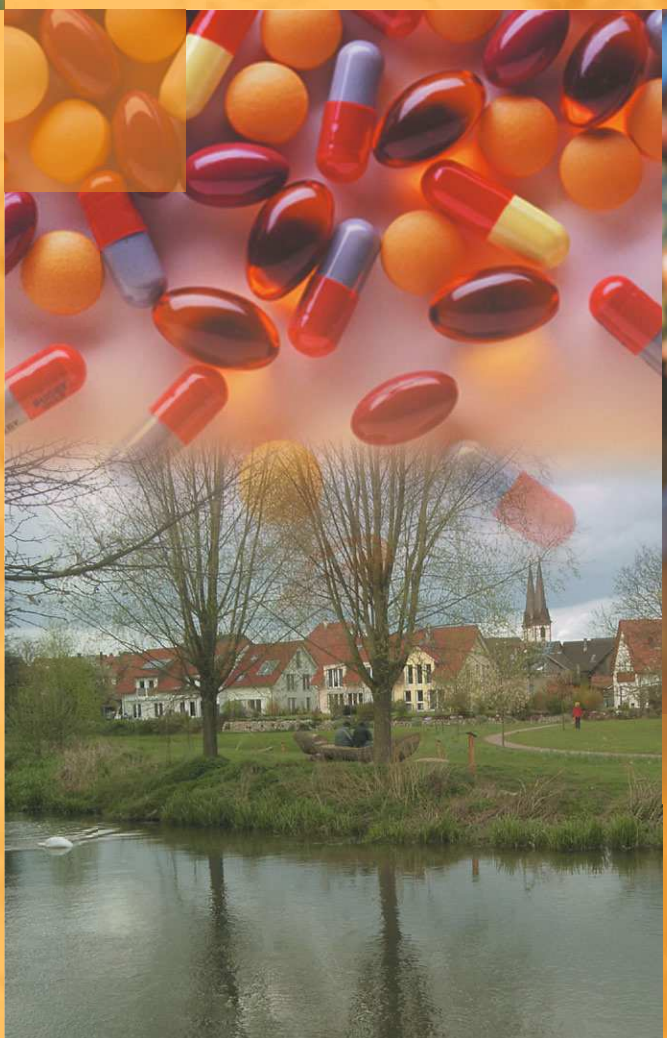
Klaus Kümmerer
Editor

Pharmaceuticals in the Environment



Sources, Fate, Effects and Risks

Third, revised and enlarged edition

Springer

Klaus Kümmerer

(Editor)

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Sources, Fate, Effects and Risks

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(Editor)

Pharmaceuticals in the Environment

Sources, Fate, Effects and Risks

Third edition
With 108 Figures and 62 Tables

Springer

Editor

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Foreword

When the first green wave appeared in the mid and late 1960s, it was considered a feasible task to solve pollution problems. The visible problems were mostly limited to point sources, and a comprehensive "end of the pipe technology" (= environmental technology) was available. It was even seriously discussed in the US that what was called "zero discharge" could be attained by 1985.

It became clear in the early 1970s that zero discharge would be too expensive, and that we should also rely on the self purification ability of ecosystems. That called for the development of environmental and ecological models to assess the self purification capacity of ecosystems and to set up emission standards, considering the relationship between impacts and effects in the ecosystems. This idea is illustrated in Fig. 0.1. A model is used to relate an emission to its effect on the ecosystem and its components. The relationship is applied to select a good solution to environmental problems by application of environmental technology.

Meanwhile, it has been disclosed that what we could call the environmental crisis is much more complex than we initially thought. We could, for instance, remove heavy metals from wastewater, but where should we dispose the sludge containing the heavy metals? Resource management pointed towards recycling to replace removal. Non-point sources of toxic substances and nutrients, chiefly originating from agriculture, emerged as new threatening environmental problems in the late 1970s. The focus on global environmental problems such as the greenhouse effect and the decomposition of the ozone layer added to the complexity. It was revealed that we use as much as about 100 000 chemicals, which may threaten the environment due to their more or less toxic effects on plants, animals, humans and entire ecosystems. In most industrialised countries comprehensive environmental legislation was introduced to regulate the wide spectrum of different pollution sources. Trillions of dollars have been invested in pollution abatement on a global scale, but it seems that two or more new problems emerge

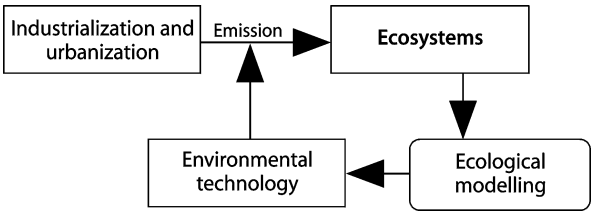

Fig. 0.1. The strategy applied in environmental management in the early 1970s is illustrated. An ecological model is used to relate an emission to its effect on the ecosystem and its components. The relationship is applied to select a good solution to environmental problems by application of environmental technology

for each problem that we solve. Our society does not seem geared toward solving environmental problems, or is there perhaps another explanation?

Recently, standards for environmental management in industries and green accounting have been introduced. The most widely applied standards today for industrial environmental management are the ISO 14000-series. These initiatives attempt to analyse our production systems to find new ways and methods to make our production more environmentally friendly. More than 100 countries have backed up the international standards for effective management of environmental impacts.

Figure 0.2 illustrates how complex environmental management is today. The first figure shows that a simultaneous application of environmental technology, ecotechnology, cleaner technology and environmental legislation is needed in environmental management.

Environmental technology offers a wide spectrum of methods that are able to remove pollutants from water, air and soil. These methods are particularly applicable to coping with point sources.

Cleaner technology explores the possibilities of recycling by-products or the final waste products or attempting to change the entire production technology to obtain reduced emissions. It attempts to answer the pertinent question: couldn't we produce our product using a more environmentally friendly method? It will to a great extent be based on environmental risk assessment, LCA and environmental auditing. The ISO 14000-series and risk reduction techniques are among the most important tools in the application of cleaner technology. The environmental risk assessment of chemicals is in this context a very important tool, as it results in a quantification of the environmental risk.

Ecotechnology covers the use of ecosystems to solve pollution problems, including the erection of artificial ecosystems. It also encompasses the technology that is applicable to the restoration of more or less deteriorated ecosystems. The mentioned classes of technologies cover a wide spectrum of methods. We have, for instance, many environmental technological methods to cope with different wastewater problems, and to

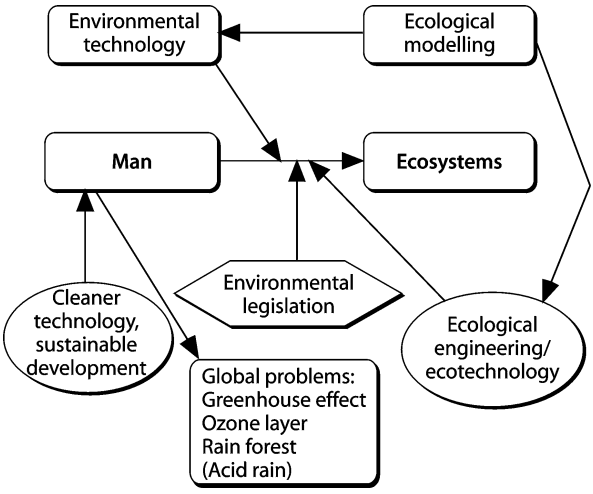

Fig. 0.2. The use of environmental models in environmental management, which, today, is very complex and must apply environmental technology, cleaner technology and ecotechnology. Models are used to select the right environmental management strategy. In addition, the global environmental problems, which also require the use of models as a synthesizing tool, play an increasing role

select the right method or most often the right combination of methods, a profound knowledge of the applicability of the methods and of the processes and characteristics of the ecosystem receiving the emission is necessary.

Environmental legislation and green taxes may be used in addition to these classes of technology. They may in principle be used as regulating instruments in every step of the flow from raw materials and energy to final waste disposal of the used product.

The 20th century has introduced more than 100 000 chemicals that are used in our every day life, either in households, industries or agriculture. We have "blindly" introduced these chemicals without realising the consequences for the environment and directly and indirectly for human health. EU started to list these chemicals in the late 1970s, and since the mid 1980s it has been compulsory to set up an environmental risk assessment for all new chemicals. It was the idea, meanwhile, to make environmental risk assessments for the chemicals already in use, but it is going very slowly, and at the present rate, we shall not be able finish ERAs for all the applied chemicals in this century. Probably, it is necessary to speed up the evaluation of the chemicals in use, for instance by forging a closer cooperation between the environmental agencies and the chemical industry, in order to obtain a realistic picture of the environmental risk associated with the many chemicals we apply today.

It is strange that drugs were not included when a compulsory environmental risk assessment was introduced for new chemicals, because drugs have properties that cause suspicion about environmental effects. Drugs are

- biologically active.
- often mobile as the water solubility is high relative to the molecular weight. This is particularly true for metabolites of the drugs that can be found in urine and therefore also in the wastewater.
- not readily biodegradable.

Drugs have, in other words, properties that make them environmentally interesting.

Today, an environmental risk assessment is required for all new medical compounds used in veterinary drugs, but it is expected that this will also be required for human drugs in the near future.

This volume focuses on what we know but also what we don't know about drugs, or rather what we ought to know to understand the occurrence, the fate and the effect of the about 4 000 medical compounds that we are using in the drugs applied today. What basic knowledge do we have today about drugs to be able to set up ERAs for the medical compounds?

Recently (February 2000) Chemosphere published a special issue on "drugs in the environment." This issue contained several interesting papers on these topics. This volume is, however, the first book to review "drugs in the environment." A book can, of course, give more detailed information than scientific papers, and also make links to what is known more generally about chemical compounds in our environment. The publication of this book is therefore an important step forward in our effort to

1. understand the environmental occurrence and processes of drugs,
2. quantify their effects and risks and

3. properly abate the associated pollution problem by trying to give an answer to the following two pertinent questions:
   - Which medical compounds should be phased out and substituted by other compounds?
   - Could we solve some of the problems with environmental or cleaner technology? How?

At least a decade will pass before we have a proper overview of the many environmental problems that are associated with medical compounds discharged into the environment. At that time – ten or fifteen years from now? – we may have substituted the most environmentally harmful chemicals with other compounds as a concluding result of the performed ERAs. The focal point is, however, that we have a realistic knowledge about the risk involved in the use of medical compounds and can phase out the most risky compounds. This process has already started – slowly but surely – because the medical industry is very concerned today about the fate and effect of antibiotics and recommendations on which antibiotics to use from an environmental point of view can already to a certain extent be given today.

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Preface to the Third Edition

Pharmaceuticals in the environment are still a "hot bed" of interest. Since the publication of the first edition in 2001 and the second edition in 2004 the focus of research has changed again. In the meantime, we have learned quite a lot about the fate, effects, and risks associated with the presence of pharmaceuticals in the aquatic and terrestrial environment. Anyway, there is still an increasing need for knowledge of pharmaceuticals in the environment.

The input and fate of parent compounds and the relative importance of different sources including compounds that up to now have only been scarcely investigated are still of interest. However, metabolites of human and animal metabolism are coming into focus. The same holds for products of transformation of parent compounds and metabolites in the environment, such as dead-end transformation products of biodegradation, oxidation or photolysis.

As it has been extensively demonstrated that the active compounds are present in the environment some of the interest in this field of research has moved from analysis of the compounds, which is still undertaken to effect studies to more extensive fate studies in the lab and in field trials. It has been found that environmental concentrations can cause effects in wildlife if proper tools are applied for effect assessment. The question of mixture toxicity has gained more and more attention. It has been learned that classical tests may underestimate effects and risks. The significance of antibiotic resistance in the environment is still not clear.

The long-awaited guideline for environmental risk assessment for human pharmaceuticals in the European Union has been in force since December 2006. Accordingly, more work has been done in the field of risk assessment and risk management. For compounds already on the market that constitute by far the biggest share this guideline is only applicable in case of the need for a renewal of the license. As for risk management strategies to eliminate pharmaceuticals from waste water or from the effluent of sewage treatment plants have been proposed and investigated. A tremendous amount of literature can now be found describing technical management measures such as oxidative or photolytic effluent treatment, filtering techniques, and application of charcoal. It has been learned however, that each of these approaches has its specific shortcomings. Therefore, additional approaches such as including people handling and using the compounds, and focussing on the properties of the compounds ("green pharmacy") have come into focus.

Accordingly, the 3rd edition has been largely changed again in comparison with the previous ones to address these new issues and the new lines of discussions and new findings. As in the previous editions, it gives an overview of the present state of knowledge presenting typical results and lines of discussion. Like the previous editions, this

one doesn't claim to give a complete overview including the fully detailed body of knowledge of pharmaceuticals in the environment. Rather, it addresses important and typical topics. In contrast, it highlights the most important questions and issues related to the presence of pharmaceuticals in the environment. It also provides many new findings that raise new questions and confirm earlier results.

This edition contains four major parts. In the first part, specifics of pharmaceuticals that distinguish them from "classical" micro-pollutants are addressed. In the second part, new findings on sources, occurrence and fate of pharmaceuticals in the environment are presented. In the third part, an overview of the current state of knowledge of effects of pharmaceuticals in in aquatic and terrestrial environmental is given. New, promising approaches to the study of the effects of pharmaceuticals in the environment are described. The fourth part addresses risk assessment issues starting with the EU guideline and practical experiences of its application. Shortcomings of the EU guidelines are discussed in several contributions. A brief description of the state of regulation of chemicals in Japan is also included. The final part is dedicated to risk management. As advanced STP effluent treatment as a management approach has already been addressed in the second edition and no generally new findings have been published since this time, only little space has been devoted to it. Instead mainly non technical approaches are presented here that are also of importance and are often overseen.

Accordingly, most of the contributions have replaced the ones of the second edition. They are addressing these new foci of research. The remaining ones have been updated. As a result, the 3rd edition is not only a revised and updated one but an additional new volume in a "series" of pharmaceuticals in the environment. The 1st and 2nd edition are still valuable sources of information and should be used together with this 3rd edition. Research needs are addressed within each chapter. Therefore, a separate chapter on research needs was omitted in this edition.

Again the edition of the book would have not been possible without the support of my co-workers in the research group of the Applied Environmental Research Section at the Department of Environmental Health Sciences at the Medical Center at Freiburg University. Special thanks to Radka Alexy, Petra Heiberger and Andreas Längin for their support of my daily routine, which gave me the necessary time to edit a book in such a dynamic field. Numerous discussions with colleagues, with contributors to the book and other people have been stimulating. Thank you to all those people who created the opportunity for discussion, the exchange of ideas and the sharing of results on the role of pharmaceuticals in the environment. This, as well as the encouraging comments and overwhelmingly positive feedback received for the second edition from many experts in the field encouraged the publisher and myself to publish a third edition. Thank you to Christian Witschel and his team from Springer Verlag Heidelberg who strongly supported the idea and helped to make the third edition possible. Thank you also to all the authors who gave up their precious time to contribute to this book.

A big thank you again to my wife Isolde, and my children Sarah and Yannik, with whom I was able to spend precious family time and without whose patience and encouragement neither this nor the preceding editions of the book would ever have been completed.

Klaus Kümmerer
Freiburg, January 2008

Preface to the Second Edition

The first edition of "Pharmaceuticals in the Environment" was sold out within two years. This is quite surprising for a book on such a specialised topic. Obviously, pharmaceuticals in the environment, their fate, effects, and the risks associated with their presence there are a "hot bed" of interest.

Since publication of the first edition, so much literature on the topic has been published in journals and proceedings that it is hard to keep an overview. Most of these papers have been of an analytical nature. The majority deal with the detection of pharmaceuticals in the aquatic environment, while others describe methods used to analyse pharmaceuticals in soil and the results of these analyses. The proportion of publications describing the occurrence and fate of pharmaceuticals in soils has increased since publication of the first edition. A minority of papers describe and assess the effects of pharmaceuticals on organisms in the aquatic environment and in the soil. The initiation of resistance and the selection of resistant bacteria in the environment has been addressed and intensively discussed. However, the significance of this topic is not yet clear. Furthermore, strategies to eliminate pharmaceuticals from waste water or from the effluent of STPs have been proposed and investigated. Introduction of restrictions relating to environmental aspects of pharmaceuticals are being discussed within the scope of EU regulatory procedure.

I have taken the opportunity provided by a second edition to revise and extend the book according to the enlarged body of knowledge on as yet unresolved, as well as newly emerging issues related to the input, occurrence and fate of pharmaceuticals in the environment, as well as the risks which they pose. The new edition gives an overview of the present state of knowledge with respect to typical results and lines of discussion. Like the first edition, this one makes no claim to give a complete overview of the state of the art of pharmaceuticals in the environment. Rather, it addresses important and typical topics and highlights the most important questions and issues related to the presence of pharmaceuticals in the environment. It also provides many new findings which raise new questions and confirm earlier results. The increased number of contributions and authors gathered in the second edition reflects with greater number of papers published, and of issues addressed, as well as the growing number of people from academia, official bodies and companies involved in the topic. It also reflects the intensified and ongoing discussions and the increased public awareness. Thus, in character, the second edition is more that of a general summary than was the case with the first edition.

The second edition of the book would have not been possible without the support of my co-workers in the research group of the Applied Environmental Research Sec-

tion of the Freiburg University Hospital Institute of Environmental Medicine and Hospital Epidemiology. Special thanks to Radka Alexy for her support in my daily routine, which gave me the necessary time to edit a book in such a dynamic field. I greatly acknowledge the support of Franz Daschner, Director of the Institute of Environmental Medicine and Hospital Epidemiolgy. Numerous discussions with colleagues, with contributors to the book and other people have been stimulating. Thank you to all those people who created the opportunity for discussion, the exchange of ideas and the sharing of results on the role of pharmaceuticals in the environment. This, as well as the encouraging comments and positive feedback received to the first edition from many experts in the field encouraged the publisher and myself to publish a second edition so soon after the first edition. Thank you to Christian Witschel and his colleagues from Springer-Verlag Heidelberg, who strongly supported the idea and helped make the second edition possible. Thank you also to all the authors who gave up their precious time to contribute to this book.

A big thank you also to my wife Isolde, and my children Sarah and Yannik, with whom I was able to spend precious family time and without whose patience and encouragement neither this nor the first edition of the book would ever have been completed.

Klaus Kümmerer
Freiburg, November 2003

Preface to the First Edition

All of us use pharmaceuticals for ourselves or for our pets, in husbandry, in agriculture or in aquaculture. But who knows what will happen to the compounds after their administration or use? Are they distributed in the environment or are they eliminated beforehand? What are the possible effects and risks for humans and the environment in connection with the emission of pharmaceuticals into the environment? Pharmaceuticals, diagnostic aids as well as disinfectants used in medicine enter municipal sewage and the aquatic environment. Drugs and growth promoters used in veterinary medicine and husbandry are excreted by animals and emitted into soil via manure or can be part of the runoff from soils after heavy rain fall, which then passes into surface water. Drugs used in aquaculture are passed directly into surface water. Some, such as X-ray contrast media, are excreted completely unchanged, while others are metabolised either into metabolites, which are still active or inactive metabolites. Outdated medications or their remnants are sometimes disposed of down household drains or as (household) waste. The fate, occurrence and effects of pharmaceuticals in the aquatic and terrestrial environment is still mainly unknown.

The disposal of pharmaceuticals in the environment means that a huge number of different substances in different amounts, products and modes of action have to be considered. Therefore, it is difficult to obtain an appropriate overview on the ongoing research. It is even more difficult to identify the most important questions for a systematic approach. The information available is still scarce and not sufficient for sound assessment and decision-making. For this reason, the European Science Foundation (ESF), located in Strasbourg (France), commissioned the workshop "Pharmaceuticals in the Environment." It was held in July 1999 in Freiburg (Germany). The core of the book consists of issues discussed and explored in depth during this workshop. Some other authors, not present at the workshop, have been added.

The book does not claim to give a complete review of the state of the art related to pharmaceuticals in the environment. There is a lot of literature, symposia, international networking and research organising on EDSs. This is still lacking for pharmaceuticals other than hormones. This book gives a short review of the fate, occurence and effects of pharmaceuticals using examples of some typical compounds to highlight the most important questions and issues related to pharmaceuticals in the environment. Input, occurence, fate and effects as well as the possible risks and their assessment are addressed. The book also gives an introduction to this new field of environmental chemistry, ecotoxicology and environmental hygiene.

This book would not have been realised without the workshop "Pharmaceuticals in the Environment" commissioned by the European Science Foundation (ESF). Dr. A. Moth-

Wiklund and her team from the Life and Environment Standing Commitee (LESC) at the ESF always gave good support whenever necessary. All the participants of the workshop contributed to the lively discussions and the identification of the important questions of research in the future. The contributors to this volume were very patient with the editor. The workshop and the book would have not been realised without the support of the director of the Institute of Environmental Medicine and Hospital Epidemiology at the Freiburg University Hospital, Prof. Dr. med. Franz Daschner, and of all my co-workers in the field of pharmaceuticals in the environment. Tina Kümpel and Birgit Stadel helped with the manuscripts. Dr. Witschel from Springer Verlag (Heidelberg) created the opportunity to publish this book. Special thanks to my wife and my children for their encouragement and their support.

Thank you!

Klaus Kümmerer
Freiburg, January 2001

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Part I
General Aspects

Pharmaceuticals in the Environment – A Brief Summary

K. Kümmerer

1.1 Parent Compounds, Metabolites and Transformation Products

1.1.1 Parent Compounds

Pharmaceuticals and disinfectants are classified according to their purpose (e.g., antibiotics, analgesics, anti-neoplastics, anti-inflammatory substances, antihistamines, X-ray contrast media, surface disinfectants, etc.).

Pharmaceutically active compounds (sometimes called active pharmaceutical ingredients, APIs) are complex molecules with different physicochemical and biological properties and functionalities. They are developed and used because of their more or less specific biological activity and are most notably characterized by their ionic nature. The molecular weights of the chemical molecules range typically from 200 to 1 000 Dalton. Such APIs are called small molecules. These are the ones researched and detected in the environment nowadays.

Some medicines contain molecules on a protein basis ("biopharmaceuticals")[1]. Their environmental relevance is not yet clear and they are not the focus of environmental research and risk management. They are not included in this book. One view is to say they are not of relevance because they are closely related to natural products and are therefore quickly biodegraded or are denatured, i.e., inactivated in the environment. The other view is that naturally occurring compounds are not easily biodegraded in every case, while modified natural compounds are even less so. Furthermore, it is known for example that the prions' protein structures[2] are very stable.

[1] Biopharmaceuticals are medical drugs produced using biotechnology by means other than direct extraction from a native (i.e., non-engineered) biological source. Examples are proteins (including antibodies) and nucleic acids. The first and well known example was recombinant human insulin. Small molecule drugs are not typically regarded as biopharmaceuticals by the industry.

