin, potassium and fluid leakage from cells | Vasogenic brain edemas, indirect neuronal excitotoxicity | 0.5 | – | 30 | Mouse neutralization test |
| *Staphylococcus aureus* | Enterotoxins | SE | AMedP-6(B) | Emetic, toxic shock syndrome | Emesis, T-cell stimulation, cytokine release | 30 | – | 25 | ELISA |
| Algae/plankton | | | | | | | | | |
| | Saxitoxin/ paralytic shellfish toxin | STX/ PST | AMedP-6(B)<br>CWC | Neurotoxic, sodium channel blockage | Flaccid paralysis | 6 | – | 0.3 | Mass spectrometry |
| Plant | | | | | | | | | |
| *Ricinus communis* | Ricin | – | AMedP-6(B)<br>CWC<br>Cat B (CDC) | AB toxin, inhibition of protein synthesis, cytotoxic | Tachycardia, hypotension, seizures, multiorgan dysfunction | 3 | Vaccine in development | 65 | In vitro bioassay |
| *Abrus* sp. | Abrin | – | | AB toxin, inhibition of protein synthesis, cytotoxic | Multiorgan dysfunction | 0.04 | – | 65 | n.d. |

(*continued*)

**Table 2** (continued)

| Origin | Name | Short | Listing | Main effect | Main pathophysiology | $LD_{50}$ | Specific prophylaxis/ treatment | Size (kD) | Gold standard detection |
|---|---|---|---|---|---|---|---|---|---|
| Fungus | | | | | | | | | |
| *Fusarium* sp. | Trichothecene | | AMedP-6(B) | Inhibition of protein synthesis | | | | | LC-MS/MS |
| | Deoxynivalenol | DON | | Inhibition of protein synthesis | Emesis | 10E7 | | 0.3 | LC-MS/MS |
| | T-2 mycotoxin | T 2 | | Inhibition of protein synthesis | Aleukia, cancerogenic | 10E3 | – | 0.47 | LC-MS/MS |

Abbreviations: $LD_{50,}$ oral human $LD_{50}$ (μg/kg)

toxins, and cholera toxin are classified as category B (second priority) agents. As listed in Table 2, biological toxins are also considered in the NATO handbook on the medical aspects of NBC defensive operations (AMedP-6(B)), and most officially in the Chemical Weapons Convention.

In a military scenario, ricin and the botulinum neurotoxins are – besides the causative agents of anthrax or pneumonic plague – also considered as high-risk agents for bioterroristic or warfare activities. Risk-ranking respects the dimension of damage and the probability of an intentional event associated with the respective substance in a given scenario.

Low-dose pharmaceutical drugs containing botulinum neurotoxin (Botox) are commercially produced for the medical treatment of various neurological syndromes (Dysport®, Ipsen Biopharmaceuticals; Myobloc® Solstice Neurosciences; Botox®, Allergan). Moreover, in recent years, the cosmetics industry has established a fairly new market for botulinum neurotoxin due to its effect of wrinkle reduction. Every year, around 75 billion dollars are reaped with such products, which has given rise to large-scale non-licensed production of Botox drugs that are distributed via the internet. Illegal Botox production plants have settled in China, India, and the successor states of the former Soviet Union and might become a potential toxin source for bioterrorists. Ricin was researched for its ability to kill tumor cells during cancer treatment. However, pharmaceutical products have never emerged from scientific approaches.

## Risk Management

### Biological Weapons Convention (BWC)

The BWC is an international agreement on the prohibition of the development, production, and stockpiling of bacteriological (biological) and toxin weapons and on their destruction. It was implemented in 1975 as a first multilateral disarmament agreement based upon the 1925 Geneva Protocol. It lacks the listing and ranking of possible agents. So far, 170 member states have signed the convention. It does not include verification regimes, and therefore cannot prosecute noncompliance.

### Chemical Weapons Convention (CWC)

#### Organization for the Prohibition of Chemical Weapons (OPCW)

Since 1997, the OPCW, located in The Hague, Netherlands, has been authorized to execute the controls and sanctions regarding the CWC as the official implementing body. Today, the organization comprises 188 member states and is directly responsible to the United Nations committee. OPCW received the Nobel Peace Prize in the year 2013.

To fulfill its tasks, the OPCW is comprised of several organs: the Technical Secretariat regulates administration, controls verification of international CWC

implementation, and coordinates routine inspections. In return, decisions are made by the Executive Council and the Conference of the States Parties. They resolve questions of policy and matters arising between the States Parties on technical issues or on interpretations of the Convention.

Two of the biological toxins are listed in Annex B, Schedule 1, Numbers 7 (saxitoxin) and 8 (ricin).

## National Regulations: Installation of Preparedness Standards

### Laboratory Safety

As regulated in the CWC, the production, acquisition, and handling of more than 100 g of a listed agent per year require permission. For the time being, only a few biological toxins are available in small amounts in the free market for research, analytical, or therapeutic issues.

Regarding safety at work on biological toxins in Germany, a national Committee on Biological Agents establishes or adapts the rules, which are officially released by the Federal Ministry of Labour and Social Affairs as Technical Rules for Biological Agents (TRBA). The most basic documents are the following TRBAs: "Protective Measures for Specific and Non-specific Activities involving Biological Agents in Laboratories" (TRBA 100) and "Basic Measures to be taken for Activities involving Biological Agents" (TRBA 500). Accordingly, handling of biological toxins is allowed in laboratories at containment level 1 (toxins) or a higher containment level corresponding to the risk group of an associated organism (e.g., level 2 for *Clostridium botulinum* strains). According to the international Globally Harmonized System of Classification and Labeling of Chemicals (GHS), tagging of vials containing biological toxins is required by use of a pictogram and a signal word (i.e., "Danger" or "Hazard"). Additionally, an individual material safety data sheet is required for each substance or mixture that mandatorily lists all hazard and precautionary statements.

### Risk Management

Besides international regulations to reduce the stockpiles of chemical warfare agents, national regulations are necessary to reduce health risks for the general population and emergency personnel. As the risk for terrorist attacks with chemical warfare agents or similar substances rises, toxicity estimates and exposure guidelines have been recently updated to ensure a more realistic national preparedness. In the United States, acute exposure guidelines (AEGLs) have been developed and published (Watson et al. 2006). AEGLs were calculated for vapor exposure (10 min–8 h). AEGL-1 has been defined as a threshold where first mild symptoms are noticed: miosis for nerve agents. On the other hand, AEGL-3 vapor concentrations may induce severe life-threatening health effects. The published data (Table 3) can be used for planning and risk management to counteract terrorist attacks with chemical warfare agents.

**Table 3** AEGL values (mg/m$^3$) for selected chemical warfare agents (Watson et al. 2006)

| | | Sarin (GB) | Tabun (GA) | Soman (GD) | Cyclosarin (GF) | VX |
|---|---|---|---|---|---|---|
| AEGL-1 | 10 min | 0.00690 | 0.00690 | 0.00350 | 0.00350 | 0.00057 |
| | 30 min | 0.00400 | 0.00400 | 0.00200 | 0.00200 | 0.00033 |
| | 1 h | 0.00280 | 0.00280 | 0.00140 | 0.00140 | 0.00017 |
| | 4 h | 0.00140 | 0.00140 | 0.00070 | 0.00070 | 0.00010 |
| | 8 h | 0.00100 | 0.00100 | 0.00050 | 0.00050 | 0.00007 |
| AEGL-2 | 10 min | 0.08700 | 0.08700 | 0.04400 | 0.04400 | 0.00720 |
| | 30 min | 0.05000 | 0.05000 | 0.02500 | 0.02500 | 0.00420 |
| | 1 h | 0.03500 | 0.03500 | 0.01800 | 0.01800 | 0.00290 |
| | 4 h | 0.01700 | 0.01700 | 0.00850 | 0.00850 | 0.00150 |
| | 8 h | 0.01300 | 0.01300 | 0.00650 | 0.00650 | 0.00100 |
| AEGL-3 | 10 min | 0.38000 | 0.76000 | 0.38000 | 0.38000 | 0.02900 |
| | 30 min | 0.19000 | 0.38000 | 0.19000 | 0.19000 | 0.01500 |
| | 1 h | 0.13000 | 0.26000 | 0.13000 | 0.13000 | 0.01000 |
| | 4 h | 0.07000 | 0.14000 | 0.07000 | 0.07000 | 0.00520 |
| | 8 h | 0.05100 | 0.10000 | 0.05100 | 0.05100 | 0.00380 |

## Laboratory Standardization Approaches

Since 2012, an expert laboratory network has been constituted for the Establishment of Quality Assurance for the Detection of Biological Toxins of Potential Bioterrorism Risk (EQuATox). The project is funded by the Seventh European Framework Programme for Research (FP7). It aims at the establishment of a laboratory network using equal standards for the detection and identification of CBRN agents within the EU, and the sharing of expertise on toxins within the food, agricultural, and biodefense sector. EQuATox enables the mutual transfer of techniques, wet-lab trainings in the partner labs, and the establishment of proficiency testings on biological toxin detection within the EU.

## Pharmacy

The availability and development of antidotes against chemical warfare agents is a continuous challenge. For several chemical warfare agents, e.g., mustard, no specific antidote exists in spite of decades of research. In recent years, new technologies were developed, allowing a deeper insight into the mechanism of toxicity and new approaches are under investigation possibly enabling improved wound healing. In other cases, e.g., nerve agents, new autoinjectors containing an oxime, atropine, and benzodiazepam are under development. As commercial interest in antidote development generally is very low, national financial support is crucial to sustain research efforts and to allow development of new devices, e.g., autoinjectors or new promising approaches to improve therapy.

During World War II, toxoid vaccines were investigated by the United States to protect researchers working on the production of biological warfare agents. Since then, further vaccines against biological toxins have been developed, among them

the pentavalent PBT vaccine (CDC) against five serotypes of botulinum neurotoxin, the RiVax™ Ricin Toxin Vaccine (Soligenix), and a candidate vaccine against staphylococcal enterotoxin B (USAMRIID).

A very limited number of heterologous antitoxin products are available for the treatment of botulism (e.g., Botulismus-Antitoxin Behring, Novartis). Besides the few specific treatment options, therapy relies on supportive measures and in most cases requires intensive care facilities.

A network of specific poison control centers is available throughout European countries. They are associated to local hospitals and store antitoxins and provide expertise regarding the treatment of intoxications.

### Decontamination

Decontamination of body parts after exposure to chemical warfare agents or biological toxins is accomplished by cleaning with soap and water. Pharmaceutical products such as Reactive Skin Decontamination Lotion (RSDL) may be used for decontamination of skin surfaces contaminated with chemical warfare agents or biological toxins with skin absorption (trichothecene group) (Table 2). Wounds and lesions may be flushed with physiological solutions. For the decontamination of equipment, protein-denaturing dilutions of sodium or calcium hypochlorite may be used.

## References

Balali-Mood M, Hefazi M, Mahmoudi M, Jalali E, Attaran D, Maleki M, Razavi ME, Zare G, Tabatabaee A, Jaafari MR (2005) Long-term complications of sulphur mustard poisoning in severely intoxicated Iranian veterans. Fundam Clin Pharmacol 19:713–721

Watson A, Opresko D, Young R, Hauschild V (2006) Development and application of acute exposure guideline levels (AEGLs) for chemical warfare nerve and sulfur mustard agents. J Toxicol Environ Health 9:173–263

## Recommended Reading

Aas P (2003) The threat of mid-spectrum chemical warfare agents. Prehosp Disaster Med 18(4):306–312

Franz DR, Jaax NK Ricin toxin. In: Medical aspects of chemical and biological warfare. Borden Institute, Chapter 32

Ketchum JS, Sidell FR Incapacitating agents. In: Medical aspects of chemical and biological warfare. Borden Institute (Chap 11)

Middlebrook JL, Franz DR Botulinum toxins. In: Medical aspects of chemical and biological warfare. Borden Institute (Chap 33)

Ulrich RG, Sidell S, Taylor TJ, Wilhemsen CL, Franz DR Staphylococcal enterotoxin B and related pyrogenic toxins. In: Medical aspects of chemical and biological warfare. Borden Institute (Chap 31)

Wannemacher RW, Wiener SL Trichothecene mycotoxins. In: Medical aspects of chemical and biological warfare. Borden Institute (Chap 34)

Wheelis M, Rózsa L, Dando M (2006) Deadly cultures: biological weapons since 1945. Harvard University Press, Cambridge, MA, pp 284–293, 301–303

# Part VI

# Addendum: Tables and Lists

The addendum contains sections with important background information. The checklist answers concisely frequently asked questions in connection with pending risk regulation processes. A tabular presentation of important limit values - together with the previous chapters - will help the reader to estimate risks and hazards in specific situations. Finally the glossary defines important terms and concepts related to regulatory toxicology.

# Checklist: Toxicological Risk Assessment in Practice

Michael Schwenk and H. Paul A. Illing

## Contents

Checklist and Comments ..... 872
References ..... 874

**Abstract**

The checklist gives brief practical hints for all those who are occasionally or professionally involved in risk assessment, risk management, and risk regulation. Further details to each topic can be found in the relevant chapters of this book.

M. Schwenk (✉)
Formerly Medical School, Hannover, Germany
e-mail: mike.schwenk@gmx.net

H.P.A. Illing
Centre for Occupational and Environmental Health, University of Manchester, Manchester, UK

Paul Illing Consultancy Services Ltd, Heswall, Wirral, UK
e-mail: paul@sherwood37.demon.co.uk

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_22, 

## Checklist and Comments

| Checklist | Comments |
|---|---|
| **Which are the steps of the risk regulation process?** | |
| The IPCS document (IPCS, 1994) identifies these as:<br>**Risk assessment** (in 4 steps):<br>Hazard identification<br>Hazard characterization (including dose–response relationship)<br>Exposure assessment<br>Risk characterization<br>**Risk management**<br>Risk evaluation<br>Emission and exposure control<br>Risk monitoring | Risk assessments are made on the basis of a scientific examination of toxicity and exposure, leading to a risk characterisation. The risk management process is aimed at developing an appropriate response to the hazard (regulatory, technical, legal). Risk (or risk-benefit) evaluation, the first step in risk management, establishes a qualitative or quantitative relationship between risks and benefits of exposure to an agent and the influence of possible control measures on that evaluation. It may be necessary to examine relative risk and benefit for different agents used for the same purpose. |
| **What data on toxic properties are needed for risk assessment?** | |
| **Chemistry**<br>Basic physical and chemical properties.<br>Structure-activity relationships (if available) for the test substance and related substances.<br>**Identification of toxic effects**<br>Animal testing results (acute, subacute, and chronic toxicity; carcinogenicity; and toxicity to reproduction).<br>Evidence of irritation and sensitization.<br>Genotoxicity.<br>Results from in vitro tests.<br>Biochemical mechanism of action.<br>Experience in humans.<br>**Toxicodynamics**<br>Dose–response relationships (size of response).<br>Rates of development and duration of effects.<br>**Toxicokinetics**<br>Absorption rates (oral, inhalation, dermal)<br>Distribution, half-life<br>Metabolites<br>Routes and rates of elimination<br>Experience with humans | By proper assessment of the physicochemical properties (“insoluble . . .”), it is often possible to get a first estimate of the risk level.<br>Data quality (this includes whether appropriate protocols and audit procedures were employed) must be considered. For chemical assessment, Klimisch gradings are often used (see Klimisch et al. 1997)<br>The overall picture will emerge only from the sum of all available information.<br>If in doubt, additional information must be asked from poison control centers and manufacturers.<br>Toxicokinetic data are often ignored in risk assessments – which is a fault. |

(*continued*)

| | |
|---|---|
| **What information is provided by the dose–response relationship?** | |
| Shows threshold above which effects can be observed (NOAEL/LOAEL/BMD). Large steepness of the dose–response relationship means reduced safety margin. Shape of the curve influences values obtained by extrapolation to low doses (e.g., unit risk). | Non-sigmoidal dose–response relationship increases the uncertainty in extrapolation to low concentrations.<br>NOAEL values of different studies often differ as they are the dose below the dose at which effects were seen and therefore depend on the dose intervals between doses in the study. They also depend on what parameters were measured in the studies. If in doubt, it should be checked as to whether one of the studies is better suited for a particular risk assessment. |
| **How is an exposure assessment made?** | |
| **External exposure**<br>Measurement or estimation of the extent of external exposure (in the intake, in the medium [air, water, food basket], or, using more complicated models, in the input to the medium [e.g., water] from the source [e.g., outlet sewer of chemical factory/sewage treatment works]).<br>Observe all routes of exposure (oral, inhalation, dermal).<br>Consider sensitive persons.<br>**Internal exposure**<br>Calculation of the assumed maximum uptake on the basis of (worst case) scenarios.<br>Probabilistic assessment of the different routes of intake.<br>Measurement of the internal concentration (human biomonitoring). | Exposure estimates can be extremely uncertain. Scenarios (models) should be clearly set out and estimates calculated according to standardized procedures. Estimates should not contain multiple "worst-case" assumptions (if the $P$ value of 0.1 [i.e., 1 in 10 will show the effect] is applied three times, this gives a $P$ value of 0.001 [1 in 1,000]). Monte Carlo analysis is essential in these circumstances.<br>Human biomonitoring is a very good method for internal exposure assessment. |
| **Which safety factors are often used?** | |
| Usual safety factor for extrapolation for a threshold effect from a good animal data to a general human population = 100 (depends on circumstances).<br>US-EPA and other regulatory agencies often use safety factors up to 10,000 (see e.g., IPCS 1994). | Depending on the size of the selected safety factors, risk assessments can vary enormously even when the experimental data base is identical. This can easily lead to dispute. |
| **Why does epidemiology rarely find a threshold value?** | |
| Large uncertainty in the estimation of exposure.<br>Large uncertainty of the effects at low doses.<br>High interindividual variability. | Lack of thresholds in epidemiological studies may be artificially caused by the multiplication of several uncertainty factors. |
| **Who belong to the vulnerable groups?** | |
| Pregnant women (organogenesis of the child), infants, and children (organ development, toxicokinetics).<br>Elderly and sick people (low functional reserves, low repair capacity).<br>Allergic people (hypersensitivity). | Often, sensitive groups are given special regulatory protection in various laws (occupational safety, baby food, allergens, etc.). This must be considered in the risk management process. |

(*continued*)

| | |
|---|---|
| **What else must be considered in risk management?** | |
| Protection philosophy of the respective areas.<br>Guideline values and their rationale. Are they applicable?<br>Verification of measurement results.<br>Quality assurance of the process. | The safety philosophy may be for good hygiene practice, precautionary, or danger-oriented<br>In order that a risk assessment finds acceptance, it is important to understand the origin of existing regulations as well as the present state of scientific interpretation of the toxicological data. |
| **What does "traffic light principle" mean in regulation** | |
| **Green**: no effect and no action required.<br>**Yellow**: slightly below threshold level.<br>Adequate action: monitoring.<br>**Red**: above the threshold of action. Swift action to reduce exposure. | Multistage systems such as the traffic light system are more flexible. Where only a single limit value exists, a brief or minor overrun may cause action or legal consequences, even if the excess is toxicologically irrelevant. |
| **When is a disease due to toxic substances?** | |
| Causality can be assumed if exposure levels and exposure duration were sufficient and the response spectrum (the affected organ, expression) characteristic for a compound.<br>The rarer the symptoms occur in daily life, the more secure a causal relationship can be assumed.<br>The criteria to be considered are given in Hill (1965) and are applicable to all toxicological data, not only epidemiological data. | The causality principle is often presumed for toxic substances. But it is not easy to prove causality. With many drugs, possible unwanted effects are often overlooked. And the dramatic health effects of smoking and alcohol are often socially trivialized and ignored.<br>Some dangerous substances produce very specific disease patterns (e.g., asbestos and mesothelioma). |
| **In which way can the modes of thinking influence the risk awareness?** | |
| **Scientific way of thinking ("objective risk")**<br>Risk assessment<br>Risk comparison<br>Risk management (technical)<br>**Emotional way of thinking by the general public (perceived risk)**<br>Risk acceptance<br>**Political way of thinking (perceived risk)**<br>Risk exaggeration (phantom risk)<br>Risk trivializing<br>Conclusion: understanding the sociological and psychological aspects of risk perception and communication is critical to effective risk management. | Many social groups (toxicologists, engineers, politicians, stakeholders, arbitrator, government representatives, etc.) are potentially involved in risk communication and risk management.<br>In this process, it often happens that different ways of thinking collide. This leads to inner discomfort and confrontation. Knowledge of the various ways of thinking of the general public, as described by psychologists and sociologists, can reduce conflict.<br>A good moderator can help overcome these hurdles.<br>Note: the eloquent charlatan and the lobbyist usually receive more credibility than the highly educated toxicologist and the regulator. |

## References

Hill AB (1965) The environment and disease: association or causation? Proc R Soc Med 58:295–300

IPCS (1994) Assessing human health risks of chemicals: derivation of guidance values for health based limits. Environmental health criteria 170. World Health Organisation, Geneva

Klimisch HJ, Andreae E, Tillman U (1997) A systematic approach for evaluating the quality of experimental and ecotoxicological data. Regul Toxicol Pharmacol 25:1–5

# Limit Values and Guideline Values in Regulatory Toxicology

Karin Heine and Alexander Eckhardt

## Contents

Reference Doses ..... 876
Acceptable Daily Intake (*ADI*) Values ..... 876
Acute Reference Dose (*Acute RfD*) ..... 876
Contaminants ..... 876
Tolerable Absorbed Doses (*TRD*) ..... 878
Reference Dose and Reference Concentration (RfD, RfC) ..... 880
Occupational Safety and Health ..... 880
Drinking Water ..... 883
WHO Guidelines for Drinking-Water Quality (WHO-GLDWQ) ..... 883
German Regulation on Drinking Water ..... 883
Air ..... 883
World Health Organization (WHO) *Air Quality Guidelines* ..... 883
*Immission Values* Based on EU Directives ..... 886
Technical Instruction on *Air Quality* Control ("TA Luft") ..... 888
Indoor Air Guideline Values ..... 888
Food ..... 891
*Maximum Residual Levels* (*MRLs*): International, European, and National Standards and Regulations ..... 891
European Maximum Levels for Certain Contaminants in Foodstuffs ..... 892
Soil ..... 893

K. Heine (✉)
Research and Advisory Institute of Hazardous Substances, FoBiG - Forschungs- und Beratungsinstitut Gefahrstoffe GmbH, Freiburg, Germany
e-mail: karin.heine@fobig.de

A. Eckhardt
Toxicology of Drinking Water and Swimming Pool Water, Federal Environment Agency, Bad Elster, Germany
e-mail: Alexander.Eckhardt@uba.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1_24, 

Body Burden ..... 894
References ..... 897
Resources: Food ..... 897
Resources: Occupational Health and Safety ..... 898
Resources: Body Burden ..... 898
Resources: Air ..... 898

**Abstract**

Depending on the matrix (e.g., water, air) and the classification of a substance (e.g., contaminant), a multitude of limits and guidelines has been defined. The motivation for defining such a limit or guideline can be different. The most important reason is to protect the population from adverse health effects such as acute chronic toxicity or cancer. Another reason could be the protection of ecosystems which could be more vulnerable than humans. Moreover, aesthetic considerations, like the taste and/or odor of drinking water, can result in limitations of chemicals. In the following chapter, definitions of and examples for limits in water, air, or occupational environments are given. These lists are by no means exhaustible.

## Reference Doses

### Acceptable Daily Intake (*ADI*) Values

The World Health Organization (WHO) defined Acceptable Daily Intake (ADI) values for pesticides, food additives, and veterinary pharmaceuticals. The ADI values form the basis for the maximum concentration of contaminations in food. These values can be considered safe, even if a lifetime exposure is assumed (see Table 1 or references mentioned "Resources").

### Acute Reference Dose (*Acute RfD*)

In order to avoid health risks caused by a single exposure exceeding the ADI, the WHO has established acute reference doses (Acute RfD, see Table 1) for some pesticides.