[2] A prion (proteinaceous infectious particle, -on by analogy to virion) is an infectious agent composed only of protein. They cause a number of diseases in a variety of animals and Creutzfeldt-Jakob disease (CJD) in humans. Prions are believed to infect and propagate by refolding abnormally into a structure which is able to convert normal molecules of the protein into the abnormally structured form. This altered structure renders them quite resistant to denaturation by chemical treatments and physical agents (proteases, heat, radiation, and formalin), making disposal and containment of these particles difficult. Prions can be denatured by subjecting them to a temperature of 134 °C in a pressurized steam autoclave (see *http://en.wikipedia.org/wiki/Prion*).

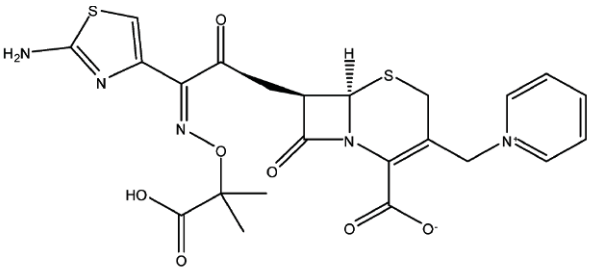
Besides the active substances, formulations may also incorporate adjuvants and in some instances pigments and dyes. They are often of minor importance to the environment. Some medicines contain endocrine disrupting chemicals as adjuvants.

Classification of small molecule APIs according to chemical structure is used mainly for the active substances within subgroups of medicines, e.g.,within the group of antibiotics or the subgroups within the antibiotics such as β-lactams, cephalosporins, penicillins or quinolones. In this case, the compounds can be treated as groups with respect to chemical behavior. Other classifications refer to the mode of action (MOA) e.g., anti-metabolites or alkylating agents within the group of cytotoxics/antineoplastics, the targets or the effects. In the case of classification according to MOA, chemical structures of molecules within the same group can be very different and therefore their environmental fate can vary, too. In this case, compounds cannot be handled as a group with respect to environmental issues.

A closely related chemical structure may be accompanied by an identical or at least a similar mode of action (e.g., β-lactam antibiotics). However, as the example of antineoplastics shows, it might also be very different: alkylating, anti-metabolic, mitosis inhibiting or intercalating substances can but need not necessarily belong to different chemical classes. Compared to most bulk chemicals, pharmaceutically active compounds are often complex molecules with special properties, e.g., dependence of $\log K_{ow}$ on pH (see Fig. 1.1, and Cunningham this book). APIs often have basic or acidic functionalities. Under environmental conditions, molecules can be neutral, cationic, anionic, or zwitterionic. Other compounds are inner salts, and additionally they can form zwitterions (Fig. 1.1). This makes their environmental behavior even more complex. Not only are different pharmaceuticals of special interest with respect to the compounds themselves, but also because of the differences in their occurrence, their fate and their effects on humans or on other target organisms such as bacteria or parasites and on non-target organisms in the environment. This can be illustrated by comparing pharmacology and eco-pharmacology (Table 1.1, Kümmerer and Velo 2006). Eco-pharmacology deals with all aspects of a pharmaceutical within the environment and emphasizes that pharmaceuticals in the environment are an issue for doctors and pharmacists. This concept is much broader than pharmacoenvironmentology, a concept which has only recently been derived from it (Rhaman et al. 2007).

1.1.2 Metabolites and Transformation Products

In recent years, it has been learned that not only the APIs are of importance but also molecules resulting from these through structural change within the human body and within the bodies of treated animals (Golan et al. 2007). The designation and meaning of "metabolite" in publications is somewhat confusing. Some authors mean compounds that result from biochemical transformations of the parent compound. Others mean products of biotic and/or non biotic transformation in sewage treatment or the environment. Therefore, I recommend using the term "metabolite" for the molecules resulting from changes of the chemical structure within the human body. Products resulting from the change of the molecular structure after excretion, i.e., in the environment, should be named "transformation product" including biotic and non biotic processes in technical facilities such as sewage treatment and drinking water



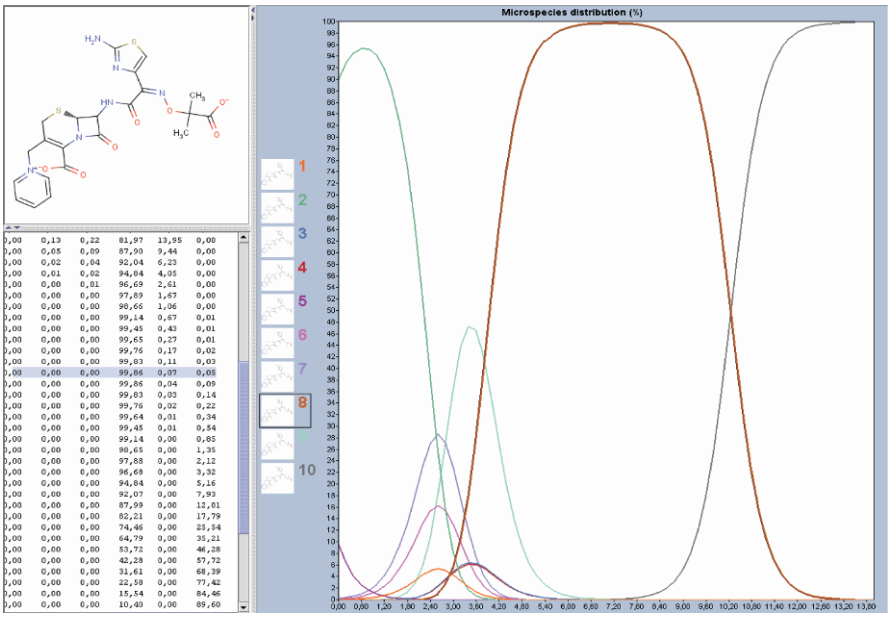


Fig. 1.1 Internal salt and zwitterionic character of ceftazidime (*top*). Depending on pH, different chemical species can be additionally formed by internal protonation, i.e., shift of protons between the basic amino functions and acidic carboxyl groups (*bottom*). (Calculator Plugins were used for structure property prediction and calculation, Marvin 4.1.1, 2006, ChemAxon, *http://www.chemaxon.com*)

treatment plants. Such processes can be specified, for example, as the photo transformation product or transformation product resulting from hydrolysis or oxidation, etc. (Fig. 1.2).

Many pharmaceuticals undergo a structural change in the bodies of humans and animals, respectively. This could be by microorganisms in the gut or by enzymes in human metabolism. The result of such a process is metabolites. Some drugs are largely metabolized before they are excreted, while others are only moderately or poorly metabolized and others yet again, such as contrast media, are excreted completely intact (Golan et al. 2007; Lienert et al. 2007). The resulting metabolites differ in both pharmacological and toxicological properties compared to their parent drugs. Phar-

Table 1.1 Pharmacology and eco-pharmacology

	Pharmacology (humans)	Ecopharmacology (environment)
Number of compounds administered	One or only a few compounds at the same time	An unknown cocktail of different compounds
Desirable physicochemical properties	Stable	Readily (bio) degradable
Administration	Targeted, on demand, controlled	Diffuse, i.e. emissions from medical care units and the community
Wanted effects/side effects	Active, wanted effects, side effects	*Wanted effects* in target organism are often most important „side effects" in the environment
Metabolism/biotransformation/ affected organisms	One type of organism	Various type organisms of different trophic levels

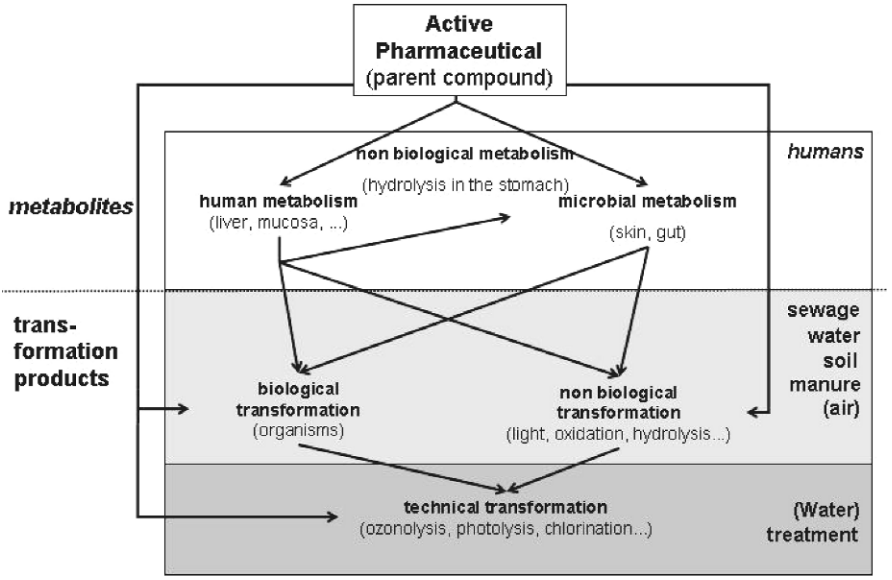


Fig. 1.2. Metabolites and transformation products

maceuticals may be administered orally or intravenously, depending on the compound itself and the medical circumstances. This also has an impact on their metabolism that is their structural change within the body of the target organism (e.g., within humans in the case of pharmaceuticals administered to humans). Metabolism may lower activity and/or enhance water solubility. However, metabolism is frequently incomplete.

Additional molecules may be formed after excretion of parent compounds and metabolites into the environment. Transformation processes can be, for example, hydrolysis and photo-oxidation or biotic ones. Structural transformations may also be a result of effluent treatment (Quiting and Zheng 1998; Ravina et al. 2002; Schröder 2002; Ternes et al. 2003; Zuehlke et al. 2004; Li et al. 2007; Trautwein et al. 2008). Many pharmaceuticals are bio-transformed by organisms such as bacteria and fungi in the environment (Haiß and Kümmerer 2006; Gröning et al. 2007). As in the process of metabolism, the chemical structure of the active molecules is changed by biotransformation, biodegradation, and non biotic transformation that in turn often results in a change in their physicochemical and pharmaceutical properties. Normally it is assumed that metabolism of APIs leads to decreased toxicity. In some cases, however, metabolism leads to more active compounds (e.g., in the case of pro-drugs). Sulfatation as a general metabolism pathway can also indirectly lead to mutagenicity. Bio-transformation and other transformation processes may lower activity or enhance water solubility. Transformation products were detected in degradability testing and in the environment.

1.1.3 Consumption and Use Patterns

There are no data available for the total use of pharmaceuticals. The consumption and application of pharmaceuticals may vary considerably from country to country (Verbrugh and de Neeling 2003; Goossens et al. 2005, 2007). If there are legislative changes imposed on the health system, it might be possible that some compounds would not be used anymore or others would gain more importance, e.g., for economic reasons. According to WHO figures, 0.4% of Japanese women of reproductive age take a contraceptive pill containing ethinyl estradiol as the main active compound, compared to 16% in North America. Some pharmaceuticals are sold over the counter without prescription in some countries, while in others they are only available by prescription. Some antibiotics such as streptomycins are used in fruit agriculture, while others are used in beekeeping. Again, the situation may vary from country to country. The heavy use of streptomycins in fruit agriculture in the US is being discussed as a possible reason for the high resistance of pathogenic bacteria against these compounds. In Germany, use of these antibiotics for this purpose has been banned. Antimicrobials are amongst the most widely used pharmaceutical compounds for animals (Boxall et al. 2003a,b; Sarmah et al. 2006). These drugs are used in animal husbandry for veterinary purposes or as growth promoters (particularly in large-scale animal farming and intensive livestock treatment).

In 2001, about 50 000 different drugs were registered in Germany, 2 700 of which accounted for 90% of the total consumption and which, in turn, contained about 900 different active substances or correspondingly 38 000 t of active compounds (Greiner and Rönnefahrt 2003). Some data for other countries are found in the second edition of this book (see 2nd edition of this book: Alder et al. for Switzerland, Zuccato et al. for Italy, Metcalfe et al. for Canada, Focazcio et al., and Sedlak et al. for the USA; for Austria see Sattelberger 1999, and for Australia see Ongerth and Khan 2004). 6 000–7 000 t a^{-1} of active substances are of potential environmental concern in Germany; that is approximately 0.45 kg per capita and year. Data for Australia are

in the same range according to data presented by Ongarth and Kahn (2004). 110 compounds are used in amounts greater than 5 t per year, which correlates to a specific per capita consumption of 0.06 kg a^{-1} in Germany (23 APIs in Australia, Ongerth and Khan 2004).

1.1.4 Manufacturers

Because of the Good Manufacturing Practice (GMP) regulations required for the manufacturing of pharmaceuticals, the frequently high value of the active substance emissions during manufacturing has been thought to be negligible. Indeed, such emissions are low in Europe and the North Americas. Only recently it has been found, however, that in Asian countries concentrations up to several mg/l can be found in effluents for single compounds (Chap. 3; Larsson et al. 2007; Li et al. 2008). To the author's best knowledge, no data are available on emissions during transport and storage.

1.1.5 Hospitals

Point sources such as hospitals are likely to be only of minor importance (Kümmerer and Henninger 2003; Heberer and Feldmann 2005; Bayerisches Landesamt für Umwelt 2005; Hädrich 2006; Heinzmann et al. 2006; Kümmerer et al. 2008a). Therefore, it is questionable whether separate treatment of hospital effluent is a valid environmental and economical goal.

1.1.6 Private Households

Outdated medicines or their remains are sometimes disposed of down household drains. In accordance with EU legislation, the discarding of unused drugs via household waste has been permitted since 1994 (EG 1993). It is reported that approximately one third of the total volume of pharmaceuticals sold in Germany (Greiner and Rönnefahrt 2003; Rönnefahrt 2005) and about 25% of that sold in Austria (Sattelberger 1999) is disposed of with household waste or down the drain. In an only recently conducted poll it has been found that 17.7% of people asked dispose of remaining and expired pills by pouring them into the toilet, while about 20% of the interviewed people do the same with liquid pharmaceuticals (Götz and Keil 2007; *www.start-project.de*). A survey carried out in the UK investigating the household disposal of unused and expired pharmaceuticals interviewed members of 400 households, predominantly from southeastern England was the basis for a conceptual model to assess the pathways of human pharmaceuticals into the environment. The model demonstrated that disposal of unused pharmaceuticals, either by household waste or via the sink or toilet, may be a prominent route that requires greater attention (Bound and Voulvoulis 2005). More than half of the patients surveyed in a study conducted in the U.S. reported storing unused and expired medications in their homes, and more than half had flushed them down a toilet. Only 22.9% reported returning medication to a pharmacy for disposal. Less than 20% had ever been given advice about medication disposal by a health

care provider (Seehusen and Edwards 2006). In a study performed in Kuwait (Abahussain et al. 2006), almost half of the respondents (45.4%) obtained medicines by prescription more than three times a year, and almost all had unwanted medicines in the home. Reasons for possessing unused medication were mostly due to a change of medication by the doctor (48.9%), or self-discontinuation (25.8%). Their most common method of disposal was to throw unwanted medicines in the trash (76.5%) or flush them down the drain (11.2%). The results of these studies suggest that there is a role for patient education about proper disposal of unused and expired medications in all countries. In some countries, product return systems are in place (see Chap. 29). In the EU and the U.S. (*http://www.whitehousedrugpolicy.gov/drugfact/factsht/proper_disposal.html*), it is legal to throw unused, unneeded or expired drugs in the trash. If the trash is incinerated, this is probably the most effective and environmentally sound way to handle the problem. If the waste is deposited in landfills, it is a bad solution as it is only a postponing of the problem. The APIs will probably show up after some years in the effluent of the landfill (see below). The U.S. FDA advises without an additional explanation that some drugs be flushed down the toilet (!) instead of thrown in the trash (*http://www.whitehousedrugpolicy.gov/drugfact/factsht/proper_disposal.html*), which is surprising because the APIs will directly end up in STPs.

1.1.7 Landfills

If compounds are disposed of with household waste, they end up on landfill sites where they can enter the landfill effluent (see Metzger in the 2nd edition of this book).

1.1.8 Animal Husbandry and Veterinary Medicine

Veterinary drugs and their metabolites are excreted with manure. Farmers use manure and sewage sludge to fertilize fields, thus the drug residues are introduced into the soil. Veterinary pharmaceuticals may reach surface water as run-off from the soil after heavy rain (Kreuzig et al. 2005; Chap. 11). The wash off from topical treatment may enter soil or ambient waters directly. Application of pharmaceuticals in aquaculture results in direct input into water and sediments.

1.2 Occurrence and Fate in the Environment

The input after use of pharmaceuticals, disinfectants, diagnostics and personal care products into the environment is the normal case. They are recognized as being an important part of chemicals that are present in low concentrations in the environment (Schwarzenbach et al. 2007). If the drugs, their metabolites and transformation products are not eliminated during sewage treatment, they may enter the aquatic environment and eventually reach the drinking water supply (Fig. 1.3). The active compounds as well as the expedients may enter the environment by different routes (Fig. 1.3) via several different non-point sources such as effluents of sewage treatment plants (STPs), waste, and landfill effluent or treatment of animals.

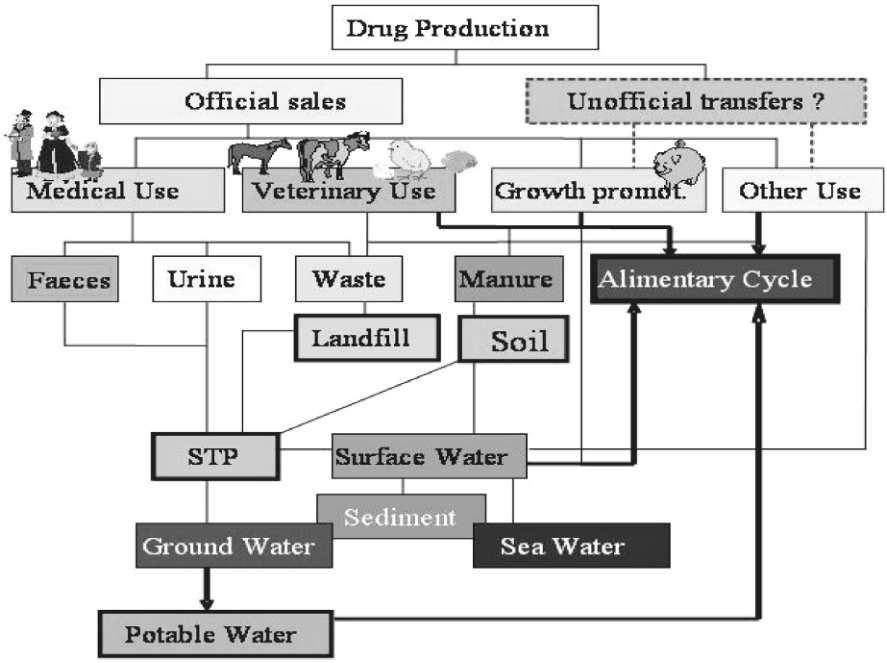


Fig. 1.3. Pathways of input and distribution of pharmaceuticals in the environment

Pharmaceuticals in the environment – namely hormones – first became a focus of scientific interest and public awareness in the 1970s (Tabak et al. 1970; Norpoth et al. 1973). The most frequent conclusion was that these hormones are not easily biodegraded. The subject generated little interest during the 1980s. Some investigations to prove the existence of drugs in the effluent of sewage treatment plants (STPs) were carried out in the mid 1980s, mainly in Great Britain (Richardson and Bowron 1985; Aherne et al. 1990). Other substances of environmental relevance such as heavy metals, polycyclic aromatic hydrocarbons or chlorinated dioxins and furans, as well as pesticides and detergents were the subject of extensive investigation during this period. Awareness of the effects of pharmaceuticals in the environment has grown since the mid 1990s.

At the same time, a discussion started about endocrine disrupting substances (EDS) – sometimes also called endocrine modulating substances – and non hormone pharmaceuticals such as lipid lowering agents (e.g., fibrates), pain killers and other substances (e.g., Stan and Linkerhägner 1992). Since then, quite a lot of activities started for the EDS beginning in the USA. In the case of pharmaceuticals, awareness and research started in Europe. These reports triggered more detailed investigations into the occurrence, fate and effects of pharmaceuticals in the environment in Europe, the USA and Canada. Meanwhile, there is a high awareness of both topics all over the world.

Some of them are used as tracers for anthropogenic impact on waters by hydrologists (Möller et al. 2000; Möller et al. 2002; Elbaz-Poulichet et al. 2002; Verplank et al. 2005; Buerge et al. 2006).

Analysis of pharmaceuticals in the environment nowadays is mostly done by LC-MS/MS, some by GC/MS-MS (Fatta et al. 2007; Perez and Barcelo 2007). Reviews of the present international state of knowledge have recently been compiled by Heberer (2002), Thiele-Bruhn (2003) and Kümmerer (2001a,b; Sarmah et al. 2006; 1st and 2nd edition of this book). Most of the studies conducted up until now describe the occurrence of the compounds in environmental compartments. Medical substances have been measured in the effluent from medical care units and sewage, as well as the effluent of sewage treatment plants, in surface water, groundwater, and in drinking water. Seasonal variations have been studied in sewage and reclaimed wastewater as well as in finished water (Loraine and Pettigrove 2006; Alexy et al. 2006). Pharmaceuticals have also been detected in the effluent from landfills (Holm et al. 1995; Metzger, 2nd edition of this book). Another source is dust (see Chap. 7).

Systematic studies of the occurrence of pharmaceuticals in the environment are now available for several countries (see this book and 2nd edition of this book). Meanwhile, there is evidence of the occurrence of some 160 different drugs in STP effluent, surface water and groundwater. Even in drinking water some APIs have been detected (see first and second edition of this book). They are also detected in the arctic environment (see Chap. 5). Compared to the free water, phase analysis of APIs is difficult in biosolids and sewage sludge, despite the fact that knowledge of pharmaceuticals in sewage sludge and biosolids is necessary for the proper understanding of fate and for risk assessment (Jones-Lepp and Stevens 2007).

The concentrations in surface waters and effluent from STPs have been shown to lie in the ng/l to µg/l range (Sacher et al. 2001; see 2nd edition; various chapters of this book). That is, the findings of recent years have been confirmed for different countries and different environmental compartments.

Drugs applied in veterinary medicine, livestock farming and aquaculture for therapeutic purposes, prophylaxis and prevention, and as growth promoters have been analyzed in manure and soil (see Chap. 7, Chap. 8, 2nd edition of this book). Veterinary pharmaceuticals are introduced into the environment when manure is spread on fields. If not degraded, these antibiotics may end up in the soil or in groundwater. By runoff they may be swept into surface water. Evidence of a wide variety of different active substances in liquid manure and in the soil has been demonstrated. The link between use of human pharmaceuticals and the terrestrial environment is the application of sewage sludge and biosolids in agriculture and for land amendment. The first studies that have investigated this transfer and the associated risks in more detail have been published only recently (Jones-Lepp and Stevens 2007; Picó and Andreu 2007).