### Contaminants

If the uptake of a contaminant is per definition of the WHO acceptable, because the contaminant is useful, these contaminations become "tolerable." Early on (since 1972) provisional tolerable weekly intake (PTWI) values were defined for metals, which accumulate in the human body due to their ubiquity. The definition of

**Table 1** Acceptable Daily Intake (*ADI*) values and acute reference doses (*RfD*) of selected pesticides and the year of their last evaluation

| Substance | ADI mg/kg/d (last evaluation) | Acute RfD mg/kg/d (last evaluation) |
|---|---|---|
| Abamectin | 0.0025 (2008) | |
| Aldicarb | 0.003 (1992) | 0.003 (1995) |
| Amitrole | 0.002 (1997) | |
| Azinphos-methyl | 0.03 (2007) | 0.1 (2007) |
| Benomyl | 0.1 (1995) | |
| Carbaryl | 0.008 (2001) | 0.2 (2001) |
| Carbofuran | 0.001 (2008) | 0.001 (2008) |
| Chlorpyrifos | 0.01 (2005) | 0.1 (2004) |
| Chlorpyrifos-methyl | 0.01 (2009) | 0.1 (2009) |
| Cyfluthrin | 0.04 (2006) | 0.04 (2006)[a] |
| Cyhexatin | 0.003 (2005)[b] | 0.02 (2005)[c] |
| Cypermethrin | 0.02 (2006) | 0.04 (2006)[d] |
| 2,4-D | 0.01 (2001) | n.l.c.n.[e] |
| Deltamethrin | 0.01 (2009) | 0.05 (2000) |
| Diazinon | 0.005 (2006) | 0.03 (2001) |
| Dichlorvos | 0.004 (1993) | 0.1 (2012) |
| Dicofol | 0.002 (1992) | 0.2 |
| Dimethoate | 0.002 (2006) | 0.02 (2003)[f] |
| Diphenylamine | 0.08 (1998) | n.l.c.n.[e] |
| Diquat | 0.002 (2001) | |
| Endosulfan | 0.006 (1998) | 0.02 (1998) |
| Ethylene thiourea | 0.004 (1993) | |
| Fenamiphos | 0.0008 (1997) | 0.003 (2002) |
| Fenvalerate | 0.02 (1986) | 0.2 (2012) |
| Ferbam | 0.003 (1996)[g] | |
| Lindane | 0.005 (2002) | 0.06 (2002) |
| Malathion | 0.3 (1997) | 2.0 (2003) |
| Mancozeb | 0.03 (1993)[h] | |
| Maneb | 0.03 (1993)[h] | |
| Methamidophos | 0.004 (2002) | 0.01 (2002) |
| Methomyl | 0.02 (2001) | 0.02 (2001) |
| Metiram | 0.03 (1993)[h] | |
| Paraquat | 0.005 (2003) | 0.006 (2003) |
| Parathion | 0.004 (1995) | 0.01 (1995) |
| Parathion-methyl | 0.003 (1995) | 0.03 (1995) |
| Permethrin | 0.05 (1999) | 1.5 (2002) |
| Propineb | 0.007 (1993) | |
| Propoxur | 0.02 (1989) | |
| Pyrethrin | 0.04 (2008) | 0.2 (2003) |

(*continued*)

**Table 1** (continued)

| Substance | ADI mg/kg/d (last evaluation) | Acute RfD mg/kg/d (last evaluation) |
|---|---|---|
| 2,4,5-Trichlorphenoxyacitic acid | 0.03 (1981) | |
| Thiabendazole | 0.1 (1997) | 0.3 (2006) |
| Thiophanate-methyl | 0.08 (2006) | n.l.c.n. (2006)[e] |
| Thiram | 0.01 (2003) | |
| Zineb | 0.03 (1993)[h] | |
| Ziram | 0.003 (1996)[g] | |

[a]Group acute RfD with beta-cyfluthrin
[b]Group ADI with azocyclotin
[c]For women of childbearing age
[d]Group acute RfD with alpha- and zeta-cypermethrin
[e]No limit considered necessary
[f]Sum of dimethoate and omethoate
[g]Group ADI for ferbam and ziram
[h]Group ADI for mancozeb, maneb, metiram, and zineb

a tolerable weekly intake is motivated by the fact that one increased intake per week caused, for example, by certain foods is irrelevant. An example for this can be the uptake of methylmercury via fish. Later on this concept was extended to substances (e.g., mycotoxins) that are not accumulating in the human body or that can diffuse into foods from packaging materials. In some cases the limit was referred to a daily intake (PMTDI = provisional maximum tolerable daily intake). For pesticides which are banned but still contaminants of food products, the original ADI value was transferred into a PTDI value (provisional tolerable daily intake). MTDI values (maximum tolerable daily intake) were derived for substances that are essential for human nutrition but for which the range between physiological needs and toxic dose is very small, e.g., copper or iron. *TDI* values (tolerable daily intake) are the basis of the WHO's drinking-water guidelines. Table 2 depicts tolerable intake values of some chemicals.

## Tolerable Absorbed Doses (*TRD*)

For chemicals that are also important contaminants in soil, the so-called tolerable absorbed doses ("tolerierbare resorbierte dosis"; TRD) were derived by the Research and Advisory Institute for Hazardous Substances ("Forschungs- und Beratungsinstitut Gefahrstoffe," FoBiG) based in Freiburg, Germany. The values given in Table 3 are calculated for safe, lifetime exposure, either after oral or respiratory intake. In order to compare those values with other limits like ADI, the absorption rate has to be taken into account.

**Table 2** Tolerable intake values of selected chemicals and the year of their last evaluation

| Substance | Type of limit | Amount [mg/kg/d] or [*mg/kg/week*] | Last evaluation |
|---|---|---|---|
| Acrylonitrile | | n.s.l.r[a] | 2000 |
| Aflatoxins | | ALARA[b] | 1998 |
| Aldrin (sum of Aldrin + Dieldrin) | PTDI[c] | 0.0001 | 1994 |
| Aluminum | PTWI[d] | *1* | 2007 |
| Arsenic | PTDI | 0.0003 | 2011 |
| Cadmium | PTWI | *0.007* | 2000 |
| Chlorine | TDI[e] | 0.15 | 1993 |
| Chloral hydrate | TDI | 0.0016 | 1993 |
| Chlorobenzene | TDI | 0.0867 | |
| Copper | MTDI[f] | 10 | 2003 |
| Cyanide, free | TDI | 0.012 | 1993 |
| 2,4-DB | TDI | 0.03 | 1993 |
| DDT | PTDI | 0.01 | 2000 |
| *o*-Dichlorobenzene | TDI | 429 | 1993 |
| *p*-Dichlorobenzene | TDI | 107 | 1993 |
| 1,1-Dichloroethene | TDI | 0.017 | 2005 |
| trans-1,2-Dichloroethene | TDI | 0.017 | 2003 |
| 1,2-Dichloropropane | TDI | 0.014 | 2003 |
| Di(2-ethylhexyl)adipate | TDI | 0.28 | 2003 |
| Di(2-ethylhexyl)phthalate | TDI | 0.025 | 1993 |
| Dichloromethane | TDI | 0.006 | 1993 |
| Dichlorprop | TDI | 0.0364 | 1993 |
| Dieldrin | PTDI | 0.0001 | 1994 |
| Epichlorohydrin | TDI | 0.00014 | 2003 |
| Ethylbenzene | TDI | 0.0971 | 2003 |
| Formaldehyde | TDI | 0.15 | 1993 |
| Heptachlor | PTDI | 0.0001 | 1994 |
| Heptachlor epoxide | PTDI | 0.0001 | 1994 |
| Hexachlorobutadiene | TDI | 0.0002 | 2003 |
| Iodine | PMTDI[g] | 0.017 | 1988 |
| Iron | PMTDI | 0.8 | 1983 |
| Lead | PTWI | 0.025 | 1993 |
| MCPA | TDI | 0.0005 | 1993 |
| Mercury | PTWI | 0.005 | 1993 |
| Methylmercury | PTWI | *0.0033* | 1998 |
| Ochratoxin A | PTWI | *0.0001* | 2001 |
| Patulin | PMTDI | *0.0004* | 1995 |
| Pentachlorophenol | TDI | 0.0003 | 1998 |
| Polychlorinated dibenzodioxins/-furans + PCB | PTMI[h] | 70 pg/kg/month | 2001 |

(*continued*)

**Table 2** (continued)

| Substance | Type of limit | Amount [mg/kg/d] or [*mg/kg/week*] | Last evaluation |
|---|---|---|---|
| Styrene | TDI | 0.0077 | 1993 |
| Tetrachloroethene | TDI | 0.0014 | 1993 |
| Tetrachloromethane | TDI | 0.0014 | 2003 |
| Tin | TWI | *14* | 2005 |
| Toluene | TDI | 0.223 | 2003 |
| 2,4,5-TP (fenoprop) | TDI | 0.003 | 1993 |
| Tributyltin oxide | TDI | 0.00025 | |
| Trichloroacetic acid | TDI | 0.0325 | 2003 |
| 1,2,4-Trichlorobenzenes[i] | TDI | 0.0077 | 2003 |
| 1,1,1-Trichloroethane | TDI | 0.6 | 2003 |
| Trichloroethene | TDI | 0.00146 | 2005 |
| Trifluralin | TDI | 0.0075 | 1993 |
| Xylenes | TDI | 0.179 | 1993 |
| Zinc | PMTDI | 1 | 1982 |

[a]No safe level recommended
[b]As low as reasonably achievable
[c]Provisional tolerable daily intake
[d]Provisional tolerable weekly intake
[e]Tolerable daily intake
[f]Maximum tolerable daily intake
[g]Provisional maximum tolerable daily intake
[h]Provisional tolerable monthly intake
[i]All isoforms

### Reference Dose and Reference Concentration (RfD, RfC)

The US Environmental Protection Agency (US-EPA) has established dose limits for oral (RfD) or respiratory (RfC) uptake of many chemicals. Based on these values, the US limits for air, water, and food were defined. Listing all of these would exceed the scope of the book. The list can be found on the internet at http://www.epa.gov/iris.

## Occupational Safety and Health

According to the World Health Organization (see WHO in "Resources"), "occupational health deals with all aspects of health and safety in the workplace and has a strong focus on primary prevention of hazards. The health of the workers has several determinants, including risk factors at the workplace leading to cancers, accidents, musculoskeletal diseases, respiratory diseases, hearing loss, circulatory diseases, stress related disorders and communicable diseases and others." Therefore, the European Union (EU) established the Occupational Safety and Health

**Table 3** Tolerable resorbed dose (*TRD*) of selected chemicals from Eikmann et al. (2010)

| | Oral intake | | Respiratory intake | |
|---|---|---|---|---|
| Substance | [µg/kg/d] | Absorption [%] | [µg/kg/d] \| [µg/m$^3$] | Absorption [%] |
| Aldrin | 0.08 | 100 | 0.1 \| 0.7 (P) | 50 |
| Antimony | 0.07 (P) | 20 | – \| 0.08 | |
| Arsenic | 0.8 | 100 | 1 \| 50 | 30 |
| Benzene | 10 | 100 | 7 \| 50 | 50 |
| Beryllium | 0.015 (P) | 1 | | |
| Cadmium | 0.025 (P) | 5 | 0.035 (P) \| – | |
| Chlorobenzene | 70 | 100 | 60 \| 400 | 50 |
| Chromium (VI) | 5 | | 0.014 \| 0.050 | |
| Copper | 25 (P) | 50 | | |
| Cyclohexane | | | 400 \| 5600 | 25 |
| Cyanides | 10 | 100 | 10 \| 50 (gas)<br>70 (particle) | 70 |
| DDT | 1 (P) | 100 | | |
| o-Dichlorobenzene | 900 | 100 | 500 \| 2,900 | 60 |
| p-Dichlorobenzene | 300 | 100 | 300 \| 1,800 | 60 |
| 1,2-Dichloroethane | 190 | 100 | 200 \| 5,600 | 75 |
| Dichloromethane | 60 (P) | 100 | 150 \| 1,000 | 60 |
| 2,4-Dichlorophenol | 9 | 100 | | |
| 1,2-Dichloropropane | 25 (P) | 100 | | |
| Di-2-(ethylhexyl)-phthalate (DEHP) | 30 | 60 | | |
| 1,3-Dinitrobenzene | 1 | 100 | 1 \| 7 | 50 |
| Diphenylamine | 20 | 100 | | |
| Ethylbenzene | 300 (P) | 100 | 700 \| 5,000 | 50 |
| Hexachlorobenzene | 0.030 | 100 | | |
| α-Hexachlorocyclohexane | 0.100 | 100 | 0.025 \| 0.088 | 100 |
| β-Hexachlorocyclohexane | 0.020 | 100 | 0.005 \| 0.02 (P) | 100 |
| γ-Hexachlorocyclohexane | 0.330 | 100 | 0.080 \| 0.3 | 100 |
| Hexachlorocyclohexane, mixture[a] | 0.020 | 100 | 0.005 \| 0.02 | 100 |
| n-Hexane | | | 100 \| - | 20 |
| Lead | 1 (P) | 50 (children) | 1 \| 9 (P) | 40 |
| Mercury, inorg. | 0.015 | 7 | 0.030 \| 130 | 80 |
| Mercury, org. | 0.05 | 100 | | |
| Nickel | 0.08 | 6 | - \| 0.170 | |
| Nitrobenzene | 2 (P) | 100 | 2 \| 7 | 100 |
| 2-Nitrotoluene | 45 | 100 | | |
| 3-Nitrotoluene | 85 | 100 | | |
| 4-Nitrotoluene | 15 | 100 | | |
| *N*-Methyl-*N*-2,4,6-tetranitroaniline | 15 | 100 | 15 \| 100 | 50 |

(*continued*)

**Table 3** (continued)

| Substance | Oral intake [µg/kg/d] | Absorption [%] | Respiratory intake [µg/kg/d] \| [µg/m$^3$] | Absorption [%] |
|---|---|---|---|---|
| PCB | 0.015 | 100 | | |
| PCDD/F | 0.000 001 | | | |
| *N*-Nonane | | | 500 \| 5,800 | 30 |
| Pentaerythritol tetranitrate (PETN) | 3 | 100 | 3 \| 21 | 50 |
| Phenol | | | 15 \| 50 | 100 |
| Styrene | 260 | 100 | 260 \| 100 | 70 |
| Tetrachloroethene | 20 (P) | 100 | 30 \| 200 (P) | 50 |
| Toluene | 200 (P) | 100 | 100 \| 700 | 50 |
| Trichloromethane | 10 (P) | 100 | | |
| 1,2,4-Trinitrobenzene | 15 (P) | 100 | 5 \| 18 (P) | 100 |
| 1,1,1-Trichloroethane | 500 (P) | 100 | 500 \| 5,800 | 30 |
| Trichloroethene | | | 80 \| 560 (P) | 50 |
| 1,3,5-Trimethylbenzene, all isoforms | | | 100 (P) \| 600 | 60 |
| 2,4,6-Trinitrotoluene | 0.5 | 100 | 0.5 \| 2.3 | 75 |
| Vanadium | 0.150 | 3 | – \| 1 ($V_2O_5$) | |
| Vinyl chloride | 2 | 100 | 2 \| 18 | 40 |
| Xylenes | 150 | 100 | 200 \| 1,000 | 65 |

*P* provisional, due to large uncertainties
[a] $\sum(\frac{\alpha HCH}{5} + \beta HCH + \frac{\gamma HCH}{16})$

Agency to protect workers from occupational health hazards. Its abbreviation is "EU-OSHA" to be distinguishable from its sister agency in the USA, which is referred to as "OSHA". Every country of the EU and the EFTA (European Free Trade Association) has its own so-called focal points, which make up the national partners of EU-OSHA. Five priority groups were formed to satisfy the special needs of some of the most vulnerable workers: young workers, women, people with disabilities, migrant workers, and ageing workers. To facilitate the implementation of EU directives on a national basis, the EU-OSHA passed several guidelines for topics such as workplaces, personal equipment, chemical agents, or physical hazards (see "Resources"). In Germany the Federal Agency for Occupational Safety and Medicine (AGS 2013) has passed several technical guidelines (TRGS 900, 903, and 905) to implement the directive 67/548/EEC on classification, packaging, and labelling of dangerous substances. The guideline TRGS 900, for example, regulates the limits of exposure against some 350 chemicals (AGS 2012). Similar regulations were passed in every member state of the EU, the USA (for link see "Resources"), and other countries. In all regulations different aspects of toxicology, i.e., acute and chronic exposure or carcinogenic potential, are considered. Although lists of substances that are regulated in Europe and the USA are quite similar, the limits can vary considerably as can be seen from Table 4.

**Table 4** Workplace limits of selected chemicals in Germany (TRGS 900) and the USA (OSHA)

| Substance | TRGS 900 Concentration [mg/m$^3$] | US-OSHA Concentration [mg/m$^3$] |
|---|---|---|
| Sulfur dioxide($SO_2$) | 2.5 | 13.0 |
| Carbon disulfide | 30.0 | 2.0 |
| Carbon monoxide | 35.0 | 55.0 |
| Dichloromethane | 260.0 | 2.0 |
| Styrene | 86.0 | 2.0 |
| Tetrachloroethylene | 138.0 | 2.0 |
| Toluene | 190.0 | 2.0 |

## Drinking Water

### WHO Guidelines for Drinking-Water Quality (WHO-GLDWQ)

The WHO recommends limits for organic as well as inorganic chemicals in its "Guidelines for Drinking-water Quality," which are not legally binding (see Table 5 or WHO 2008). Especially in countries without national regulations on drinking water, they can be used as guidelines. They are mostly derived from toxicological data, and the quality of the water bodies themselves is given only limited consideration.

### German Regulation on Drinking Water

The German drinking water regulation ("Trinkwasserverordnung"; TrinkwV) was ratified in February 2001 and entered into force on 1st January 2003. It was last modified on 5th December 2012. Most of the limits are based on the guidelines of the WHO. Exceptions are the limits for pesticides. The WHO states a limit for each pesticide, whereas the German drinking water regulation sets an additional limit for the sum of all pesticides (i.e., 0.5 µg/l; see Table 5).

## Air

### World Health Organization (WHO) *Air Quality Guidelines*

Clean air is a basic need for human well-being and health. The World Health Organization (WHO) therefore first published its Air Quality Guidelines for Europe in 1987, and a second edition was issued in 2000 (WHO 2000). Within these guidelines, guideline values for various inorganic, organic, and so-called classical pollutants (i.e., nitrogen dioxide, ozone, particulate matter, and sulfur dioxide) for air outdoors were established. Moreover, guideline values for indoor air pollutants were provided. In 2005 a global update became available for the classical pollutants (WHO 2006). Air quality guideline values are provided within Table 6.

**Table 5** Comparison between the WHO guidelines on drinking-water quality and the limits in the German drinking water regulation (TrinkwV)

| Parameter | WHO [mg/l] | TrinkwV [mg/l] |
|---|---|---|
| Inorganic | | |
| Aluminum | | 0.200 |
| Ammonia | | 0.500 |
| Antimony | 0.020 | 0.0050 |
| Arsenic | 0.010[a] | 0.010 |
| Barium | 0.7 | |
| Beryllium | 0.01 | |
| Boron | 0.5 | 1.0 |
| Cadmium | 0.0030 | 0.0030 |
| Chloride | | 250 |
| Chromium | 0.050 | 0.050 |
| Copper | 2.0 | 2.0 |
| Cyanide | 0.07 | 0.050 |
| Fluoride | 1.5 | 1.5 |
| Iron | | 0.2 |
| Lead | 0.010 | 0.010[b] |
| Manganese | 0.4 | 0.050 |
| Mercury | 0.0010 | 0.0010 |
| Nickel | 0.020 | 0.020 |
| Nitrate (as $NO_3^-$) | 50 | 50 |
| Nitrite (as $NO_2^-$) | 0.20 | 0.50 |
| Selenium | 0.010 | 0.010 |
| Sodium | 200 | 200 |
| Sulfate | | 250 |
| Uranium | 0.030 | 0.010 |
| Organic | | |
| Acrylamide | 0.0005[c] | 0.0001 |
| Benzene | 0.010[c] | 0.0010 |
| Tetrachloromethane | 0.004 | |
| 1,2-Dichlorobenzene | 1.0 | |
| 1,4-Dichlorobenzene | 0.3 | 0.0030 |
| 1,2-Dichloroethane | 0.030 | |
| 1,1-Dichloroethene | 0.030 | |
| 1,2-Dichloroethene | 0.050 | |
| Di(2-ethylhexyl)adipate | n.l.c.n. | |
| Di(2-ethylhexyl)phthalate | 0.008 | |
| Dichloromethane | 0.02 | |
| EDTA | 0.60 | |
| Epichlorohydrin | 0.00040 | 0.00010 |
| Ethylbenzene | 0.30 | |
| Hexachlorobutadiene | 0.0006 | |
| Monochlorobenzene | n.l.c.n. | |

(*continued*)

**Table 5** (continued)

| Parameter | WHO [mg/l] | TrinkwV [mg/l] |
|---|---|---|
| Nitrilotriacetic acid | 0.20 | |
| Polyaromatic hydrocarbons (PAH) | 0.00070 | 0.00010[d] |
| Benzo[a]pyrene | 0.7[c] | 0.000010 |
| Styrene | 0.020 | 0.010 |
| Tetrachloroethene + trichloroethane | 0.040 | |
| Toluene | 0.7 | |
| Tributyltin oxide | | |
| Trichlorobenzene (sum) 1,2,4-Trichlorobenzene | n.l.c.n. | |
| 1,1,1-Trichloroethane | n.l.c.n. | |
| Vinyl chloride | 0.00030[c] | 0.00050 |
| Xylenes | 0.5 | |
| Pesticides | | |
| Alachlor | 0.02 | 0.00010[e] |
| Aldicarb | 0.01 | 0.00010 |
| Aldrin/dieldrin | 0.000030 | 0.000030 |
| Atrazine | 0.002 | 0.00010 |
| Carbofuran | 0.007 | 0.00010 |
| Chlordane | 0.0002 | 0.00010 |
| Chlortoluron | 0.03 | 0.00010 |
| 2,4-D | 0.03 | 0.00010 |
| 2,4-DB | 0.09 | 0.00010 |
| DDT | 0.001 | 0.00010 |
| 1,2-Dibromo-3-chloropropane | 0.001 | 0.00010 |
| Dichlorprop | 0.1 | 0.00010 |
| Fenoprop | 0.009 | 0.000030 |
| Heptachlor + heptachloroepoxide | n.l.c.n. | 0.00010 |
| Hexachlorobenzene | n.l.c.n. | 0.00010 |
| Lindane | 0.002 | 0.00010 |
| MCPA | 0.002 | 0.00010 |
| Mecoprop | 0.01 | 0.00010 |
| Methoxychlor | 0.02 | 0.00010 |
| Pentachlorophenol | 0.009 | 0.00010 |
| Permethrin | n.l.c.n. | 0.00010 |
| Propanil | t.m. | 0.00010 |
| 2,4,5-T | 0.009 | 0.00010 |
| Disinfecting agents | | |
| Chlorine | 5.0 | |
| Disinfection by-products | | |
| 2,4,6-Trichlorophenol | 0.02 | 0.01 |
| Bromate | 0.01 | |
| Chloral hydrate | 0.01 | |
| Chlorite | 0.7 | |
| Dibromoacetonitrile | 0.07 | |

(*continued*)

**Table 5** (continued)

| Parameter | WHO [mg/l] | TrinkwV [mg/l] |
|---|---|---|
| Dichloroacetic acid | 0.05 | |
| Dichloroacetonitrile | 0.02 | |
| Formaldehyde | 0.90 | |
| Trihalomethanes | 6 | 0.050 |
| Chloroform | 0.20 | |
| Bromoform | 0.10 | |
| Bromodichloromethane | 0.060 | |
| Dibromochloromethane | 0.10 | |
| Trichloroacetic acid | 0.20 | |
| Trichloroacetonitrile | i.d. | |

*n.l.c.n.* No limit considered necessary; *t.m.* More toxic metabolites, but insufficient data on them; *i.d.* Insufficient data

[a]Additional risk for skin cancer $10^{-4}$

[b]Limit of 0.025 mg/l until 11/30/2013, 0.010 mg/l from 12/01/2013

[c]Additional risk for cancer $10^{-5}$

[d]Sum of the following substances: benzo[b]fluoranthene, benoz[k]fluoranthene, benzo[ghi] perylene, indeno[1,2,3-cd]pyrene

[e]General limit for pesticides in German drinking water, when no lower value is necessary, sum 0.5 µg/l

[f]For authorities wishing to establish a total THM standard to account for additive toxicity, the following fractionation approach could be taken: the sum the value of each THM divided by this guideline value has to be $<1$

## *Immission Values* Based on EU Directives

Within the European Union there are directives dealing with several air pollutants (e.g., nitrogen dioxide, particulate matter, lead, and sulfur dioxide), which provide immission limit values. They are mostly based on the recommendations of the WHO (see above) and are designed to protect human health from harmful environmental influences. In Germany the respective legislation is based on the Federal Immission Control Act ("Bundes-Immissionsschutzgesetz," BImSchG, last updated in 2011). Limit values were defined earlier within the 22nd Regulation of Federal Immission control (22. BImSchVO as of 26th October 1993). In 1996 the Framework Directive 96/62/EC entered into force and subsequently various sub-directives ("daughter directives" in Annex I of Framework Directive; 1999/30/EC; 2000/69/EC; 2002/3/EC; 2004/107/EC), in which immission limit values were described to ensure protection of human health, as well as protection of the environment (ecosystems and vegetation). For implementation of revisions made, the European Directive 2008/50/EC entered into force (21st May 2008). Afterwards in Germany the 22nd amended BImSchVO was withdrawn and newly regulated on a national level via the 39th Regulation of Federal Immission Control (39. BImSchVO as of 2nd August 2010, see Table 7). In case of carcinogenic substances (e.g., benzene), the limit values are set to an extra risk of $1{:}1 \times 10^{-6}$.

**Table 6** Air quality guideline values as provided in the second air quality guidelines for Europe, the global update in 2005 and guidelines for indoor air quality: selected pollutants (WHO 2010)

| Substance | Concentration [$\mu g/m^3$, if not otherwise indicated] or unit risk (UR)[a] | Averaging period |
|---|---|---|
| **Classical pollutants** | | |
| Sulfur dioxide ($SO_2$) | 20 | 24 h |
| | 500 | 10 min |
| Nitrogen dioxide ($NO_2$) | 40 | Year |
| | 200 | 1 h |
| $PM_{10}$[b] | 20 | Year |
| | 50 | 24 h[c] |
| $PM_{2.5}$[b] | 10 | Year |
| | 25 | 24 h[c] |
| Ozone[b] | 100 | Daily maximum 8-h mean |
| **Organic pollutants** | | |
| Acrylonitrile | UR: $2 \times 10^{-5}$ (lung) | |
| Benzene | UR: $6 \times 10^{-6}$ (blood: leukemia) | |
| Carbon disulfide | 100 | 24 h |
| Carbon monoxide | 10 $mg/m^3$ | 8 h |
| | 30 $mg/m^3$ | 1 h |
| | 60 $mg/m^3$ | 30 min |
| | 100 $mg/m^3$ | 15 min |
| 1,2-Dichloroethane | 700 | 24 h |
| Dichloromethane | 450 | 1 week |
| | 3,000 | 24 h |
| Formaldehyde | 100 | 30 min |
| Polycyclic aromatic hydrocarbons[d] | UR: $9 \times 10^{-2}$ (lung) | |
| Styrene | 260 | 1 week |
| Tetrachloroethylene | 250 | Year |
| Toluene | 260 | 1 week |
| Trichloroethylene | UR: $4.3 \times 10^{-7}$ (lung, testis) | |
| Vinyl chloride | UR: $1 \times 10^{-6}$ (lung and other sites) | |
| **Inorganic pollutants** | | |
| Arsenic | UR: $1.5 \times 10^{-3}$ (lung) | |
| Asbestos | At a concentration of 500 fibers[e]/$m^3$, the following ranges of lifetime risk estimates are made:<br>$10^{-6}$ to $10^{-5}$ (lung cancer in a population where 30 % are smokers)<br>$10^{-5}$ to $10^{-4}$ (mesothelioma) | |
| Cadmium | 0.005 | Year |
| Chromium (VI) | UR: $4 \times 10^{-2}$ (lung) | |
| Hydrogen sulfide | 150 | 24 h |
| Lead | 0.5 | Year |

(*continued*)

**Table 6** (continued)

| Substance | Concentration [μg/m$^3$, if not otherwise indicated] or unit risk (UR)[a] | Averaging period |
|---|---|---|
| Manganese | 0.15 | Year |
| Mercury | 1 | Year |
| Nickel | UR: $4 \times 10^{-4}$ (lung) | |
| Platinum | | |
| Refractory ceramic fibers | UR: $1 \times 10^{-6}$ (fiber/l)$^{-1}$ (lung) | |
| Vanadium | 1 | 24 h |
| **Indoor air pollutants** | | |
| Environmental tobacco smoke | UR: approximately $1 \times 10^{-3}$ | |
| Man-made vitreous fibers | See above (refractory ceramic fibers, for most other MMVF data are considered inadequate to establish AQG) | |
| Radon | Reference level: 100 Bq/m$^3$ (a) | Year |
| | 300 Bq/m$^3$ (b) | Year |
| | UR[f]: $0.6 \times 10^{-5}$ per Bq/m$^3$ (nonsmokers) | |
| | UR[f]: $15 \times 10^{-5}$ per Bq/m$^3$ (smokers) | |
| Naphthalene | 10 | Year |

[a]Unit risk: Cancer risk estimates for lifetime exposure to concentration of 1 μg/m$^3$
[b]Air quality guideline values (AQG)
[c]24 h concentration: 99th percentile (3 days/year)
[d]Based on benzo[a]pyrene
[e]Fibers measured by optical methods
[f]Excess lifetime risks (by the age of 75 years) for lung cancer of lifelong nonsmokers or current smokers (15–24 cigarettes/day)
(1) To minimize health hazards due to indoor radon exposure
(2) Applies only if the Reference Level mentioned in (a) cannot be reached under the common country-specific conditions

## Technical Instruction on *Air Quality* Control ("TA Luft")

Based on the Federal Immission Control Act ("Bundes-Immissionsschutzgesetz," BImSchG, last updated in 2011), an administrative act was issued. This was the technical instruction on air quality control ("TA Luft" as of 24th July 2002, which replaces the "TA Luft" from 1986). Within this updated administrative act, emission and immission values are provided. They are used to control for construction and operation of industrial sites, which are subjects of approval. Emission values are precautionary values in order to protect against detrimental environmental influences. Immission values shall protect from hazard to human health (see Table 8) as well as from major disturbance or disadvantages.

## Indoor Air Guideline Values

Besides the WHO air quality guideline values indicated above (see Table 6), which can be used for indoor air as well, national committees are in place to establish

**Table 7** Immission limit values and target values for protection of human health taken from 39th BImSchVO, which represents national implementation of current European legislation

| Substance | Concentration [µg/m$^3$] | Averaging period | Allowed frequency of excess per year |
|---|---|---|---|
| Sulfur dioxide ($SO_2$) | 125 | Day | 3 |
| | 350 | 1 h | 24 |
| Nitrogen dioxide ($NO_2$) | 40 | Year | - |
| | 200 | 1 h | 18 |
| $PM_{10}$ | 40 | Year | - |
| | 50 | Day | 35 |
| $PM_{2.5}$[a] | 25 | Year | |
| Ozone[b] | 120 | Daily maximum 8-h mean | 25 |
| Lead | 0.5 | Year | |
| Benzene | 5 | Year | |
| Carbon monoxide (CO) | 10,000 | Daily maximum 8-h mean | |
| Arsenic[c] | 6 ng/m$^3$ | Year (total content in $PM_{10}$ fraction) | |
| Cadmium[c] | 5 ng/m$^3$ | | |
| Nickel[c] | 20 ng/m$^3$ | | |
| Benzo[a]pyrene[c] | 1 ng/m$^3$ | | |

[a]Target value, which will be valid as immission limit value from 1st January 2015. For the immission limit value, there is a tolerance margin of 5 µg/m$^3$, which will be cut down yearly about one-seventh part starting from 1st January 2009. At 1st January 2015 the tolerance margin is zero. Starting from 1st January 2020, further reduction of $PM_{2.5}$ exposure is foreseen
[b]Target value, which will be the long-range target value without allowance of excess
[c]Target values which will be valid from 1st January 2013 onwards

guideline values for indoor air (see Table 9). In Germany, since 1993 an ad hoc working group exists. This ad hoc working group consists of technical experts from Indoor Air Hygiene Commission (IRK) of the Federal Environment Agency and the Permanent Working Group of the Highest State Health Authorities ("Arbeitsgemeinschaft der Obersten Landesgesundheitsbehörden," AOLG). Until now, guideline values for organic compounds, hydrocarbons used as solvents, mercury vapors, and for the inorganic gases carbon monoxide and nitrogen dioxide have been derived. There are two categories of guideline values: *RW-I* and *RW-II* (RW = "Richtwert" = guide value).

RW-I is a precautionary guideline. It is anticipated that even with lifelong exposure below the RW-I concentration, no adverse effects on human health are to be expected. If the threshold is exceeded, additional and non-usual burden might be the case. If the measured concentration is between RW-I and RW-II, precautionary measures shall be taken (either by changing the consumer behavior or by technical or construction measures). RW-I guideline values can be used as target values for remediation purposes.

RW-II values are effect-related values, which are derived from current toxicological and epidemiological data on the respective substance taking assessment

**Table 8** Immission limit values for protection from hazard to human health according to "TA Luft"

| Substance | Concentration [μg/m$^3$] | Averaging period | Allowed frequency of excess per year |
|---|---|---|---|
| Sulfur dioxide ($SO_2$) | 50 | Year | - |
| | 125 | 24 h | 3 |
| | 350 | 1 h | 24 |
| Nitrogen dioxide ($NO_2$) | 40 | Year | - |
| | 200 | 1 h | 18 |
| $PM_{10}$ | 40 | Year | - |
| | 50 | 24 h | 35 |
| Lead[a] | 0.5 | Year | |
| Benzene | 5 | Year | |
| Tetrachloroethylene | 10 | Year | |

[a]Lead and its inorganic compounds as part of particulate matter ($PM_{10}$), given as Pb

**Table 9** Guideline values from the ad-hoc working group and other committees for indoor air

| Substance | RW-II[a] [μg/m$^3$] | RW-I[a] [μg/m$^3$] | Year of designation by ad hoc working group |
|---|---|---|---|
| Ethylbenzene | 2,000 | 200 | 2012 |
| Alkylbenzene ($C_9$–$C_{15}$) | 1,000 | 100 | 2012 |
| Cresols | 50 | 5 | 2012 |
| Phenol | 200 | 20 | 2011 |
| 2-Furaldehyde | 100 | 10 | 2011 |
| Cyclic dimethylsiloxanes D3–D6 (total sum guideline) | 4,000 | 400 | 2011 |
| Benzaldehyde | 200 | 20 | 2010 |
| Benzyl alcohol | 4000 | 400 | 2010 |
| Monocyclic monoterpenes *(lead substance d-limonene)* | 10,000 | 1,000 | 2010 |
| Aldehydes ($C_4$–$C_{11}$, saturated acyclic aliphatic) | 2,000 | 100 | 2009 |
| $C_9$–$C_{14}$-Alkanes/ isoalkanes (dearomatized) | 2,000 | 200 | 2005 |
| Naphthalene | 20 | 2 | 2004 |
| Bicyclic terpenes *(lead substance α-pinene)* | 2,000 | 200 | 2003 |
| Tris(2-chloroethyl) phosphate (TCEP) | 50 | 5 | 2002 |
| Mercury (as metallic vapor) | 0.35 | 0.035 | 1999 |

(*continued*)

**Table 9** (continued)

| Substance | RW-II[a] [µg/m$^3$] | RW-I[a] [µg/m$^3$] | Year of designation by ad hoc working group |
|---|---|---|---|
| Styrene | 300 | 30 | 1998 |
| | 350 (½ h) | | |
| Nitrogen dioxide ($NO_2$) | 60 (7 day) | - | 1998 |
| Dichloromethane | 2,000 (24 h) | 200 | 1997 |
| Carbon monoxide | 60,000 (½ h) | 6,000 (½ h) | 1997 |
| | 15,000 (8 h) | 1,500 (8 h) | |
| Pentachlorophenol (PCP) | 1 | 0.1 | 1997 |
| Toluene | 3,000 | 300 | 1996 |
| Substance | Guideline value [µg/m$^3$] | Remarks | |
| Formaldehyde | 125 | Recommended by German Federal Board of Health, 1977 (confirmed by ad hoc working group in 2006) | |
| Tetrachloroethylene | 100 | Legally binding value! (2. "BImSchV," 1990; Regulation to limit emission of volatile halogenated organic compounds) | |
| Radon | Existing buildings: 400 Bq/m$^3$; | European Commission Recommendation (90/143/Euratom) | |
| | Future constructions: 200 Bq/m$^3$ | | |
| Polychlorinated biphenyls (PCBs) | < 0.3 (nonhazardous) | Recommended by German Federal Board of Health, 1990 (partially confirmed by ad hoc working group in 2007) | |
| | > 3 (immediate actions to minimize exposure)[b] | | |

Resources: Umweltbundesamt, 2013 http://www.umweltbundesamt.de/gesundheit/innenraumhygiene/richtwerte-irluft.htm

[a]Usually, these guidelines are given as long-term values. Averaging periods deviating from this are given in brackets, e.g., ½ h

[b]Valid only if the only source for exposure is spacing material

factors into account. Depending on the mode of action, the RW-II values might refer to short-term (RW-II-K) or long-term values (RW-II-L). In case that the RW-II value is reached or even exceeded, an acute need for action is indicated.

Overall, there is only one legally binding indoor guideline value, which was established for tetrachloroethylene.

## Food

### *Maximum Residual Levels* (*MRLs*): International, European, and National Standards and Regulations

As fruits and vegetables are prone to various detrimental effects (e.g., diseases and pests), pesticides are used to ward off or at least minimize these negative

consequences. On the other hand, it must be granted that plant protection products have no adverse effects on human health. Therefore, maximum residual levels (MRLs) are in place in order to prevent consumers from adverse health effects. Within Europe Maximum Residue Levels are defined as "the upper legal levels of a concentration for pesticide residues in or on food or feed based on good agricultural practices and to ensure the lowest possible consumer exposure." Most of the values are based on the ALARA principle (as low as reasonably achievable). If the MRL within one foodstuff is exceeded, trading might be forbidden, even if there is no adverse health effect yet.

The WHO and the FAO (Food and Agriculture Organization of the United Nations) jointly publish the *CODEX Alimentarius*, which provides international food standards for pesticides (also for veterinary drugs and food additives, see "Resources"). The CODEX standards contribute to the safety, quality, and fairness of the international food trade. They are only recommendations but may often serve as a basis for national legislations. The standards are not listed here as they can easily be searched within the given internet source.

In Europe, in 2005 a harmonized regulation was being introduced with Regulation (EC) No 396/2005 which entered into force in 2008. The text of the regulation is given on the homepage of the Federal Office of Consumer protection and food safety, which includes the currently valid MRLs in Annex II, IIIA, and IIIB. As the list of regulated substances is quite extensive and would not fit into this chapter, we refer to the list as provided within the regulation. Moreover, an EU Pesticides Database was established in order to perform searches for MRLs (for link see "Resources"). In cases where no specific MRL was determined, a general level of 0.01 mg/kg has to be met.

Even though the European Regulation overrules the former, valid German Regulation on maximum residual levels ("Rückstands-Höchstmengenverordnung"; RHmV, 1994, last updated in 2010), which was the national implementation of various former EU Directives (i.e., 90/642/EEC, 93/57/EEC, 93/58/EEC), this regulation still is in force concerning certain areas; for example, for safeners and synergists which are relevant co-formulants in plant protection products, or in case of category 11 (fish, fish products, shellfish, molluscs, and products of other freshwater or seawater fish). In case of group 12 (exclusive use as feeding stuff), still the German Regulation on Feeding stuff ("Futtermittelverordnung," FuttMV, 1981, last updated in 2012) is in place.

## European Maximum Levels for Certain Contaminants in Foodstuffs

Contaminants are substances that have not intentionally been added to food. In general they negatively influence the quality of food and may cause harm to human health in certain cases. Basic principles are therefore regulated within Council Regulation 315/93/EEC, stating that food containing unacceptable amounts of contaminants based on considerations for the human health shall not be placed on the market, the level shall be kept as low as reasonably achievable (ALARA principle),

and maximum levels must be set. These maximum levels are given in the Commission Regulation (EC) No 1881/2006, which entered into force in 2007 and was amended afterwards several times. Currently, there are community measures for the following contaminants: mycotoxins (aflatoxins, ochratoxin A, fusarium toxins, patulin), metals (cadmium, lead, mercury, inorganic tin), dioxins and PCBs, polycyclic aromatic hydrocarbons (PAH), 3-MCPD, and nitrates. Even though only a small number of contaminants are regulated, the list is quite extensive as foodstuff description is detailed, and therefore here it is referred to the list included in the Commission Regulation (EC) No 1881/2006 and its amendments.

Resources: European Commission

## Soil

Germany adopted a legislation to protect soil in 1998 (so-called Bundes-Bodenschutzgesetz; BBodSchG). Based on this legislation the federal regulation on soil conservation and contaminated sites was adopted in 1999 ("Bundesbodenschutz-verordnung"; BBodSchV). Within this regulation there are *precautionary values*, *trigger values*, and *action values* (i.e., "Vorsorge, Prüf- und Maßnahmenwerte"). These values are designed to apply for different routes of exposure (i.e., soil to humans, soil to plants, soil to groundwater). The values established for direct interaction (soil to humans) are the most relevant from the toxicological point of view (see Table 10). These values always consider different exposure scenarios (i.e., playground, residential area, park and recreation area, industrial sites) and are usually derived from the *TRD* values (tolerable absorbed dose; cf. paragraph on Tolerable Absorbed Doses (*TRD*)). In case of carcinogenic substances, an additional risk of $> 5 \times 10^{-5}$ is assumed to be no more tolerable under defined exposure assumptions for trigger as well as action values.

When a trigger value is exceeded, a site-specific assessment has to be performed, in order to investigate if a detrimental effect to soil quality or even a hazardous waste site may be present. This site-specific determination allows the respective authority to decide if further action to reduce exposure is necessary.

In general, if action values specified for a certain exposure scenario (e.g., playground) are exceeded, it is assumed that detrimental effects to soil quality occurred or a hazardous waste site was identified, and an immediate need for action to reduce exposure exists. Action values are only available for polychlorinated dibenzodioxins or dibenzofurans.

A volume entitled "Calculation of guidance values for assessment of hazardous waste sites" is dedicated to the protection of soil and was published by the Federal Environment Agency (Bachmann et al. 2007). This supplementary volume includes the underlying calculations for all legally binding trigger values presented in Table 10. Moreover, references for further trigger values are given for approximately 50 additional substances, relevant for hazardous waste sites, especially abandoned military sites.

**Table 10** Trigger and action values for substances mentioned in annex 2 of the "BBodSchV" (exposure pathway: soil to humans)

| Trigger values [mg/kg dry weight] | | | | |
|---|---|---|---|---|
| Substance | Playground | Residential area | Park and recreation area | Industrial site |
| Arsenic | 25 | 50 | 125 | 140 |
| Lead | 200 | 400 | 1,000 | 2,000 |
| Cadmium | 10[a] | 20[a] | 50 | 60 |
| Cyanides | 50 | 50 | 50 | 100 |
| Chromium | 200 | 400 | 1,000 | 1,000 |
| Nickel | 70 | 140 | 350 | 900 |
| Mercury | 10 | 20 | 50 | 80 |
| Aldrin | 2 | 4 | 10 | - |
| Benzo[a]pyrene | 2 | 4 | 10 | 12 |
| DDT | 40 | 80 | 200 | - |
| Hexachlorobenzene | 4 | 8 | 20 | 200 |
| Hexachlorocyclohexane (HCH mixtures or beta HCH) | 5 | 10 | 25 | 400 |
| Pentachlorophenol (PCP) | 50 | 100 | 250 | 250 |
| Polychlorinated biphenyls ($PCB_6$)[b] | 0.4 | 0.8 | 2 | 40 |
| Action values [ng l-TEQ/kg dry weight][c] | | | | |
| Polychlorinated dibenzodioxins/ dibenzofurans (PCDD/F) | 100 | 1,000 | 1,000 | 10,000 |

[a]In garden plots, which can be occupied by children and are used for growing food plants, a guidance value of 2 mg cadmium per kg dry weight has to be applied
[b]In case total amount of PCBs are determined, the measured values have to be divided by 5
[c]Total amount of 2,3,7,8–TCDD toxicity equivalents (according to NATO Committee on the Challenges of Modern Society)

## Body Burden

The Human Biomonitoring Commission is part of the German Federal Environment Agency ("Umweltbundesamt," UBA). Members are recruited from Federal and Regional authorities, as well as from Academia (universities, institutes of hygiene, medicinal clinics). The work of the commission was to establish various reference and human biomonitoring values for different toxic substances in various body fluids, which are further exemplified below (Table 11, 12, and 13).

The reference value describes the exposure of individuals or population groups compared to the ubiquitous background exposure (they are checked and updated continuously). The reference value for a specific chemical is established only on a statistical basis, after having collected a representative series of measurements (from a defined group of the general population). These values therefore have not been derived with respect to protecting human health.

In contrast to that, Human Biomonitoring (HBM)-I and HBM-II values are based on toxicological and epidemiological data. According to the current opinion of the

**Table 11** Reference values and Human Biomonitoring (*HBM*) values for various substances in urine or blood

| Substance and matrix | Reference values | | Human biomonitoring (HBM) values | | |
|---|---|---|---|---|---|
| | Subpopulation | Reference value | Subpopulation | HBM-I | HBM-II |
| Lead (blood) | Children (3–14 year)<br>Females (18–69 year)<br>Males (18–69 year) | 35 µg/l<br>70 µg/l<br>90 µg/l | Children ≤ 12 years and females of reproductive age<br>Other persons | Suspended | Suspended |
| Cadmium (urine) | Nonsmoking<br>Children (3–14 year)<br>Adults (18–69 year) | 0.2 µg/l<br>0.8 µg/l | Children and adolescents<br>Adults | 0.5 µg/l<br>1 µg/l | 2 µg/l<br>4 µg/l |
| Mercury (urine) | Without amalgam fillings<br>Children (3–14 year)<br>Adults (18–69 year) | 0.4 µg/l<br>1.0 µg/l | Children and adults | 7 µg/l<br>5 µg/g Crea | 25 µg/l<br>20 µg/g Crea |
| Mercury (blood) | Fish consumption ≤ 3 times/month<br>Children (3–14 year)<br>Adults (18–69 year) | 0.8 µg/l<br>2.0 µg/l | Children and adults[a] | 5 µg/l | 15 µg/l |
| Thallium (urine) | Children (3–14 year)<br>Adults (20–29 year) | 0.6 µg/l<br>0.5 µg/l | General population | 5 µg/l | - |
| Pentachlorophenol (serum) | Adults | 12 µg/l | General population | 40 µg/l | 70 µg/l |
| Pentachlorophenol (urine) | Children (3–14 year)<br>Adults (18–69 year)[c] | 2.0 µg/l[b]<br>5 µg/l | General population | 25 µg/l<br>20 µg/g crea | 40 µg/l<br>30 µg/g crea |
| ∑ of the DEHP metabolites 5oxo- and 5OH-MEHP (urine) | Children (3–14 year)<br>Adults (20–29 year) | 280 µg/l<br>50 µg/l | Children aged 6–13<br>Women of childbearing age<br>Males ≥ 14 years of age and remaining general population | 500 µg/l<br>300 µg/l<br>750 µg/l | -<br>-<br>- |
| ∑ PCB (138 + 153 + 180) (serum *x*2) | See Table 12 | | Babies, small children, and women of childbearing age | 3.5 µg/l | 7 µg/l |

(*continued*)

**Table 11** (continued)

| Substance and matrix | Reference values | | Human biomonitoring (HBM) values | | |
|---|---|---|---|---|---|
| | Subpopulation | Reference value | Subpopulation | HBM-I | HBM-II |
| Bisphenol A (urine) | Children (3–5 year)<br>Children (6–14 year)<br>Adults (20–29 year) | | Children<br>Adults | 1,500 μg/l<br>2,500 μg/l | - |

*Crea* creatinine

[a]Derived from females in reproductive age. The use is recommended for other groups

[b]No reference value, but should there be analytical reliable and confirmed concentrations above the mentioned value, a special exposure must be expected

[c]Subpopulation only refers to adults who were living in homes where no wood preservatives had been used

**Table 12** Reference values for persistent organic pollutants in whole blood [μg/l]

| | | | | | | | DDE | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Germany | |
| Age (years) | PCB 138 | PCB 153 | PCB 180 | ∑ PCB (138 + 153 + 180) | β-HCH | HCB | East | West |
| 7–14 | 0.3 | 0.4 | 0.3 | 1.0 | 0.3 | 0.3 | 1.4 | 0.7 |
| 18–19 | 0.4 | 0.6 | 0.3 | 1.1 | 0.3 | 0.4 | 3 | 1.5 |
| 20–29 | 0.6 | 0.9 | 0.6 | 2.0 | 0.3 | 0.5 | 5 | 2 |
| 30–39 | 0.9 | 1.6 | 1.0 | 3.2 | 0.3 | 1.0 | 11 | 4 |
| 40–49 | 1.4 | 2.2 | 1.6 | 5.1 | 0.3 | 2.5 | 18 | 7 |
| 50–59 | 1.7 | 2.8 | 2.1 | 6.4 | 0.5 | 3.3 | 31 | 8 |
| 60–69 | 2.2 | 3.3 | 2.4 | 7.8 | 0.9 | 5.8 | 31 | 11 |

**Table 13** Reference values for some polychlorinated biphenyls (*PCB*) and organochlorines in human breast milk [mg/kg fat]

| Total DDT (applies only to women in western Germany) | ∑ PCB (138 + 153 + 180) | β-HCH | HCB |
|---|---|---|---|
| 0.5 | 0.5 | 0.07 | 0.06 |

HBM committee, HBM-I values represent the concentration of a chemical in a defined biological material, below which no adverse health effect is expected and therefore no actions have to be taken. In case the *HBM-I value* is exceeded, but is still lower than the *HBM-II value*, further measurements have to be performed, and the possible source of exposure should be identified. Moreover, the exposure to the source should be minimized. The HBM-I value thus represents a verification or control value.

HBM-II values represent the concentration above which a high possibility of an adverse health effect exists, thus resulting in acute need for action (i.e., reduction of exposure and biomedical care (advice)). The HBM-II value therefore represents an intervention or action value.