Little is known about the occurrence, fate and activity of metabolites. An important question to be addressed is whether the glucoronides, methylates, glycinates, acetylates, and sulfates are still active and whether they can be cleaved by bacteria during sewage treatment and in the environment. This would result in the active compound being set free again. In Germany, for example, 31% of the 2 429 million tons of dry ac-

tivated sludge produced are utilized in agriculture. This application may vary from country to country. If they are not sorbed and biodegraded, the compounds may seep through the soil and enter groundwater. Runoff due to rain after the application of manure to agricultural land seems to be an important factor in the input of veterinary pharmaceuticals into surface waters. A limited number of investigations deal explicitly with the importance of different sources (e.g., Kümmerer 2001a; Kümmerer and Henninger 2003; Kümmerer et al. 2008a).

The predominant fate processes for pharmaceuticals in the different environmental compartments are sorption (e.g., tetracyclines and quinolones) and (bio)degradation. Photodegradation (e.g., quinolones and sulfonamides) and hydrolysis (e.g., for some β-lactams) can also be significant.

1.2.1 Elimination by Adsorption and Complexation

The disappearance of a substance does not indicate biological or photochemical degradation. An important pathway for elimination is sorption of pharmaceuticals. It depends on the extent of neutral and ionic species present and the characteristics of the target particles. Sorption may have an impact on the spread and (bio)availability of pharmaceuticals in the environment (particle bound transport) and their removal during wastewater treatment. Some antibiotics, e.g., tetracyclines, are known to have a tendency to bind to soil particles or to form complexes with the ions present (Marengo et al. 1997; Plate 1991; Rabølle and Spliid 2000; Tolls 2001; Boxall 2002; Thiele-Bruhn 2003; ter Laak et al. 2006a,b). Therefore, the disappearance of a substance does not necessarily indicate biological or photochemical degradation.

The sorption of antibiotics is especially affected by the amount and nature of free and suspended particles in the water phase and SOM (soil organic matter) and soil minerals and distribution coefficients (K_d) (Thiele-Bruhn 2003). Binding to particles or the formation of complexes may cause a loss in detectability, as well as a loss in antibacterial activity. The loss of antibacterial activity, for example, was demonstrated for an aquaculture antimicrobial in seawater driven by the formation of complexes with magnesium and calcium present in marine water (Lunestad and Goksøyr 1990). Tetracyclines are able to form complexes with double cations, such as calcium or magnesium. This finding is not only interesting from a degradation point of view, but it also underlines the problematic nature of applying such potentially inactive antibiotics in aquaculture, and especially in marine fish farming as it clearly shows the necessity of using considerably more antibiotics for treating fish in marine water. The $\log K_{ow}$ is not sufficient for the assessment of sorption and distribution behavior of antibiotics. In general, the sorption behavior of antibiotics depends heavily on the chemical structure of the compounds. In contrast to highly lipophilic "classical" environmental contaminants such as PCBs or chlorinated pesticides such as aldrine, dieldrine or DDT, which are not ionizable, antibiotics are complex chemical molecules which may contain acidic and basic groups within the same molecule (see Fig. 2.1, Cunningham this book). Ionic interactions are possible sorption mechanisms. Therefore, sorption or distribution between two phases such as water and sludge or water and soil ($\log K_D$) depends on pH. This is illustrated for ceftazidime in Fig. 1.1 as an example. Solubility,

hydrophobictiy, distribution, and sorption of ciprofloxacin are pH dependent. Some antibiotics also contain planar aromatic structures, which are favorable for intercalation, for example into the layers of some clay minerals. Therefore, the sorption of such compounds depends not only on the $\log K_{ow}$ that is the lipophilicity of the sorbed molecule but is also governed by pH, redox potential, the stereo chemical structure, and the chemical nature of both the sorbent and the sorbed molecule. This is a fundamentally new aspect compared to most bulk chemicals and to some of the classical environmental contaminants such as polychlorinated biphenyls. The acid constants of ciprofloxacin are 6.16 and 8.63. At a pH of 7.04, the iso-electric point of ciprofloxacin, the molecule carries both a negative and a positive charge, i.e., it is neutral as an entity despite the charges within the molecule. The $\log K_{ow}$ of ciprofloxacin at pH 7.04 is calculated to be about –1.74 and was experimentally determined to be –0.28 (Howard and Meylan 1995). This means the molecule is highly water soluble and only a small proportion should partition into sludge or sediments. Contrary to what would be expected from $\log K_{ow}$ it was found that ciprofloxacin sorbs well onto active sludge or sediments, for example (Wiethan et al. 2000; Golet et al. 2002). Normally this is expected for compounds with a $\log K_{ow}$ above 3 or 4. Some compounds such as quinolones or tetracyclines are eliminated by more than 50% by sorption to sewage sludge. Antibiotics may diffuse into biofilms, present in sewage pipes, sludge flocks or stones in rivers and lakes. This may result in a biased risk estimate, as concentration in such "reservoirs" may be much higher than in the free water phase. The effects and behavior of antibiotics in such biosolids with high bacterial density and special conditions has not yet been investigated. It is not known how strongly the antibiotics are sorbed to sludge, particulate matter, biosolids such as sewage sludge, and sediments, and under what circumstances they are (bio)available and active after sorption. Little is known about the conjugates and other metabolites in this respect.

1.2.2 Biodegradation

Substances reaching the environment may undergo different reactions resulting in partial or complete transformation (see Chap. 1.1.2) and/or degradation (mineralization if the degradation to carbon dioxide, sulfate, nitrate, and other inorganic compounds is complete) of the parent compound. The potential of chemicals to undergo biotransformation is an important aspect of assessing their environmental fate and the risk they present. As outlined above, several different non biotic processes may lead to the removal of chemicals from sewage, surface water, groundwater, and soil. Biologically mediated processes (i.e., biotransformation) can also result in the partial transformation or total mineralization of chemicals in the aquatic environment.

Organic compounds are used by microorganisms for energy and as building blocks for their growth. Some compounds are biodegraded without any energy gain, provided that another compound is available whose biodegradation supplies the necessary energy. Sometimes total degradation does not take place and the process is stopped before mineralization has been completed. These intermediates, i.e., the stable products of biotransformation, can be even more stable than the parent compounds. They often also vary in their toxicity and have a potential for accumulation compared to the parent compound. Bacteria and fungi are the two groups of organisms that are best

able to degrade organic compounds. Fungi are particularly important in soils but do not usually play an important role in the aquatic environment. Therefore, in sewage treatment plants (STPs), surface, ground and seawater bacteria are assumed to be responsible for most biodegradation processes.

Laboratory testing of biodegradation is used for several reasons such as to obtain comparable results within a reasonable period as well as to save cost in the evaluation of the biodegradability of chemicals. The handling that is the pre-adaptation and acclimation of the bacteria often has a significant impact on the test result that is the classification of a chemical. Pre-adapted bacteria normally give better biodegradation results. Since antibiotics are designed to be active against bacteria, this point is of particular importance for the biodegradability testing of antibiotics. Monitoring bacterial toxicity before conducting a biodegradation test is essential if false negative results are to be avoided. The question is which test and which parameters should be used for toxicity assessment. If a compound is toxic against some of the bacteria, a selection of certain bacteria may take place that is in toxicity tests as well as in biodegradability tests. The change of diversity changes within and between bacterial populations in the test system depends on the activity profile of the test compound. Such a change happens within a time scale which is related to the generation time of the bacteria; that is, the selection process is different for every bacterial species. At least the standardized respiration inhibition test failed for some antibiotics (Kümmerer et al. 2004) depending on the test period and the test compound. Halling-Sørensen (2000b) reported difficulties with the nitrification inhibition test. Use of a complex inoculum in the nitrification inhibition test resulted in dose-effect curves that cannot be interpreted. In some cases, enhancement of nitrification occurred, in some there was no effect, while in others the process was inhibited. Conducting the test with a single species resulted in the usual s-shaped dose-effect curves. Monitoring such changes is possible either by counting colony forming units or by other means such as chemotaxonomy, or the use of a toxicity control (Kümmerer et al. 2008b). Different parameters are used for each method by which different groups of bacteria of varying activity are monitored. Therefore, one cannot speak of *the* bacterial toxicity of a compound. The toxic effects have to be specified in relation to the method and the experimental setting used to measure them.

It must be assumed that microbial degradation will be slower in surface water than in the sewage system due to its lower bacterial density and lower diversity. In our own investigations, more than twenty compounds representing the most important groups of antibiotics were found not to be readily biodegradable (Al-Ahmad et al. 1999; Kümmerer et al. 2000; Alexy et al. 2004).

1.3 Effects

1.3.1 Single Compounds

The active ingredients of medications have been selected or designed because of their activity against organisms. Within the last years, effects on other organisms such as fish have been studied. For most human medicines analyzed, acute effects to aquatic

organisms are unlikely, except for spills. Thus it is to be expected that the following properties will be crucial for their environmental impact:

- Effective against bacteria;
- Effective against fungi;
- Effective against (non) target higher organisms;
- Sometimes persistent.

Information available on the effects of the active substances on organisms in the aquatic and terrestrial environment is increasing but still too low (Fent et al. 2006). High concentrations of some compounds, i.e., in the g per liter range have been found to produce acute effects in environmental organisms. In the meantime, effects on *Daphnia*, algae and bacteria have also been demonstrated using low concentrations in chronic tests. Most often these studies covered antibiotics (Holten Lützhöft et al. 1999; Halling-Sørensen 2000a; Backhaus and Grimme 1999; Al-Ahmad et al. 1999; Kümmerer et al. 2000; Boxall et al. 2004). For investigated pharmaceuticals, chronic lowest observed effect concentrations (LOEC) in standard laboratory organisms are about two orders of magnitude higher than maximal concentrations in STP effluents (Fent et al. 2006). For diclofenac, the LOEC for fish toxicity was in the range of wastewater concentrations (Schwaiger et al. 2004; Triebskorn et al. 2004; Triebskorn et al. 2005; Hoeger et al. 2007), whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured STP effluent concentrations (Fent et al. 2006). In surface water, concentrations are lower and so are the environmental risks. However, targeted ecotoxicological studies are lacking almost entirely and such investigations are needed for focusing on subtle environmental effects (Fent et al. 2006).

1.3.2 Mixtures

All risk assessment is based on single compounds. However, it has been found that mixtures might exhibit different effects than single compounds (Silva et al. 2002; Pomati et al. 2007). This new field of ecotoxicology is just at the beginning, and much has to be learned in the future (see Chaps. 16 and 17). Additionally, it has been found that standardized test may underestimate the effects (Kümmerer et al. 2004).

1.3.3 Indirect Effects

It has also been found that detrimental effects may happen if the transfer of compounds occurs within the food web: Between 2000 and 2003, high annual adult and subadult mortality (5–86%) of the oriental white-backed vulture and resulting population declines (34–95%) were associated with renal failure and visceral gout. A direct correlation of residues of the anti-inflammatory drug diclofenac with renal failure was found. Diclofenac residues and renal disease were reproduced experimentally in oriental white-backed vultures by direct oral exposure and through feeding vultures diclofenac-treated livestock (Oaks et al. 2004). Evidence from studies of one of these species strongly implicates mortality caused by ingestion of residues of the veterinary non-

steroidal anti-inflammatory drug diclofenac as the major cause of the decline. Other findings show that veterinary use of diclofenac is likely to have been the major cause of the rapid vulture population declines across the subcontinent (Swan et al. 2006; Taggart et al. 2007).

Another example of indirect effects of antibiotics was reported by Hahn and Schulz (2007). Results of food selection experiments with *Gammarus pulex* demonstrated clear preferences for leaves conditioned in the absence vs. those conditioned in the presence of two antibiotics, oxytetracycline and sulfadiazine.

1.4 Risk Assessment

The risk of adverse effects on humans through ingestion of pharmaceuticals contained in drinking water seems to be negligible. Thus, the risks posed to humans from pharmaceuticals in the environment seem to concern environmental hygiene rather than toxicology and pharmacology. The maximum possible intake within a normal lifespan (2 liters drinking water per day over 70 years) is far below the dosages used in therapy (Christensen 1998; Kümmerer and Al-Ahmad 1998). However, this statement relies on some assumptions: (*i*) that effects and side effects during therapeutic use (short term, high dosage) are the same in quality and quantity as during a life-long ingestion (long-term ingestion, low dosage), (*ii*) that the effects are the same for fetuses, babies, children, healthy adults and elderly people, (*iii*) that the risk imposed by a single compound is comparable to the one imposed by a mixture. As for the last point, it has been found that elderly people who take in several different medications at a time suffer more often from unwanted side effects of drugs during therapy. How to extrapolate data from high dose short-term ingestion during therapy to low dose long-term ingestion, i.e., "medication" via drinking water, is still an unresolved issue in toxicology and in ecotoxicology.

Publication of the EU guideline on environmental risk assessment for human pharmaceuticals was seen in 2006 (see Olejniczak and Spindler, 2nd edition; Chap. 20 of this book). The shortcomings and experiences from application in practice are addressed in this book by Knacker et al. (Chap. 23), Metcalfe et al. (Chap. 11) and Montforts (Chap. 24).

Additionally, in general the possible effects of only a few compounds have been studied until now. Data enabling a sound assessment for metabolites and transformation products are missing. Furthermore, up to now, risk assessments have been undertaken for single substances only and not for mixtures (see Chaps. 16 and 17). Some of the APIs have carcinogenic, mutagenic or reproductive toxic effects ("CMR-compounds"). It is unclear whether such compounds should be treated as "however"-compounds (see Chap. 20).

Besides toxicity, the property of persistence is of particular importance for the assessment of the environmental significance of substances. Persistent organic pollutants (POPs)[3] increase the potential for long-term and hence varied effects, and the

[3] The Stockholm Convention is a global treaty to protect human health and the environment from persistent organic pollutants (POPs). POPs are chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife. POPs circulate globally and can cause damage wherever they travel. In implementing the Convention, Governments will take measures to eliminate or reduce the release of POPs into the environment (*http://www.pops.int/*).

longer the exposure lasts, the higher the risk for multiple contamination of the ecosystem. This cannot be tested in advance with the presently available test systems (Cairns and Mount 1992). Standard tests are often used for effect assessment and biodegradability testing (e.g., according to OECD series 200 and 300) developed for bulk chemicals. It is unclear to what extent the test systems have to be modified to obtain reliable results (Kümmerer et al. 2004).

1.5 Risk Management

Pharmaceuticals are ubiquitous water and soil contaminants that may have subtle detrimental effects on aquatic organisms, and possibly also on human health. The risks of pharmaceuticals, or pharmaceutically active compounds, remain poorly understood. Awareness of the presence of pharmaceuticals in the environment, coupled with evidence of effects, however, suggest that precautionary management action to reduce the release of pharmaceuticals into the environment should be considered.

Combinations of management strategies will likely be most effective in mitigating the risks presented by pharmaceuticals. In a study, the scope of the issue and possible management strategies were examined from the perspectives of expert stakeholders, drawn from government, academia, and the pharmaceutical and consulting industries involved in scientific research or policy and management activity from Canada, the United States, and Europe (Doerr-MacEwen and Haight 2006). Twenty-seven interviewees were asked about their views on management strategies such as pharmaceutical-return programs, advanced effluent treatment and incentives for the development of "green" pharmaceuticals. Interviewees believed that advanced wastewater treatment technology, education of medical professionals to reduce over prescription, pharmaceutical-return programs coupled with public education, and requirements for all municipalities to have a minimum of secondary wastewater treatment were the most effective management strategies to reduce the environmental impacts of pharmaceuticals.

Some of the aspects of the measures and activities which should be taken have already been discussed above; others are discussed within specific chapters in this book.

1.6 Conclusion

It should be pointed out that although new and essential knowledge has been published since the second edition of this book, data are still too scarce to allow us to undertake a complete and sound risk assessment and proper risk management. There is still an urgent need to close the gaps in our knowledge.

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Special Characteristics of Pharmaceuticals Related to Environmental Fate

V. L. Cunningham

2.1 Introduction

An important consideration when assessing the environmental fate of pharmaceutical compounds is that, as a class, they generally possess characteristics that make them different than conventional industrial chemical pollutants. Some of these attributes include the following:

- Tendency of the parent neutral compound and associated salts to form polymorphic solid states;
- Introduced into the environment subsequent to human metabolism;
- Large, chemically complex molecular structure;
- Generally ionizable with multiple ionization sites spread throughout the molecule.

These characteristics need to be kept in mind when establishing the environmental form to be evaluated (parent, metabolite(s), other), designing fate and effects testing strategies, interpreting test results, predicting environmental fate, and carrying out environmental risk assessments.

2.2 Solid State Chemistry of Pharmaceuticals

Many active pharmaceutical ingredients exist as solid forms, often as salts, with a tendency to form polymorphs. Polymorphs arise when molecules of a compound stack in the solid state in distinct ways. Although identical in chemical composition, polymorphs can have very different properties. They are distinguishable by various analytical techniques, especially X-ray powder diffraction. In addition, solids may form solvates and hydrates, also called pseudopolymorphs. Polymorphs, including pseudopolymorphs, usually differ in bioavailability, solubility, dissolution rate, chemical and physical stability, melting point, color, filterability, density and flow properties, etc. They also tend to convert from less stable to more stable forms with the rate of conversion depending on the required activation energies. Experimental difficulties may therefore be encountered because of these different physical chemical properties, and attempts to correlate pharmaceutical experimental results with relationships derived from less complex solid materials may lead to erroneous conclusions. Similarly, active pharmaceutical ingredients may be prepared as various salt forms. These salt forms, too, may vary significantly in physical chemical properties, particularly water solubility (Haleblian 1975; Bryn 1982).

Because active pharmaceutical ingredients do exist in these different solid state forms (polymorphic and pseudopolymorphic crystalline and salt forms), and these forms possess different physical chemical properties, the methodology commonly used in water solubility experiments may be inappropriate. Data from these studies, besides being difficult to interpret, are sometimes equivocal. An example of the variance in water solubility of various polymorphic and salt forms of rifampicin is shown as an example in Table 2.1.

As these data show, the water solubility of rifampicin varies by a factor of 8 (or almost an order of magnitude) depending on the crystalline state of the material. Using an experimentally determined water solubility value in environmental modeling may result in errors if the water solubility does not actually represent a fundamental property of the molecule but rather a particular solid form. In addition, none of the data above necessarily indicate the actual solubility of the parent compound in water at environmental pH levels. Use of any of these data to predict fate or transport may lead to significant error. The solubility of a given solute in a particular solvent (in this case water) is the concentration of the saturated solution at equilibrium with the solid. In cases where the original crystalline solid cannot reform under equilibrium conditions (e.g., for hydrates: anhydrous forms or other solvates), or where the original polymorph is not the thermodynamically stable form, experimental anomalies may be encountered. From a practical standpoint, the use of the simplest salt form and the most thermodynamically stable polymorph should be considered for water solubility studies. These issues may also affect testing strategies for environmental effects, where solubility constraints imposed by the use of particular solid forms may underestimate potential toxicity.

2.3 Metabolism

Since active pharmaceutical ingredients are generally ingested, they may be extensively metabolized. Some consideration must therefore be given to the actual compound(s) excreted into wastewater treatment plants (WWTPs) and ultimately released into the environment. Studies on parent compounds may not adequately address the chemical, physicochemical, pharmacological or toxicological differences associated with

Table 2.1 Solubility of rifampicin crystal forms in water at 30 °C (Henwood et al. 2001)

Crystal form	Solubility (mg ml^{-1})
Amorph II	0.195
Acetone solvate	0.732
Monohydrate	0.874
Amorph I	0.897
Dihydrate	0.982
Form II	1.472
2-pyrrolidone solvate	1.576

these metabolites. In order to carry out a relevant environmental risk assessment, it is important that the chemical species that is likely to enter the environment be identified. Biodegradability studies simulating WWTPs may be very useful in determining the species entering the environment after biotransformation or biodegradation through microbial metabolism of the substrate or other mechanisms. However, it is also critical to identify the compound entering the WWTP from human use. This implies that human metabolism must be considered in determining the appropriate compound for fate (and effects) testing.

Drug metabolism refers to chemical alterations of a drug in vivo. For the purposes of this report, focus is on the metabolism of active pharmaceutical ingredients by humans. In general, active pharmaceutical ingredients are metabolized and form more polar and water-soluble derivatives that have reduced pharmacological activity compared to the active pharmaceutical ingredient and are rapidly excreted. In some cases, however, the administered compound may be a pro-drug, and be first metabolized to the active pharmaceutical ingredient before being further metabolized to less active forms.

Drugs are metabolized by a variety of mechanisms. Reactions such as oxidations, reductions and hydrolyses are referred to as Phase I reactions. Conjugations, considered as Phase II reactions, are a subset of drug metabolism mechanisms. They may occur when the drug contains a group, usually OH, COOH, NH_2 or SH, which is suitable for combining with a natural compound provided by the body to form readily excreted water-soluble polar metabolites (La Du et al. 1979). Because of the general availability of glucose in biological systems, glucuronide formation is one of the more common routes of drug metabolism and quantitatively may account for a major share of metabolites. The reaction involves the condensation of the drug or its biotransformation product with D-glucuronic acid. Several types of drugs tend to form glucuronides, including alcohols, phenols, carboxylic acids, amines and certain thiols as well as normally occurring substrates such as steroids. In general, glucuronide formation diminishes the biological and pharmacological activity of a drug. Conjugates may also be formed with sulfate (La Du et al.1979; Ballie 2002).

Administered parent compound may be excreted:

- Unchanged;
- As a glucuronide or sulfate conjugate;
- As a "major" metabolite;
- As a complex mixture of many metabolites.

Where the parent active compound is excreted essentially unchanged, the test scenario is straightforward and the degradation of the parent compound in the environment is measured. However, where the parent undergoes significant biotransformation (drug metabolism) in the patient prior to excretion, judgment must be exercised as to the appropriate chemical species for testing. In general, metabolism may occur in several ways, including relatively simple conjugation to more polar glucuronides or sulfates, transformation to structurally similar metabolites which may have partial activity or be inactive, or transformation to a number of structurally related as well as unrelated species.

There is evidence that glucuronides, which are the simplest and most common form of conjugated pharmaceutical compounds excreted by humans, are capable of being deconjugated to the parent compound during municipal sewage treatment. The glucuronidase enzyme is present to such an extent in the fecal coliform bacteria that are so prevalent in WWTPs that its occurrence can be used as in indicator of fecal coliforms in environmental waters. Published research has demonstrated such de-conjugation in estrogenic compounds (Kozak 2001). Therefore, in the case where glucuronides are the primary drug metabolite, they should be considered to be the same as the parent compound and the environmental fate and effects of the parent compound should be investigated. For structurally related major metabolites, decisions as to testing strategies may depend on the characteristics of the parent compound itself. Where testing has been carried out on the parent, and where data support the position that the parent will be significantly degraded or biotransformed (and deactivated) in a WWTP, the environmental fate of the structurally related major metabolite may be deduced by analogy. However, where the parent is recalcitrant, and where the major metabolite represents a large percentage of the drug dose, separate testing on the metabolite may be prudent. Other cases may need to be decided on a case by case basis.

2.4 Molecular Structure

In general, pharmaceuticals are comparatively large and chemically complex molecules. When considering the issue of pharmaceuticals in the environment, popular articles, and even some scientific reports, appear to refer to pharmaceuticals as a class, as if they were a somewhat homogeneous group of compounds with the characteristic of pharmacological activity. Perhaps this tendency comes about because of other well-known classes of pollutants, such as PAHs (polycyclic aromatic hydrocarbons) or dioxins, where different members of the class differ primarily by number, type and position of substitutents. For example, "dioxins" as a class are all variations on the basic dioxin backbone (Fig. 2.1).