## References

Ausschuss für Gefahrstoffe (AGS) (2012) Technische Regeln für Gefahrstoffe – Arbeitsplatzgrenzwerte (TRGS 900). Ausgabe: zuletzt gewändert und ergänzt: GMBl 2012 S 715–716 [Nr. 40]. Bundesanstalt für Arbeitsschutz und Arbeitsmedizin online. http://www.baua.de/de/Themen-von-A-Z/Gefahrstoffe/TRGS/TRGS-900.html

Bachmann G, Oltmanns J, Konietzka R, Schneider K, Rück F, Albrecht E (2007) Berechnung von Prüfwerten zur Bewertung von Altlasten ergänzbares Handbuch 4. Erg.Lfg. IV/07, hrsg. vom Umweltbundesamt Berlin Erich Schmidt Verlag, Berlin

Eikmann T, Heinrich U, Heinzow B, Konietzka R (2010) Gefährdungsabschätzung von Umweltschadstoffen. Ergänzbares Handbuch toxikologischer Basisdaten und ihre Bewertung Erich Schmidt Verlag, Berlin

World Health Organization (WHO) (2000) Air quality guidelines for Europe WHO regional publications, vol 91, 2nd edn, European series. Regional Office for Europe, Copenhagen

World Health Organization (WHO) (2006) Air quality guidelines for Europe. Global update 2005. WHO Regional Office for Europe, Copenhagen

World Health Organization (WHO) (2008) Guidelines for drinking-water quality. incorporating 1st and 2nd Addenda, vol 1. Recommendations, 3rd edn. Geneva. http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/

World Health Organization (WHO) (2010) WHO guidelines for indoor air quality: selected pollutants http://www.euro.who.int/en/what-we-publish/abstracts/who-guidelines-for-indoor-air-quality-selected-pollutants

## Resources: Food

Bundesinstitut für Risikobewertung (BfR). www.bfr.bund.de/cm/343/pflanzenschutzmittel-wirkstoffe-adi-werte-und-gesundheitliche-trinkwasser-leitwerte.pdf. Accessed 14 Mar 2013

European Food Safety Authority (EFSA): Maximum residual levels http://www.efsa.europa.eu/en/pesticides/mrls.htm. Accessed 14 Mar 2013

EU Pesticides Database. http://ec.europa.eu/sanco_pesticides/public/index.cfm. Accessed 27 Mar 2013

World Health Organization (WHO): Inventory of IPCS and other WHO pesticide evaluations and summary of toxicological evaluations performed by the Joint Meeting on Pesticide Residues (JMPR) through 2010. www.who.int/foodsafety/chem/jmpr/publications/jmpr_pesticide/en/index.html. Accessed 14 Mar 2013

World Health Organization (WHO): Summary Report from the 2012 Joint FAO/WHO Meeting on Pesticide Residues (JMPR). www.who.int/foodsafety/chem/jmpr/summary_2012.pdf. Accessed 14 Mar 2013

Codex Alimentarius, International Food Standards. http://www.codexalimentarius.org/standards/en/. Accessed 14 Mar 2013

European Commission. Food contaminants – Introduction. http://ec.europa.eu/food/food/chemicalsafety/contaminants/index_en.htm. Accessed 14 Mar 2013

## Resources: Occupational Health and Safety

World Health Organization (WHO). Occupational health. http://www.who.int/topics/occupational_health/en/. Accessed 14 Mar 2013
European Agency of safety and Health at Work (EU OSHA). http://osha.europa.eu/en/legislation/guidelines. Accessed 27 Mar 2013
U.S. Department of Labor, Occupational Safety & Health Administration (OSHA). Occupational safety and health standards. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992. Accessed 14 Mar 2013

## Resources: Body Burden

European legislation. http://eur-lex.europa.eu/en/index.htm. Accessed 14 Mar 2013
For more reference values please see www.umweltbundesamt.de/gesundheit-e/monitor/definitionen.htm. Accessed 14 Mar 2013
HBM-Commitee. http://www.umweltbundesamt.de/gesundheit-e/monitor/index.htm. Accessed 14 Mar 2013

## Resources: Air

Umweltbundesamt, Gesundheit und Umwelthygiene. Richtwerte für die Innenraumluft. http://www.umweltbundesamt.de/gesundheit/innenraumhygiene/richtwerte-irluft.htm. Accessed 14 Mar 2013

# Appendix

# Regulatory Toxicology: Glossary

Franz-Xaver Reichl and Gisela Degen

**Abuse** Improper and excessive use of drugs or stimulants (e.g., alcohol, tobacco) and the use of pharmaceutical products without medical indication or in exceeding doses.

**Acceptable Risk** This is a risk management term (for a given risk level). Risk levels used for risk evaluations can only be sociopolitically established rather than scientifically substantiated. Numerous criteria have to be taken into account apart from risk perception, e.g., severity of health damage, the possible extent of damage (type of damage and/or number of persons affected), relation to other comparable risks, direct benefit, and actual and possible risk reduction measures. According to a concept adopted in 2007 by the German Committee on Hazardous Substances (AGS), an *acceptable* and a *tolerable* risk level serves to derive exposure-risk relations for carcinogenic chemicals at the workplace. This concept for setting risk-based occupational exposure limits is linked to a set of risk reduction measures.

**Accumulation** Enrichment of a substance in a medium or environmental compartment. Bioaccumulation is the successive enrichment of a repeatedly administered chemical in an organism when the half-life is very long due to minor metabolization and slow excretion. Accumulation occurs often in specific organs, e.g., cadmium in kidney, lead in bone, and PCDD in fat tissue. Body burden.

**Acute Toxicity** Adverse effect occurring within a short time of exposure (up to max. 14 days) after a single (high) dose (see also (Sub)chronic Toxicity).

**Acute Toxicity Test** Test with an observation time up to 14 days after a single dose. The toxic class method (for estimating LD50) requires a clearly reduced number of animals.

F.-X. Reichl (✉)
LMU Munich, Munich, Germany
e-mail: reichl@lmu.de

G. Degen
Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany
e-mail: degen@ifado.de

F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1, 

**Adaptation** Compensatory change in an organism, in response to certain environmental conditions, which occurs without disruption of the biological system and without exceeding the homeostatic capacities of its response.

**Added Risk** Difference between the incidence of an adverse effect on a treated group of organisms or a group of exposed humans and a control group.

**Additive Effect** An effect which is the simple sum of the effects of two or more chemicals acting independently (see also Combined Chemical Effects).

**ADI (Acceptable Daily Intake)** Dose of an agent (amount expressed on a body mass basis) to which an individual in a (sub)population may be exposed daily over its lifetime without an appreciable health risk. The WHO sets ADI values for food additives and tolerable daily intakes (TDI) for contaminants; they are calculated by division of the *NO(A)EL* with a *safety factor* (see also Reference Dose and TDI).

**Adverse Effect** "Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences. Decision on whether or not any effect is adverse requires expert judgement" (according to IPCS/WHO 1994). A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge.

**Agent** Something (chemically, physically, or biologically active principle) capable of producing an effect.

**Aggrieved Party** Any natural person or legal entity or rather a group of persons whose interests or values can be affected (derogated) by the impact of risks or by risk reduction measures.

**Agonist** Chemical that can activate a receptor similar to a physiological mediator.

**AGS** Ausschuss für Gefahrstoffe, the German Committee on Hazardous Substances. The AGS gives advice to the German Federal Ministry of Labour and Social Affairs regarding regulation of workplace chemicals.

**AGW** Arbeitsplatzgrenzwert: A health-based OEL for a substance in workplace air set by the German Committee on Hazardous Substances (AGS) according to criteria of BekGS 901 and published in the TRGS 900. The AGS evaluates OELs proposed by the German MAK commission, by SCOEL, and by other scientific advisory bodies (DECOS, TLV committee).

**ALARA** Acronym for "as low as reasonably achievable"; a term from the US Nuclear Regulatory Commission. It means "making every reasonable effort to maintain exposures to ionizing radiation as far below the dose limits as practical, consistent with the purpose for which the licensed activity is undertaken, taking into account the state of technology, the economics of improvements in relation to state of technology, the economics of improvements in relation to benefits to the public health and safety, and other societal and socioeconomic considerations, and in relation to utilization of nuclear energy and licensed materials in the public interest." The ALARA principle is a regulatory tool in

the risk management of substances (when a regular risk assessment is not available) (see also Precautionary Principle).

**Allergen** Any substance that can cause an allergy. Antigens which cause an allergic reaction (hypersensitivity of type I) by stimulating immunoglobulin E (IgE) responses upon contact with skin and/or mucous membranes. Allergens are often compounds, polypeptides, or proteins, the sensitizing potential of which depends on chemical structure and the presence of allergenic determinants (epitopes).

**Allergy** An (acquired) hypersensitivity disorder of the immune system against environmental (normally harmless) substances. Allergic reactions (to an allergen) involve excessive activation of mast cells and basophils by IgE antibodies. Symptoms occur on the skin, in mucous membranes, and in the respiratory tract (e.g., urticaria, eczema, edema, conjunctivitis, hay fever, asthma) of sensitized individuals.

**Ames Assay** In vitro assay (developed by Bruce Ames) for the detection of mutagenic effects of chemicals in bacteria (Salmonella test strains). As it reveals mutagenic effects of, e.g., cigarette smoke components and of a high percentage of known mutagenic carcinogenic substances, the Ames assay is usually a starting point in genotoxicity testing.

**Aneuploidy** Deviation from the number of the normal (euploid) chromosome set by one or several chromosomes.

**Annoyance** An unpleasant (mental) state that is characterized by such effects as irritation and distraction. Annoyance can result from (various) environmental stimuli (e.g., noise, odor) perceived as unpleasant or pestering by the recipient(s). The property of being easily annoyed is called irritability.

**Antagonism** The property of a chemical to counteract the effect of another; e.g., in the case of co-exposure to two chemicals when the resulting effect is less than the simple sum of their independent effects (see also Antagonist).

**Antagonist** Chemical (or drug) which fits into the inactive conformation of a receptor and thereby diminishes or prevents its activation by another chemical, an agonist.

**Antigen** A substance which elicits a specific immune response (e.g., formation of antibodies) when introduced into an organism.

**Antioxidants** Substances that inhibit or prevent oxidation processes which result in undesirable changes of biomolecules.

**Antitoxins** Antibodies (often immunoglobulins of the IgG class) which can neutralize toxins of microbial, plant, or animal origin (e.g., snake venom).

**Application** Administration of substances to an organism. Common routes of application are: p.o. = per os (via the gastrointestinal tract), s.c. = subcutaneous (injection under the skin), i.m. = intramuscular (in the skeletal muscle), i.p. = intraperitoneal (injection in the abdominal cavity), i.v. = intravenous (injection in veins).

**Assessment Endpoint** Qualitative/quantitative expression of a specific factor (a response) with which a risk may be associated through an appropriate risk assessment.

**Assessment Factor** Numerical adjustment used to extrapolate from experimentally determined dose-response relationships to estimate the agent exposure below which an adverse effect is not likely to occur (see also Safety and Uncertainty Factor).

**Atopic Persons** Individuals with a predisposition for developing an allergy.

**Background Burden/Exposure** Substance concentrations in biological samples of humans as a result of normal conditions (without known additional exposure).

**BAT Value** German "Biologischer Arbeitsstoff-Toleranzwert" for biological tolerance value (BLV) for occupational exposures; defined as the maximum permissible quantity of a chemical substance or its metabolites or the maximum permissible deviation from the norm of biological parameters induced by these substances in exposed humans. As with MAK values, BAT values are established on the assumption that persons are exposed at work for at most 8 h daily and 40 h weekly (see also BEI and EKA).

**BEI** Biological Exposure Indices: used in the USA analogous to BAT values in Germany.

**Benchmark Approach/Dose** Adjustment of a mathematical model to the data obtained in a study for the dose-response relationship. The benchmark approach is an instrument to determine a *point of departure* for quantitative risk assessments. The dose that leads to an effect with a certain likelihood can be estimated for a defined frequency (for quantal data) or a defined effect measure (for continuous data), i.e., a benchmark response (BMR). This dose is referred to as benchmark dose (BMD). A $BMD_{10}$ indicates the dose at which there is a 10 % risk that the effect concerned would likely occur. The reliability of assessing dose-response relationships is quantified by specifying a confidence interval. The value of the lower (generally 90 % or 95 %) confidence interval is referred to as benchmark dose lower bound (BMDL).

**Bioactivation** Conversion of xenobiotics (e.g., by enzymes) to biologically reactive, toxic, or carcinogenic metabolites.

**Bioassay** An assay for determining the potency (or concentration) of a substance that causes a biological change in experimental animals and living systems.

**Bioavailability** The fraction of a chemical or drug that can be absorbed by the body through the gastrointestinal system, the pulmonary system, or the skin and is systemically available. By definition, when a medication is administered intravenously, its bioavailability is 100 %. Upon administration by other routes, its bioavailability generally decreases (due to incomplete absorption and first-pass metabolism) or may vary from person to person.

**Biocide** A selectively acting toxic substance that is used to destroy harmful organisms (see *Pesticide*).

**Biological Limit Values** For occupational health purposes, special human biomonitoring (limit) values have been established, such as Biologischer Arbeitsstoff-Toleranzwert (BAT) and Biologische Leitwerte (BLW) by the German MAK commission or Biological Exposure Indices (BEI) by the AGCIH. These values are meant to allow evaluating the risk to an individual's health that

results from exposure to a substance at the workplace (by inhalation and/or dermal uptake) and to protect the health of the employee at the workplace.

**Biomonitoring** In a broader sense, all biological monitoring methods used to investigate the (complex) relationship between external and internal exposure and, thereby, the potential adverse health and environmental effects. In ambient monitoring, living organisms are used as "sensors" in water/sediment quality surveillance and compliance to detect changes in an effluent or water body and to indicate whether aquatic life may be endangered. In health monitoring, biomonitoring is a general term for the following subcategories: (a) biological monitoring applying biomarkers of exposure such as internal dose or body burden, (b) biochemical effect monitoring with biomarkers of effective dose (e.g., adduct levels and also tissue dose), (c) biological effect monitoring with biomarkers of effect (e.g., micronuclei), and (d) clinical parameters – biomarkers of disease. Most common in human biomonitoring are studies with biomarkers of exposure and biochemical effects aimed to establish distribution of exposure among the general population (including trends and changes in exposure), identify vulnerable groups and populations with higher exposures, identify new chemical exposures, and identify environmental risks at specific contaminated sites or at workplaces.

**Biotransformation** Enzymatic conversion of xenobiotics in an organism; metabolization (biotransformation) usually results in products that are less toxic, more water soluble, and readily excreted from the organism. But with some chemicals, biotransformation results in bioactivation and thus an increased toxicity.

**Body Burden** The total amount of a substance in the body. Some substances build up in the body because they are stored (e.g., in fat or bone) or because they leave the organism very slowly. In such cases, the blood concentration does not reflect the amount stored in the body. Body burden must be measured with independent methods.

**Brownfields** Sites or soil polluted with hazardous substances (e.g., abandoned or existing waste deposits and/or production sites).

**Cancer** Disease which results from the development of a malignant tumor and its spreading into surrounding tissues.

**Carcinogen** An agent capable of inducing cancer. A substance or mixture (e.g., coal tar) which causes tumors (cancer) in animals or humans.

**Carcinogenesis** The development of cancer, a disease of heritable, somatic mutations affecting cell growth and differentiation, characterized by an abnormal, uncontrolled growth of cells. In chemical carcinogenesis, several steps are defined: initiation, promotion, and progression. Any chemical which can cause cancer is said to be carcinogenic.

**Carcinogenicity Test** A form of chronic toxicity testing directed to detect carcinogenic effects of chemicals: The test substance is applied to rodents for 18–24 months, usually five times a week, at several dose levels. The appearance of tumors is assessed upon necropsy and compared to the frequency in a nonexposed control group.

**Cell Culture** The maintenance and propagation of previously isolated cells in a suitable nutrient (culture) medium. Cell cultures are used for various in vitro toxicity tests. Other than primary cells, cell lines can be kept in culture for long periods and passaged numerous times.

**Chromosomal Aberration** An abnormality in chromosome number or structure.

**Chromosome** The heredity-bearing gene carrier in the cell nucleus, composed of DNA and protein.

**Chronic Toxicity** Refers to long-term adverse effects in an organism after dosing of a toxicant over an extended time period. Long-term effects relate to changes in, e.g., growth, reproduction, or the ability to survive. Examples in humans are cardiovascular diseases and cancer from smoking and liver disease from alcohol abuse.

**Chronic Toxicity Test** Experimental studies with repeated application of a substance over an extended period of time (at least 3 months), usually at several dose levels, to gain information on, e.g., organ toxicity, tumor formation, and dose-effect relationships.

**Clastogens** Agents which cause chromosomes to break. This may be a result of direct damage to the DNA or by indirect mechanisms, e.g., inhibition of topoisomerases.

**Clearance** The process of losing a substance from the body. Total clearance ($Cl_{tot}$) is a measure for the ability of an organism to eliminate a given substance by both renal and extrarenal clearance. Renal clearance ($Cl_R$) is a function of glomerular filtration, secretion from the peritubular secretion of the nephron, and reabsorption from the nephron back to these blood vessels. Another major route for elimination of foreign compounds is their uptake by liver cells and secretion into bile.

**CLP** Acronym for Regulation on **C**lassification, **L**abelling, and **P**ackaging of chemical substances and mixtures (see also ECHA and REACH).

**Combined Chemical Effects** Chemicals that act by the same mode of action and/or at the same target cell or tissue often act in a (potency-corrected) "dose-additive" manner. Where chemicals act independently, by discrete modes of action or at different target cells or tissues, the effects may be additive ("effects additive" or "response additive"). Alternatively, chemicals may interact to produce an effect, such that their combined effect "departs from dose additivity." Such departures comprise "synergy," where the effect is greater than that predicted on the basis of additivity, and "antagonism," where the effect is less than that predicted on the basis of additivity. Related terms are "mixture toxicity," additive effect, antagonism, and synergism.

**Compartment** In pharmaco- and toxicokinetics, a compartment is a defined volume of body fluids. Major body compartments are blood plasma, interstitial fluid, fat tissue, and intracellular and transcellular fluid. With the exception of blood, where the volume is rather well defined, other "compartments" are of less distinct size, because the volume for distribution of a given substance can comprise various body fluids and tissues. In pharmaco-/toxicokinetics, "compartments" are separated entities which have a defined volume and defined

rates of influx and efflux. These interact with each other in a dynamic way. There are one-, two-, or multi-compartment mathematical models. The models are a practical approach to a much more complex reality.

**Concentration-Effect Relationship** Relationship between the exposure, expressed in concentration, of a given organism, system, or (sub)population to an agent in a specific pattern during a given time and the magnitude of a continuously graded effect to that organism, system, or (sub)population.

**Concern Level** Concentration of an environmental chemical expected/suspected to cause harm to a population in field experiments.

**Congeners** Substances whose structure, function, or origin is similar to others and may match the same structure-activity relationship (SAR). Examples are polyhalogenated dibenzodioxins and -furans (dioxins) which can have diverse toxicological properties.

**Consumer Protection** All areas of legislation and policy which serve to protect citizens (private persons) who are buying or consuming goods or demanding services. Protecting the health of consumers involves mainly issues of food safety, product safety (e.g., personal care products, detergents and household cleaners, textiles, toys), and other consumer goods.

**Contamination** In general, the presence of a minor or unwanted constituent (contaminant) in a material (physical or body tissue), the environment, at a workplace, etc. In food and medicinal chemistry, the term contamination usually refers to the presence of toxic substances or pathogens.

**Course of Action** Variants of possible actions in risk management to reduce risks, including the option for nonaction (for minimal risks). In the case of action, there may be also options for different risk reduction measures.

**Cross-reactivity** Immunological reaction of specific antibodies or specifically sensitized T-lymphocytes with compounds having similar or identical determinants as the so-called homologous antigen.

**Cytochrome P-450** A family of heme containing enzymes that transfer oxygen to chemicals (old term mixed-function oxidases) involved in phase I reactions of xenobiotics. They are located on microsomes and have a light absorption peak near 450 nm.

**Cytotoxicity** Ability of an agent to cause disturbance to cellular structure or function, often leading to cell death.

**DDT** Dichloro-diphenyl-trichloroethane (Chlorphenotan). Contact insecticide, now widely banned because of its high persistence in the environment and accumulation in the food chain.

**Decontamination** Removal of hazardous substances, e.g., from materials, from soil, or from dead and living tissues.

**Default** Statistically supported standard value or assumption that is to be used in the absence of substance-specific or species-specific data. A default is a means to describe systems despite incomplete knowledge of their characteristics.

**Deposition** Sedimentation of solid, liquid, or volatile particles in the organism.

**Desoxyribonucleic Acid** That constituent of cells which stores the hereditary information of an organism in the form of a sequence of nitrogenous bases.

Much of this information relates to the synthesis of proteins. Damage of DNA can result in a mutation.

**Detergent** A cleaning or wetting agent which possesses polar and nonpolar functional groups or surfaces allowing interaction with nonpolar molecules making them miscible with a polar solvent.

**Detoxification** (a) A process which renders a toxic molecule less toxic by biotransformation, removal, or the masking of active functional groups, and (b) the treatment of patients suffering from poisoning in order to reduce the probability or severity of harmful effects.

**Dioxin(s)** Systematic term for a twofold unsaturated six-membered ring system with two oxygens in the ring. Dioxin is used colloquially for the group of polychlorinated dibenzodioxins (PCDD) and sometimes also the polychlorinated dibenzofurans (PCDF); in the first group, there are 75, and in the latter, 135 isomers (congeners). The most famous dioxin, the "Seveso-poison," i.e., 2,3,7,8-tetrachlordibenzo[1,4,]dioxin (2,3,7,8-TCDD) is far more toxic than all other congeners.

**Disinfectants** Substances/preparations used to reduce or eliminate (pathogenic) microorganisms on skin and other surfaces. Examples are ethanol, phenol, soaps, and tensides which act against bacteria.

**Distribution** Dispersal of a xenobiotic and its derivatives throughout an organism or environmental matrix, including tissue binding and localization. In toxicokinetics, this includes the passage of a substance from one compartment (e.g., blood, extracellular fluid) to another (e.g., fat tissue), moving towards an equilibrium.

**Dose** Total amount of an agent administered to, taken up, or absorbed by an organism, system, or (sub)population. Administered doses are often given in mg/kg of body weight.

**Dose-Effect Relationship** The (functional) relationship between the dose and the magnitude of a continuously graded effect in an organism, system, or (sub) population (see also Dose-Response Relationship).

**Dose-Response Relationship** Relationship between the total amount of an agent (the dose) and responses in an organism, system, or (sub)population in reaction to that agent (see also Dose-Effect Relationship).

**Dust** Fine, dry powder consisting of inorganic particles (e.g., ash, clay, rock chip, sand) and/or organic material (e.g., fungal spores, microorganisms, mites, feather or plant fragments, sooty particles), matter lying on the ground or on surfaces or carried in the air. Dusts are generated by work processes such as cutting, crushing, detonation, grinding, and handling of organic and inorganic matter such as coal, grain, metal, ore, rock, and wood, but may also occur naturally (e.g., pollens, volcanic ashes, sandstorms). The term "airborne dust" often refers to airborne particulate matter ranging in diameter from 1 to 100 μm, which differs in deposition in the respiratory tract. Very small particles (fine and ultrafine, less than 5 μm) are of concern as they deposit in the tracheobronchal and alveolar regions. Fibrous dusts, such as asbestos and other such materials, have been shown to present special health problems primarily related to the shape of the particles (see also Fibers).

**EC50** Effective concentration which affects 50 % of a test population after a specified exposure time.

**ECHA** European Chemical Agency (in Helsinki, Finland) with the task of implementing the EU's chemical legislation. The mission of ECHA is to manage all REACH and CLP tasks by carrying out or coordinating the necessary activities, ensure a consistent implementation at the community level, and provide member states and the European institutions with the best possible scientific advice on questions related to the safety and the socioeconomic aspects of the use of chemicals.

**Ecotoxicology** The study of effects of toxic chemicals on biological organisms, mainly at the population, community, and ecosystem levels. A multidisciplinary field which integrates toxicology and ecology, with the aim to predict the effects of pollution and to gather information as to the best course of action to restore already affected ecosystems. Ecotoxicology differs from environmental toxicology in that it integrates the effects of stressors across all levels of the biological organization, whereas environmental toxicology focuses upon effects at the level of individual species and the occurrence and fate of anthropogenic chemicals in the environment.

**ECVAM** European Centre for the Validation of Alternative Methods.

**ED50** Dose that affects a designated criterion in 50 % of the population observed. Also known as median effect concentration/dose.

**Effect** A change in the state or dynamics of an organism, system, or (sub)population caused by exposure to an agent.

**EIA** Environmental Impact Assessment.

**EINECS** European Inventory of Existing Commercial Substances.

**EKA Value** Exposure equivalents for carcinogenic substances at the workplace (see also BAT Value).

**Elimination** The combined process of metabolism and excretion which results in the removal of a substance from an organism.

**ELINCS** European List of Notified Chemical Substances.

**Embryotoxicity** Damage to the embryo (the undeveloped animal or individual), e.g., by chemicals, which results in early death, delays in development, impaired organ function, or malformations (see also Teratogenicity).

**Emission** Release of a substance (or radiation) from a source, including discharges into the wider environment.

**Endogenous** Arising within or derived from the organism.

**Environmental Health** A branch of public health related to all aspects of the natural and built environment that may affect human health. The term "environmental hygiene" is used synonymous with environmental health.

**Environmental Impact Assessment** EIA: A procedural step – introduced by an amendment to Federal Act on EIA in 2001 in Germany – for a screening process ("case-by-case examination") in order to determine whether for a given project (e.g., new streets, train tracks) an EIA is required in the authorization procedure. With regard to chemicals, toxicity testing in nonmammalian species is part of the required hazard assessment under ecological aspects.

**Environmental Medicine** A field of medicine which studies the interaction between environment and human health and the role of the environmental factors in causing or mediating disease in patients (i.e., in a clinical setting) and, thereby, differs from environmental health.

**Environmental Protection** A wide range of societal and individual "measures" aimed to prevent or remediate interferences with ecosystems, e.g., in raising consciousness by information campaigns, labeling of "eco-friendly" products, or setting of standards for hazardous chemicals and pollutants or use restrictions and bans of particularly hazardous agents (such as DDT, vPvB).

**Environmental Toxicology** A field of toxicology which focuses upon the occurrence and fate of anthropogenic chemicals in the environment and the effects of pollutants at the level of individual species (see also Ecotoxicology).

**EPA** Environmental Protection Agency (in the USA, also in Denmark and elsewhere; in Germany, UBA).

**Epidemiology** The study of the incidence, distribution, and causes of disease or the statistical study of categories of persons and the patterns of diseases from which they suffer in order to determine the events or circumstances causing the diseases.

**Epoxide** Highly reactive metabolites with the ability to bind to cell components. Epoxides are held responsible for the toxic and carcinogenic effects of, e.g., polycyclic aromatic hydrocarbons and certain other organic compounds.

**Excretion** Removal of a substance or its metabolites from an organism by the discharge of biological material, including urine, feces, expired air, mucus, milk, eggs, and perspiration.

**Existing Chemicals** Chemicals which have been available in an EC member state between 1971 and 1981 – prior to the introduction of legal obligations for testing them for hazardous properties – and have been listed in EINECS (see also REACH).

**Exogenous** Resulting from events or derived from materials external to an organism.

**Expert Judgment** The opinion of an authoritative person on a particular subject.

**Exposure** The concentration or amount of a particular agent that reaches a target organism, system, or (sub)population at a specific frequency for a defined duration.

**Exposure Assessment** Evaluation of the exposure of an organism, system, or (sub)population to an agent (and its derivatives). Exposure assessment is a key step in the process of risk assessment.

**Exposure Routes** External routes by which a chemical exposure of the organism can occur (e.g., by inhalation, dermal contact, or oral intake) from air, food, water, or soil.

**Extrapolation** An estimation of a numerical value of an empirical (measured) function at a point outside the range of data used to calibrate the function or the use of data derived from observations to estimate values for unobserved entities or conditions. . . .

**Fecundity** (1) Potential to produce offspring frequently and in large numbers, and (2) in demography, the physical ability to reproduce. A lack of fecundity is called sterility.

**Fertility** In humans and mammals, the natural capability to produce offspring. Fertility in a stricter sense differs from fecundity which is defined as the potential for reproduction and influenced by gamete production (sperm, eggs), fertilization, and carrying a pregnancy to term.

**Fetotoxicity** Damage to mammals in the womb, after completion of organogenesis. In humans, this stage is reached after about three months of pregnancy. Prior to this, the developing mammal is in the embryo stage (see also Embryotoxicity).

**Fibers** In relation to health, particles with a diameter $<3$ μm, length $>5$ μm, and aspect ratio (length to width) greater than or equal to 3 to 1 are classified as "fibers" (WHO 1997). Examples of fibers include asbestos (comprising two groups of minerals: the serpentines, e.g., chrysotile, and the amphiboles, e.g., crocidolite – "blue asbestos"). Other examples include synthetic fibrous materials, such as rock wool (or stone wool) and glass wool, as well as ceramic, aramid, nylon, and carbon and silicon carbide fibers.

**First-Pass Effect** The metabolism that an ingested compound undergoes in its passage through the gut and liver before reaching the systemic circulation.

**Fly Ash** (Or flue-ash): Residues generated during combustion of coal which comprise fine particles that rise with the flue gases. Depending on the specific type of coal burnt, fly ash can contain highly toxic materials (arsenic, heavy metals, along with dioxins and PAH); these are concentrated in filter devices (which have to be treated as hazardous waste).

**Food Chain** A sequence of links in a food web between different trophic species, starting, for instance, with basal species such as producers of fine organic matter (plants) and continuing with consumer organisms (herbivores and carnivores). Persistent lipophilic substances (e.g., pesticides) can accumulate in the course of the food chain (see Bioaccumulation).

**Genotoxicity** Ability to cause damage to the genetic material or an adverse effect in the genome, e.g., a gene mutation, chromosomal aberration, or aneuploidy. Genotoxicity is a broader term and refers also to processes which alter the genetic material, yet are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but no direct evidence of mutation), e.g., increases in unscheduled DNA synthesis or sister chromatid exchange, DNA strand breaks, DNA adduct formation, as well as tests for mutagenicity.

**GHS** The Global Harmonized System for classification and labeling of chemicals (CLP) of the UN, implemented under Regulation (EC) No. 1272/2008.

**GLP** Good Laboratory Practice, a quality assurance system.

**Guidance Value** Value, such as concentration in air or water, which is derived after allocation of the reference dose among the different possible media (routes) of exposure. The aim of the guidance is to provide quantitative information from risk assessment to risk managers to enable them to make decisions (see also Reference Dose).

**Half-life** The time interval (commonly denoted as $t_{1/2}$) that corresponds to a concentration decrease by a factor of 2. After five half-lives, the blood level will be 3 % of the initial concentration, a decrease due to metabolism or

excretion. Compounds with a long half-life can accumulate upon repeated intake. Environmental half-life data generally reflect the rate of disappearance from a medium without identifying the mechanism of chemical loss.

**Half-life, Biological** Time interval after administration of a substance to an organism in which half of the originally present dose is eliminated, i.e., metabolized or excreted.

**Harm** Physical injury or mental damage; actual or potential ill effects or danger. For example, "smoking when pregnant can harm your baby."

**Hazard** (1) A potential source of harm. (2) The inherent property of an agent or a situation having the potential to cause adverse effects in organisms or individuals.

**Hazard Assessment** A process designed to determine the possible adverse effects of an agent (or situation) to which an organism could be exposed. The process includes hazard identification and hazard characterization. The process focuses on hazard in contrast to *risk assessment* where exposure assessment is a distinct additional step.

**Hazard Characterization** The qualitative and, wherever possible, quantitative description of the inherent properties of an agent (or situation) having the potential to cause adverse effects. It is a stage in hazard assessment and the second step in risk assessment (see also Concentration/Dose-Effect Relationship).

**Hazard Identification** The identification of the type and nature of adverse effects that an agent can elicit in an organism. Hazard identification is the first stage in the process of hazard assessment and the first in the process of risk assessment.

**Hazardous Situation** Circumstances of danger for a given object (see also Risk).

**HBM Value** Human biomonitoring values I and II

**HCH** Acronym for six chlorine substituted cyclohexanes; the most common form is gamma-HCH (lindane).

**HCp (HC5) Value** Hazardous concentration for p % (5 %) of the species, derived by means of statistical extrapolation for acceptable levels in ecotoxicology.

**Health** As defined by the WHO, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity.

**Health Damage/Impairment** All temporary or permanent undesirable changes triggered by, e.g., chemicals, radiation, accidents, or lifestyle factors.

**Health Protection** All measures taken to protect the health and well-being of the population against harmful factors, such as hazardous chemicals, infectious agents, and radiation. Preventive health protection aims to keep the possible impact low by means of exposure reduction and education measures.

**Health Risk** The probability (likelihood) that damage to health will occur in a population with exposure to a harmful agent or factor. This depends upon the intensity and duration of exposure to a hazardous compound or factor and its activity/affectivity.

**HPVC** High Production Volume Chemical.

**Human Equivalent Dose** The human concentration (for inhalation exposure) or dose (for other routes of exposure) of an agent that is believed to induce the same magnitude of toxic effect as the experimental animal species concentration or

dose. This adjustment may incorporate toxicokinetic information on the particular agent, if available, or use a default procedure, such as assuming that daily oral doses experienced for a lifetime are proportional to body weight raised to the power of 0.75.

**Human Biomonitoring Values I and II (HBM I and II)** The Human Biomonitoring (HBM) Commission of the German Federal Environmental Agency (UBA) defines two different types of HBM values: HBM I and HBM II. HBM I describes the concentration in the body matrix of a substance below which, according to the Commission's current assessment, no adverse health effect should be expected. The HBM II value represents the concentration above which there is an increased risk for adverse health effects; it is thus regarded an intervention or "action" level. HBM I and II values are set on the basis of health risk assessments and are thereby clearly distinct from reference values ($RV_{95}$). RV values (derived according to a defined statistical method from a series of analytical results) are statistical descriptions of the ranges of concentrations typically seen in a specified reference population, but which have no direct relationship to health effects or risk assessment.

**i.v.** Intravenous administration (injection).

**ICH** The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. A source for toxicity test guidelines in drug development.

**Immunotoxicity** Adverse effects of chemicals (or other agents) on components/function of the immune system.

**In Vitro/In Vitro Test** In glass; refers to studies and tests in the laboratory usually involving isolated organs, tissues, cells, or biochemical systems.

**In Vivo/In Vivo Test** Within the living organisms; refers to studies and tests of chemicals in laboratory animals.

**Incidence** The number of newly diagnosed cases of a certain disease within a given period of time; epidemiological measure to characterize disease trends in a certain population.

**Incidence Rate** The ratio of new cases within a population to the total population at risk given a specified period of time.

**Incorporation** The entry/uptake of a (hazardous) substance in an organism or a compartment (e.g., lung, gastrointestinal tract).

**Interest Group** Parts of the population organized as a group that holds and presents a common view.

**Interspecies Dose Conversion** The process of extrapolating from animal doses to human equivalent doses.

**Intervention Value** A value discussed in the context of chemical residues and contaminants of food which is lower than the respective (maximal) limit values. When the intervention value is exceeded, appropriate measures should be taken to reduce emissions of this substance in the environment and thereby prevent contamination of food commodities.

**Intolerance** (Med.) Varied (nonallergic) responses to drugs (drug sensitivity) or food ingredients (e.g., lactose). . . .

**Intoxication** (Poisoning): Impact of usually chemically defined, toxic agents. These substances may also be of mineral, plant, animal, or viral origin. They can "enter" the body via the gastrointestinal tract, the respiratory system, the intact skin, but also via wounds or injection. The severity of an illness depends upon the toxicity, the amount (dose), the duration of exposure, and the susceptibility of the afflicted individual. Poisoned persons often show typical symptoms.

**Invasion** Passage of an incorporated substance into the circulation (e.g., blood or lymph); resorption and distribution in the body.

**IPCS** International Programme on Chemical Safety of the WHO.

**IRIS** Acronym for Integrated Risk Information System. More information (list of substances and documents/reviews) is available on the EPA page: http://www.epa.gov/iris/intro.htm

**Irritants** Substances which cause local reactions (e.g., erythema) on skin or mucous membranes upon prolonged contact due to irritant properties. Agents with corrosive properties (e.g., strong acids or bases) cause more severe damage.

**JECFA** Joint FAO/WHO Expert Committee for Food Additives (and Veterinary Drugs and Contaminants). An international committee that sets ADI and TDI values.

**Latency Period** The time between first exposure to an agent and manifestation or detection of a health effect of interest.

**LC50 (Lethal Concentration)** Concentration of an agent in the surrounding atmosphere, respectively for aquatic organisms in water, which results in the death of 50 % of the exposed individuals.

**LD50 (Lethal Dose)** The median lethal dose that is estimated to cause death of 50 % of the test organisms.

**Limit Values** Limit values (maximal permissible concentrations) for hazardous chemicals are set to protect humans and the environment against harmful effects. Examples of toxicologically founded limit values are maximal residual levels for pesticide contaminants in food or MAK values and other OELs for industrial chemicals at the workplace. Limit values are quantitative standards where noncompliance triggers legal consequences while a "guidance" value has to be observed only when this is possible.

**Lindane** 1,2,3,4,5,6-Hexachlocyclohexane (gamma-HCH), a pesticide.

**Linear Dose Response** A pattern of frequency or severity of biological response that varies directly with the amount of dose of an agent.

**LO(A)EL (Lowest Observed (Adverse) Effect Level)** LOEL: In a study, the lowest dose or exposure level at which a statistically or biologically significant effect is observed in the exposed population compared with an appropriate unexposed control group. LOAEL: The lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

**LOEC** The lowest concentration at which a statistically or biologically significant effect is observed in the exposed population compared with an appropriate unexposed control group.

**MAC and MAK Value** Regulatory value (MAC) defining the concentration which if inhaled daily (for workers: 8 h/day over a working week of 40 h; for the general population: 24 h/day) does not appear capable of causing appreciable harm in the light of the present knowledge. In Germany, MAK values (*Maximale Arbeitsplatz-Konzentration*) for volatile chemicals and dusts are proposed by the Senate Commission of the German Research Foundation Council (DFG) on the basis of toxicological data and workplace-related observations. The MAK Commission also draws up proposals for BAT values (biological tolerance values) and develops procedures to analyze chemical substances in the air and in biological materials. The list of MAK and BAT values is published annually and presented to the German Federal Ministry of Labour and Social Affairs. The Ministry's Committee on Hazardous Substances (AGS) subsequently reviews the proposals and makes recommendations for their inclusion in the Hazardous Substances Ordinance.

**Malignant Tumor** An abnormal growth of tissue which can invade adjacent or distant tissue.

**Margin of Exposure (MOE)** Ratio of the no observed adverse effect levels (the NOAEL or other point of departure) for the critical effect to the theoretical, predicted, or estimated dose or concentration. The MOE is a tool used by risk assessors to consider possible safety concerns arising from the presence in food and feed of substances which are both genotoxic (i.e., which may damage DNA, the genetic material of cells) and carcinogenic (see also Margin of Safety (for noncarcinogenic substances)).

**Margin of Safety (MOS)** For some experts, the MOS has the same meaning as the MOE (margin of exposure), while for others, the MOS means the margin between the reference dose and the actual exposure dose or concentration.

**Mechanism of Action** The interpretation of pharmacological or toxicological effects on the basis of biochemical and molecular data; a sufficient understanding of the molecular basis for an effect so that causation can be established. Compare Mode of Action.

**Medicine** The applied science or practice of the diagnosis, treatment, and prevention of disease.

**Metabolic Activation** See Bioactivation.

**Metabolism** In toxicology, the term refers to the conversion of xenobiotics by endogenous enzymes (see Biotransformation).

**Metabolite** The product from a biotransformation process of xenobiotics.

**Minimal Risk Level** An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. The substance-specific estimates, which are intended to serve as screening levels, are used by the US Agency for Toxic Substances and Disease Registry (ATSDR) health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites.

**Mode of Action** A postulated MoA for a chemical is a plausible sequence of key events leading to an observed effect, supported by robust experimental

observations and mechanistic data. Both MoA and mechanistic data can be important elements in chemical risk characterization, e.g., with regard to the question whether an adverse effect is thresholded or not.

**MOE** See Margin of Exposure.

**Monitoring** Repeated measurements, observations, and evaluation of specified properties of the environment, in order to define the current state and establish trends over time. Surveys and surveillance are used to achieve this objective and/or study the situation after taking measures to reduce risk, e.g., from contaminants.

**Morbidity** The state of disease or illness within a population; the morbidity rate is given as incidence and prevalence. Compare also Mortality.

**Mortality (Rate)** The state of death; mortality rate is a measure of the number of deaths (in general or due to a specific cause) in a population, scaled to the size of that population, per unit of time.

**MRL** See Minimal Risk Level.

**Mutagenicity** The ability of a substance (or agent) to induce a *mutation*.

**Mutagenicity Testing** Studies on mutagenic properties of chemicals: Several in vitro assays (tests in bacteria, such as Ames assay and mammalian cells) or in vivo assays are used for the identification of agents that can induce or increase the frequency of mutation in an organism (see also Genotoxicity).

**Mutation** A change in the amount or structure of the genetic material (DNA) of cells or organisms. Changes may involve a single gene or gene segment, a block of genes, or chromosomes. Mutagenicity refers to the induction of permanent transmissible changes either in somatic cells or in germ cells (see also *Genotoxicity*).

**N(O)EC** No (observed) effect concentration. The highest concentration of a material or substance in a toxicity test that has no statistically significant adverse effects on the exposed population of test organisms compared with the control group.

**NAS** National Academy of Science in the USA.

**Necrosis** Cell death or death of areas of tissue, usually surrounded by healthy tissue. Necrosis (caused by trauma or toxic chemicals) results in an unregulated digestion of cell components. It differs from apoptosis, a programmed and targeted cause of cell death in multicellular organisms.

**Neurotoxicity** Toxicity on any aspect of the central or peripheral nervous system. Adverse effects may be observed as functional changes (behavioral or neurological abnormalities) or as neurochemical, biochemical, physiological, or morphological changes.

**NOAEL** No observed adverse effect level. The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control group; some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects.

**Nocebo** In medicine, a nocebo reaction is the harmful, unpleasant, or undesirable effect seen in a subject who received an inert tablet (e.g., sugar pill) and may be also observed in persons with exposure to chemicals at irrelevant levels.

Nocebo responses are due to the subject's pessimistic belief and expectation that the inert drug (or the chemical) will produce negative consequences. The opposite of *placebo*.

**NOEL** No observed (adverse) effect level. An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control group.

**Notified Substance** According to REACH, a substance for which a notification has been submitted and which could be placed on the market in accordance with Directive 76/548/EEC. In the EC, "new chemicals" were those produced since 1981 and not listed on the EINECS.

**Objective (Protection Goal)** The goal to be reached with respect to a desired level of protection. The framework for risk regulation often sets the objective, i.e., a level of protection adequate for averting a danger (i.e., hazard control) or for prevention.

**Occupational Disease** An official list of diseases which have been recognized to be caused by specific influences at the workplace. The list is used – along with several criteria for causation (i.e., occupational history, conditions at the workplace, exposure measurements) – in legal procedures to establish (or refute) cases claiming compensation.

**Occupational Medicine** Applied science dealing with occupational diseases, workplace accidents, questions of occupational hygiene, and toxicology.

**Occupational Safety** All measures to protect workers against accidents and diseases in conjunction with the workplace. Important elements of occupational safety are on-the-job safety requirements and company medical doctors. OEL and MAK values limit exposure to hazardous chemicals.

**Odds Ratio (OR)** A relative measure of the difference in exposure between the diseased (cases) and not diseased (controls) individuals in a case-control study. The OR is interpreted similarly to the relative risk.

**OEL** Occupational Exposure Limit; expressed as concentration of a substance in the air at the workplace.

**Oral Slope Factor** An upper bound, approximating a 95 % confidence limit, on the increased cancer risk from a lifetime oral exposure to an agent. This estimate, usually expressed in units of proportion (of a population) affected per mg/kg-day, is generally reserved for use in the low-dose region of the dose-response relationship, i.e., for exposures corresponding to risks less than 1 in 100.

**Overadditive Effects** See Synergism.

**PAH** Polycyclic aromatic hydrocarbons.

**PBPK** Physiologically Based Pharmacokinetic Model. A model that estimates the dose to a target tissue or organ by taking into account the rate of absorption into the body, distribution among target organs and tissues, metabolism, and excretion.

**PCB** Polychlorinated Biphenyls. An important group of environmental chemicals, some of which tend to persist in the environment and to accumulate in the human body.

**PCDD** Acronym for poly-chlorinated dibenzo-1,4-dioxins; a class of 75 chemicals with several highly toxic compounds. The best known example of these

ubiquitously found "environmental chemicals" is 2,3,7,8-tetrachloro-dibenzo-1,4-dioxin, colloquially termed dioxin.

**PCDF** Polychlorinated dibenzofurans (see also PCDD and Dioxin).

**PCP** Pentachlorophenol, previously widely used for preservation of wood, textiles, and leather and as disinfectant.

**PEL** Permissible Exposure Limit. In the USA, a legal limit for exposure of an employee to a chemical substance or a physical agent. PEL values, established by OSHA, are usually expressed in ppm or $mg/m^3$ (see also MAC/MAK values).

**per os (p.o.)** Administration via the mouth, e.g., by gavage (bolus) or with food.

**Pesticide(s)** Chemicals used in agriculture and other areas to control the severity and incidence of pests and diseases. Pesticides (or "biocides") are used to control bacteria, fungi, algae, higher plants, nematodes, molluscs, mites and ticks, insects, rodents, and other organisms. The generic term is also used to cover bactericides, fungicides, algicides, herbicides, nematocides, molluscicides, acaricides, insecticides, and rodenticides.

**Pharmacodynamics** See Toxicodynamics.

**Phase-I (Reactions)** First step in biotransformation. Modification of a molecule (drug or other chemical) by oxidation, reduction, hydrolysis, hydration, dechlorination, or other reactions which are catalyzed by enzymes, mainly xenobiotic metabolizing enzymes of the endoplasmic reticulum (microsomal) or cytosolic enzymes (see also Cytochrome P450).

**Phase-II (Reactions)** Step in the biotransformation of a substance or its phase-I reaction product by enzyme families which catalyze either acetylation, glucuronidation, sulfation, or conjugation with glutathione. This results in the formation of more water-soluble metabolites which can be excreted in urine or bile.

**Placebo** Latin: "I shall please." A simulated treatment or an inert drug given in medical research as control treatment. Interestingly, patients receiving a placebo may feel or show an (actual) improvement in their condition, a phenomenon known as the "placebo effect." The opposite phenomenon is known as nocebo effect.

**Point of Departure (POD)** The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD), or a NOAEL or LOAEL for an observed incidence or a change in response level (see also Benchmark Approach/Dose).

**Point Source** Emission source(s), either single or multiple, which can be quantified by means of location and the amount of substance emitted per source and emission unit (e.g., amount per time).

**Poison** Compound of which a certain dose can elicit toxic effects and, possibly, death. A poison may be a mixture of various toxic substances: Natural poisons (e.g., bee or snake venom) are often secretion products and consist of numerous components, i.e., toxins and/or (toxic) enzymes as well as poisonous plant extracts.

**Pollution** Release to or presence in the environment of a chemical, physical, or biological agent (a pollutant) that has the potential to damage the health of humans or other organisms.

**Polymorphism** The existence of interindividual differences in DNA sequences coding for one specific gene. The effects of such differences may vary, from fully intact protein to inactive protein or lack of synthesis of this protein. In the context of pharmacology and toxicology, genetic polymorphisms of xenobiotic metabolizing enzymes are of special interest, as certain (iso)enzymes which are expressed at higher or lower levels or missing in individuals may predispose them to adverse effects (see also Susceptibility and Biotransformation).

**POP(s)** Persistant organic pollutant(s). PCDDs, PCDFs, and PCBs are persistent organic pollutants (POPs) under the Stockholm Convention on Persistent Organic Pollutants (http://www.pops.int/) and are omnipresent in the global environment.

**ppb** A unit of measure expressed as parts per billion. Equivalent to $1 \times 10^{-9}$, e.g., µg per kg.

**ppm** A unit of measure expressed as parts per million. Equivalent to $1 \times 10^{-6}$, e.g., mg per kg.

**Prevalence** The proportion of disease cases that exist within a population at a specific point in time, relative to the number of individuals within that population at the same point in time.

**Prioritization** Setting priorities in the sequential risk assessment of (numerous) agents under consideration according to their perceived importance.

**Probability** A quantitative statement about the likelihood of a specific outcome. Probability values can range from 0 to 1.

**Proliferation** Multiplication, i.e., an increase by frequent and repeated reproduction or growth by cell division.

**Promotion** Phase of proliferation of carcinogen-initiated cells in the context of carcinogenesis.

**Promotor** An agent that is not carcinogenic in itself, but when administered after an initiator of carcinogenesis stimulates the clonal expansion of the initiated cell to produce a neoplasm.

**PTWI (Provisional Tolerable Weekly Intake)** The weekly dose of a contaminant which an individual may ingest over its lifetime without an appreciable health risk, according to current knowledge (thus provisional). The WHO sets PTWI values for food contaminants (see also ADI and TDI).

**QSAR** Quantitative structure-activity relationship. A quantitative relationship between a biological activity (e.g., toxicity) and one or more molecular descriptors that are used to predict the activity (see also Structure-Activity Relationship).

**REACH** Registration, Evaluation, Authorisation and Restriction of Chemicals – the new chemical legislation of the European Union (Regulation No. 907/2006) which came into force in 2007. It replaces about 40 directives and regulations and erases the former regulatory distinction between newly notified substances

and existing chemical substances. REACH does not touch special regulations for drugs, biocides, radioactive compounds, and food and feed additives.