In contrast, pharmaceuticals do not represent any sort of homogenous group of compounds. They vary widely, in molecular weight, structure, functionality(ies), salt forms, polymorphs, etc. Some examples of typical pharmaceuticals are shown in Fig. 2.2a,b. Even a cursory look will show some of the diversity of these compounds.

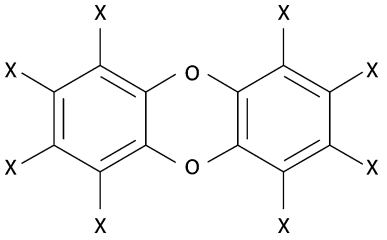
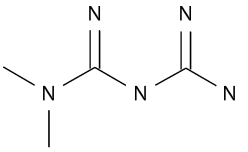
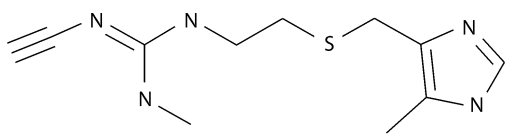


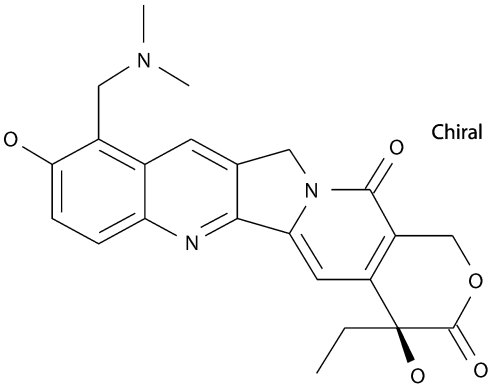
Fig. 2.1. "Dioxin"



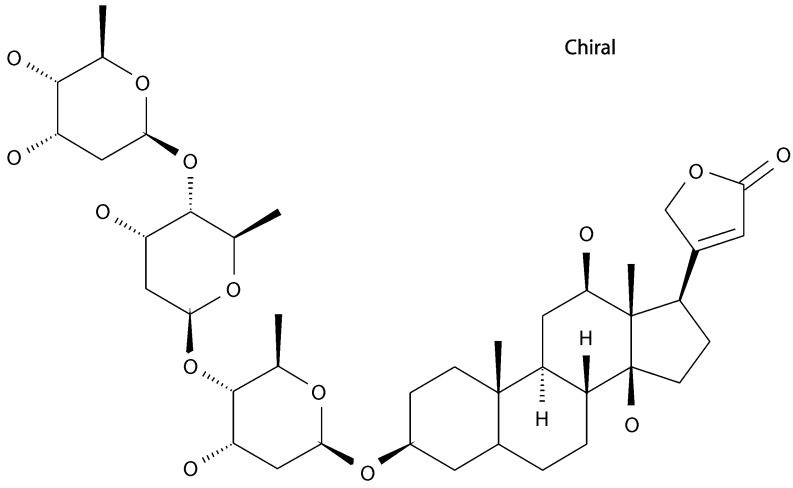
Metformin (MW = 129.17)



Cimetidine (MW = 252.34)



Topotecan (MW = 421.46)



Digoxin (MW = 780.95)

Fig. 2.2. Structures of selected pharmaceuticals (metformin, cimetidine, topodecan, digoxin)

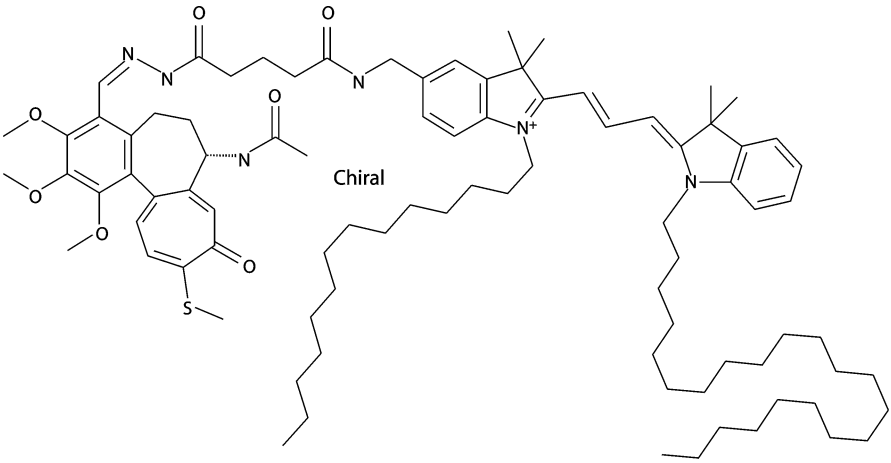


Fig. 2.2b. Structures of selected pharmaceuticals (cyclosporin)

2.5 Ionization

As comparatively large and chemically complex molecules, the heteroatom content and multifunctional composition of pharmaceuticals makes them, among other things, polar, ionizable molecules, and these properties are arguably profoundly dependent upon and influenced by solution pH. Mathematical relationships and models based on smaller, neutral, hydrophobic molecules may not adequately describe the environmental partitioning and chemical equilibria or dynamics of pharmaceuticals (Lyman 1990; Karcher 1990). In particular, the octanol/water distribution coefficient (D_{ow}) and the octanol/water partition coefficient (K_{ow}) (derived by correction for ionization) need to be carefully evaluated with regard to multiple ionization sites. Models based on LogK_{ow} may not adequately describe the chemical equilibrium (a) or dynamics of these compounds. In addition, even the use of LogD_{ow} may lead to errors in calculating or estimating partitioning to solids such as biomass, soils, and sediments. The conventionally modeled mechanism, sorption to organic matter in the solid state, ignores the fact that an active pharmaceutical ingredient may possess a charge state(s) that leads to more complex ionic, ion pairing or complexation mechanisms. These additional mechanisms are simply not accounted for in a simple non-polar partitioning model.

2.5.1 Dissociation Constant

The dissociation constant is an equilibrium constant that describes the degree of ionization of a compound at a known pH. Chemically, it is represented as follows:

$$HA \longleftrightarrow H^+ + A^-$$

$$K_a = [H^+][A^-]/[HA]$$

When $K_a = [H^+]$, one half of the compound will be dissociated, and at this value, the pK_a (the negative logarithm of K_a) is termed the acid dissociation constant. The analogous constant, the base dissociation constant, refers to the dissociation of the conjugate acid of a base. The base association constant pK_b is 14 – pK_a. Stated differently, when the pH of the system is equal to the pK_a, there will be a 50:50 mixture of ionized and un-ionized species present in solution. If, as is characteristic of many active pharmaceutical ingredients, there is more than one ionizable functional group, then several equilibrium constants can be written for the compound, and each must be considered separately. The degree of ionization is therefore controlled by the pH of the solution containing the compound, and the ionized and un-ionized species may have very different properties. The significance of the dissociation constant is this relationship between pK_a and pH and the resulting distribution of the drug substance in the environment. The degree of ionization of the drug substance at a particular pH will affect its availability to biological organisms, its chemical and physical reactivity, and its ultimate environmental fate. For example, an ionized molecule will generally have greater water solubility and will be less likely to partition to lipid-like substances than its non-ionized form. Ionic charge will also affect the potential of a molecule to participate in environmental ion exchange processes that are ubiquitous in soil and sludge systems. Knowledge of the pK_a will assist experimentalists in their design of appropriate sorption and ecotoxicity studies and in accurately interpreting the results from these studies.

2.5.2 Octanol/Water Distribution Coefficient

The octanol/water distribution coefficient (D_{ow}) has historically been used in environmental assessments to estimate other parameters, such as water solubility, soil/sediment adsorption coefficients, and bioconcentration factors for aquatic life. Many other relationships have been derived to estimate other parameters as well.
The octanol/water distribution coefficient (D_{ow}) is defined as the ratio of the concentration of a chemical in two phases, *n*-octanol (a surrogate for lipids) and water, when the phases are in equilibrium with one another and the test chemical is in dilute solution in both phases. The *n*-octanol water distribution coefficient indicates the tendency of an organic chemical to

- Partition into lipids or fats;
- Sorb to particulates such as soils or sediments;
- Sorb to biomass and sludges;
- Distribute among the various environmental compartments.

The *n*-octanol/water distribution coefficient is given by

D_{ow} = (concentration in *n*-octanol)/(concentration in water)

$$\mathrm{Log}D_{ow} = \log_{10}(D_{ow})$$

It can also be used to predict the bioconcentration potential in aquatic and terrestrial organisms and to estimate the amount of sorption to soils, sediments, biomass and sludges. These processes are major factors in determining the movement of chemicals in the biosphere. However, in most cases, these relationships were derived from and applied to neutral industrial chemicals and pesticides. They do not appear to be as applicable to pharmaceutical compounds, which are primarily large, complex, multifunctional organic compounds that are ionized in the aquatic environment at environmentally relevant pH levels. To understand appropriate areas of applicability, a fundamental understanding of the aqueous chemistry of the compound in question is necessary. The relationship becomes more complex with ionizable compounds since the un-ionized species will be the predominant species to partition into octanol from water, with the ionized species predominantly remaining in the aqueous phase. Therefore the pH at which measurements are made is important since this governs the degree of ionization. For ionizable compounds, D_{ow} is usually determined at pH values of 5, 7, and 9. For environmental risk assessments, the value at pH 7 is usually used.

LogD_{ow} values less than one indicate that a chemical is unlikely to significantly bioconcentrate or sorb onto organic matter. LogD_{ow} values equal to or greater than three indicate that the chemical may bioconcentrate or sorb significantly. The n-octanol/water distribution coefficient (D_{ow}) may be corrected for the ionization of the compound so that only the concentration of the un-ionized species is considered. The n-octanol/water partition coefficient is given by (Hansch 1995)

$$K_{ow} = D_{ow}(1 + 10(\mathrm{abs}(\mathrm{pH} - \mathrm{p}K_a))$$

$$\mathrm{Log}K_{ow} = \mathrm{Log}_{10}(K_{ow})$$

LogK_{ow} is often represented as Log$P$. For non-ionizable chemicals, Log$P$ = LogD_{ow}. When using this conversion for large, ionizable chemicals such as pharmaceuticals, Log$P$ discounts the often-significant solubility of the ionized species in the octanol phase. For these molecules, use of LogD_{ow} is preferred.

When referring to information in the literature on the use of Log$P$ or LogK_{ow}, care should be taken to determine whether the reported value is the corrected or uncorrected value. Use of the corrected value for an ionizable compound will result in values that represent only the un-ionized species and overestimate the hydrophobicity of the compounds, and hence their potential bioaccumulation potential. Also, many ecotoxicity models use LogK_{ow}, which may over-predict toxicity for ionizable compounds. Note: Many computer programs which are used to generate logK^{ow} values, calculate only the un-ionized species and should be corrected to the logD^{ow} before data assessment, analysis and use in environmental risk assessment models.

2.5.3 Sludge Sorption/Desorption ($K_{biomass}$ or K_p)

Since many organic chemicals are treated in wastewater treatment plants, the tendency of the chemical to sorb to the biosolids is an important factor to be evaluated. The

biosolids/water distribution coefficient, $K_{biomass}$ or $K_{p,}$ is the ratio of the concentration of a chemical in two phases, biosolids and water, when the solid phase is biomass and the phases are in equilibrium with one another and the test chemical is in dilute solution in both phases. Biomass or sludge adsorption studies are generally run at a biomass concentration in the range of a few grams per liter. This approximates the aeration basin biomass concentrations typically found in wastewater treatment plants.

The ability to estimate the sorption of a pharmaceutical to solids in various media is critical to understanding its environmental fate. Unfortunately, many of the methodologies and relationships suggested for determining this important parameter were, like Log*P* discussed above, derived from studies with neutral, hydrophobic compounds such as pesticides and industrial chemicals. For these classes of compounds, the primary driver for partitioning behavior is its hydrophobicity, or lipophilicity, and most of the relationships explicitly relate the distribution coefficient to the organic carbon content of the solid (Environmental Assessment Technical Assistance Document 1987), for example, for soils, a K_d is defined as

$$K_d \quad \frac{\text{chemical sorbed}}{\text{chemical in solution at equilibrium}}$$

and another coefficient, K_{oc} as

$$K_{oc} \quad \frac{K_d}{\%\ \text{organic carbon}}$$

or

$$K_{oc} = \frac{\mu\text{g chemical sorbed / g soil / g soil organic carbon}}{\text{mg chemical dissolved at equilibrium / g solution}}$$

The assumption is that the partitioning of the chemical will be essentially solely into the organic fraction of the solid. While this assumption appears useful when dealing with neutral, hydrophobic compounds, for large, multi-functional ionic compounds such as many pharmaceuticals, the partitioning behavior is more complex. In addition, the terrestrial compartment is not the typical site of release for human pharmaceuticals into the environment. Rather, the introduction of most human pharmaceuticals from use is through WWTPs.

For biosolids such as WWTP sludges, models (Barton 1991) have been reported that allow sludge/water distribution coefficients to be estimated from octanol/water partition coefficients. For example, using linear regression of experimental values, Barton (1991) reported

$$\text{Log}K_{oc} = 1.00\text{Log}K_{ow} - 3.21$$

where K_{oc} is the partition coefficient based on fractional organic carbon content of the solids (l/g) and K_{ow} is the octanol/water partition coefficient of the compound. The biosolids partition coefficient, K_p was then determined by

$$K_p = f_{oc} K_{oc}$$

where f_{oc} is the fraction of organic carbon in the solids (g/g). A value of 0.531 for f_{oc} has been suggested. However, as noted above, the relationships, and the utility of the relationships are in general not applicable to polar, ionized pharmaceuticals. This is primarily related to the fact that the relationships are based on the assumption that the primary mechanism for sorption is hydrophobic interaction, and hence dependent on the amount of organic carbon present in the solid. A recent review (Tolls 2001) of the sorption of veterinary pharmaceuticals to soil reported that prediction of K_{oc} by LogK_{ow} leads to significant underestimation of K_{oc} and K_d. The authors hypothesize that a number of hydrophobicity independent mechanisms such as cation exchange, cation bridging, surface complexation and hydrogen bonding appear to be involved (certainly for ionized compounds, direct ionic interaction or even ion-pairing may be operative). They conclude that use of organic carbon normalization is conceptually inappropriate for pharmaceutical compounds and fails to describe sorption behavior accurately.

Many of the conventional partitioning models are based on relatively simple, neutral, and often hydrophobic molecules. These models generally are not suited for predicting environmental partitioning behavior of APIs, which are often large, complex, multifunctional, ionizable organic compounds. For this reason, a series of regression equations were developed to estimate the sludge/water partition coefficient, K_p, from the log of the octanol-water distribution coefficient (LogD_{ow}) for different chemical classes of APIs. These regression equations were developed from experimentally determined K_p and LogD_{ow} values for 17 APIs. The data were separated into subsets based on chemical functionality, and empirical relationships were derived for each subset. The chemical functionalities examined were: acidic, doubly acidic, basic, and zwitterionic. Although the data set is very limited, it is suggestive that this approach may be useful in deriving useful models for estimating K_p. A two data point regression (first regression in Table 2.2) leaves much to be desired, but it illustrates that the relationships are different for the different functional types, and that for estimating the K_p, the appropriate equation should be used.

The regression equations derived for these relationships are summarized in Table 2.2).

Table 2.2. Regression equations for LogK_p and LogD_{ow} for different functionalities

Functionality	Regression equation	*n*	R^2
Acidic	$\text{Log}\,K_p = 0.46 \log D_{ow} + 0.42$	3	0.94
Doubly acidic	$\text{Log}\,K_p = 0.32 \log D_{ow} + 1.70$	3	0.94
Basic	$\text{Log}\,K_p = 0.56 \log D_{ow} + 2.2$	8	0.90
Zwitterionic	$\text{Log}\,K_p = 0.58 \log D_{ow} - 0.37$	3	0.89

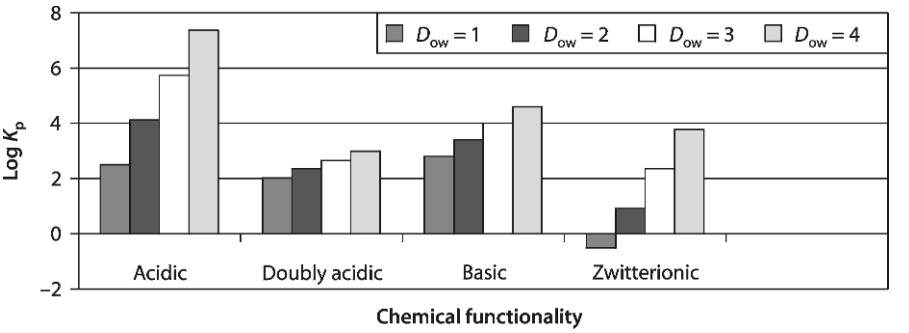


Fig. 2.3. Dependence of LogK_p on LogD_{ow} and chemical functionality

For neutral compounds, the LogK_p = LogD_{ow}, by definition. A graphical representation of the dependence of LogK_p on LogD_{ow} and chemical functionality is shown in Fig. 2.3.

These results suggest that great care must be taken in applying environmental fate models derived from neutral hydrophobic compounds to ionizable, hydrophilic APIs. These preliminary correlations also suggest that molecular descriptors such as chemical functionality may be useful in addition to LogD_{ow} when deriving more accurate predictive models. Additional experimental LogK_p and LogD_{ow} data are needed to further refine this hypothesis and develop models more applicable to this class of compounds.

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Part II

Sources, Occurrence and Fate

Drug Production Facilities – An Overlooked Discharge Source for Pharmaceuticals to the Environment

D. G. J. Larsson

3.1 Introduction

Until 2005 there were at least 255 scientific articles published, reporting the detection of human pharmaceutical residues in the environment, according to a recent survey (Williams and Cook 2007). None of these studies have explicitly investigated whether drug manufacturers could be significant point sources for pharmaceuticals to the environment[1]. A reasonable way to interpret the lack of incitement to perform such studies is that there has been a rather widespread consensus that the direct contribution from pharmaceutical production facilities is relatively unimportant.

The production of pharmaceuticals involves a series of steps, often involving several different companies and production sites, in order to produce a finished pharmaceutical product. Production could be said to begin when the raw materials are gathered and transported. This can be oil, minerals etc., which are required in order to synthesize the small inorganic or organic molecules that are common building blocks in the synthesis of intermediates and finally active ingredients. Often, the production facilities for these raw and intermediate materials are separate from the sites where the actual production of the active pharmaceutical ingredients (APIs) occurs. The APIs may be assembled into pills at special formulation plants, with packaging taking place at yet other sites. At each stage in the production process, as with most types of chemical production, there is a risk that chemical waste enters the environment unless appropriate measures are taken. This paper will only deal with the potential release of APIs and not with other chemicals.

Production facilities need to follow environmental standards. To what degree emissions are regulated varies between countries and sites. However, the release of the APIs themselves is rarely regulated. For the direct discharges of treated liquid waste streams to surface waters or indirect discharges to municipal sewage treatment sites, compliance limits often regulate concentrations of organic solvents, common water quality parameters such as pH, biological oxygen demand, chemical oxygen demand, suspended solids, total organic carbon, nitrates, phosphates, some heavy metals and at times a measure of acute toxicity (*www.eper.cec.eu.int*; *www.epa.ie*). This basic level of control stands in contrast to the strong focus on the environmental fate and toxicological effects of the APIs post consumer use (USFDA 1998; EMEA 2006).

[1] Refers to the papers included in the review. One of these papers reported that the investigated sewage treatment works also received waste from drug manufacturers, but it was not clear if the manufacturers produced any of the five drugs that were analyzed in the effluents (Soulet et al. 2002).

3.2 Regional Perspectives

The majority of the analyses of pharmaceutical residues in the environment have been performed on samples from Europe and North America. Indeed, Europe and the USA dominate the world sales market, suggesting that these regions would have the highest loads of drugs entering the environment via municipal sewage effluents. On the other hand, sewage treatment is often more advanced in Western countries, which could compensate for their larger use of medicines, at least for those drugs that are removed in sewage treatment works to some extent. Actual detection data of APIs in sewage effluents and waterways from Asia, Africa and South America are still so limited that the extent of the release of drugs is difficult to estimate in these regions. If we turn to production, the situation is rather different. While Asia, Africa and Australia together only stood for 8% of the global pharmaceutical market in 2004 (Green 2006), India and China alone accounted for 42.4% of the world's generic API production (CPA 2006). This figure had increased to 44.6% in 2005 and is estimated to reach 60% by 2010 (CPA 2006). In Europe, Italy and Spain are the largest producers of APIs, although their share of the global API production is rapidly decreasing (CPA 2006). Thus, if production plants are indeed important point sources for pharmaceuticals to the environment, it could be argued that countries like India or China would be at highest risk. However, with the sparse data available today, we can not exclude any country from the possibility that some of their production facilities release significant amounts of APIs.

3.3 Major Release of Drugs from Indian Manufacturers

In a recently published study, we investigated the potential release of fifty-nine APIs in the effluent from a common effluent treatment plant receiving process water from about 90 bulk drug industries near Hyderabad, India (Larsson et al. 2007). Twenty-three drugs were found at concentrations above 1 $\mu g\ l^{-1}$ in the treated effluent. Eleven of these exceeded 100 $\mu g\ l^{-1}$, with ciprofloxacin levels reaching 31 $mg\ l^{-1}$ (Larsson et al. 2007; Table 3.1). To the best of our knowledge, these levels exceed by far all reported levels of APIs in municipal effluents. All six analyzed fluoroquinolones were present at levels known to be toxic to microorganisms. The total amount of ciprofloxacin released during one day was estimated at 45 kilograms, which equals the total use in Sweden (population 9 million) over a five-day period.