**Reference Dose** Acronym: RfD. "An estimate (with uncertainty perhaps spanning an order of magnitude) of a daily oral exposure to the human population (including sensitive subpopulations) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used" (WHO/IPCS 2004) (see also Reference Values).

**Reference Values (RfD or RfC and $RV_{95}$)** An estimate of an exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a BMDL, a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used. Reference value, a generic term as used by EPA (2002), is not specific to a given route of exposure. EPA develops numerical toxicity values for the reference dose (RfD) and reference concentration (RfC), usually for noncancer health assessments. The term "reference value" is also used in human biomonitoring: Here, $RV_{95}$ are statistical descriptions of the range of concentrations typically seen in a specified reference population, but which have no direct relationship to health effects or risk assessment. But, $RV_{95}$ are an important tool for prevention to assess whether populations (or individuals) are more exposed when compared to the environmental background exposure. Compare also Human Biomonitoring Values I and II (HBM I and II).

**Relative Risk** The relative measure of the difference in risk between the exposed and unexposed populations in a cohort study. The relative risk is defined as the rate of disease among the exposed divided by the rate of disease among the unexposed. A relative risk of 2 means that the exposed group has twice the disease risk as the unexposed group.

**Reproductive Toxicology** The study of the adverse effects of chemicals (and medicinal drugs) on the embryo, fetus, neonate, and prepubertal animal and the adult reproductive and neuroendocrine systems. Reproductive toxins comprise both agents which impair the fertility of adult organisms and/or those which can adversely affect the developing organisms (see also Embryotoxicity and Teratogenicity).

**RfD** See Reference Dose.

**Risk** The probability of an adverse effect in an organism, system, or (sub)population caused under specific circumstances, e.g., by exposure to an agent and/or a situation.

**Risk (Human Health)** The Regulation EC 178/2002 defines risk as "a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard." For some areas, different definitions have been established in legislation or by relevant international authorities. Although most definitions of "risk" have a common core (probability of adverse effects resulting from ...), they differ in various disciplines (e.g., epidemiology, economics, sociology, toxicology).

**Risk Analysis** This term is not well defined in toxicology. Some consider it as a process consisting of three components: risk assessment, risk management, and risk communication.

**Risk Assessment (Human Health)** The evaluation of scientific information on the hazardous properties of environmental agents (hazard characterization), the dose-response relationship (dose-response assessment), and the extent of human exposure to those agents (exposure assessment). The product of the risk assessment is a statement regarding the probability that populations or individuals so exposed will be harmed and to what degree (risk characterization).

**Risk Characterization** The integration of information on hazard, exposure, and dose response to provide an estimate of the likelihood that any of the identified adverse effects will occur in exposed people. The *Codex Alimentarius* definition: "The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment."

**Risk Communication** An interactive process of exchanging information and views on risks between scientific experts, risk managers (at federal or state agencies), consumers, and the interested public.

**Risk Evaluation** Establishment of a qualitative or quantitative relationship between risks and benefits of exposure to an agent, involving the complex process of determining the significance of the identified hazards and estimated risks to the system concerned or affected by the exposure as well as the significance of the benefits brought about by the agent. It is an element of risk management. Risk evaluation is synonymous with risk-benefit evaluation.

**Risk Management (Human Health)** A decision-making process that accounts for political, social, economic, and engineering implications together with risk-related information in order to develop, analyze, and compare management options and select the appropriate managerial response to a potential chronic health hazard.

**Risk Management Measures (RMMs)** Measures in the control strategy for a hazardous substance that reduce the emission of and exposure to a substance, thereby reducing the risk to human health and the environment.

**Risk Perception** An integral part of risk evaluation. The subjective perception of the gravity or importance of the risk based on an individual's knowledge of different risks and the moral and political judgment attached to them and their importance.

**Safety** Practical certainty that adverse effects will not result from exposure to an agent under defined circumstances. It is the reciprocal of risk. Toxicological "safety" is defined as a high probability that adverse effects will not result from exposure to a chemical under specific conditions of quantity and manner of use.

**Safety Factor** Composite (reductive) factor by which an observed or estimated NOAEL is divided to arrive at a criterion or standard that is considered safe or without appreciable risk (see also Assessment Factor and Uncertainty Factor).

**SAR** Structure-activity relationship.

**SCOEL** The Scientific Committee for Occupational Exposure Limit values with the mandate to advise the European Commission on occupational exposure limits for chemicals at the workplace.

**Sensibility** An acute perception or responsiveness of an individual toward something, such as the emotions of another person or to environmental factors, e.g., noise or chemicals.

**Sensitivity** Personal susceptibility of an individuum to a stimulus. The concept "multiple chemical sensitivity" assumes that some individuals are more susceptible to low-dose toxic effects of chemicals than others.

**Sensitivity and Specificity** Statistical terms used to describe the ability of a test to correctly identify true positives and negatives. For instance, in medical diagnostics, test sensitivity is the ability of a test to correctly identify those with the disease (true positive rate), whereas test specificity is the ability of the test to correctly identify those without the disease (true negative rate).

**Short-Term Exposure Limits** Acronym: STEL. For a number of workplace chemicals, peak exposure or ceiling limits for short-term exposure are set, in addition to the 8-h time-weighted average (TWA) exposure limits (MAK or TLV values). According to STEL, exposure should not be longer than 15 min and should not be repeated more than four times per day. The "excursion" factor ($\geq 1$) by which the STEL can exceed the TWA depends upon the chemical concerned and takes into account certain properties such as irritation.

**Smog** A word coined for air pollution from smoke and fog. Smog is a problem in many cities, aggravated by inversion weather conditions (which traps pollution close to the ground), and continues to harm human health. When limit values for smog are reached, warnings are given to the public.

**SMR** Standardized mortality ratio. This is the relative measure of the difference in risk between the exposed and unexposed populations in a cohort study. The SMR is similar to the relative risk in both definition and interpretation. This measure is usually standardized to control for any differences in age, sex, and/or race between the exposed and reference populations. It is frequently converted to a percent by multiplying the ratio by 100 (see also Mortality Rate).

**Specificity** The term has context-dependent meanings. In chemistry, the ability to identify and quantify the target analyte in the presence of chemically similar interfering compounds. In medicine, the ability to exclusively detect persons with a particular disease; ratio of persons with a negative test result to nondiseased persons.

**Standard** An environmental quality standard is the limiting concentration of a chemical (or other adverse condition, e.g., pH) permitted in a given compartment (soil, effluent, water). All standards are established for regulatory purposes and set on the basis of a judgment of a number of criteria involved: The standard is dependent on the use (e.g., drinking water or agricultural water for irrigation), the subject of protection, and the objective (protection goal).

**STEL** See Short-Term Exposure Limit.

**Stochastic Effects** The term stochastic indicates that the occurrence of effects so named would be random (with a probability $<1$ and $>0$), meaning – even for an individual – there is no threshold of dose below which the effect will not occur and the chance of experiencing the effect increases with increasing dose. Hereditary effects and cancer induced by radiation are considered to be stochastic effects.

**Stress-Strain Concept** Concept in occupational medicine that describes how mechanical stress at the work-place will result in an overstraining of the musculoskeletal system. This concept is also useful for nonmechanical stress factors, such as exposures to toxicants or radioactive compounds and the resulting effects on health.

**Structural Alert** A molecular (sub)structure associated with the presence of a biological activity (e.g., genotoxicity).

**Structure-Activity Relationship** SAR: The correlation between the chemical or 3D structure of a molecule and its physicochemical properties or its biological activity. SAR analysis can help to determine chemical groups responsible for evoking a biological effect. This allows (targeted) modification of the effect or potency of a bioactive compound (e.g., a drug) by changing its chemical structure. The method has been refined to build mathematical models for the prediction of quantitative relationships between structure and biological activity (see QSAR).

**Subchronic Exposure (Toxicity Study)** Repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10 % of the life span in humans (more than 30 days up to approximately 90 days in typically used laboratory animal species) (see also Chronic Toxicity Study).

**Subject of Protection** The target object (e.g., human population, subgroup, or environment) to be protected by risk reduction measures.

**Susceptibility** The state or fact of being likely or liable to be influenced or harmed by a particular thing. In epidemiology or toxicology, susceptible individuals are members who are more prone to develop an illness than the (average) population at risk.

**Symptoms** Signs of disease. They are usually characteristic of a specific type of disease and also of a specific toxic agent.

**Synergism** A phenomenon in which the toxicity of a mixture of chemicals is greater than that which would be expected from the total toxicity of the individual chemicals present in the mixture (see also Combined Chemical Effects).

**Systemic Toxicity** Toxic effects as a result of absorption and distribution of a toxicant to a site distant from its entry point.

**Target Organ** The biological organ(s) most adversely affected by exposure to a chemical, physical, or biological agent.

**TCCD** Acronym for trichlorodibenzo[1,4]dioxin(s) or for tetrachloro-dibenzo [1,4]dioxin(s); mostly for 2,3,7,8-tetrachlorodibenzo[1,4]dioxin (see Dioxins).

**TCDF** Acronym for trichlorodibenzofuran(s) and for tetrachlorodibenzofuran(s) (see Dioxin).

**TDI** Tolerable Daily Intake; analogous to acceptable daily intake (ADI). The term "tolerable" is used for agents that are not deliberately added, such as contaminants in food.

**TEF** See Toxicity Equivalency Factor.

**Teratogen** Agent which when administered prenatally to the mother induces permanent structural malformations or defects in the offspring. The most widely known example of a teratogen is thalidomide (Contergan) which can cause severe malformations of internal organs, but mainly of extremities (limbs).

**Teratogenicity** Structural developmental defects due to exposure to a chemical agent during formation of individual organs.

**Threshold** The dose or exposure below which no deleterious effect is expected to occur.

**Threshold Limit Value (TLV)** Values for occupational exposure to airborne substances published by the American Conference of Governmental Industrial Hygienists (ACGIH). TLVs represent the average concentration in $mg/m^3$ for an 8-h workday and a 40-h work week to which nearly all workers may be repeatedly exposed, day after day, without adverse effect.

**Tolerable Risk** A risk which may be tolerated for transient exposure, but requires further risk reduction measures to achieve lower risk levels (see Acceptable Risk).

**Toxicant** An agent or material capable of producing an adverse response (effect) in a biological system, seriously injuring structure and/or function or causing death.

**Toxicity** Inherent property of an agent to cause an adverse biological effect. Toxicity is a general term for all undesirable or detrimental health effects of a substance and depends upon dose and properties of the substance. Based on the effect, one distinguishes between, e.g., organ toxicity, carcinogenicity, mutagenicity, embryotoxicity, and teratogenicity.

**Toxicity Equivalency Factor** TEF. Factor used in risk assessment to estimate the toxicity of a complex mixture, most commonly a mixture of chlorinated dibenzo-*p*-dioxins, furans, and biphenyls; in this case, TEF is based on relative toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

**Toxicity Tests** Determination of the effect of a substance on a group of selected organisms under defined conditions. A toxicity test usually measures either the proportion of organisms affected (quantal) or the degree of effect shown (graded or quantitative) after exposure to specific levels of a stimulus (concentration or dose, or mixture of chemicals) for a given period of time.

**Toxicodynamics** The determination and quantification of the sequence of events at the cellular and molecular levels leading to a toxic response to a chemical agent. In the context of medicinal drugs, this is referred to as pharmacodynamics.

**Toxicokinetics** The field of toxicology dealing with the determination and quantification of the time course of absorption, distribution, biotransformation (metabolism), and excretion of hazardous chemicals in the body. In the context of medicinal drugs, this is referred to as pharmacokinetics.

**Toxicology** The discipline of the adverse effects of chemical substances on living organisms. Basic toxicology characterizes the type of effects observed at different doses as well as the cellular, biochemical, and molecular mechanisms of

action and the toxicokinetics. Clinical toxicology deals with the diagnosis and treatment of human and animal intoxications. Regulatory toxicology sets rules with the aim to prevent unwanted effects of chemicals.

**Toxin(s)** Natural poison(s); toxic organic substance(s) produced by a living organism, e.g., mycotoxins of fungi, phytotoxins of plants, or venoms of animals, often agents with partly highly specific mechanisms of action.

**Toxinology** The science that deals with toxins of plant, animal, and microbial origin. Toxin.

**Transcriptomics** Techniques available to identify the mRNA from actively transcribed genes, e.g., used to compare patterns in treated and untreated cells/organisms.

**Transfer** Term in environmental toxicology for the passage of a substance (e.g., cadmium) from one medium (e.g., soil) in plants.

**Tumor** An abnormal, uncontrolled growth of cells (synonym: neoplasm). A benign tumor is defined as a tumor that does not spread to a secondary localization, but may impair normal biological function through obstruction or may progress to malignancy later.

**UBA** German: Umweltbundesamt, the Federal Environmental Protection Agency.

**Uncertainty** Imperfect knowledge concerning the present or future state of an organism, system, or (sub)population under consideration. Lack of knowledge about variability in specific parameters in a risk assessment. Uncertainty is not the same as variability. For example, a risk assessor may be very certain that different people drink different amounts of water but may be uncertain about how much variability there is in water intakes within the population. Uncertainty can often be reduced by collecting more and better data, whereas variability is an inherent property of the population being evaluated. Variability can be better characterized with more data, but it cannot be reduced or eliminated. Efforts to clearly distinguish between variability and uncertainty are important for both risk assessment and risk characterization (see also Uncertainty Factors).

**Uncertainty Factor(s)** One of several, often tenfold, default factors used in operationally deriving the RfD and RfC from experimental data. The factors are intended to account for (1) variation in susceptibility among the members of the human population (i.e., interindividual or intraspecies variability); (2) uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty); (3) uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) uncertainty associated with extrapolation when the database is incomplete.

**Unit Risk** The upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 μg/L in water, or 1 $\mu g/m^3$ in air. The interpretation of unit risk would be as follows: If unit risk $= 2 \times 10^{-6}$ per μg/L, then two excess cancer cases (upper bound estimate) are expected to develop per 1,000,000 people if exposed daily for a lifetime to 1 μg of the chemical per liter of drinking water.

**Validation** Process by which the reliability and relevance of a particular approach, method, process, or assessment is established for a defined purpose. Different parties define "reliability" as establishing the reproducibility or outcome of the approach, method, process, or assessment over time. "Relevance" is defined as establishing the meaningfulness and usefulness of the approach, etc., for a defined purpose. Validation is a process required, e.g., for alternative test methods to replace for toxicity tests in animals.

**Virtually Safe Dose** The VSD, derived from the unit risk for a carcinogenic chemical, denotes the dose which could cause one additional case of cancer in 1,000,000 persons with lifetime exposure. In practical terms, VSD denotes a "safe" dose.

**vPvB** Acronym for "very persistent, very bioaccumulative." There are restrictions for compounds with such properties due to high concerns for the environment and human health.

**Weight-of-Evidence (WoE) for Carcinogenicity** A system used by the US EPA (and others) for characterizing the extent to which the available data support the hypothesis that an agent causes cancer in humans. The approach outlined in EPA's guidelines for carcinogen risk assessment (2005) considers all scientific information in determining whether and under what conditions an agent may cause cancer in humans and provides a narrative approach to characterize carcinogenicity rather than categories. Five standard weight-of-evidence descriptors are used as part of the narrative.

**WHO** World Health Organization. An independent organization of the United Nations (UN) with advisory functions, e.g., publication of guidance documents on hazardous chemicals in air, water, and food and recommendations on maximal levels in food commodities (see also ADI and PTWI).

**Xenobiotic(s)** A term for man-made (manufactured) chemical(s) not produced in nature and not normally considered a constituent component of a specified biological system. With regard to the latter, also phytochemicals (of natural origin but "foreign" for the mammalian organism) can be considered as xenobiotics for humans.

## References

The glossary was compiled using many other references. Some passages were quoted word for word from these references.

AGS/Committee on Hazardous Substances (2008) Guide for the quantification of cancer risk figures after exposure to carcinogenic hazardous substances for establishing limit values at the workplace. http://www.baua.de

Angerer J, Aylward L, Hays SM, Heinzow B, Wilhelm M (2011) Human biomonitoring assessment values: Approaches and data requirements. Int J Hyg Environ Health 214:548–560

Duffus JH, Nordberg M, Templeton DM (2007) IUPAC Glossary of terms used in toxicology, 2nd edition. Pure Appl Chem 79:1153–1344

ECHA/European Chemicals Agency (2008) Guidance on information requirements and chemical safety assessment. http://echa.europa.eu/about/reach_en.asp

EFSA/European Food Safety Agency (2012) Scientific Opinion on Risk Assessment Terminology. EFSA J 10(5):2664 [64 pp]; doi:10.2903/j.efsa.2012.2664. http://www.efsa.europa.eu/en/efsajournal/pub/2664.htm

Foth H, Hayes W (2008) Concept of REACH and impact on evaluation of chemicals. Hum Expert Toxicol 27:5–21

Integrated Risk Information System (IRIS) Glossary. Access via EPA terminology services; http://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=

International Programme on Chemical Safety (IPCS). Glossary of terms for use in IPCS publications. http://www.ilo.org/public/english/protection/safework/cis/products/safetytm/glossary.htm

REACH. http://www.reach-clp-helpdesk.de

Van Leeuwen CJ, Vermeire T (eds) (2007) Risk assessment of chemicals – an introduction, vol 2. Springer, Dordrecht

WHO (2009) Assessment of combined exposures to multiple chemicals. IPCS harmonization project document nr. 7. ISBN 978 92 4 156383 3

# Index


**A**
Abrin, 863
Absolute risk, 590
Absorption, 142, 143, 149, 151–153, 159, 522–524, 805, 806, 808
Absorption, distribution, metabolism and excretion (ADME), 10, 75, 135, 270, 418
Acceptable, 238, 239, 246, 247, 248, 785, 792
Acceptable risk, 608–609, 611
Acceptable daily intake (ADI), 432, 446, 630, 644, 834, 837–838, 876, 877–878
Accident, 740, 741, 745, 746
Accreditation, 60–62
Accumulation, 142, 144, 150, 152, 159
Acetaminophen, 427, 428
Acetylcholinesterase, 859
ACGIH. *See* American Conference of Governmental Industrial Hygienists (ACGIH)
Action values, 893, 894, 897
Acute, 5, 19
Acute toxicity, 83–85, 526–527
Acute exposure guidelines levels (AEGLs), 866, 867
Acute reference doses (Acute RfD), 876, 877–878
Adaptive response, 94, 401
Added risk, 240
Addiction, 713, 714
Additional risk, 886, 893
Additives, 408, 633, 638–639, 645, 814–816, 818–820, 822
Adducts, 398, 401–404
Adequacy, 782, 785, 786, 791, 792, 794
ADI. *See* Acceptable daily intake (ADI)
Adjustment factors, 460
ADME. *See* Absorption, distribution, metabolism and excretion (ADME)
Adverse, 87, 449–454
  health effects, 164, 169–171, 628–633, 644, 645, 646, 700, 736
  reactions, 512, 518
Adverse outcome pathway (AOP), 118–119
Adversity, 450–454
Advisory Board, 753, 754
Advocacy, 25
AEGLs. *See* Acute exposure guidelines levels (AEGLs)
Aflatoxin $B_1$, 538–542, 545, 552, 553
Agencies, 23, 24
Agency for Toxic Substances and Disease Registry (ATSDR), 313
Agenda 21, 34, 35, 476
Agents, 740, 742, 744
Air pollution, 505, 506
Air quality, 622
Air quality guidelines (AQG), 654, 883, 887–888
ALARA. *See* As low as reasonably achievable (ALARA)
Alarm, 743
Alert system, 43
Algorithms, 125
Alkaloids, 814–816, 821–823
Allergens, 571, 572, 832, 838, 839, 840–843
Allergies, 530, 531, 571–572
Allometric scalin, 244
Alternative compounds, 50
Alternative hypothesis, 214, 215
Alternative tests, 184
Alveolar, 142, 150, 152, 154, 155, 157
Ambient air, 612, 651, 654, 655
Ambient monitoring, 570, 686
Amden, 184, 185
American Conference of Governmental Industrial Hygienists (ACGIH), 652
Ames assay, 101, 102, 216–218


F.-X. Reichl, M. Schwenk (eds.), *Regulatory Toxicology*,
DOI 10.1007/978-3-642-35374-1, 

Analytes, 325, 326, 329–334, 336–338, 340–348, 350–352
Analytical chemistry, 321–353
Analytical epidemiology, 196–197
Angiogenesis, 112
Aniline, 146, 147
Animal, 183–186, 188
  care, 487, 489
  data, 579, 580
  experimentation, 482, 488
  species, 144, 145, 149
  studies, 90, 93, 238–240
  tests, 783, 785, 787
  welfare, 83, 84, 183
Animal Welfare Act (AWA), 487
Antagonism, 408
Anthropometric data, 300
Antibody, 483
Antibody responses, 420
Antidotes, 745, 746, 859, 867
AOP. *See* Adverse outcome pathway (AOP)
Apoptosis, 402, 404, 426
AQG. *See* Air quality guidelines (AQG)
Area under the curve (AUC), 142, 146, 150, 158, 235, 236
As low as reasonably achievable (ALARA), 440, 443, 465, 614, 633, 643, 892
Assessment factors, 433, 791–792
Atmosphere, 38
Atomic spectroscopy, 337, 346–347
Atopic, 572
Atropine, 859, 867
ATSDR. *See* Agency for Toxic Substances and Disease Registry (ATSDR)
Attorneys, 728
AUC. *See* Area under the curve (AUC)
Audit process, 24
Aum Shinrikyo cult, 856
Authenticity, 761
Authorities, 22–24, 749–752, 755, 758, 762–763
Authorizations, 610, 612, 613, 781, 793, 794
Authorizations list, 664
Autoinjectors, 867
Avoidability, 628, 632, 634, 646

**B**
Background, 440, 443, 444, 445
Background exposures, 439–446, 628
Banning, 50
Bans, 618
Baobab tree, 834
Batrachotoxin, 862
BAT values. *See* Biological tolerance values (BAT values)
BAuA, 43, 44
Beer-Lambert law, 347, 352
Behavior scenario, 292, 299–302
Benchmark, 362
Benchmark dose (BMD), 245–250, 359–374
Benefit/risk, 53, 484–485
Benzodiazepam, 867
Berzelius, J., 323
BEs. *See* Biomonitoring equivalents (BEs)
BfR, 43, 44
Bibliographical databases, 313, 314, 316
Bilirubin, 722
Binary, 549, 563
Bio-accumulative, 783, 790
Bioanalytic, 859–860
Bioavailability, 151, 158
Biocidal products, 665–668
Biocidal products regulation (BPR), 666–668
Biocide, 705, 707, 708
Bioinformatics, 178
Biologicals, 414, 416, 418, 419, 421
Biologicals warfare agents, 856, 861, 862, 867
Biological tolerance values (BAT values), 444, 446
Biological Weapons Convention (BWC), 856, 865
Biologics, 414–421
Biomarkers, 572–574, 687–692, 701, 702
Biomedical studies, 487
Biomonitoring, 8, 9, 570, 574, 859–860, 894, 895–896
Biomonitoring equivalents (BEs), 694, 700–701
Biopharmaceuticals, 414
Biosensors, 350, 351
Biostatistics, 214
Biotherapeutics, 77–80
Biothreat, 861, 862
Biotransformation, 109–110, 134–137
Birth defects, 721
Bitter orange, 816
Blepharospasm, 860
Blind reading, 92–93
Blood flow, 151, 152, 155, 273, 275
BMD. *See* Benchmark dose (BMD)
Body on a chip, 120
Body surface, 235
Bone marrow, 724
Botanicals, 813–823
Botulinum, 856
Botulinum neurotoxin (Botox), 856, 862, 865, 868

Boyle, R., 323
BPR. *See* Biocidal products regulation (BPR)
Bridging principles, 798–800
Buehler test, 105
BVL, 43–44
BWC. *See* Biological Weapons Convention (BWC)