The release of such high levels of antibiotics from production plants is problematic in at least three ways. First of all, the antibiotics may impair the general efficiency of effluent treatment by killing microorganisms or preventing them from growing. Secondly, the antibiotics are very likely to affect the local environment, including microbial ecosystems, downstream from the treatment plant. Thirdly, and perhaps most importantly, the release raises serious concerns about an accelerated rate of microbial resistance development. At the investigated treatment plant near Hyderabad, raw human sewage (inevitably containing human pathogens) is added at approximately 20% of the total load to sustain the microbial populations within the treatment plant. Also, some of the generated sludge is recirculated. Adapting microorganisms within a treat-

Table 3.1. Top eleven active pharmaceutical ingredients analysed in effluent samples from PETL, a common effluent treatment plant near Hyderabad serving about 90 bulk drug manufacturers. Drugs were analysed using LC-MS/MS monitoring at least two specific fragment ions per substance when possible and quantified using a four-point calibration. Data from two samples taken on consecutive days are presented (reprinted from Larsson et al. 2007, with permission from Elsevier)

Active ingredient	Type of drug	Range (µg l^{-1})
Ciprofloxacin	Antibiotic-fluoroquinolone	28 000–31 000
Losartan	Angiotensin II receptor antagonist	2 400– 2 500
Cetirizine	H_1-receptor antagonist	1 300– 1 400
Metoprolol	β_1-adrenoreceptor antagonist	800– 950
Enrofloxacin	Antibiotic-fluoroquinolone (veterinary use)	780– 900
Citalopram	Serotonin reuptake inhibitor	770– 840
Norfloxacin	Antibiotic-fluoroquinolone	390– 420
Lomefloxacin	Antibiotic-fluoroquinolone	150– 300
Enoxacin	Antibiotic-fluoroquinolone	150– 300
Ofloxacin	Antibiotic-fluoroquinolone	150– 160
Ranitidin	H_2-receptor antagonist	90– 160

ment plant by recirculating sludge is normally desired, but this procedure can be strongly questioned for effluents containing high levels of antibiotics. In this case, the recirculation of sludge leads to an active selection for microorganisms that can tolerate the exposure to therapeutic levels of antibiotics. If resistant pathogens develop, they may eventually spread widely beyond the treatment plant, making existing antibiotics inefficient, thereby forcing us to develop new and more efficient drugs at an even higher pace. The issue of resistance development is most serious for poor people in developing countries who cannot afford newer, usually more expensive, patented medicines that may be more efficient. Thus one can argue that the release of antibiotics from production facilities could be a human health issue which concerns everyone, regardless of national borders.

3.4 The Value of APIs Going Down the Drain

How can it be economically feasible that such large volumes of drugs go down the drain? We have pointed out that the eleven most common drugs released during twenty-four hours would represent a price at a Swedish pharmacy greater than 100 000 Euro as final products. However, the production cost for the APIs would apparently be much lower (Larsson et al. 2007). But how much lower? A report by Networth Stockbroking Ltd (2005) describes the development of the export price for ciprofloxacin from India, the most abundant drug detected in the effluent. The export price in October 2004 corresponded to only about 1.5% of a generic final product's

sales price in Sweden (*www.lfn.se*). Profit margin for the API producer is included in the 1.5%; therefore, actual production costs were less than 1.5% of the final sales price to consumers. Note that the patent has expired for ciprofloxacin; thus, the costs for drug development are now no longer included in the sales price. Clearly the ratio between production costs of the API and the sales price of the final product may vary greatly between different APIs, between different products and between different markets. Nevertheless, with the data from ciprofloxacin at hand, taking into account the potential investments required to produce a clean effluent, it appears reasonable that the current economical incentives are not by themselves sufficient for preventing some APIs from following the wastewater.

3.5 More Evidence of Release of APIs from Production Units

How representative is this study for other production units in Asia or elsewhere? At least two other publications, not included in the review by Willams and Cook (2007), suggest that our findings are not the result of an isolated problem.

Already in 1993, a paper was published on a spectrophotometric method to detect phenols and salicylic acid in effluents from pharmaceutical industries (Bisarya and Patil 1993). The authors reported levels of 2 270 ppm (2.27 g l^{-1}!) of salicylic acid in the effluent from a plant producing acetylsalicylic acid (aspirin) in Bangalore, India. Although salicylic acid in this case was a by-product of the production of the acetylsalicylic acid, salicylic acid is itself an antipyretic API, which historically preceded the now much more common acetylsalicylic acid. This study has until today gone largely unnoticed in the field of pharmaceuticals in the environment.

In 2006, a study was published on the release of estrogens from a Chinese plant producing contraceptives (Cui et al. 2006). The average concentration of ethinylestradiol in the effluent was 51 ng l^{-1} as determined by LC/MSMS. Although this level is much lower than the detected concentrations of many other drugs in effluents from sewage treatment works, such high levels of ethinylestradiol have not been demonstrated previously in any regular sewage effluent. The potency of ethinylestradiol to aquatic vertebrates makes the Chinese finding highly significant. Indeed, Kidd et al. (2007) showed that a tenth of this concentration is sufficient to make a fish population collapse. At concentrations below one nanogram per liter, ethinylestradiol feminizes male fish, reduces egg fertilization rates and alters hepatic gene expression (Örn et al. 2003; Parrot and Blunt 2005; Gunnarsson et al. 2007).

Although publicly available studies on the potential releases from European or American production facilities are still sorely lacking, there is a growing dataset on the levels of pharmaceutical residues in the surface waters of these regions. It is perceivable that this dataset would have some potential to reveal if releases greatly exceeding the amounts released from sewage treatment works were very common from European and/or American plants. To the best of my knowledge, data in favor of that hypothesis has not been presented, except in one case from the river Rhein. A water quality monitoring program in Switzerland reported unexpectedly high concentrations of venlafaxin, an antidepressant. The source could be tracked back to an upstream pharmaceutical plant, which apparently took measures to reduce their release (AUE 2004).

Some European and American pharmaceutical companies have recently pointed out during discussions at public scientific meetings and in the media that the release of APIs from their plants is nothing like the reported case in India. In order to generate a better picture of the situation, it would be valuable if the analytical data and methods they refer to would be made publicly available.

China is currently struggling with many environmental issues as a consequence of its rapid economical growth. The Chinese authorities recently threatened to close down 117 pharmaceutical production plants because of serious pollution issues unless the industries rapidly improve their operations (China Daily 2007). This threat also included China's largest penicillin plant. Indeed, it should be acknowledged that there are many ways in which a drug factory could cause pollution, not only through the release of APIs. Whether the pollution from the 117 Chinese industries also included a known release of APIs is not clear to the author.

3.6 Some Management Issues

The knowledge that some production plants cause a significant environmental impact adds to the complexity of how we could manage environmental risks with pharmaceuticals. For example, in the Swedish voluntary environmental labeling system, products are classified based on the environmental properties of the APIs they contain and the predicted exposure to aquatic organisms based on the current usage in Sweden (*www.fass.se*). However if some production plants pollute more than others, a natural consequence is that some products are less environmentally friendly than others, even though they contain the same amount of the same API. Taking the environmental impact of production into account in any labeling system is a great challenge for the future.

Today, it is very difficult for both prescribers and patients to figure out where the API of a given product is produced. And even in cases where this information can be tracked, there is negligible data on the potential environmental release of APIs from the production plants. Therefore, there are currently very limited possibilities for the prescriber or the patient to make informed decisions based on the environmental impact from the production of a given medicine/brand. As long as this information is not readily accessible to consumers, there are fewer incentives for producers to take environmental responsibility seriously throughout the entire production chain.

The Swedish Association of the Pharmaceutical Industry has recently taken an initiative to investigate the possibilities to change GMP (Global Manufacturing Practice) to include environmental pollution limits for API production (Stenberg 2007). The future will tell us if this initiative is successful and/or if there are other, more efficient means to manage the issue of API-release from production plants.

Acknowledgements

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Substance Flows Associated with Medical Care – Significance of Different Sources

K. Kümmerer · A. Schuster

4.1 General Considerations[1]

4.1.1 Substance Flows

Pharmaceuticals and disinfectants are amongst the most important groups of chemical substances in modern life to prevent health threats caused by microorganisms. In human medicine, pharmaceuticals, disinfectants and diagnostics are used in hospitals, but they are also used in the home. After excretion, they end up in municipal sewage. The presence of pharmaceuticals, their metabolites and transformation products in the environment are clearly established now. Reviews of the present international state of knowledge have recently been compiled (see Chap. 1 of this book).

Discussions about proper and effective risk management strategies started only recently. However, knowledge on sources is indispensable for sound risk assessment, risk management and risk reduction. In this context, one has to know the size of substance flows associated with the different sources of pharmaceuticals such as households and hospitals. This knowledge is of utmost importance to be able to tackle the problem properly and efficiently. For example, for the allocation of investments one has to decide whether one should focus on separate treatment of hospital effluents, household effluents or municipal sewage that collects both of them. However, only little is known about sources and attributed substance flows. Balances are necessary on the different temporal and spatial scales (Kümmerer and Hofmeister 2008; Kümmerer et al. 2008) on a local, regional and national level.

As for the input of Active Pharmaceutical Ingredients (APIs) resulting from production, it is generally assumed that these are negligible because of the regulations for Good Manufacturing Practice (GMP) and because of the economical value of the compounds. However, a recent study by Larsson and coworkers has shown that this is not the case if production is in developing countries. The authors found concentrations up to several mg/l of pharmaceuticals in the effluent of a production plant (Larsson et al. 2007; Li et al. 2008; Larsson this book). Data for other APIs are not available but might look the same. More studies are needed to assess the significance of these findings.

[1] Use and fate of antibiotics is extensively described in this book by Kümmerer in the chapter on antibiotics. Therefore, they are not covered in this chapter.

If it is necessary to take measures for risk management, one has to know the relative importance of different sources for pharmaceuticals in the environment to take the proper and most effective measures. In this chapter, the substance flows of some pharmaceuticals used in human medicine (such as cytotoxics, antibiotics, anesthetics, diagnostics and disinfectants) resulting from their use in hospitals and private households is discussed exemplarily as the main pathways for the introduction of human pharmaceuticals into the aquatic environment.

4.1.2 Use Patterns

The consumption and application of pharmaceuticals may vary considerably from country to country (Verbrugh and de Neeling 2003; Goosens et al. 2005; for antibiotics see Kümmerer this book). Reasons are different legislation and different "philosophies," i.e., education of doctors, pharmacists and patients. Additional reasons are cultural and political ones. Antibiotics, for example, are sold over the counter without prescription in some countries (OTC pharmaceuticals) while in other countries they are only available by prescription. According to the World Health Organization (WHO) figures, only 0.4% of Japanese women of reproductive age take a contraceptive pill containing ethinyl estradiol as the main active pharmaceutical ingredient (API), compared with 16% in North America. Legislative changes within the health system may alter the use of single APIs or even the whole level of consumption. In Germany, between 2003 and 2004 (Schwabe 2006), one single health statute dropped the turnover of statutory health insurance financed proprietary medicinal drugs by 2 500 million Euro (–10.2%, see Fig. 4.1).

Pharmaceuticals can be prescribed by office-based practitioners. They are also used and administered in hospitals. After administration, pharmaceuticals are excreted and released into the aquatic environment via wastewater effluent. Some of the pharmaceuticals taken in hospitals are also excreted at home, e.g., because of a long half time in the human body (up to several days in some cases such as with cytotoxics, Eitel et al. 2000) or hospital-based ambulatory care.

Unused drugs are sometimes disposed of down drain in households (Götz and Keil 2007), and unless they are degraded or eliminated during sewage treatment, traces may enter the aquatic environment and eventually reach the drinking water supply. It was also hypothesized that antibiotics and disinfectants may disturb the wastewater treatment process and the microbial ecology in surface waters. Furthermore, STPs could spread resistance by throughput of already resistant bacteria or even by own selection – caused by the antibiotic substances present.

4.2 The Most Important Sources

4.2.1 Economical Data

Only a limited number of investigations deal explicitly with the relative importance of different sources. For Germany, nationwide data on the total consumption of phar-

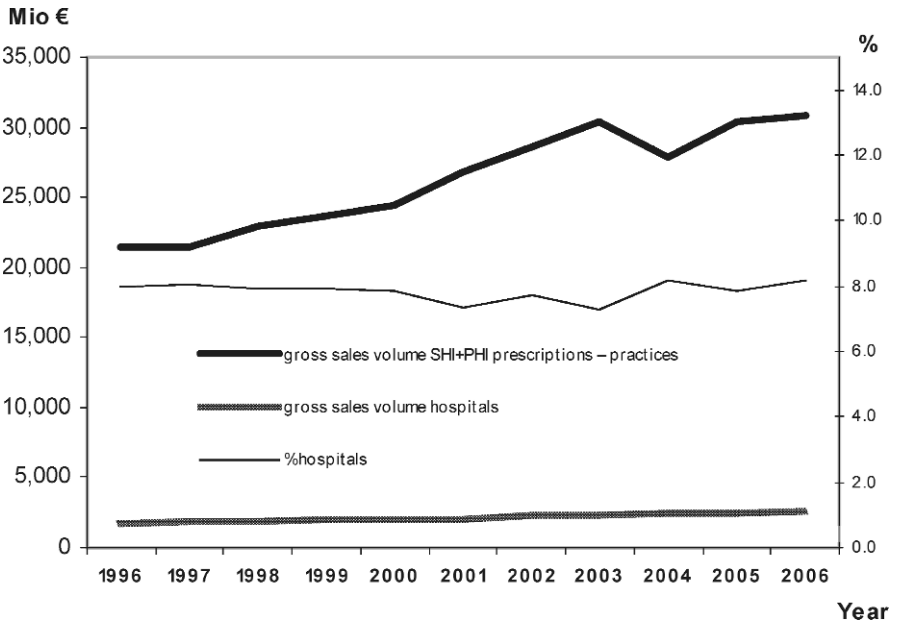


Fig. 4.1. Costs of pharmaceuticals in Germany (*right axis*: sales volume in millions of Euros, right scale% share of hospitals)

maceuticals according to the different sectors are not available for the general public. Most data are available for antibiotics as for the discussions about antibiotic resistance (e.g., Kümmerer 2001, Kümmerer and Henninger 2003, Alexy et al. 2006, Kümmerer this book). In Germany, the economic value of pharmaceuticals sold in 2003 was around 36.1 billion Euro according to the Federal Statistical Office (*www.gbe-bund.de*). Thereof 8.1% were spent by acute-care-hospitals and 0.4% by rehabilitation or prevention-care-hospitals; whereas 91.5% were prescribed by office-based practitioners (Fig. 4.1, left scale).

Since 1996, the figure shows a general increase of costs of pharmaceuticals with a relatively constant share of hospitals of 7.8%[2] (Fig. 4.1, right scale). As can be seen in Fig. 4.2, the treated cases are more or less constant, whereas the number of hospitals is decreasing, and the average residence time is decreasing, too (Germany 8.9 days, Italy 7.4 days, Great Britain 7.1 days, France 5.9 days, USA 4.8 days in 2004, *www.gbe-bund.de*) – as well as the total number of in-patient treatment days. These data indicate a low and even decreasing significance of hospital wastewater as a source of pharmaceuticals in the environment in Germany[3].

[2] Note the decrease of costs in 2004 due to new legislation (see Schwabe 2006).
[3] The ongoing substitution of in-patient care by ambulatory care in hospitals should also be considered (no data available).

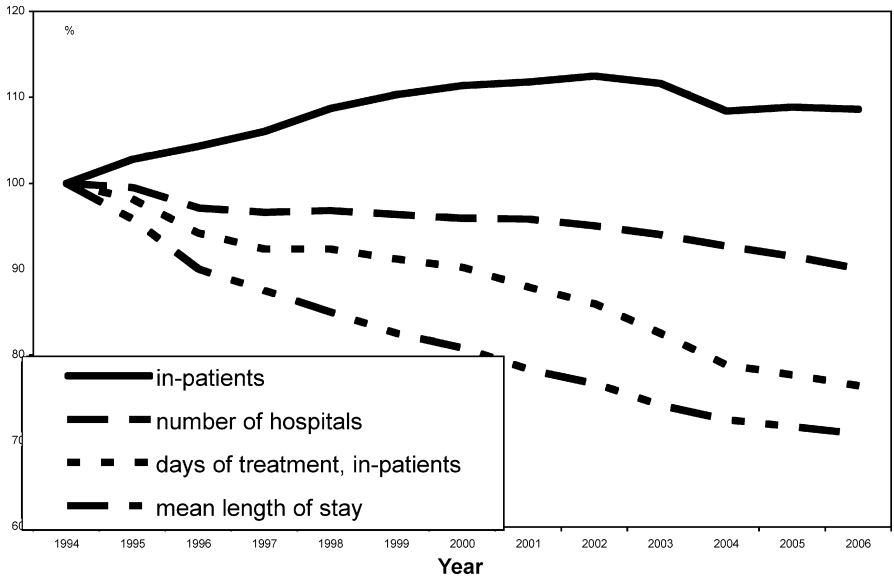


Fig. 4.2. Trend of some characteristic numbers for German hospitals (1994–2006, 1994 = 100%)

4.2.2 Accounting of Pharmaceuticals

The result of a nationwide balance on the use of pharmaceuticals in Germany in 2006 is shown in Table 4.1[4].

In another study consumption of pharmaceuticals on a regional basis was compared in respect to the importance of households and hospitals for the input of pharmaceuticals into wastewater (Table 4.2). The study was based on the consumption of all hospitals (nine of different size and medical service spectrum) and a total of 350 000 inhabitants living in the catchment of the sewage treatment plant that received all effluents. It was found that hospitals account for less than 5% of almost all of the several hundred APIs that have been balanced (see Fig. 4.3). As for the use of pharmaceuticals, this study also showed that there are compound-specific differences as far as the specific consumption (per capita and year) and the ranking by total amount used.

For the anti-epileptic drug carbamazepine and the nonsteroidal anti-inflammatory drug diclofenac in a study conducted by Heberer and Feldmann (2005), it was found that in total, 2.0 kg of carbamazepine per week (105 kg per year) and 4.4 kg of diclofenac per week (226 per year) were discharged into Berlin's surface water. The corresponding municipal STP treats household sewage from approximately one million inhabitants and large amounts of hospital effluents (approximately 12 060 hospi-

[4] The data represent proprietary medicinal products, prescribed by office-based physicians and financed by statutory health insurance.

Table 4.1. Input of top ranking APIs into sewage in Germany in 2005, threshold 6 t

API, INN	Input (t)
Metformin	757.4
Piracetam	120.1
Amoxicillin and enzyme inhibitor	113.4
Mesalazine	58.1
Amoxicillin	52.5
Allopurinol	39.6
Phenoxymethylpenicillin	38.5
Gabapentin	36.5
Hydrochlorothiazide	32.2
Irbesartan	27.2
Ranitidine	25.0
Furosemide	24.0
Valsartan	22.8
Tramadol	19.5
Diclofenac	16.2
Erythromycin	15.5
Simvastatin	15.0
Methotrexate	13.4
Theophylline	13.1
Levodopa and decarboxylase inhibitor	12.4
Lisinopril	12.2
Sotalol	11.9
Metoprolol	11.3
Levetiracetam	11.1
Sevelamer	10.2
Captopril	9.6
Cefaclor	8.7
Enalapril	8.6
Paracetamol	8.5
Colestyramine	8.4
Atenolol	8.3
Verapamil	8.2
Bezafibrate	7.8
Ciprofloxacin	7.7
Amantadine	7.0
Acetylsalicylic acid	6.9
Aciclovir	6.5
Valproic acid	6.5
Sulfamethoxazole and trimethoprim	6.5

Only proprietary medicinal products, prescribed by office-based physicians and financed by statutory health insurance are included, excretion rate is accounted.

Table 4.2. Consumption of selected pharmaceuticals on a nationwide and a local level (Kümmerer et al. 2008, excretion rate not included)

Ranking	Germany				Catchment AZV Breisgauer Bucht			
	API	Consumption (t)	Per capita and year (g)	Share (%)	API	Consumption (t)	Per capita and year (g)	Share (%)
1	Metformin	757.4	9.18	62.2	Metformin	3 191.9	9.39	48.6
2	Piracetam	120.1	1.46	9.9	Metoprolol	444.1	1.31	6.8
3	Metoprolol	115.8	1.40	9.5	Hydrochlorothiazide	213.7	0.63	3.2
4	Verapamil	44.5	0.54	3.6	Furosemide	153.2	0.45	2.3
5	Hydrochlorothiazide	33.6	0.41	2.8	Atenolol	145.5	0.43	2.2
6	Furosemide	25.7	0.31	2.1	Verapamil	142.2	0.42	2.2
7	Pentoxifylline	25.6	0.31	2.1	Pentoxifylline	133.8	0.39	2.0
8	Simvastatin	18.0	0.22	1.5	Lithium	133.6	0.39	2.0
9	Triamterene	12.1	0.15	1.0	Piracetam	123.1	0.36	1.9
10	Spironolactone	9.9	0.12	0.8	Melperone	110.5	0.32	1.7
11	Isosorbide dinitrate	9.5	0.12	0.8	Triamterene	93.5	0.27	1.4
12	Atenolol	8.7	0.11	0.7	Amiodarone	90.9	0.27	1.4
13	Acarbose	7.6	0.09	0.6	Chlortalidon	83.6	0.25	1.3
14	Amitriptyline	6.8	0.08	0.6	Amitriptyline	78.1	0.23	1.2
15	Doxepin	5.6	0.07	0.5	Sulpiride	70.1	0.21	1.1
16	Amiodarone	5.3	0.06	0.4	Acarbose	60.1	0.18	0.9
17	Sulpiride	4.6	0.06	0.4	Spironolacton	59.3	0.17	0.9
18	Melperone	3.5	0.04	0.3	Isosorbide dinitrate	57.5	0.17	0.9
19	Lithium	3.5	0.04	0.3	Simvastatin	55.9	0.16	0.9

tal beds). The largest share by far was found in the household sewage. For antibiotics, is has also been found that hospitals are by far a less significant source.

The consumption of antibiotics was 412 t in Germany in 1998. Thereof 277 t were emitted unchanged into wastewater (Kümmerer and Henninger 2003). The share of hospitals vs. private households (due to prescriptions by practitioners) is shown in Fig. 4.4.

The volume of cytotoxics used lies far below other drugs such as antibiotics (approximately 400 t per year in Germany for medical purposes) or the single compound metformin (757 t per year). In Germany, the anticipated annual average concentrations of most intensively used cytotoxics in wastewater are a few ng l^{-1}. This is in the same range as those actually measured (Steger-Hartmann et al. 1996; Buerge et al. 2006; Lenz et al. 2007). Measured levels in surface water are even lower (Buerge et al. 2006). However, based on consumption data, a water quality model was set up for fluorouracil in

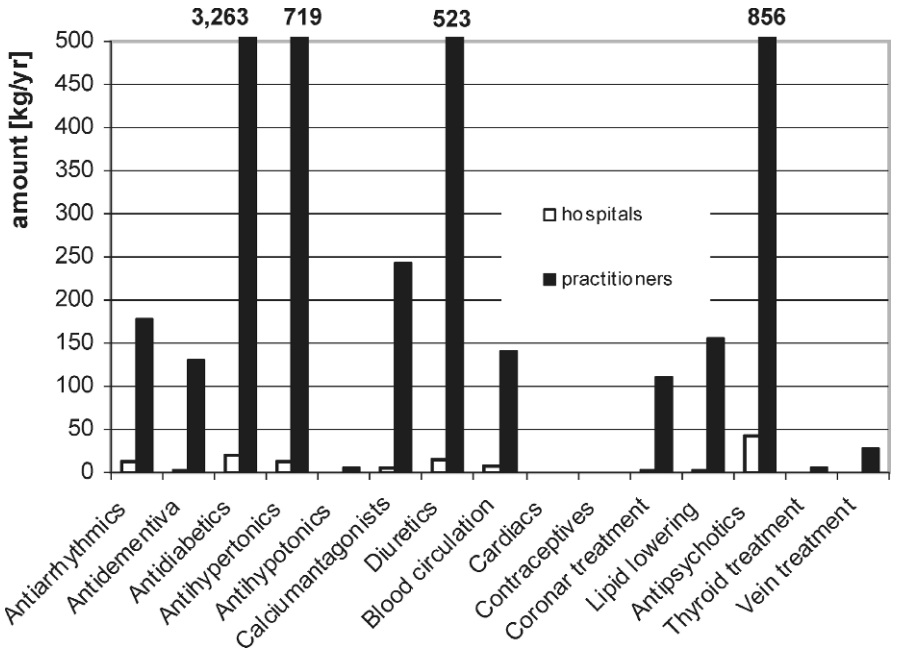


Fig. 4.3. Consumption of pharmaceuticals in private households and hospitals in the catchment of a STP (650 000 inhabitant equivalents, 350 000 inhabitants, 9 hospitals of different size and medical service spectrum including one of the biggest hospitals in Germany) (Kümmerer et al. 2008)

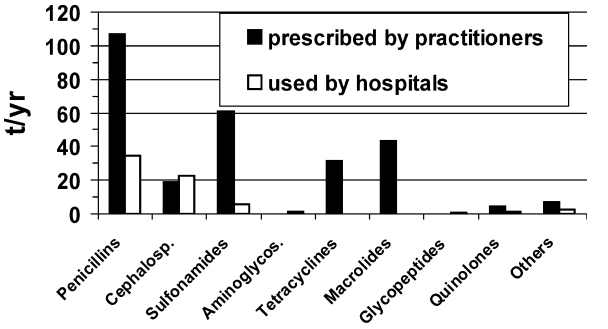


Fig. 4.4. Consumption of antibiotics in private households and hospitals (Kümmerer and Henninger 2003)

the Aire and Calder catchment in Northern UK. The study predicts 5–50 ng l^{-1} concentrations for long stretches of this catchment under low flow conditions (Johnson et al. 2008). Data for the use of cytotoxic compounds in Germany (for examples see Fig. 4.5) show that the compounds that are most often used are exclusively used outside hospitals (Schuster et al., unpublished results).

One has to be aware that most cytotoxics have a half-life in the human body of several days (Eitel et al. 2000). In modern anti-cancer therapy, patients may start the treatment in a hospital and leave the hospital after a day if there are no complications. In

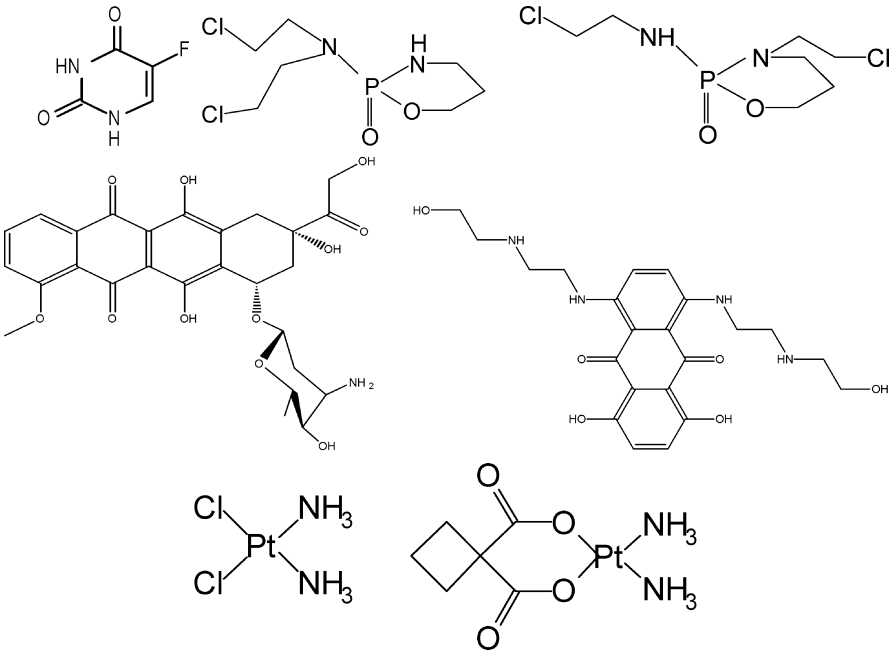


Fig. 4.5. Formulas of some cytotoxic compounds, *from left to right and top to bottom*: fluorouracil, cyclophosphamide, ifosfamide, epirubicin, mitoxantrone, cisplatin and carboplatin

such an "ambulant" anti-cancer therapy, cytotoxics are administered in the hospital, but most of the dosage is excreted at home. That is, by far the main share of cytotoxics is excreted outside hospitals into municipal sewage.

Fluorouracil is used as a cytotoxic but also for the local treatment of warts. Dermatological and oncological indications of fluorouracil depend on the same molecular mechanism of action. The platinum-containing compounds carboplatin and cisplatin form the same active intermediates (Figs. 4.5, 4.6) and therefore have to be seen together since carboplatin is hydrolyzed to cisplatin under appropriate conditions. Cisplatin is stable if the Cl^--concentration is >0.3% (m/v). Hydrolysis of cisplatin leads to aquo-complexes (Fig. 4.7). *Cis*-diammineaquachloroplatinum(II) is the most toxic compound (Daley-Yates and Mc Brien 1984, 1985). This compound may also react further to form other monomeric and oligomeric chloro-aquo complexes of different toxicity. Phosphate reacts fast with platinum-aquo complexes. Together, carboplatin and cisplatin consumption in Germany add up to about 172 kg (0.4% by weight of total amount of cytotoxic APIs used in 2006 in Germany).

Platinum is discharged into the various environmental compartments from a variety of sources (Lustig et al. 1997; Helmers and Kümmerer 1999). Cars equipped with catalytic converters are a major source. Hospital effluents contain platinum from the excreta of patients treated with the cytostatic agents cisplatin and carboplatin (Kümmerer et al. 1999) and also oxaliplatin. After administration of the cytostatic agents, the platinum is excreted and thus reaches the municipal sewer system. The

Fig. 4.6. Transformation of carboplatin (*left*) into cisplatin (*right*)

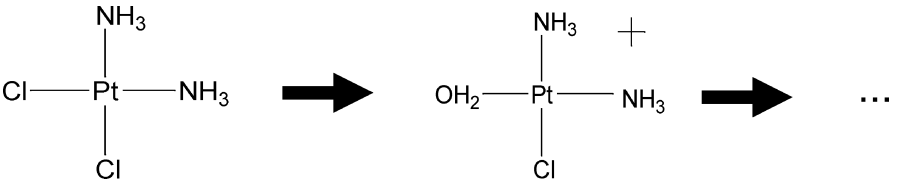


Fig. 4.7. Hydrolysis of cisplatin to aquo complexes

concentrations in 2 h mixed wastewater samples were between 20 and 3 580 ng l^{-1}, with a daily average of between <10 and 660 ng l^{-1} for a maximum size hospital. Absolute emissions are lower in smaller hospitals offering fewer health care services. The specific emissions per bed and year differ less than the concentrations, with values ranging from 14 mg/bed and year (low medical service spectrum) to 150 mg/bed and year (maximum medical service spectrum, Fig. 4.8). In 1996, total platinum emissions into the public sewage systems via hospitals were approximately 14.3 kg a^{-1} in Germany. This corresponds to 12% of the total volume of emissions from cars and hospitals, while it is estimated that in the Netherlands and Austria the total volume was 6% and 3.3%, respectively (Kümmerer et al. 1999). Emissions from other sources cannot be quantified (Helmers and Kümmerer 1999).

4.2.3 Disinfectants, Diagnostics and AOX

For quaternary ammonium compounds (QACs) (Fig. 4.9), which are widely used for surface disinfection, consumption data show the same picture (Fig. 4.10).

Only recently it has been found that QACs are weakly mutagenic (Ferk et al. 2007). Again the hospitals are a smaller source when compared to others for the introduction of compounds related to medical care into municipal sewage (Fig. 4.10).

The role of hospitals as contributors to AOX in urban wastewater cannot be ignored. Brominated organic compounds do not contribute substantially to AOX in hospital effluents. Chlorinated or iodinated compounds play a much more important role with respect to AOX. A separate determination of AOCl, AOBr and AOI in partial effluent streams helps to identify possible sources of AOX emission and enables the introduction of measures to reduce such emissions to be envisaged. In particular, the times of AOX peak loads can be identified in a daily concentration profile and ascribed to spe-

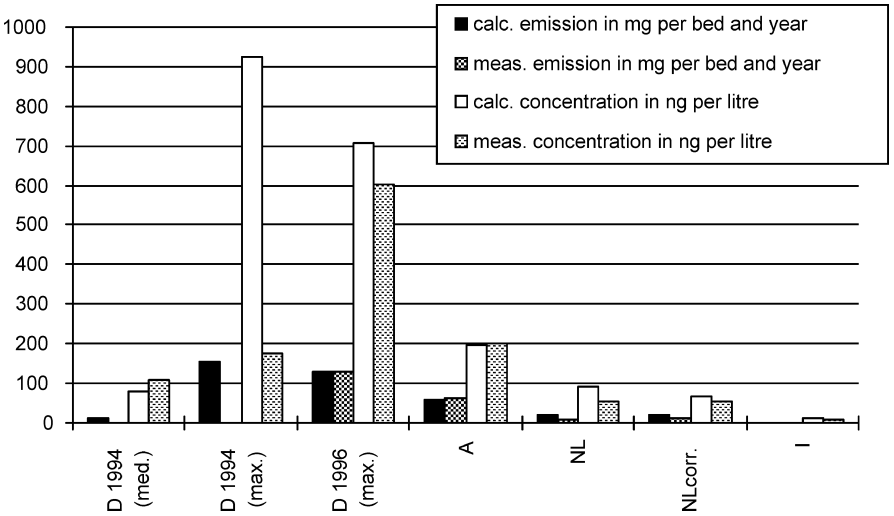


Fig. 4.8. Measured and calculated concentrations and specific amounts of platinum emitted by the European hospitals investigated (*max.*: hospital of maximum medical service spectrum; *med.*: hospital of medium medical service spectrum)

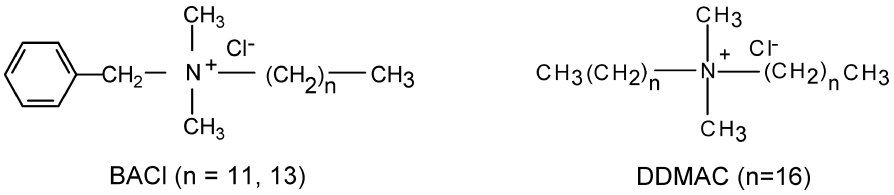
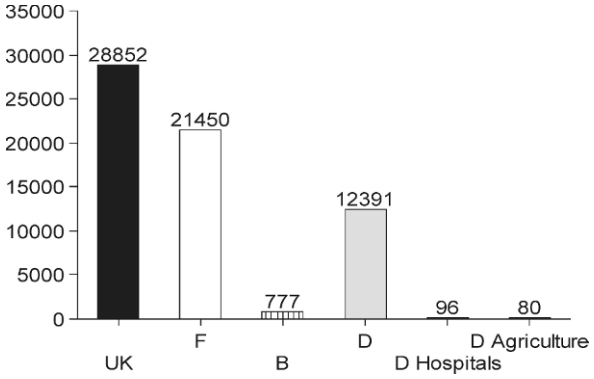


Fig. 4.9. Formulas of two quaternary ammonium compounds (QACs) frequently used as disinfectants: benzalkonium chloride (*left*) and didecyldimethylammonium chloride (DDMAC) (*right*)

Fig. 4.10. Application of QACs in different European countries; specific ratios are: UK 3.2 (0.48 kg per capita and year), F 2.4 (0.35 kg per capita and year), B 0.5 (0.074 kg per capita and year), D 1.0 (0.15 kg per capita and year). *Data source:* German Federal Statistical Office (1999), own balances and Huschek (2004)



cific activities and processes in the hospitals (Haiß et al. 1998). A surprisingly high AOX-proportion of municipal wastewater was identified as AOI, i.e., organic iodine. Also, unlike the AOCl, it showed a pronounced weekly progression with minimal values on weekends (Oleksy-Frenzel et al. 1995). In general, the proportion of AOI fluctuated between 23 and 53% of the total volume of AOX. The proportion of AOI was particularly high when effluents from hospitals were discharged (Drewes and Jekel 1997). Iodized X-ray contrast media have been identified as an important source of the AOX (Gartiser et al. 1994, 1996; Erbe et al. 1998; Haiß et al. 1998). Worldwide consumption of these compounds is estimated to be 3,460 t per year; German consumption is to be about 10% of this (*http://www.kompetenz-wasser.de/fileadmin/user_upload/pdf/forschung/RKM/ Schuster.pdf*).

Contrast media are optimized so that they are not metabolized in the human body. The biological half-life for excretion of all iodized X-ray contrast media (Fig. 4.11) in use today is about two hours. Some of them, such as the anionic amidotrizoic acid are excreted by feces. Their half-life is longer. Amidotrizoic acid may pass water treatment and has been detected in drinking water (Seitz et al. 2006; Schittko et al. 2004). Normally, X-ray contrast media are given to patients in radiology departments of hospitals or in radiological surgeries. Once the examination is completed, the patient normally leaves the X-ray department immediately and either excretes the contrast medium in the appropriate ward or in the case of patients treated as outpatients, at home. Iodized X-ray contrast media contribute substantially towards AOX, especially in the effluent of hospitals with large radiological departments (Haiß et al. 1998). Surveys and comparisons with measurements have demonstrated that a large proportion of the AOX in hospital effluent is present as AOI (Ziegler et al. 1997; Erbe et al. 1998). AOI is not necessarily the most significant contributor to AOX in hospital effluent, but organic iodine compounds can account for about 50% of AOX pollution (Haiß et al. 1998). Since iodized X-ray contrast media are used not only in hospitals, but also in doctors' surgeries, a substantial proportion of the iodine found in the public sewage system could have been diffusely discharged through X-ray contrast media (Schuster 2006; *http://www.kompetenz-wasser.de/fileadmin/user_upload/pdf/forschung/RKM/ Schuster.pdf*). The share of hospitals in the use of iodized X-ray contrast media (Fig. 4.10) is estimated to be about 50% in Germany.

As in hospitals, outpatients are treated too; the contribution of hospitals to the iodized contrast media share in municipal wastewater is lower. It has also been found

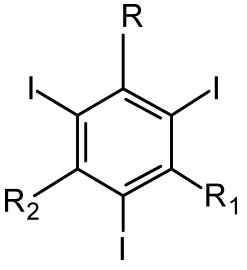


Fig. 4.11. Basic structure of iodized X-ray contrast media (R = NHR', COONR"R'"; COOH in the case of ionic contrast media)

that in some cases the share due to examinations at local radiological practitioners can be much higher than that of hospitals (Haiß 2002).

4.2.4 Gadoliniums

Besides application in medicine, gadolinium is also used in nuclear engineering and, together with other rare earth elements, in the production of color monitors. Due to its high magnetic moment, gadolinium is used in the form of organic complexes in magnetic resonance imaging (Hammond 1995). The gadolinium complexes typically used in magnetic resonance imaging (MRI) are gadodiamid and gadopentate (Fig. 4.12). Following administration, the organic complexes are excreted quickly and unchanged: >95% within 24 h (Nycomed 1995) and emitted into the public sewage systems and into surface waters via hospital effluent. The concentrations measured in hospital effluents are in the range of a few $\mu g\ l^{-1}$ to 100 $\mu g\ l^{-1}$ (Kümmerer and Helmers 2000). Falter and Wilken (1998) measured between 0.3 and 1.9 $mg\ kg^{-1}$ in water works sludge. Vivian (1986) found 0.6–2 $mg\ kg^{-1}$ in sewage sludge, while we found 1.3 ±0.05 $mg\ kg^{-1}$ ($n = 4$) of dry substance in our measurements. As these data correspond well, it may be assumed that there is no substantial enrichment in sewage sludge. The natural background concentration of gadolinium in rivers is about 0.001 $\mu g\ l^{-1}$; peak concentrations of as much as 1.1 $\mu g\ l^{-1}$ in the effluent from STPs are possible (Bau and Dulski 1996). Increased concentrations have been found in rivers in regions with a high population density. Concentrations of 0.2 $\mu g\ l^{-1}$ have been measured in rivers influenced by STP discharge (Bau and Dulski 1996). With 0.12–0.3 $\mu g\ l^{-1}$, the estimated average gadolinium concentration in German surface waters is within the range of contrast media altogether, which suggests that the concentrations well above the natural background are mainly due to the emission of contrast media containing gadolinium (Kümmerer and Helmers 2000). Emissions by hospitals and practitioners partly ac-

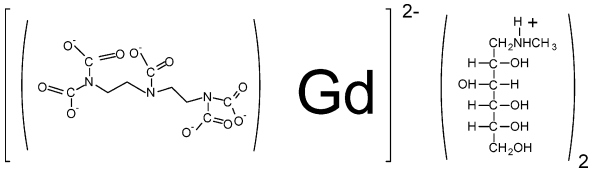


Fig. 4.12. (DTPA)Gd-dimeglumine: diethylene-triaminepentaacetate-Gd(III)-bis-(D-(–)-1-methylamino-1-desoxy-D-glucite) used as a contrast aid in magnetic resonance imaging

Table 4.3. Use of gadolinium in MRI in Germany (Kümmerer and Helmers 2000)

Basis of calculation (data from)	Total use for MRI in Germany ($kg\ yr^{-1}$)	Emissions by hospitals ($kg\ yr^{-1}$)	Predicted environmental concentration ($\mu g\ l^{-1}$)	Predicted environmental concentration attributable to hospitals ($\mu g\ l^{-1}$)
Local data Freiburg		132		0.3
Local data Berlin	1 355	741	3.1	1.7
Federal Statistical Office	1 160	484	2.6	1.1

count for this so-called anomaly (Table 4.3). In the meantime, hydrologists and geologists are using the Gd anomaly as a tracer for human impact on waters (Möller et al. 2000; Möller et al. 2002; Elbaz-Poulichet et al. 2002; Verplank et al. 2005).

In the future, it is expected that some of the Gd-containing contrast aids could be displaced by others containing micro particles of highly paramagnetic iron oxides, which are enclosed by a polymeric organic membrane.

4.2.5 Mercury and Other Heavy Metals

Mercury levels in the environment are still a research area of high interest. In spite of the fact that emissions into the aquatic environment have been successfully reduced, mercury continues to be one of the heavy metals of which the volume discharged is too high. The discharge of mercury from public health institutions is attributable to preservatives containing mercury usually found in diagnostic agents or pharmaceuticals (e.g., thiomersal) or to dermatological applications that use mercury-based derivatives as antiseptics or disinfectants (ATC-group D08AK: Mercurial products). There are as well some diuretic agents such as mercurophyllin (Craig 1986) or mersalyl. Altogether, the use of mercury in medicine is regressing.

The mercury concentrations measured in the central wastewater channel of European hospitals of different sizes were between 0.04 and 2.6 $\mu g\, l^{-1}$ (Gartiser et al. 1994; Leppold 1997). This corresponds to an annual load of approximately 220–250 g in bigger hospitals. As far as the skin disinfectant mercurochrome is concerned, it was shown that administration of this agent at Freiburg University Medical Center alone accounted for about 1–1.5% of the sludge contamination at the sewage treatment facility in 1996. Based on the volume of prescriptions collected by national health insurance companies in 1994, approximately 100 kg of mercury were emitted by use of mercuchrome in Germany, most of which is likely to have reached wastewater during or after application. At Freiburg University Medical Center, mercuchrome was eventually replaced by mercury-free alternatives (Kümmerer 1998).

The oxidizing components of cleaning or disinfecting agents help to remobilize mercury in the amalgam separators of dental treatment units (Kümmerer et al. 1997c; Stone et al. 2006). It is estimated that remobilization of mercury in amalgam separators with oxidizing disinfecting components causes an additional mean mercury load of about 32.5 kg per year in Germany. This appears to be slight when compared with other sources and the quantities actually retained by mercury separators. But the International Commission for the Protection of the River Rhine (IKSR) found in its interim report that "… *the mercury values in 1993 [in surface waters] were still twice to four times the value of the target specification … about 44% (of 1100 kg/a) of the point-to-point emissions originate from municipal wastewater (approx. 440 kg/a) …*" (IKSR 1994). The report of the International Commission for the Protection of the River Rhine expressly states also that further measures for reducing discharges from the municipal/public sector must be taken at the source, i.e., at the inlet site. Mercury has again become a focus of international research. Mercury remobilized from dental amalgam through oxidizing disinfecting components in amalgam separators accounts for 7.3% of the mercury load in public wastewater (Kümmerer et al. 1997c; Stone et al. 2006);

Table 4.4. Heavy metal loads emitted by different sources (annual load in kg, Leppold 1997)

	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Hospital[a]	0.028	6.8	11.7	0.030	0.87	20.0	2.08
SME[b]		54–281	5–19		91–250	34	180–497

[a] Maximum medical service spectrum, 1 500 occupied beds.
[b] Small and medium enterprises with specific emission related activities.

this could be reduced to 0.3% if disinfectants without oxidizing properties were used (Kümmerer et al. 1997c). Part of the mercury emitted into wastewater is also discharged together with sludge. However, the proportion and the species are unknown. Some may be emitted as highly toxic methyl-mercury (Stone et al. 2003). Other heavy metals are found in the effluent from hospitals, too. These are not attributable to hospital-specific activities or products. Data from our own research (Leppold 1997; Table 4.4) show that the annual load for a hospital offering maximum service spectrum is much lower than the ones of small and medium enterprises (SMEs) with specific activities.

Only for copper are the loads of the hospital effluent in the range of the ones of the SME. However, for zinc and copper the input from roof gutters and roofs constructed of these metals has not been accounted for.

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Pharmaceutical Residues in Northern European Environments: Consequences and Perspectives

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5.1 Background Information

Residues of pharmaceuticals in the environment as well as the potential effects associated with these contaminants are currently intensively discussed in environmental sciences. As the chapters of this book demonstrate, research on the various aspects of pharmaceuticals in the environment is a high-priority research subject for international environmental sciences. However, although the presence of many pharmaceuticals is confirmed in the aqueous, terrestrial and marine environment as well as in biota, scientific information on ecological and ecotoxicological consequences is still sparse.

From well-known legacy pollutants like persistent organic pollutants (POPs), polynuclear aromatic hydrocarbons (PAHs) as well as trace metals, we know that general ambient environmental conditions (e.g., temperature, radiation, microbiology, geology etc.) as well as physicochemical properties of the chemical play a crucial role in determining the environmental behavior of contaminants (e.g., environmental stability, degradation pathways, partitioning coefficients). In general, a significantly prolonged environmental presence of contaminants can be assumed at low ambient temperatures (as is already well documented for a large variety of chemicals in northern environments). This assumption is also valid for pharmaceutical residues and other sparsely investigated environmental contaminants (AMAP 2004).

During the past decade, northern environments have been in the focus of environmental sciences as deposition and accumulation regions for globally distributed persistent environmental pollutants such as POPs, brominated flame retardants, trace metals and organo-metal compounds (e.g., methyl mercury, etc.). The environmental significance for POPs in northern environments is comprehensively documented (Burkow and Kallenborn 2000; AMAP 2004; Berg et al. 2004; Evenset et al. 2005; Su et al. 2006; Orbæk et al. 2007).