**C**
Cadmium, 6
Caloric demand, 433, 436
Cancer, 101–103, 240, 241, 246, 247, 250
  incidence, 241, 246
  risks, 443, 446, 549, 563, 565, 566, 702
Carbon disulfide ($CS_2$), 147, 148
Carcinogenicity, 170, 237–250, 398, 443, 527, 529–530
  studies, 86–88, 103
  testing, 419
Carcinogenic, mutagenic, reproductive (CMR) toxicant, 14–15, 18, 663, 664, 671, 708, 709
Carcinogens, 409, 410, 609–612, 653, 654, 656, 658, 706, 707, 709, 713
Cardiotoxic, 111, 113
Case-by-case, 800
Case-control study, 197–198
CAS number, 316
CASRN, 799
Catastrophes, 743–745
Category B, 864–865
Causality, 454, 730, 731, 735, 736
Causality relationship, 194
CCRIS, 314
cDNA, 175
Cells, 142, 146, 151, 152, 154, 160
  cultures, 160, 166, 167
  cycle, 558, 568
  lines, 108, 164, 167–168, 170
Certification, 60, 61–62
Chance, 194, 199
Check list, 239
Chemical, biological, radiological and nuclear (CBRN) agents, 867
Chemicals, 32–45
  antagonist, 228
  properties, 26–27
  safety, 33–36, 38, 39–42
  warfare agents, 740, 742, 744, 856–858, 861, 866–868
  weapons, 38
Chemical Weapons Convention (CWC), 38, 856–861, 865–866
Chemikaliengesetz (ChemG), 705
Chicken eggs, 112
Childbearing potential, 516–517
Children, 294–297, 299, 300, 428–430, 708–710, 712, 715, 716
Chip, 175
Choleratoxin, 865
Cholesterol, 834, 836
Chromatography, 324, 330, 333–338, 341, 342, 344–345
Chromosomal aberrations, 101, 102, 111
Chronic, 5, 19, 81–88
Chronic toxicity, 169, 527–528, 805, 807, 809
Cinnamon, 820
Citizens, 748–750, 753, 755
Classification, 33, 41, 44, 45, 781, 782, 785, 787, 788, 790
  hazards, 677–682
  systems, 293
Classification, labeling and packaging (CLP), 619, 779–795, 851–852
*Claviceps purpurea*, 857
Clean Air Act, 622
Clean production, 39–40
Clean Water Act, 656
Clearance, 142, 150, 152–155
Client, 728, 729
Clinical chemist, 86
Clinical studies, 483, 487, 512, 513, 514
Clinical trials, 208–211, 484, 512–519
CLP. *See* Classification, labeling and packaging (CLP)
CLP regulation, 678–680, 798–800
CMR toxicant. *See* Carcinogenic, mutagenic, reproductive (CMR) toxicant
Codes of conduct, 62
Codex Alimentarius, 711, 819, 892
Co-exposure, 408, 409
Cohort study, 197
Colours, 815, 820, 822
Comet assay, 692, 807
Commissions, 51, 52, 54, 55
Communication, 749, 753, 758, 760–763, 765
Compartment models, 270–273, 275
Composition, 798, 799
Concentration-response, 262–264
Confidence limit, 362
Conflicts, 748, 750–755
Confounder, 197, 198, 200, 202, 204
Conservative estimates, 386
Constituents, 408, 410, 798–799

Consumers, 115, 116, 682
products, 613
protection, 42–44
Contact times (CTs), 296, 299
Contaminants, 707, 711–712, 876, 878, 892–893
Contaminated land, 620
Contergan, 737
Contract research organizations (CROs), 22, 23, 25
Control subjects, 202, 203
Control value, 697
Cosmetic Products Notification Portal (CPNP), 851
Cosmetics, 613, 671, 711–713, 849–852
Cosmetics regulation, 851
Cost-benefit analysis, 53, 586, 603–604
Court, 25, 728–731, 735, 736
Covalent binding, 162, 163
CPNP. *See* Cosmetic Products Notification Portal (CPNP)
Credibility, 646
CROs. *See* Contract research organizations (CROs)
Crosslinks, 860
Cross-sectional study, 198
CTs. *See* Contact times (CTs)
CWC. *See* Chemical Weapons Convention (CWC)
Cyclooxygenases (COX), 535
Cyclosarin, 867
CYP1, 424, 426, 428
Cytochromes, 424–428
Cytochromes P450 (CYP), 94, 109, 137, 400, 534, 535, 540, 692, 721
Cytokine, 416
Cytokine storm, 483
Cytotoxicity, 109–110

**D**

Dalton, J., 323
Damage, 478–480, 494–496
Danger, 463–467, 478–480, 495–497, 633, 646, 647, 678, 682
Danger goods, 743, 744
Databases, 124, 128, 313–316, 318
Data gaps, 783
Data quality, 58
Declaration of Helsinki, 208–210, 483, 484
Decontamination, 742, 745, 746, 868
Deep compartments, 271
Default factor, 720
Default values, 434
DEFC. *See* Division of Federal Employees Compensation (DEFC)
Degenerative, 90, 94
Democracy, 749
Deoxynivalenol, 864
Dependency, 713
Deposition, 146, 152–153, 154
Derived minimal effect level (DMEL), 653
Derived no effect levels (DNELs), 458, 653, 791, 792
Dermal, 83, 87
Dermal exposure, 296–297, 300
Detector, 333, 334, 335, 337–341, 346, 349, 350
Detoxification, 536, 540–542
Developmental toxicity, 529
Diagnosis, 850
criteria, 96
nomenclature, 96
Dichlorodiphenyltrichloroethane (DDT), 623
Diet, 360
Diet supplements, 814, 821
Diffusion, 151, 152, 153, 155, 158, 523
Disasters, 596, 597, 739–746
Disinfectants, 667
Disposition, 142, 144, 149, 291, 294–296, 300
Dissociation, 523
constant, 227
Distribution, 75, 78, 142, 143, 147, 154, 155, 157–158, 254, 256, 258, 270, 271
Division of Federal Employees Compensation (DEFC), 735
DMEL. *See* Derived minimal effect level (DMEL)
DNA adducts, 426, 427, 552–556, 558, 560, 563, 566, 567, 687, 691
DNA alkylation, 860
DNA ethylation, 555, 556, 566, 567
DNA methylation, 556, 567
DNA mismatch repair, 401
DNA repair, 401–402, 404, 554, 555, 556, 558, 559, 562, 563, 566, 567
DNA sequencing, 174
DNA strand breaks, 402, 692
DNELs. *See* Derived no effect levels (DNELs)
Documentation, 59, 62, 609, 612
Doping, 714–715
Dose, 66, 67, 69, 70
descriptor, 791
range, 85, 86, 87
response, 5, 6–10, 12, 16, 239, 240, 242–248, 547–568
response models, 361, 365–367

Dose–cancer incidence, 563–566
Downstream users, 662, 781, 790–791, 793
Draize eye test, 184
Drinking water, 471, 472–474, 629, 633, 634, 640–644, 883
Drinking water guidelines, 878, 883, 884–886
Drugs, 207–211, 713–716, 736
development, 509–519
Duration extrapolation, 433–435
Dust, 294–300, 302

**E**
e-books, 313
$EC_{50}$, 228
ECB. *See* European Chemicals Bureau (ECB)
ECHA. *See* European Chemical Agency (ECHA)
eChemPortal, 306, 313
Ecological studies, 199
Economic, 32, 40, 584, 585, 586, 587
Economic damage, 597
ECVAM. *See* EU Commission's Centre for the Validation of Alternative Methods (ECVAM)
Education, 24
Effect-researcher, 27
Efficacy, 230
EFSA. *See* European Food Safety Authority (EFSA)
Electrophilic moieties, 535
Electrophoresis, 324, 345–346
Elements, 323, 324, 346–350
Elimination, 142, 143, 150, 153, 155, 157, 159, 269–272, 275
Elimination kinetics, 150, 157
EM. *See* Environmental monitoring (EM)
EMBASE, 315
Embryo, 112, 528
Embryotoxic, 100
EMEA. *See* European Medicines Agency (EMEA)
Emergency, 763
  medicine, 850
  planning, 619, 624
  response, 742, 744
Emissions, 505, 506
Emphysema, 723, 724
Employment, 647
Endocrine active substances, 722
Endocrine disrupters, 706
Endosulfan, 707
Endpoints, 124, 125, 127–130, 177, 262, 364, 365, 369–372, 579, 580
Enterohepatic circulation, 10
Enterotoxins, 862–863, 868
Environmental contaminants, 633, 639–640, 645
Environmental disorders, 770, 775
Environmental equity, 586
Environmental-hygienic, 471, 473–474
Environmental medicine, 768, 770, 771–772
Environmental monitoring (EM), 686–687
Environmental persistent pharmaceutical pollutants (EPPPs), 715
Environmental protection, 630
Environmental Protection Agency (EPA), 179, 246, 247, 622
Enzymes, 134–138
Enzymes induction, 534
EOGRTS. *See* Extended One-Generation Reproductive Toxicity Study (EOGRTS)
EPA. *See* Environmental Protection Agency (EPA)
Epidemiology, 191–205, 238, 239, 241, 487
Epiphyses, 722
Epoxide hydrolase, 400, 426
EPPPs. *See* Environmental persistent pharmaceutical pollutants (EPPPs)
Epsilon toxin, 863
EQuATox. *See* Establishment of Quality Assurance for the Detection of Biological Toxins of Potential Bioterrorism Risk (EQuATox)
Error bands, 386
Errors, 58, 59, 382, 384–388
Erythromycin, 427
Establishment of Quality Assurance for the Detection of Biological Toxins of Potential Bioterrorism Risk (EQuATox), 867
Ethics, 751
  committee, 208–209, 482–485
  principles, 482, 484–485, 488
  review, 482, 484–486
EU Commission's Centre for the Validation of Alternative Methods (ECVAM), 184–186
EudraCT. *See* European Union Drug Regulating Authorities Clinical Trials (EudraCT)
European Chemical Agency (ECHA), 41, 44, 662–664, 667, 668, 706, 710, 781, 782, 784, 785, 787, 790, 792–794

European Chemicals Bureau (ECB), 41–42
European Food Safety Authority (EFSA), 42, 43, 612–613, 804–811, 814–819, 821, 822, 828, 831, 832, 834, 835, 837, 838, 840, 842–845
European Medicines Agency (EMEA), 674, 675, 706, 713, 715, 716
European Union (EU), 82, 84
European Union Drug Regulating Authorities Clinical Trials (EudraCT), 208
EU Scientific Committee on Occupational Limits (SCOL), 611
Evaluation, 116, 117, 119, 121, 787, 793–794
Experts, 728–731, 736, 737
  judgement, 454, 800–801
  knowledge, 308
  systems, 124–125, 127
Exposure, 4–20, 254–259, 377–393, 569–574, 607–614, 767–776, 781, 783, 786, 790–793, 814–818, 820, 822
  assessment, 5, 7–9, 19, 290, 293, 296, 299–302, 570, 571, 790, 791
  characterization, 579–580
  multiple, 236
  scenario, 289–304
Extended One-Generation Reproductive Toxicity Study (EOGRTS), 808, 809
Extraction, 323, 324, 326, 329–332, 343, 344
Extrapolations, 70, 431–438
Extra risk, 240, 241, 248, 364, 366, 886

**F**
Factual databases, 314, 316
False-negative, 214, 215, 218
False positive, 214, 215
Falsification, 214
FAO. *See* Food and Agriculture Organization (FAO)
Fast, 331, 337–340
Fatalism, 502
Fatty acid profile, 840, 844
FDA. *See* Food and Drug Administration (FDA)
FECA. *See* Federal Employees Compensation Act (FECA)
Federal Employees Compensation Act (FECA), 735
Feeding study, 837, 842, 845
Fertility, 100, 528
Fetus, 528
Fick's first law, 151
Fingerprint, 343
First-order excretion, 273
First-pass bioactivation, 147
First pass effect, 10
First responders, 744
Fitted curve, 362, 369
Fitting model, 248
Food, 360, 707, 711–713, 716
  additives, 611, 612, 711–712, 803–811, 814, 815, 818–820, 832, 836, 837, 842, 876, 892
  allergic patients, 838
  allergy, 842
  chain, 297
  classification, 299
  colour, 815, 820, 822
  constituents, 832, 835, 837, 841
  consumption, 293, 298, 301
  contact, 669–670
  monitoring, 43
  safety, 42, 43–44
  standards, 892
  supplements, 712, 814, 816–822
Food and Agriculture Organization (FAO), 35, 38, 39, 804
Food and Drug Administration (FDA), 716, 821
Foodstuffs, 892–893
Forensic toxicology, 730
Formaldehyde, 278, 279, 280, 282, 285, 286
Formulations, 421, 851
Fresenius, C.R., 324
Full model, 367, 368, 373
Functional antagonist, 228

**G**
G agents, 859
Galton board, 563, 564
Gas chromatography (GC), 280, 282, 332, 333, 336, 341–343
Gas chromatography/mass spectrometry (GC/MS), 342–343
Gastrointestinal (GI) tract, 147, 149, 152, 157
Gay-Lussac, J., 323
GC. *See* Gas chromatography (GC)
GDWQ. *See* Guidelines for drinking-water quality (GDWQ)
Gene copies, 425
Gene expression, 174–176, 558, 559, 839
Genetically modified organisms (GMOs), 827–845
Genetic engineering, 838
Genetic material, 709

Genetic polymorphisms, 171
Geneva Protocol, 856, 865
Genomics, 174, 175
Genotoxicity, 5, 10, 11, 12, 14, 19, 100–102, 400, 415, 417, 419, 529, 530, 535, 538, 540, 611, 612, 805–807, 810
  carcinogen, 398, 402–404
  effects, 170
GEP. *See* Good epidemiologic praxis (GEP)
German, 42, 43, 44, 45–46
Germline mutations, 101
GHS. *See* Globally harmonized system (GHS)
Global guidance, 654
Globally harmonized system (GHS), 496, 499, 664, 705, 781, 798
Global Reporting Initiative (GRI), 716
GLPs. *See* Good laboratory practices (GLPs)
GMOs. *See* Genetically modified organisms (GMOs)
GMP. *See* Good manufacturing practice (GMP)
Good clinical practice, 208
Good epidemiological practice, 203
Good epidemiologic praxis (GEP), 487
Good laboratory practices (GLPs), 23, 24, 59–61, 82, 83, 86, 182, 183, 326, 579, 785
Good manufacturing practice (GMP), 673
Grapefruit juice, 428
GRI. *See* Global Reporting Initiative (GRI)
Groundwater, 651, 655, 656, 658
Groups of people, 651
Guidance, 609, 613
Guidance values, 650, 654
Guidelines, 90, 91, 100, 101, 103–105, 182–187, 414–418, 420, 421, 514, 515
Guidelines setting, 654
Guidelines for drinking-water quality (GDWQ), 656, 657

**H**

Haber's rule, 144
HACCPs. *See* Hazard Assessment and Critical Control Points (HACCPs)
Half-time, 153–155
Harm, 66, 68, 494
Harmonization, 23, 28, 34, 41, 43, 70, 182–184
Hazard Assessment and Critical Control Points (HACCPs), 835
Hazards, 4, 5–6, 14, 16, 17, 19, 68, 570, 571, 572, 573, 677–682
  assessment, 579
  characterization, 578–579
  classification, 785
  index, 410, 643
  materials, 740, 780–781
  pictograms, 678–680
  prevention, 464–465
  quotients, 701, 702
  statements, 678, 679, 681
Hb adducts. *See* Hemoglobin (HB) adducts
HBM. *See* Human biomonitoring (HBM)
Health-based guidance values, 360, 362, 371, 372
Health claim, 712
Health impact assessment (HIA), 624
Health problems, 278
Health promotion, 471
Health protection, 473
Healthy volunteers, 210
Hearings, 752, 754, 758, 762–763
Helleborin, 856
Hematology, 90, 94
Hemoglobin (HB) adducts, 691–692, 694
Hepatic necrosis, 428
Hepatocytes, 109, 138
Hepatotoxic, 109–110
Hereditary diseases, 101
Heterogeneity, 378, 379, 387
High-performance liquid chromatography (HPLC), 332, 334–337
High-tech nations, 647
High-throughput, 116
Histopathological, 90–93, 97, 103, 105
History, 323–324
Hockey stick, 555, 556
Hormetic dose-effect curves, 234
Host resistance (HR) assay, 105
Hot zone, 742, 745
House dust, 292, 294, 297, 298, 299, 300, 302
Household, 602
HPLC. *See* High-performance liquid chromatography (HPLC)
HR assay. *See* Host resistance (HR) assay
Human biomonitoring (HBM), 439–446, 768, 773
  HBM–1, 440
  HBM–2, 440
  HBM-I values, 894, 896
  HBM-II values, 894, 896, 897
Humans, 510–515, 517–519
  capital approach, 604
  data, 238, 578, 579, 786, 787
  experiments, 482
  population, 563, 566, 567
  rights, 486

HVP, 17
Hybridizing, 175
8-Hydroxy–2′-deoxyguanosin (8-OHdG), 691, 692
Hygiene, 469, 471–472, 835
Hypersensitivity, 768, 769

**I**
IARC. *See* International Agency for Research on Cancer (IARC)
ICCA, 17
ICH. *See* International Conference on Harmonization (ICH)
ICP-MS. *See* Inductively coupled plasma mass spectrometry (ICP-MS)
IEC. *See* Independent Ethics Committee (IEC)
IFCS. *See* International Forum on Chemical Safety (IFCS)
ILO. *See* International Labor Office (ILO)
Immission limit values, 886, 888, 889, 890
Immunity, 402
Immunoassays, 351–353
Immunochemical tests, 843
Immunogenicity, 417, 420, 421
Immunoglobulin E (IgE) antibodies, 571
Immunohistochemistry, 97
Immunological reactions, 571
Immunotoxicity, 105, 808, 809
Important role in the future, 507
Impurities, 129
INCHEM, 313
Incidents, 682, 740, 742, 746
Independence, 762, 763
Independent Ethics Committee (IEC), 208–209
Indoor air, 277–287, 294, 295, 302, 612, 654, 655, 883, 887–891
Inducers, 90, 94
Induction, 136–138, 164, 165, 170, 426, 428
Inductively coupled plasma mass spectrometry (ICP-MS), 347, 349–350
Industry, 22–24, 116, 613
Informed consent, 209
Informed decisions, 484
Ingredients, 292, 297, 851, 852
Inhalation, 83, 142, 144, 146, 147, 148, 150, 151, 158, 290, 294–296, 299, 300, 651, 653, 654, 658
Inhalation toxicity, 144, 146, 155
INHAND. *See* International Harmonization of Nomenclature and Diagnostic Criteria (INHAND)
Initiation-promotion-model, 266
Inorganic ions, 344
Insecticide, 623
*In silico*, 123–125, 127, 128
Insolubility, 522
Inspection, 38
Intake, 82, 83, 85, 87, 88
Interactions, 6, 13, 408–410
Interface, 332, 338, 349, 350
Interindividual, 562, 567
Interindividual differences, 534
Internal dose, 270
International, 22, 23, 26, 28, 31–46, 181–188
International Agency for Research on Cancer (IARC), 14, 18, 39, 313, 707
International Conference on Harmonisation (ICH), 23, 61, 80, 100, 101, 103, 105, 110, 111, 183, 184, 415, 417–421, 514–519, 610, 674, 716
International Conference on Harmonization (ICH), 61
International Forum on Chemical Safety (IFCS), 34
International Harmonization of Nomenclature and Diagnostic Criteria (INHAND), 96
International Labor Office (ILO), 34, 35, 37–39
International Program on Chemical Safety (IPCS), 33–34, 38, 313, 459, 460
International uniform chemical information database (IUCLID), 313, 664, 782, 793
Internet, 306
Interorganization Program for the Sound Management of Chemicals (IOMC), 35
Interspecies differences, 537, 538, 541
Interspecies extrapolation, 433–436
Intraspecies extrapolation, 434, 436
Intrinsic activity, 228–230
Inverse agonist, 229
Investigator, 209
*In vitro*, 107, 108, 111–113, 129, 130, 488, 538
  assays, 97
  data, 785
IOMC. *See* Interorganization Program for the Sound Management of Chemicals (IOMC)
Ion channels, 166
Ion-exchange chromatography (IEC/IEX), 344–345
Ion selective electrode, 350, 351
IPCS. *See* International Program on Chemical Safety (IPCS)
Irritation, 112, 570
Irritation effects, 530

Isolated perfused organs, 168
IUCLID. *See* International uniform chemical information database (IUCLID)

**J**
JECFA. *See* Joint FAO/WHO Expert Committee on Food Additives (JECFA)
JMPR. *See* Joint FAO/WHO Expert Meeting on Pesticide Residues (JMPR)
Joint FAO/WHO Expert Committee on Food Additives (JECFA), 313, 432, 458, 613, 804, 810, 819
Joint FAO/WHO Expert Meeting on Pesticide Residues (JMPR), 38, 39
Journalists, 764, 765
Journals, 313, 314
Judges, 728, 730
Jurisdiction, 730, 737
Jurisprudence, 730

**K**
Keratoconjunctivitis, 861
Kidneys, 134, 142, 155, 157, 723
Klimisch category, 786
"Known knowns", 28, 601, 602

**L**
Labeling, 34, 44, 677–682, 781, 787, 790
  inventory, 680
  system, 744
Lab on a chip, 326
Laboratory animals, 424
Laboratory safety, 866
Labour Courts, 736
Lactose intolerance, 424
Land contamination, 657–658
Language, 381
LanguaL, 293, 294
Lateral flow immunoassays, 353
Lavoisier, A., 323
Law of mass action, 227, 229
Lawyer, 728
Lay people, 751
LCR. *See* Lifetime cancer risk (LCR)
$LD_{50}$. *See* Lethal dose$_{50}$ ($LD_{50}$)
Lead, 424, 426–429
Legal concentration limits, 628
Legal protection, 475, 476, 478
Legislation, 41, 44, 781, 794
Lethal, 567
Lethal dose$_{50}$ ($LD_{50}$), 232–233
Liability, 735
Life cycle, 505
Life expectancy, 451, 452
Lifetime cancer risk (LCR), 241, 246–246
Ligand binding, 551
Likelihood, 729, 730
Limit concentrations, 629
Limit risk, 246–247
Limit values, 50, 51, 440, 443, 445, 627–647, 649–658
Linear model, 263–264
Lipid solubility, 522
Liquid-liquid extraction, 331, 333
Literature, 305–308
Literature search, 312
Liver, 109–110, 134, 137, 138
Local tolerance, 417, 420
LO(A)EL. *See* Lowest observed (adverse) effect level (LO(A)EL)
Logarithmic scaling, 549, 550, 551, 552
Logit model, 265–266
Log-linear model, 264, 265
Low dose, 549, 550, 551–559, 561, 563, 565
Lowest observed (adverse) effect level (LO(A)EL), 230–231, 232, 236, 243, 433, 434, 435, 437
Lung, 142, 146, 150–155, 157
  cancer, 572
  irritants, 153
Lutein, 815
Lycopene, 834, 836–837
Lymphocyte subpopulations, 105

**M**
MABEL. *See* Minimum anticipated biological effect level (MABEL)
Macrophages, 142, 150, 152, 153, 154
Major accident, 618, 619, 624
MAK-values, 50, 54, 444, 446, 611, 652
Malformations, 100, 101, 621
Management, 748–751, 754, 755, 758, 759, 762, 763
Mantel–Bryan assumption, 563, 566
Manufacturing, 421
Margin of exposure (MoE), 9, 10, 14, 17, 239, 249–250, 302, 371, 372, 612, 810, 818
Margin of safety (MOS), 457–461, 645
Marketing, 23, 24, 27
Mass spectrometry (MS), 176, 324, 333, 337–340, 342, 343, 347, 349
Mathematical model, 382, 383

Matrix, 325, 326, 329–331, 337–339, 350, 352
Maximum residual levels (MRLs), 891–892
Maximum safe dose, 215
Maximum tolerable daily intake (MTDI), 878, 880
MCS. *See* Multiple chemical sensitivity (MCS)
Measurement errors, 384
Mechanism of action, 5, 6, 10, 11–12, 14, 19, 527, 528, 538, 539, 540, 542, 544, 545
Mechanistic, 176–178
Mechanistic information, 561
Media, 27, 28
Mediation, 753, 754
Medical devices, 45–46, 661, 667, 671–673
Medical history, 693, 728–729, 768–769
Medical study, 484, 486
Medicines, 45, 207–208, 510, 513, 514, 519, 661, 663, 665, 673–675
MEDLINE, 315, 316
Membrane fraction, 163, 166
Mercury, 37
Message, 759–762, 765
Metabolic activation, 400, 552, 553, 562, 566
Metabolism, 133–138
Metabolites, 534–536, 536, 537, 539, 542, 544, 545
Metabolomics, 176
Methemoglobin (MetHb), 147
Methylmercury, 429
Michaelis constant, 273, 275
Michaelis–Menten equation, 264, 551
Microarray, 173, 175–177
Microbiological risk, 835
Micronucleus test, 806, 807
Micronutrients, 832, 835, 840
Microsomes, 537–541
Military conflicts, 856
Minamata Convention, 37
Minimization, 465
Minimum anticipated biological effect level (MABEL), 416
Misclassification, 201
Mispairing, 554, 556
Mitochondria, 163, 166
Mixtures, 8, 13, 408–410, 678–682, 781, 787, 797–801, 814, 849–852
Model variables, 255, 256
Models, 362–373
MoE. *See* Margin of exposure (MoE)
Molecular size, 523
Monetization, 603, 605
Monitoring, 23, 24, 29
Monkey, 419
Monoamine oxidases, 535
Monooxygenases, 535
Monte Carlo simulations, 567–568
Moral responsibility, 482
Morphology, 90, 93, 169
Motor activity (MA), 104
Mouthing behaviour, 254, 297, 300
MRLs. *See* Maximum residual levels (MRLs)
MS. *See* Mass spectrometry (MS)
Mucociliary escalator, 153, 154
Multi-generation study, 171
Multimedia context, 585
Multiple chemical sensitivity (MCS), 768
Multiple risk context, 584, 585
Multiple testing, 204
Multistage, 244, 246, 247, 248
Mutagenesis, 554
Mutagenicity test, 101, 102
Mutations, 401, 402
Mutations test, 111
Mutual acceptance of data, 182, 183

**N**

NAAQS. *See* National Ambient Air Quality Standards (NAAQS)
NAEL. *See* No-adverse-effect level (NAEL)
Nanotechnology, 845
Narcotics, 46
National Academy of Sciences (NAS)-scheme, 226
National Ambient Air Quality Standards (NAAQS), 655
National Health and Nutrition Examination Survey (NHANES), 693, 702
National Institute for Occupational Safety and Health (NIOSH), 128, 313, 314, 652
National Toxicology Program (NTP), 313
Natural constituents, 634, 636–638
NCE. *See* New chemical entity (NCE)
Necropsy, 90, 91, 95
Negative control, 214
Nerve agents, 857–860, 866, 867
Nested, 198
Neuropathology, 104
Neurotoxic, 104
Newborns, 428
New chemical entity (NCE), 414–416, 418–421
New micro-fluidic, 329
NHANES. *See* National Health and Nutrition Examination Survey (NHANES)

NIOSH. *See* National Institute for Occupational Safety and Health (NIOSH)
NMR. *See* Nuclear magnetic resonance (NMR)
No-adverse-effect level (NAEL), 433–435, 437
NOAEL. *See* No observable adverse effect level (NOAEL)
No data, no market, 663
No effect level, 498
NOEL. *See* No-observed-effect level (NOEL)
Nonclinical experiment, 510
Non-genotoxic, 398
Non-genotoxic carcinogens, 14
Noni juice, 837–838
No observable adverse effect level (NOAEL), 4, 5, 6, 9–10, 12, 13, 14, 226, 232, 234, 236, 243, 244, 248, 440, 444, 445, 458–460, 527, 791
No-observed-effect level (NOEL), 214, 217, 229–232
Normal distribution, 551, 557, 563, 564, 567
Notification, 849–852
Novel foods, 827–845
NTP. *See* National Toxicology Program (NTP)
Nuclear magnetic resonance (NMR), 176
Nuclei, 163, 166
Nucleic acids, 162
Nucleophilic moieties, 535
Null hypothesis, 214, 215
Nuremberg Code, 482, 483
Nutrients, 835, 844
Nutritional equivalence, 835, 844

**O**
Occupational, 610–612, 614
  disease, 731–737
Occupational exposure limits (OELs), 6, 7, 651–653
Occupational health, 44, 695, 701
Occupational safety, 880–883
Occupational Safety and Health Administration (OSHA), 246, 313, 652, 653, 882, 883
Octanol, 75
Odds ratio, 195, 196, 198, 203
Odor, 278, 524
Odor intolerance, 768
OECD. *See* Organisation for Economic Cooperation and Development (OECD)
OECD test guidelines, 119
OELs. *See* Occupational exposure limits (OELs)
Of societal consent, 643
8-OHdG. *See* 8-Hydroxy–2′-deoxyguanosin (8-OHdG)
Omics, 173–179, 329
One-hit model, 244
OPIN. *See* Organophosphate induced delayed neuropathy (OPIN)
Opinion, 749
OPPTS, 90
Oral, 83
Oral exposure, 297–300
Organic pollutants, 278, 280–283, 287
Organisation for Economic Cooperation and Development (OECD), 14, 17, 22, 23, 60, 82, 84, 90, 100, 101, 103–105, 182–188, 708, 710, 716, 785
Organophosphate induced delayed neuropathy (OPIN), 860
Organophosphorus, 857–859, 860
Organs, 89–97
  dose, 142, 144, 149
  toxicity, 6
  weights, 90, 92, 94
Original literature, 312
OSHA. *See* Occupational Safety and Health Administration (OSHA)
Oxidative damage, 691
Oxime, 859, 867
Ozone layer, 38

**P**
Packaging, 781
Paediatric-use, 715
Panel study, 198–199
Paralytic shellfish poison, 862
Parameters, 90, 92–94
Partial agonist, 229
Participants, 484–486
Participation, 747–755
Particles, 142, 146, 150, 151–155, 157
Partition, 522
Passive smoking, 593
Pasteurisation, 833
Pathologist, 86
Pathology, 90–97
Pathology report, 92
PBT. *See* Persistent, bioaccumulative, toxic (PBT)
PBTK models. *See* Physiologically based toxicokinetic (PBTK) models
Peak concentration, 142
Pediatric patients, 517

PELs. *See* Permissible exposure limits (PELs)
People, 748–752, 754, 755
Pepsin resistance test, 843
Percutaneous, 151
Permeability, 151
Permissible exposure limits (PELs), 446, 652
Peroxisome proliferators, 94–95
Persistent, bioaccumulative, toxic (PBT), 790, 794
Persistent organic pollutants (POPs), 36, 704–707
Pest control, 665
Pesticides, 471–473, 706, 707, 709–711, 876, 877–878, 883, 886, 891, 892
Phantom risk, 498
Pharmaceuticals, 45, 414–416, 418, 419, 609–610
  pollutants, 715
  rules, 673
Pharmacovigilance, 24, 29, 673
Phase 0, 210
Phase I, 210, 535, 540, 720
Phase I trials, 515
Phase II, 210, 535, 538, 540, 541
Phase II conjugation, 721
Phase III, 210–211, 535
Phase IV, 211
Philosophy, 495
Photoirritation, 185
Phototoxicity, 108, 110, 111, 113
Phthalate, 165
pH-trap, 152
Physicochemical, 75–80
Physicochemical data, 782, 783, 785, 788, 790
Physiologically based toxicokinetic (PBTK) models, 144, 149, 150, 151, 154, 155, 157, 160, 244
Physiological toxicokinetic models, 272, 273, 275
Phytochemicals, 814
Phytosterols, 834
Pivotal studies and critical endpoints, respectively, 239
Placebo, 485, 486
Plans, 740, 742–746
Plants, 39, 42, 43–45, 813–815, 817, 819–822
  health, 42, 45
  protection, 661, 663, 665–667, 892
  sterols, 836
Plaque assay, 105
Point of departure (POD), 360, 362, 367–369, 458–460, 791–792
Poison information centers, 744
Poisoning cases, 850, 851
Poisons centers, 682, 709, 850–852
Policy makers, 748, 754
Political, 584, 586, 587, 629–632, 637–640, 643, 646
Pollutants, 408, 410, 883, 886, 887–889
Pollution, 618, 619, 654–657
Polyaromatic hydrocarbons, 426
Polymers, 663
Polymorphisms, 137, 436, 534
Poor metabolizers, 425
Popper, 214
POPs. *See* Persistent organic pollutants (POPs)
Population, 192–193, 195, 197–201
Positive control, 214, 221
Positive lists, 666, 671
Post-Launch Monitoring, 834
Potency, 227, 229, 230
Practical thresholds, 653
Preanalytics, 326, 329
Precaution, 35, 463–467, 888, 889, 893
  limit values, 631, 634, 635–640, 643
  principle, 13, 15–16, 19–20, 471, 480
  values, 640, 643–644
Precision, 202–203
Prediction analysis, 178
Prediction model, 185, 187
Predictive systems, 124–127
Predictive value, 126
Pregnancy, 516, 517, 721, 723, 724
Prerequisite, 19
Preservatives, 667, 671
Presumption of safety, 817, 818
Primary cell cultures, 108
Primary cell types, 167
Primary literature, 305–308
Principles of GLP, 60
Privacy, 484, 486
Probability, 214, 253–259, 494–497, 510, 729
Probability approach, 301
Probit model, 265–266
Product, 850–852
  categories, 292–294
  composition, 682
  databases, 851
Professional, 602, 604
Prohibition, 703–717
Propineb, 147, 148
Protection, 35, 38, 39, 41, 42–45, 50–52, 475–480, 503, 505, 507
  aims, 643, 644, 645
  equipment, 741, 743

factor, 745
level, 475–480
Proteins, 162, 164, 166
Proteins binding, 151, 155
Proteomics, 176
Provisional tolerable daily intake (PTDI), 878, 880
Provisional tolerable weekly intake (PTWI), 876, 880
Pseudomembranes, 860
Psychiatric disorder, 774
PTDI. *See* Provisional tolerable daily intake (PTDI)
PTWI. *See* Provisional tolerable weekly intake (PTWI)
Public, 51, 52, 55
Publish, 306, 307
Pulmonary infections, 150
Pumps, 166
p-value, 204

**Q**
QM. *See* Quality management (QM)
Q-PCR. *See* Quantitative polymerase chain reaction (Q-PCR)
QSAR. *See* Quantitative structure activity relationship (QSAR)
QT-interval, 111, 518–519
Quality, 58–62, 305–308, 570
criteria, 58
goals, 466, 467
guidelines, 80
Quality assurance systems (QA Systems), 57–62
Quality management (QM), 58, 59, 61, 325, 326, 343
Quantal data, 240, 247, 248, 361, 364, 366, 368
Quantitative polymerase chain reaction (Q-PCR), 175
Quantitative structure activity relationship (QSAR), 125–129
Quantum satis, 810
Questionnaire, 769

**R**
Radiation, 614
Radiolabelled compounds, 135
Radon, 593, 594
Random errors, 202–203, 386–387
Rapid Alert System, 712
Rapid metabolizer, 425, 426
Rate ratio, 195, 198
Ratio, 522
REACH. *See* Registration, evaluation, authorisation and restriction of chemicals (REACH)
Reaction mechanisms, 24
Reactive metabolites, 536
Reactive oxygen, 398, 399
Reactive Skin Decontamination Lotion (RSDL), 868
Receptor affinity, 227, 228
Receptor agonist, 228–229
Receptor antagonist, 228–229
Receptor conformation, 164
Re-evaluation, 52, 54
Reference dose (RfD), 243, 445–446, 880
Reference values ($RV_{95}$), 440, 442–445, 686, 693–699, 768, 771, 773, 774, 894, 895–896
Regeneration cost, 604
Registration, 781–784, 786, 790–795
Registration, evaluation, authorisation and restriction of chemicals (REACH), 4, 14, 17–19, 41, 44, 55, 159, 290, 292, 293, 302, 613, 619, 620, 662–665, 668, 678, 680–682, 705–709, 779–795
Registry of Toxic Effects of Chemical Substances (RTECS), 128, 314
Regulations, 50, 51, 52, 54, 55
actions, 621
different, 882
toxicology, 178, 179, 432, 438
Relative risk, 195, 196, 201, 590
Relevance, 782, 783, 785–787, 790, 791
Relevance species, 417, 419
Reliability, 254, 258
Remediation, 619, 620, 650, 657
Renal function, 722, 723
Repair, 554–556, 558, 559, 563, 566, 567
Replacement, 488
Reports, 306
Reproduction, 99, 515–517
toxicity, 171, 420, 528–529
toxicology, 99
Research, 481–489
Residual risk, 495
Responders, 560
Responsibility, 54, 55
Restrictions, 50, 703–717, 786, 794
Retention, 152–154, 157, 330, 333, 335, 336, 345
Reversed phase, 330, 335, 336
Reversible, 87, 451–453, 610, 612

RfD. *See* Reference dose (RfD)
Ricin, 856, 857, 862–863, 865, 866, 868
*Rio Declaration*, 750
Risk-friendly, 618
Risk-generating, 749
Risks, 66, 67, 68, 69, 70, 482–487, 493–499, 510, 511, 512–514, 516, 517–519, 569–574, 607–614
  acceptance, 585–586, 598
  analysis, 66–68
  assessment, 38, 39, 42, 43, 65–70, 144, 149, 150, 158, 159, 270, 275, 432–435, 437, 438, 583, 587, 618–620, 624, 632, 633, 650, 651, 658, 813–823, 850, 851, 871–874
  assessors, 754
  benefit, 601–605, 609, 610, 750–751
  benefit analysis, 236
  of cancer, 102
  characterization, 69–70, 577–582, 790–792
  communication, 757–765
  continuum, 465
  cycles, 501–507
  evaluation, 67, 68, 70, 503–505, 583–587
  experts, 758–764
  extrapolation, 238–239
  factor, 585, 586, 587
  level, 360, 366
  management, 66–68, 70, 467, 583, 584–587, 650, 704–705, 748–751, 754, 755, 758, 763, 781, 783, 790, 792–794, 856, 862–868, 872–874
  managers, 752
  minimization, 509–519
  mitigation, 41
  parameter, 239–240
  perception, 502
  profile, 591
  ratio, 195, 198, 202
  reduction, 502, 504–507
  reduction spiral, 506
  regulation, 872
  structure, 591–593
Rotterdam Convention, 36, 39
Round tables, 752–754
Route of administration, 158
RSDL. *See* Reactive Skin Decontamination Lotion (RSDL)
RTECS. *See* Registry of Toxic Effects of Chemical Substances (RTECS)
Rule of environmental hygiene (REH), 628, 631–646
$RV_{95}$. *See* Reference values ($RV_{95}$)
RW-I, 889
RW-II, 889, 891

**S**
Safety, 33–45, 66, 510, 512–514, 517, 518, 781, 786, 790–794
  evaluation, 814, 817–819, 822
  margins, 234–236, 424
  pharmacology, 108, 111, 511, 517, 518
  report, 619
  testing, 536
Safety data sheet (SDS), 678, 681–682, 744, 790, 792, 793, 850, 852
Safety factors (SFs), 242–244, 430, 431–438, 537, 611, 612
SAICM. *See* Strategic Approach to International Chemicals Management (SAICM)
Sample is insoluble in the solvent and/or only few solvents are suitable, 338
Sample size, 214–217, 221–222
Sampling, 278–287
Sandwich, 352
SAR. *See* Structure activity relationships (SAR)
Sarin, 856, 859, 860, 867
Saxitoxin, 857, 866
Scaling factors, 436
Scandals, 507, 764
SCCNFP. *See* Scientific Committee on Cosmetology and Non-Food Products (SCCNFP)
Scenarios, 289–302, 790, 792, 793
School-kids, 722
Science, 749, 750
Scientific Committee on Cosmetology and Non-Food Products (SCCNFP), 186
Scientific Committee on Occupational Exposure Limits (SCOEL), 652, 653
Scientific misbehavior, 62
Scientific rationale, 630
Scientific societies, 25–26
Scientific statements, 760
Scientists, 52–54
SCOEL. *See* Scientific Committee on Occupational Exposure Limits (SCOEL)
SDS. *See* Safety data sheet (SDS)
Secondary literature, 312
Second messenger, 164
Sedimentation, 152, 153
Selection bias, 194, 199–201

Sensitivity, 126, 719, 721–723, 768, 769, 771
  analysis, 254, 256–258, 379, 382, 386, 387, 390–392
  groups, 612
Sensitization, 530, 571–572
Sensor, 329, 350–351
Separation, 323, 325, 326, 330, 331, 333–346, 349, 350, 352
Sequential procedure, 52
Severity, 450–453
"Seveso Directives", 619
SFs. *See* Safety factors (SFs)
Side products, 633, 639
Signaling pathways, 398
Significant, 203, 204
Siloxane, 336
Simulations, 256–259, 567–568
Sink, 285–286
Skin, 146, 151–152, 296–298, 570, 571, 573, 722
Skin sensitization, 105, 125, 129
Slope factor, 247
Small and medium-sized enterprises (SMEs), 781
SMEs. *See* Small and medium-sized enterprises (SMEs)
Smog, 622
Societal acceptance, 630, 631
Society, 749, 750, 755
Socio-economic, 620, 621
Socio-economic analysis, 794
Sociological, 22, 25, 26
Software packages, 366
Software tools, 391
Soil, 298–300, 878, 893–894
  contamination, 657
  screening, 658
  trigger values, 658
Solid phase extraction, 326, 329–331, 343
Solubility, 75–77, 521–522
Soman, 858, 859
SOPs. *See* Standard operating procedures (SOPs)
Spare receptors, 228
Species, 144–146, 149, 152, 154, 158, 159, 534, 536, 537, 538
Species differences, 534
Specifications, 78, 126
Spectrum, 325, 346, 347, 348
Sponsor, 208–210
Spontaneous lesions, 92
Stability, 77, 80
Stakeholders, 618, 620, 621, 624, 748–754, 758, 759, 762
Standard, 50–54
Standard operating procedures (SOPs), 82, 138
Statement, 728, 729, 736
State-of-art, 61
State of discussion, 28
Statistics, 547–568
  fit, 367, 368
  interaction, 202
  power, 197, 202
Stem cells, 108
Stockholm Convention, 36, 39
Strategic Approach to International Chemicals Management (SAICM), 34–36, 39
Structure, 124, 125, 127, 129
  activity, 124, 125
  comparison, 124, 128
Structure activity relationships (SAR), 124, 125, 127–129
Study, 306–308
  design, 193, 194, 196–199, 202
  protocol, 82, 209, 210
Subacute, 85, 86–88
Subchronic, 85, 86–88
Subgroups, 451, 454
Subjective assessment, 452
Subjects of protection, 475–478
Subpopulations, 720
Substances of very high concern (SVHC), 706
Sulfur mustard, 860, 861
Superagonist, 416
Surveillance, 611, 612, 629, 636, 645, 646
Susceptibility, 423, 424, 428–430, 560–566, 568, 570, 571, 572, 574, 692
Susceptibility factors, 563, 564, 566
Sustainability, 466, 644, 716
Sustainability development, 34, 35, 507
SVHC. *See* Substances of very high concern (SVHC)
Sweeteners, 711–712
Symptoms, 740, 768–770, 774–776
Synergy, 408, 410
Systematic errors, 199, 386, 387
Systemic toxicity, 570, 571–574

**T**

T25, 248–249
Table 3, 866
Tabun, 859, 860
TA Luft. *See* Technical Instruction on Air Quality Control (TA Luft)
Target organ, 5, 6, 9, 144, 149, 150
Task force, 744

TCDD. *See* Tetrachlorodibenzo-p-dioxin (TCDD)
TD50, 249
TDI. *See* Tolerable daily intake (TDI)
TDI value, 630, 644
TDM. *See* Toxikodynamic modeling (TDM)
Technical completeness, 793
Technical Instruction on Air Quality Control (TA Luft), 888, 890
Technology, 504, 507
Temperatures, 280, 281, 283–286
Temporal scale, 385
Teratogenic, 99, 100
Teratogenic effects, 529
Testimonies, 729
Testing, 5, 6, 14, 16, 19
  data, 782
  designs, 159
  of difference, 840
  of equivalence, 840
  guidelines, 41, 60
  hypotheses, 204
  method, 784–785
  models, 514
Tetrachlorodibenzo-p-dioxin (TCDD), 709, 710
TGD 2003,
TGN1412, 416, 483
Thalidomide, 99, 621–622
Theranostics, 160
Therapeutic proteins, 420
Therapeutic ratio, 234
Thin-layer chromatography, 336, 343–344
Threshold limit values (TLVs), 50, 570, 572, 610–611, 652
Threshold of Toxicological Concern (TTC), 16
Thresholds, 9–11, 16, 51, 218, 222, 238, 239, 242–244, 249, 397–405, 444–445, 541–545, 549–553, 555–559, 561–563, 566, 568, 579, 628, 630, 634, 635, 646, 650, 652–654, 656
  of concern, 630, 631, 644–646
  of danger, 465–467
  values, 458–461
TI. *See* Tolerable intake (TI)
Time budgets, 299
Tissues, 90–91
  burdens, 270, 272, 275
  sections, 97
  slices, 168
TLVs. *See* Threshold limit values (TLVs)
T–2 mycotoxin, 864
3T3 Neutral Red Uptake 186 Phototoxicity Test (3T3 NRU-PT), 185–187
3T3 NRU-PT. *See* 3T3 Neutral Red Uptake 186 Phototoxicity Test (3T3 NRU-PT)
Toddlers, 300
Tolerable absorbed doses (TRD), 878, 881–882, 893
Tolerable daily intake (TDI), 878, 880
Tolerable intake (TI), 654
Tolerance, 608, 609, 612, 629, 632–636, 640, 876, 893
  doses, 469
  intake, 612
  limits, 55
  risk, 246, 478
  thresholds, 628, 630, 631, 633, 634
Tonnage, 782–784
Top down, 748, 749
ToxCast, 129, 130
Toxic dose$_{50}$ ($TD_{50}$), 232–234
Toxicity, 779–795
  pathways, 117–120, 188
  studies, 360–362, 364, 365, 367, 369–370, 372, 415, 417–420
Toxicodynamic factor, 460
Toxicodynamic interactions, 409
Toxicogenomics, 174–179
Toxicokinetics, 5, 6, 10–12, 19, 141–160, 269–276, 415, 417–419
  factor, 460
  interactions, 409
Toxicological information, 33, 41
Toxicologists, 86, 748
Toxicology, 469–474
Toxicology report, 727–737
Toxicovigilance, 850, 851
Toxikodynamic modeling (TDM), 261–268
Toxins, 856, 861, 862, 865–868
TOXLINE, 313–317
TOXNET, 314
ToxRTool, 307
Toys, 7, 8, 12, 13, 661, 670–671
Transboundary, 37
Transcriptomics, 175
Transfer factor, 384, 386
Transgenic animals, 419
Transition period, 782
Transparency, 52, 748, 750, 753, 754
Transporters, 166
Transport systems, 157
TRD. *See* Tolerable absorbed doses (TRD)
Trichothecene, 860, 868
Trichothecene mycotoxins, 862
Trigger values, 893, 894
Truth, 728

TTC. *See* Threshold of Toxicological Concern (TTC)
TTCA, 147, 148
Tumorigenesis, 404
Tumor promoters, 170, 398–399
Tumor risk, 611
Tuskegee Syphilis Study, 483
Two-generation, 100
Type I error, 214, 215, 222
Type II error, 214, 215, 222

**U**
UBA, 44–45, 442
UD. *See* Unit dose (UD)
Ultraviolet (UV) radiation, 593
Unacceptable, 504, 506
Unavoidability, 454, 628, 629, 632, 633, 634, 636, 638–639
UNCED. *See* United Nations Conference for Environment and Development (UNCED)
Uncertainties, 4–5, 8, 9, 12–15, 19, 145, 158, 159, 253–256, 258, 299, 302, 360, 364, 368, 369, 371–373, 377–393, 441, 495–497, 578, 580–582, 748, 749, 751, 753
Uncertainties factors, 433, 438, 441, 445
UNDP. *See* United Nations Development Program (UNDP)
UNEP. *See* United Nations Environment Program (UNEP)
UNIDO. *See* United Nations Industrial Development Organization (UNIDO)
Unit dose (UD), 241
United Nations Conference for Environment and Development (UNCED), 34, 35
United Nations Development Program (UNDP), 35, 40–41
United Nations Environment Program (UNEP), 34, 35, 39
United Nations Industrial Development Organization (UNIDO), 35, 39–40
Unit risk (UR), 241, 441, 479, 498, 887–888
Unknown risks, 511
Unscheduled DNA, 101, 102
Unwanted effects, 609, 610
UR. *See* Unit risk (UR)
US EPA, 120, 121
Users, 682
US Food and Drug Administration (US-FDA), 610, 613
US National academy of Sciences (NAS), 67–69
Utility analysis, 605

**V**
V agents, 859
Vapor concentration, 523
Vapour pressure, 284
Variabilities, 127, 253–258, 299, 301, 378–393, 432, 433, 436, 437, 458, 460, 461, 496, 497, 580–581
Ventilation, 280, 281, 283, 284, 286
Verification, 859–860, 865–866
Vesicants, 860–861
Veterinary drugs, 39, 44
Veterinary pharmaceuticals, 876
Veterinary use, 715
Vienna Convention, 38
Vinyl acetate, 541–545
Virtual Embryo, 121
Virtual Liver, 120–121
Virtually safe dose (VSD), 246
Volatile, 523, 524
Volume of distribution, 154, 157–158, 271, 561
Voluntary agreements, 621
Volunteers/patients, 510, 511, 513–519
VSD. *See* Virtually safe dose (VSD)
Vulnerable population, 578, 580
VX, 859, 860

**W**
Warning, 678, 681
Warning values (WVs), 644
Waste, 37, 40, 41, 44
Water, 650, 651, 655–658
  management, 44
  solubility, 534, 535
Weight of evidence (WoE), 308, 581, 782, 787, 798, 800–801
WELs. *See* Workplace exposure limits (WELs)
WHO. *See* World Health Organization (WHO)
WHO guidelines, 472, 473–474
Williams-test, 215
Willingness to pay, 604
Witnesses, 728
WoE. *See* Weight of evidence (WoE)
Worker, 610, 611
Workplace, 44, 610, 611

Workplace exposure limits (WELs), 652
World Health Organization (WHO), 82, 84, 242, 248, 249, 290, 293, 432–435, 437, 654–657, 707–711, 804, 809, 876, 878, 880, 883, 884–888, 892
World Summit on Sustainable Development (WSSD), 35
Worst case, 256, 258, 296, 297, 301, 302
WSSD. *See* World Summit on Sustainable Development (WSSD)

**X**
Xenobiotics, 424–429
Xenobiotics metabolism, 534–537

**Z**
ZEBET, 110, 185, 186
Zero-order kinetics, 157
Zero-risk, 608
Zinc, 147