Due to low annual average ambient temperature and the special seasonal daylight conditions in northern regions, suitable conditions for deposition and prolonged environmental stability of anthropogenic pollutants are obvious. Thus extended half-lives, i.e., reduced (bio)degradation, are resulting in elevated environmental levels as well as increased exposure potential for organisms and humans of the northern regions. The following parameters demonstrate why the northern regions may be especially sensitive to anthropogenic pollutant exposure including pharmaceutical residues.

5.1.1
Daylight Conditions

In northern regions, the seasonality of the light conditions is evident. 24 h light during the summer period changes rather quickly into the lack of light during the winter season above the Arctic Circle (66° N latitude). This constellation has a tremendous effect on the photochemical degradation of some anthropogenic pollutants. However, not only the duration of irradiation but also the intensity and spectrum are of importance for the effectiveness of photochemical transformation processes. In Tromsø, Norway, the sun is below the horizon during the period between November 25 and January 19; however, the UV-light (e.g., $\lambda \sim 315$ nm) winter relevant for photodegradation is considerably longer: October to March (Engelsen et al. 2005). The UV winter length increases with increased latitude.

5.1.2
Temperature Conditions

The low annual average temperature (including low peak temperatures during summer seasons) of the northern regions is the main reason why microbiological degradation processes are negligible compared to middle latitude regions. In general, low densities of Arctic microbial communities are found in Arctic sediments and waters demonstrating this postulation (Ravenschalg et al. 2001; Bano and Hollibaugh 2002; Brinkmeyer et al. 2003). This environmental condition reduces microbiological degradation in water, on soils and sediment surfaces, thus, often results in a considerably prolonged half-life of these compounds in the cold polar environments.

5.1.3
Demographics

The human population structure in northern Scandinavia is characterized by a decentralized, scattered distribution of minor settlements with a few cities as cultural and social centers. This scattered population profile is different from the structures in middle European countries and has considerable consequences also for the release of pharmaceutical residues into the environment. Installation of modern small scale/ medium scale STPs is usually not affordable for the small communities of the North. However, in Sweden, more than 85% of all households are connected to a sewage treatment plant (STP) (Lindberg, pers. communication).

Although the usage of pharmaceuticals is not documented as much as in densely populated regions, the lack of these modern STP installations in larger settlements/ cities in northern Scandinavia has been resulting in surprisingly high release rates of selected pharmaceuticals into the environment (Weigel et al. 2004; Vasskog et al. 2006, 2008).

5.2
Quantification of Pharmaceutical Substances in Swedish, Norwegian and Finnish Aqueous Samples

In general, the determination of pharmaceutical substances in low-contaminated aqueous environmental matrices regardless of sample origin is based today mainly on solid

phase extraction (SPE) and liquid chromatography (LC), or gas chromatography (GC) often after derivatization. Chromatographic separation methods are combined with mass selective detection (MS) for identification and sensitive quantification (see Table 5.1).

Quantification by internal standard (IS) calibration allows the determination of ultra trace levels of pharmaceutical residues of interest; however, they are often associated with interferences by matrix components. Commercially available SPE cartridges, usually polymeric based, offer extraction yields usually above 50% (Lindberg et al. 2005; Lindqvist et al. 2005; Vieno et al. 2006). SPE sample preparation of a 2 l water sample, most often 1 l or less, in combination with LC-MS/MS separation and quantification methods results usually in a limit of quantification (LOQ) in the low ng/l range. In case chemical derivatization combined with GC/MS quantification is applicable for reliable quantification, the LOQ will decrease further (sometimes ten-fold) compared to LC-MS/MS methods. The often higher LOQs in LC-MS/MS is, among other reasons, attributed to signal suppression effects in the electrospray ion source, caused by interfering matrix components (Lindberg et al. 2005; Vieno et al. 2006).

The relatively new extraction technique liquid phase microextraction (LPME) is capable of producing better LOD and LOQ than SPE for certain compounds; however, it is an equilibrium extraction technique and the recovery is dependent on each compound's distribution coefficient between the sample and the organic solvent used. Additionally, ion suppression in LC-MS/MS seems to be avoided with LPME (Ho et al. 2007; Vasskog et al. 2008).

IS calibration, based on surrogate standard quantification and target substance peak area ratio, is a popular, time efficient and validated quantification method used to improve the accuracy and precision of the trace-analytical method. The IS is added before the extraction and, thus, subjected to the same matrix and chemical/physical alteration of the sample throughout the sample preparation process as the target substance. The behavior of the IS during extraction and instrumental analysis should be closely correlated to that of the target substance. Pharmaceuticals within the same class, but not expected to be present in the samples, are suitable as alternatives (Lindberg et al. 2005; Lindqvist et al. 2005). The use of isotope labeled target substances such as IS is highly recommended since it will also have the same retention time during the LC or GC separation and experience identical signal suppression effects. Sampling collection and sample preparation in polar regions are often associated with considerable economic and logistic efforts; thus special care and comprehensive quality control measures have to be employed in order to ensure scientifically reliable concentration data.

5.3 Environmental Levels of Pharmaceuticals in Northern European Environments

Recently, an initial Norwegian screening of pharmaceutical residues in major sewage treatment plants (STPs) has been published (SFT 2007a). Very little has so far been published about the broader distribution of these pharmaceutical residues in the Nordic countries, although a number of more recent surveys have shown that this may also pose an environmental challenge in Nordic countries (Swedish Medical Products Agency 2004).

Table 5.1. A brief summary of the analytical methodology used for quantification of pharmaceutical substances in various Swedish, Norwegian and Finnish waters

Compounds	Extraction	IS[a]	LC-MS/MS	GC-MS	Reference[d]
	SPE		LOQ[b] (ng l^{-1})	LOQ[c] (ng l^{-1})	
Antibiotics					
Amoxicillin	Isolute ENV+	Cephalexin	74		Lindberg et al. (2005)
Ampicillin	Isolute ENV+	Cephalexin	60		Lindberg et al. (2005)
Cefadroxil	Isolute ENV+	Cephalexin	77		Lindberg et al. (2005)
Ciprofloxacin	Isolute ENV+	Enrofloxacin	6		Lindberg et al. (2005)
	Oasis HLB	Enrofloxacin	163		Vieno et al. (2006)
Doxycycline	Isolute ENV+	Demeclocycline	64		Lindberg et al. (2005)
Metronidazole	Isolute ENV+	2-M-5 nitroimid[e]	33		Lindberg et al. (2005)
Norfloxacin	Isolute ENV+	Enrofloxacin	7		Lindberg et al. (2005)
	Oasis HLB	Enrofloxacin	78		Vieno et al. (2006)
Ofloxacin	Isolute ENV+	Enrofloxacin	6		Lindberg et al. (2005)
	Oasis HLB	Enrofloxacin	18		Vieno et al. (2006)
PCV[f]	Isolute ENV+	Cephalexin	82		Lindberg et al. (2005)
SMX[g]	Isolute ENV+	Sulfamethazine	80		Lindberg et al. (2005)
Trimethoprim	Isolute ENV+	Diaveridine	8		Lindberg et al. (2005)
Antiepileptics					
Carbamazepine	Oasis HLB	D-carbamazepine[h]	3.5		Vieno et al. (2006)
	Oasis HLB	$^{15}N_2$-caffeine		N.d.[i]	Weigel et al. (2004)
Anti inflammatory and anti rheumatics					
Diclofenac	Oasis MCX	Fenoprop	5		Lindqvist et al. (2005)
	Oasis HLB	D_3-mecoprop		0.09	Weigel et al. (2004)
Ibuprofen	Oasis MCX	Fenoprop	5		Lindqvist et al. (2005)
	Oasis HLB	D_3-mecoprop		0.07	Weigel et al. (2004)
Ketoprofen	Oasis MCX	Fenoprop	25		Lindqvist et al. (2005)
Naproxen	Oasis MCX	Fenoprop	25		Lindqvist et al. (2005)
Beta blockers					
Acebutolol	Oasis HLB	Alprenolol	6.4		Vieno et al. (2006)
Atenolol	Oasis HLB	Alprenolol	49		Vieno et al. (2006)
Metoprolol	Oasis HLB	Alprenolol	21		Vieno et al. (2006)
	Oasis HLB	Not used[j]	N.d.		Weigel et al. (2004)
Propranolol	Oasis HLB	Not used	N.d.		Weigel et al. (2004)
Sotalol	Oasis HLB	Alprenolol	19		Vieno et al. (2006)

Table 5.1. *Continued*

Compounds	Extraction	IS[a]	LC-MS/MS	GC-MS	Reference[d]
	SPE		LOQ[b] (ng l^{-1})	LOQ[c] (ng l^{-1})	
Lipid modifying agents					
Bezafibrate	Oasis MCX	Fenoprop	5		Lindqvist et al. (2005)
Clofibric acid	Oasis HLB	D_3-mecoprop		0.24	Weigel et al. (2004)
Antidepressants[k]					
Citalopram	Varian Bond Elut ENV	N-7084[n]	0.2	0.05	Vasskog et al. (2006)
Fluvoxamine	Varian Bond Elut ENV	N-7084	0.2	0.05	Vasskog et al. (2006)
Fluoxetine	Oasis HLB/Varian Bond Elut ENV	N-7084	0.2	0.05	Weigel et al. (2004); Vasskog et al. (2006)
Paroxetine	Oasis HLB/Varian Bond Elut ENV	N-7084	0.2	0.05	Weigel et al. (2004); Vasskog et al. (2006)
Sertraline	Oasis HLB/Varian Bond Elut ENV	N-7084	0.2	0.05	Weigel et al. (2004); Vasskog et al. (2006)
Clomipramine	LPME	M-methyl-paroxetine	0.05		Ho et al. (2007)
Amitriptyline	LPME	M-methyl-paroxetine	0.03		Ho et al. (2007)
Nortriptyline	LPME	M-methyl-paroxetine	0.02		Ho et al. (2007)
Mianserin	LPME	M-methyl-paroxetine	0.1		Ho et al. (2007)
Doxepin	LPME	M-methyl-paroxetine	0.04		Ho et al. (2007)
Metabolites					
C-ibuprofen[l]	Oasis HLB	D_3-mecoprop		0.69	Weigel et al. (2004)
H-ibuprofen[m]	Oasis HLB	D_3-mecoprop		0.42	Lindberg et al. (2005)
Erythromycine[-H_2O]	Isolute ENV+	demeclocycline	160		Lindberg et al. (2005)

[c] Limit of quantification (maximum or only value reported in paper), all substances underwent methylation with methyl chloromethanoate (except for carbamazepine).
[e] 2-methyl-5 nitroimidazole.
[f] Phenoxymethyl penicillin.
[g] Sulfamethoxazole.
[h] Dehydrocarbamazepine.
[i] No data.
[j] Semi-quantitative results by external calibration and no correction of extraction yields.
[k] Mostly selective serotonine re-uptake inhibitors.
[l] Carboxylated-ibuprofen.
[m] Hydroxylated-ibuprofen.
[n] For structure information see publications Vasskog et al. (2006), Weigel (2003), and Weigel et al. (2004).

One of most intensively investigated regions for pharmaceutical residues in northern Scandinavia is the area around Tromsø, Norway. The first investigation by Weigel and coworkers (Weigel et al. 2004) detected selected pharmaceuticals in STP influent and effluent water and seawater (see Table 5.1).

The concern regarding the possible impact of environmental residues of selective serotonin reuptake inhibitors (SSRI) has grown considerably during the past years (Fong 1998). Also around Tromsø, SSRI residues were investigated comprehensively. An improved analytical method was developed by Vasskog et al. (2006) for STP effluent water. This new validated analytical procedure allowed the trace-level quantification of all five government-approved SSRI compounds in Norway including citalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline (see Table 5.1). Other antidepressants like clomipramine, amitriptyline, nortriptyline, mianserin and doxepin have also been found in STP effluents in Tromsø, however, in far lower concentrations than the SSRIs (Ho et al. 2007).

This first survey on pharmaceutical residues in Tromsø illustrated that application patterns seem to be reflected in the distribution and concentration values of pharmaceutical residues in the receiving aqueous environment (Weigel et al. 2004). Thus, the presence of the target chemicals was shown to be influenced mainly by the discharges from the university hospital and a psychiatric ward of the local university hospital (see Fig. 5.1). Caffeine, ibuprofen and its degradation products as well as diclofenac were also found in the seawater around Tromsø (Weigel et al. 2004). Due to the large emission rates for compounds like Ibuprofen (incl. transformation products) and caffeine, the strong tidal currents around the island had surprisingly little dilution effects on the overall concentration levels (ng/l range) in the adjacent waters around the STP outlet.

After the above described scientific investigations leading to validated trace analytical methods for the quantitative determination of pharmaceutical residues in northern European aqueous samples (Table 5.1), Swedish authorities and research groups have performed several campaign-based surveys of drug residues in the Swedish environment during the last few years. The results of a comprehensive survey in Sweden have been published recently (Woldegiorgis et al. 2007). The Stockholm County Council carried out a survey of sewage sludge and effluent investigating levels of antibiotics in three large sewage treatment plants in Bromma, Henriksdal and Käppala in Stockholm (Wennmalm and Gunnarsson 2005). At the same time, aqueous samples from Lake Mälaren and Lake Saltsjö were examined.

Only low levels of antibiotics were found in the effluent (about 10 ng l^{-1}), compared with slightly higher amounts of sulfamethoxazole (max. 130 ng l^{-1}), trimethoprim (max. 470 ng l^{-1}) and metronidazole (max. 80 ng l^{-1}). In Mälaren and Saltsjö, only norfloxacin, ofloxacin and trimethoprim were found, and then in very small amounts (max. 10 ng l^{-1}).

Scientific efforts to quantify the degradation of pharmaceuticals in STP plants are a challenging task. In Finland, Vieno et al. (2006) reported the content of certain pharmaceuticals in STP outlet water to be 20–120% of inlet water for the same pharmaceuticals.

A survey was also carried out at the Rheumatic Hospital in Spenshult, Sweden, where drug residues at the hospital's own treatment plant had been studied. It was shown

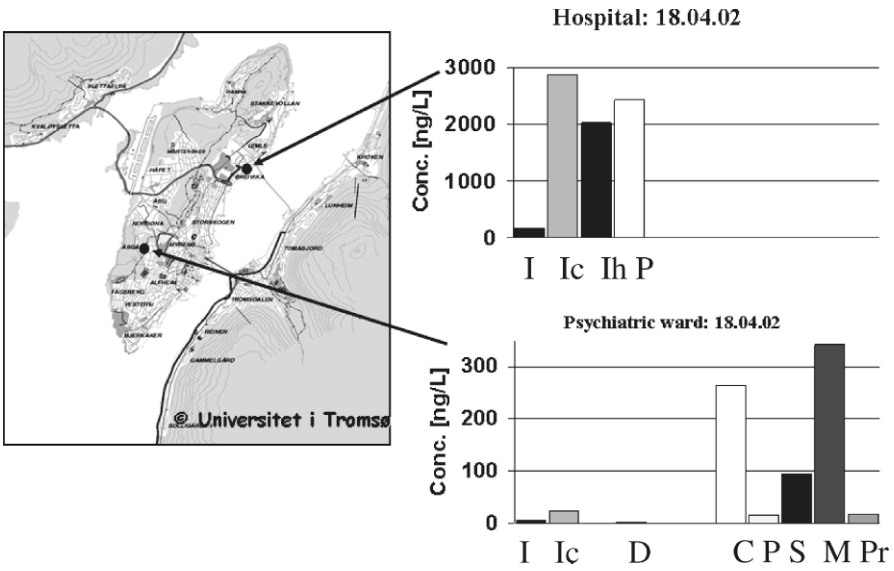

Fig. 5.1. Concentration levels (ng l^{-1}) of selected pharmaceuticals in sewage effluent of from a psychiatric ward (*lower graph*) and the university hospital (*upper graph*). Figure modified according to Weigel et al. (2004). Abbreviations: *I* = ibuprofen, *Ic* = carboxylated ibuprofen, *Ih* = hydroxylated ibuprofen, *P* = paroxetine, *C* = carbamazepine, *S* = sertraline, *M* = metoprolol, *Pr* = propranolol

that eleven of the fourteen drugs looked for could be detected in the incoming sewage. The most predominant compound found was Ibuprofen (77–116 mg l^{-1}). This survey also showed that the levels of drugs leaving the treatment plant were below 1 mg l^{-1}. The degree of treatment, i.e., the proportion of drug that the treatment plant was able to remove, was shown to vary between 0 and 99% for the various drugs analyzed (Apoteket 2006).

In another Swedish survey on antibiotic residues in the effluents and sludge, twelve types of antibiotics were monitored in five municipal sewage treatment plants. The most frequently detected antibiotics, fluoroquinolone substances, trimethoprim and doxycycline, were present in concentrations in ng/l range for sewage water and in mg/kg range for sludge. In addition, the results showed that penicillins and cephalosporines are readily degradable, since only a small number of substances from these classes of drugs were detectable in only a few of the samples (Lindberg et al. 2005).

Based upon trace analytical and ecotoxicological assessment studies on the presence of various pharmaceuticals in the north European environment published so far (including human and veterinary drugs), the Norwegian Pollution Control Authorities (SFT) recently completed a literature study recommending a suit of pharmaceuticals for continuous monitoring in aqueous samples (SFT 2007b). The reviewed pharmaceuticals were evaluated for potential monitoring purposes based upon their esti-

mated or known occurrence in the aquatic environment (PEC = predicted environmental concentrations or MEC = Measured environmental concentration), potential to harm aquatic organisms or ecosystems (PNEC = predicted no effect concentration), known environmental stability, bioaccumulation potential, previously reported occurrence, and the methods available for reliable quantification. Based upon these comprehensive evaluations, the following list of priority pharmaceutical compounds is recommended for continuous monitoring in aqueous samples from the north European environments (Table 5.2). For evaluation of this comprehensive literature study, please consult the currently published SFT-report (SFT 2007b).

5.4 Variable Degradation Rates

Already during the first study on distribution and fate of selected pharmaceutical residues in Tromsø STP effluent and adjacent seawater, surprising differences in pattern and concentration distribution were identified (Weigel 2003; Weigel et al. 2004) most likely due to different environmental conditions compared to middle latitude regions. The comparison of the ibuprofen patterns in Tromsø (including the major transformation product hydroxylated and carboxylated ibuprofen) with published data from Germany revealed an interesting feature (Weigel et al. 2004). The carboxylated transformation product (Ibu-COOH) seems to be significantly more stable in the cold seawater environment around Tromsø annual average temperature 4–6 °C compared to middle latitude environments annual water temperature ~+5 °C.

After human excretion, the carboxylated metabolite (Ibu-COOH) is predominant; however, after microbial degradation in the receiving waters, the hydroxylated transformation product (Ibu-OH) seems to dominate in middle latitude aqueous environments (Weigel et al. 2003). This documented environmental transformation, however, is severely altered in the marine seawater around the STP outlet in Tromsø and may thus be considered a first indication of reduced transformation capacity in cold aqueous northern environments (Weigel 2003).

In a currently finalized comparative Norwegian study, the occurrence of SSRI antidepressants in STP effluent from Oslo (450 000 inhabitants), Tromsø (60 000 inhabitants) and Longyearbyen (Svalbard, 2 000 inhabitants) was investigated (Vasskog et al. 2008). The Oslo sewage treatment plant VEAS (Vestfjorden Avløpsselskap) is the most advanced state-of-the-art high throughput sewage treatment plant in Norway, producing 121.5 million m^3 water annually (2007) from around 200 000 households/industrial sources in and around Oslo. VEAS has an advanced treatment system with flocculation, sedimentation, nitrification and denitrification steps. Tromsø STP filters only (mechanical sieving) the sewage from around 20 000 households before releasing the water phase into the adjacent seawater of the Tromsø sound. However, no sewage treatment is performed for the sewage from Longyearbyen (Svalbard) prior to release into the Adventfjord seawater on Spitsbergen (Main Island of the Svalbard archipelago). The comparison of the three sampling sites indicates that there is no significant difference in efficiency between these different treatments when it comes to removal of SSRIs from wastewater (Vasskog et al. 2008).

Table 5.2. Recommendations for monitoring in aqueous samples based upon a national Norwegian survey on environmental risk posed by pharmaceutical residues (SFT 2007b)

Residues from human use of pharmaceuticals			
Estradiol	Sulfamethoxazole	Carbamazepine	Trimethoprim
Amoxicillin	Atorvastatin	Metoprolol	Phenoxymethylpenicillin
Penicillin G	Naproxen	Glucosamine	Citalopram
Ciprofloxacin	Ibuprofen	Metformin	Ezetimibe
Ethinylestradiol	Allopurinol	Alendronic acid	Diazepam
Propranolol	Amitriptyline	Verapamil	Fluticasone
Paracetamol	Tetracycline	Simvastatin	Furosemide
Fluoxetine	Pivmecillinam	Carvedilol	Midazolam
Diclofenac	Ofloxacin	Bicalutamide	and Clarithromycin
Estriol	Oxytetracycline	Oxazepam	
Sertraline	Erythromycin	Paroxetine	
Veterinary drug residues			
Ivermectin	Flunixin	Danofloxacin	Trilostane
Penicillin procaine	Clavulanic acid	Medroxyprogesterone	Lufenuron
Pyrantel	Ceftiofur	Phenylpropanolamine	Clomipramine
Sulfadoxine	Toltrazuril	Cyclosporine	Aglepristone
Phenylbutazone	Benzathine	Cefadroxilb	Benazepril
Metamizol	Xylazine	Marbofloxacin	Ibafloxacin
Cephalexin	Propofol	Selamectin	and Pimobendan
Febantel	Prednisolone	Flubendazole	
Guaifenesinb	Eprinomectin	Tepoxalin	
Clindamycin	Carprofen	Vedaprofen	

In addition, in this study clear indications were found that selected pharmaceuticals may experience significantly longer half-life times in Northern environments compared to recipient seawater in middle latitude regions (Vasskog et al. 2008). A set of nine different SSRI antidepressants and their transformation products were analyzed and quantified in sewage effluent and adjacent seawater samples. In the receiving seawater from Tromsø and Oslo (VEAS) only citalopram and paroxetine were identified from the total of ten analyzed SSRI-related compounds, whereas in the cold seawater environment close to the sewage outlet in Longyearbyen, paroxetine (1.4 ±0.4 ng l^{-1}), fluvoxamine (0.8 ±0.3 ng l^{-1}) and Sertraline (below LOQ) were all detected. Unfortunately, due to data security restrictions, no information on local usage patterns is available. Since the human population in Tromsø is considerably larger than in Longyearbyen and, in addition, the psychiatric section of the university hospital is actively applying antidepressants during therapy, the sewage influent SSRI levels from the city of Tromsø have to be assumed considerably higher compared to Longyearbyen. (Vasskog et al. 2008). It seems, thus, that the presence of quantifiable amounts of paroxetine, sertraline and fluvoxamine in the receiving seawater in Longyearbyen is a clear signal of increased environmental stability of these compounds in the cold arctic aqueous environment (Vasskog et al. 2008).

5.5 Transformation Under Cold Environmental Conditions

The presence of a chemical in the environment is governed through the direct releasing sources, indirect diffuse sources and the half-life in the respective environment. For pharmaceutical residues, STPs are considered as the predominant sources for environmental contamination. Thus, the quality of the treatment procedure (retention and removal properties) and the efficacy of the (bio)transforming and degrading environment in the receiving waters are crucial parameters for the ecotoxicological potential of the respective pharmaceutical identified. In addition, especially for antibacterial and antiviral drugs, the incomplete removal and consequently, the continuous release into an aqueous environment may result in the development of microbial or viral resistance with severe consequences for human populations and the environment at concentrations in the higher ng/l range. A general overview about environmental consequences of microbial resistance has been presented earlier in a comprehensive review (Kümmerer 2004, this book). Fick et al. (2007) investigated the removal properties of standard STP procedures for the antiviral drug oseltamivir. The authors concluded that modern STPs are not designed for the effective removal of oseltamivir. The direct release of oseltamivir from a STP may, thus be the result. However, the incomplete retention of oseltamivir may also cause the development of resistance for the Influenza A Virus. Few studies are available that have identified the causal factors underlying the degradation and inactivation of antimicrobials present in the environment. Since antimicrobials represent numerous and distinct chemical classes, their degradation kinetics is likely to vary considerably. Both physical and biological factors contribute to their degradation (Maki et al. 2006). For instance, the first generation beta-lactic antibiotics (penicillins) are generally regarded to be short-lived in the environment due to the ubiquitous presence of microbially-produced enzymes (β-lactamases) (Henriques et al. 2006; Lorenzo et al. 2008).

There is only a limited amount of information available on the physical factors leading to the degradation of antimicrobial substances. A first study on the temperature-dependent biodegradation of penicillin-G (benzyl penicillin), a β-lactam type of antibiotic drug, using the Zahn-Wellens test (OECD 302B) has recently been reported. Penicillin-G is one of the most used antimicrobial drugs applied in a variety of human and veterinary treatments worldwide. The Zahn-Wellens test of biodegradation was performed under controlled laboratory conditions at 5 °C, 12.5 °C and 20 °C. The results showed that the degradation time increased about two-fold from 5 to 20 °C (Helland et al. 2008). Furthermore, the major transformation products through hydrolysis and subsequent carboxylation are significantly more stable under low temperature conditions (5 °C) compared to 20 °C (Helland et al. 2008). Although temperature may be an important factor, the relevance of such study to natural conditions remains unclear as microbially-produced β-lactamases are considered the main degradation factor of β-lactam antibiotics. Microbial species composition varies in different aquatic environments; hence variable β-lactamase activity will be present.

Fluoroquinolones are degraded photolytically in aquatic systems and also removed by binding to particulate organic carbon (Cardoza et al. 2005). It is reasonable to assume that a reduction in particulate organic matter content and photodegradation reduce the removal of fluoroquinolones in the winter season.

5.6 Antimicrobial Residues Under Cold Environmental Conditions

Antimicrobial drug residues are continually released into aquatic environments from anthropogenic sources including raw or filtered sewage and from STP (Halling-Sørensen et al. 1998). The release into seawater of various antibiotics used in agriculture for treatment of livestock is likely of only moderate importance if the manure is applied to agricultural soil (Hirsch et al. 1999). Clay-rich sediments are known to bind and immobilize a range of chemical substances including some antimicrobials. Data on the annual usage of various antimicrobials in human and veterinary medicine can be found in yearly published reports for Norway (NORM/NORMVET 2006), for Denmark (DANMAP 2006), and clinical usage data for Sweden (SWEDRES 2006).

Many antimicrobials are semi-synthetic or synthetic derivatives of naturally occurring antibiotics and are observed to be persistent over time in the environment. Their environmental persistence can rationally be explained by the fact that they have been specifically developed to retain activity and resist degradation by the biochemical activities of the human body and exposed microbes. The majority of the dose of antibiotics consumed by humans is excreted chemically unchanged (Hirsch et al. 1999). The subsequent incomplete removal of antimicrobial substances in STPs and the resulting continuous release into the aqueous environment may lead to the development of microbial resistance with severe consequences for human populations and the environment. The exact concentration of seasonal fluctuations and persistence of pharmaceutically-produced antimicrobial substances remain unclear for most natural environments and need further scientific elucidations.

The relationship between bacterial exposure to antimicrobials and resistance development is exceedingly complex and needs scientific consideration of a range of structure specific, microbiological and environment specific factors. Moreover, various classes and types of antimicrobials are likely to induce resistance development at different speeds and in different types of bacterial populations. Thus, broadly-oriented studies are necessary to determine the lower limits of biologically relevant concentrations of antibiotics in the environments (Alonso et al. 2001). As antimicrobials do have selective effects on bacterial populations also well under their minimal inhibitory concentration (MIC) (defining 100% growth inhibition of the bacterial population), further studies should be conducted to determine the long-term effect of the exposure of bacterial populations to sub-lethal doses (microgram range) of antimicrobial residues (Kümmerer 2004). Although only a few relevant studies are available, bacterial populations in many naturally occurring environments not intentionally exposed to antimicrobials are known to carry antimicrobial resistance determinants (Esiobu et al. 2002; Henriques et al. 2006). The potential impact of the continual environmental release of antimicrobial substances in northern Europe is therefore a shift in bacterial populations from being predominantly sensitive to pharmaceutically-produced antimicrobials to predominantly resistant phenotypes. Such a shift could have an impact on clinically relevant microbial pathogens, since various bacterial communities have been proven to possess efficient ways of transferring genetic information (resistance determinants).

5.7 Perspectives and Consequences

During the past ten years, a series of national and international research and screening studies have confirmed that pharmaceuticals are also present in the aquatic northern Scandinavian environment. Also in Scandinavian countries, STPs (in Sweden at least 85% of the population is connected to municipal STPs) and direct sewage release into the aqueous environment are considered the chief source for pharmaceutical residues in the aquatic environment. Agricultural sources seem minor compared to middle European regions, due to the volume of agricultural activities in the Nordic countries (Llorens Abando et al. 2007).

As already outlined earlier, the absence of modern STP installations in larger settlements/cities in northern Scandinavia has obviously resulted in relatively high release rates of selected pharmaceuticals into the environment (Weigel et al. 2004; Vasskog et al. 2006, 2008). As a direct consequence, the combination of lower elimination/degradation rates and reduced retention due to a lack of suitable sewage treatment may even result in comparable or even higher concentrations in the aqueous environment as reported from middle latitude regions with better STP infrastructure.

Pharmaceuticals are designed to express a specific biochemical function at low level concentrations as a part of an integrated therapeutic procedure. This biochemical effect, desirable during therapy, may cause unwanted ecotoxicological effects when the compound is released into the environment. In the Nordic environments, pharmaceutical residues are released into low-temperature aqueous environments. Low biodegradability and, thus, prolonged residence time must be expected for the majority of the pharmaceuticals entering the aquatic system.

The environmental and ecotoxicological consequence of the continuous release may thus be different compared to temperate regions of the globe.

Impact on the human populations due to consumption of contaminated fish and invertebrates caught locally in the release environment or through exposure to resistant microbial communities cannot be excluded.

However, the scientific results so far available through published papers and reports must be considered as indication only. Comprehensive environmental studies on the fate and distribution of pharmaceuticals applied in high volumes and released into the Nordic environment under cold northern climate conditions should be given high priority by national and international authorities. This is also necessary to ensure that local food sources can also be harvested by the future generation without any concern for health and well-being.

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Antibiotics in the Environment

K. Kümmerer

6.1 Introduction

Antibiotics are among the most important groups of pharmaceuticals used. Antimicrobial drugs can be used for either prophylaxis (prevention) or treatment of disease caused by microorganisms such as bacteria and fungi. The term antibiotic stands often for drugs that combat any of these microorganisms. In this chapter the term "antibiotic" refers only to drugs that kill or inhibit bacteria. Antibiotics that are sufficiently nontoxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases of humans, animals and plants. They are used extensively in human and veterinary medicine as well as in aquaculture for the purpose of preventing or treating microbial infections, while in livestock farming they are used to promote the growth of animals. Some antibiotics are also used in growing fruit and in bee keeping. The classic definition of an antibiotic is a compound produced by a microorganism that inhibits the growth of another microorganism. Over the years, this definition has been expanded to include synthetic and semi-synthetic products. Antibiotics are grouped based on chemical structure or the mechanism of inhibition of microorganisms.

Antibiotics are used in medicine, veterinary medicine, farming, and aquaculture for the prevention and treatment of diseases. Consumption in prevention and therapy related to animals is largely determined by modern animal breeding and fattening methods and conditions. Some compounds may be used for purposes other than human or veterinary medicine: Some antibiotics such as streptomycins are used in fruit crops, while others are used in beekeeping. Again, the situation may vary from country to country.

Internationally comparable data on antibiotic consumption is scarce, and whatever information is available is heterogeneous. Country specific consumption for groups of antibiotics in DDDs can be found for Europe on the ESAC homepage (*http://www.esac.ua.ac.be/main.aspx?c=*ESAC2&n=1066l*). Use patterns may be different in different countries (see Table 6.1). The relative importance of the different use patterns in different countries is not known. In the USA, for instance, use of streptomycin is widespread in fruit crops, whereas it is banned for this purpose in Germany.

Antibiotics are excreted after administration. They are only partially eliminated in sewage treatment plants, and residual amounts can reach surface waters, groundwater or sediments. Although they have been used in large quantities for some decades, until recently the existence of these substances in the environment was accorded only little attention. It is only in recent years that a more complex investigation of antibiotic substances has been undertaken in order to permit an assessment of the envi-

Table 6.1. Antibiotics in the aquatic environment: data on use, input and concentration in different countries (given concentration are maximum concentrations for different compounds

Region/ country	Total volume used in human medicine (t yr^{-1})	Volume used in human medicine (gram per capita)	Thereof in hospitals (%)	Unused medicaments	Measured in sewage up to (µg l^{-1})	Measured in surface water up to (µg l^{-1})	Reference
World wide	100 000–200 000	?	?	?	?	?	Wise (2002)
EU + CH	8637	22.4	?	?	?	?	FEDESA (2001)
USA	4860?	17?	70?	?	1.9	0.73	Union of Concerned Scientists (2001); Kolpin et al. (2002)
CDN	?	?	?	?	?	0.87[a]	Miao et al. (2004)
CH	34.2	4.75	20–40?	?	0.57	0.2	Alder et al. 2nd edition this book
D	411	4.95	25	20–40?	6	1.7	Kümmerer and Henninger (2003); Rönnefahrt (2005)
DK	40	7.4	?[b]	?	5?[c]	?	Gurdabassi and Daalsgard (2001); Bager and Emborg (2000)
A	38	4.7	?	20–30?	?	?	Sattelberger (1999)
NL	40.9	3.9	20	?	4.4	0,11–0.85	Sacher and Stocks (2003); Verbrugh and de Neeling (2003); Ministerie van Verkeer en Waterstaat (2003)
I	283	4.88	?	?	0.85	0.25	Castiglioni et al. (2004a,b); Calamari et al. (2003); Zuccato et al. (2000)

[a] Undiluted.
[b] Data only available as daily defined dose.
[c] Only sulfamethizol and penicillin V which have not been measured or detected in other studies.
?: Data not available or quality/significance not clear.

ronmental risks they may pose. Within the last decade, an increasing number of studies covering their input, occurrence, fate and effects have been published. There is still a lack of understanding and knowledge of antibiotics in the environment despite the numerous studies performed. In recent years, more data and broader knowledge have become available. In contrast to the properties and effects wanted from their therapeutic application, these same properties are often disadvantageous for target *and* nontarget organisms in the environment.

Antibiotics are a diverse group of chemicals that can be divided into subgroups such as β-lactams, quinolones, tetracyclines, macrolides, sulfonamides and others. The active compounds of antibiotics are often complex molecules which may possess different functionalities. Under environmental conditions, these molecules can be neutral, cationic, anionic, or zwitterionic (Fig. 6.1). Because of the different functionalities within one molecule, their physicochemical and biological properties may change with pH levels.

Ciprofloxacin, for example, possesses both basic and acidic functionalities. The acid constants are 6.16 and 8.63. At a pH of 7.04, the isoelectric point of ciprofloxacin, the molecule carries both a negative and a positive charge, i.e., it is neutral as an entity despite these charges within the molecule. Solubility, hydrophobictiy and hydrophilicity are pH dependent. Compared to most industrial chemicals and other environmental contaminants, pharmaceutically active compounds – especially antibiotics – are often complex molecules with special properties e.g., dependence of $\log K_{ow}$ or $\log K_D$ on pH.

This chapter starts with an analysis of the present state of knowledge concerning the input, occurrence, fate and effects of antibiotics on the environment; it addresses

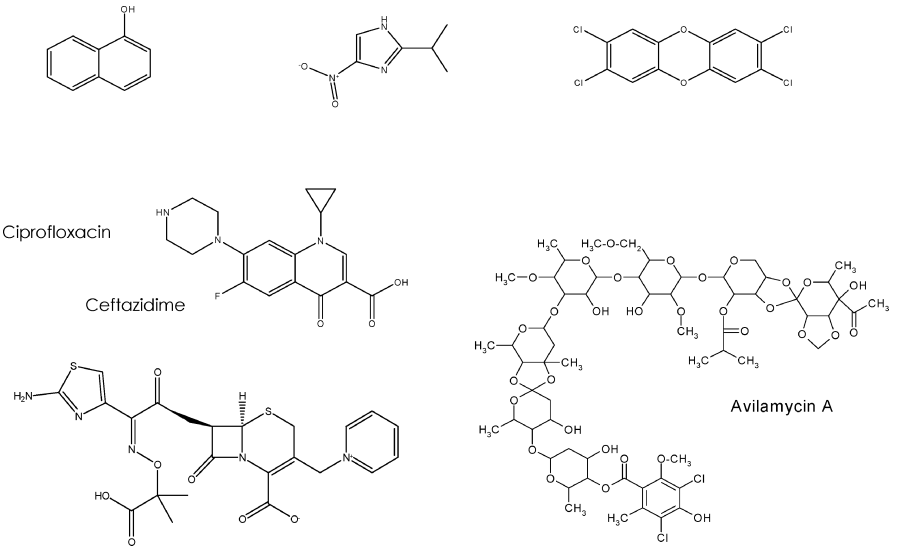

Fig. 6.1. Bulk chemicals and "classical" environmental contaminants (examples are given in the *top row*) have a relatively simple chemical structure compared to pharmaceuticals such as antibiotics (*bottom*)

important open questions, and names some significant issues which must be tackled in the future for a better understanding of the behavior of antibiotics in the environment and the risks associated with their occurrence.

Active substances discharged with liquid manure can be washed off from the top soil after rain. Furthermore, direct discharge, especially from poultry processing, meat processing, aquaculture and from pets (e.g., aquariums) is also possible and can contribute towards an increase in the total concentration of antibiotics in sewage and surface water. Since an extensive review of veterinary pharmaceuticals in the environment has only been recently published (Sarmah et al. 2006), the focus in the following will be more on human antibiotics than on veterinary ones.

6.2 Sources

6.2.1 Natural Background

The question of natural background concentrations of antibiotics is important for the risk assessment of antibiotics. Several antibiotics such as some β-lactams, streptomycins, aminoglycosides and others are produced by soil bacteria. The group of actinomycetes includes many soil bacteria such as streptomycetes. Streptomycetes produce antibiotics. The antibiotic activity from local soil samples is variable and takes many samples to find a few that produce zones of inhibition. To the authors' best knowledge there have been no findings of tetracyclines in soils which have not been fertilized with manure containing tetracyclines. The concentration has always been below the detection limit in untreated soils used as the control when studying the input and fate of tetracyclines in soils. This situation may be different in tropical soils as the bacteria producing tetracycline occur naturally in higher densities in such soils.

Bacterial density is much lower in the free water phase compared to sewage sludge or soil to expect measurable concentrations of antibiotics of natural origin. One may assume that sediments resemble soils since they are also solid media with both aerobic and anaerobic compartments. In both soils and sediments, bacteria are less mobile than in the free water phase, and bacterial density is higher. Up to now, there has been no report of such production of antibiotics in sediments or the aquatic environment. A conclusion on this open question cannot be drawn as long as we do not have any results for antibiotics naturally occurring in sediments and their concentrations.

6.2.2 Production

Emissions from production plants have been qualified as of minor importance. Only recently, however, it has been found that in Asian countries concentrations up to several mg l^{-1} can be found in effluents for single compounds (Larsson et al. 2007; Li et al. 2008; Larsson this book).

6.2.3 Human Medicine

250 different antibiotic and antimycotic substances are used in medicine and veterinary medicine in Germany (Kümmerer and Henninger 2003). Antibiotic prescription rates vary markedly between countries (Mölstad et al. 2002). Hospitals, in contrast to general expectation, are not the main source of antibiotics in municipal sewage. Community use is reported to be 70% in the UK (House of Lords 1998) and 75% in the U.S. (Wise 2002). In Germany, about 75% of antimicrobials are used in the community and 25% in hospitals (Kümmerer and Henninger 2003). For Oslo it was found that less than 10% of certain analyzed antibiotics were from hospitals (Thomas et al. 2007). Different usages of single compounds are quite common. Vancomycin, for example, is heavily used in the U.S., whereas in Germany it is only used in cases where all other possible compounds prove ineffective due to resistance. Wise (2002) estimated antibiotic consumption worldwide to lie between 100 000 and 200 000 t per annum. In 1996, about 10 200 t of antibiotics were used in the EU, of which approximately 50% were applied in veterinary medicine and as growth promoters. According to data supplied by the European Federation of Animal Health (FEDESA 2001) in 1999, 13 216 t of antibiotics were used in the European Union and Switzerland, of which 65% were applied in human medicine. For the EU in total 22 g per capita and year would result from the use in medicine. For the U.S. about 17 g per capita and year can be calculated from the available data for use in human medicine (see 2nd edition of this book).

Data on the use of antibiotics in different countries are available from different sources but mostly as DDD[1]. Country-specific consumption for groups of antibiotics in DDDs can be found for Europe on the ESAC homepage (*http://www.esac.ua.ac.be/main.aspx?c=*ESAC2&n=1066l*). The volume of use usually only refers to a nationwide scale, and analytical data do not cover local volume of use. Cars et al. (2001) obtained data for non-hospital antibiotic sales for 1997 from the fifteen member states and analyzed these according to the Anatomic Therapeutic Chemical (ATC) classification system and expressed them as defined daily doses per 1 000 people per day. Sales of antibiotics varied more than four-fold: France (36.5), Spain (32.4), Portugal (28.8), and Belgium (26.7) had the highest sales, whereas the Netherlands (8.9), Denmark (11.3), Sweden (13.5), Germany (13.6), and Austria (12.4) had the lowest. There was also profound variation in use of different classes of antibiotics. In another study (Vaccheri et al. 2002), antibiotic consumption was 16.5 DDD/1 000 inhabitants/day in Ravenna (Italy) and 10.4 DDD/1 000 inhabitants/day in Funen (Denmark). Italian children received a greater amount (four-fold in DDDs) of antibiotics than Danish ones. In Italy, injectable antibiotics (third generation cephalosporins or aminoglycosides) accounted for 4% of total DDDs and 11% of exposed subjects. In Funen, use of injectable antibiotics was negligible. The bulk of prescriptions (90% of total DDDs) were made up of eight (out of thirty-eight) different antibiotics in Denmark, mainly narrow-spectrum penicillins and macrolides (1st: phenoxymethylpenicillin), and of eighteen (out of sev-

[1] Daily defined dose, see Chap. 1, this book.

enty-four) antibiotics in Italy, mainly broad-spectrum penicillins, macrolides, fluoroquinolones and cephalosporins. These examples show that at least on the level of specific compounds, general data on the consumption of antibiotics may be misleading. An evaluation on a case-by-case basis may be necessary to assess substance flows of antibiotics.

If antibiotics are sold over the counter (OTC), i.e., without any prescription consumption may still be higher. According to different legislation and differing degrees of importance ascribed to the use of antibiotics, reliable data providing information on the total use and the use patterns of antibiotics and the per capita consumption only exist for a few countries. In most countries, β-lactam antibiotics, including the subgroups of penicillins, cephalosporins and as a marginal fraction carbapenems and others make up the largest share of human-use antibiotics, and account for approximately 50–70% of total antibiotic use. In most countries, sulfonamides, macrolides, and fluoroquinolones follow in order of decreasing use (*http://www.esac.ua.ac.be/main.aspx?c=*ESAC2&n=1063*). In human medicine, use was between 3 and 5 g per capita and year in Germany according to more reliable published data. Expressed on a per capita basis, local sales amounted to 2.6 g per capita in a small German municipality, compared to consumption of 4.95 g per capita nationwide in Germany in 1998 (Alexy et al. 2006; Fig. 6.2).

Excretion rates for the unchanged active compound cover a broad range (10–90%, ceftazidime for example less than 10%). A summary of the amounts used and compound-specific excretion rates show that three quarters of the amounts used are excreted unchanged into wastewater (detailed data in Kümmerer and Henninger 2003). On average, if the volume for all the antibiotics used is totaled, the metabolic rate is 30% (Kümmerer and Henninger 2003).

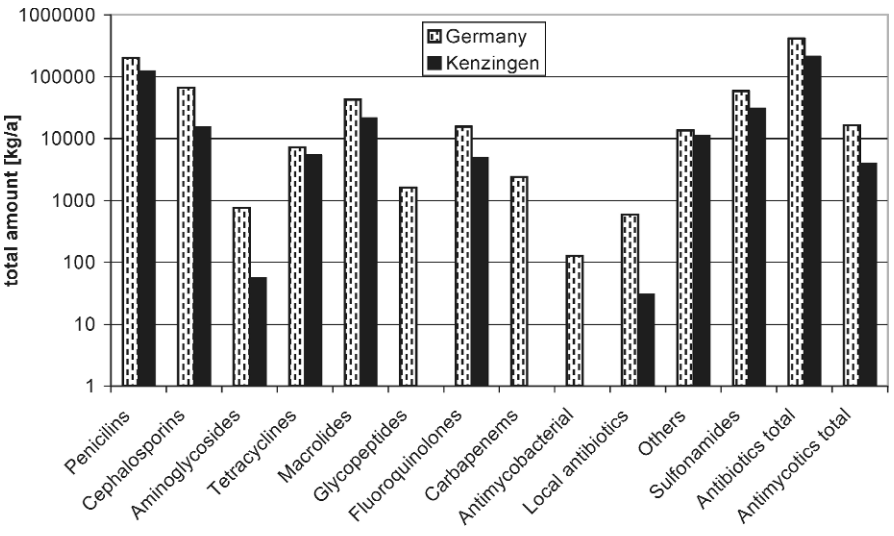

Fig. 6.2. Nationwide consumption of antibiotics (groups) calculated on the basis of data collected in Kenzingen (21.1 kg a^{-1}, thereof 13.5 kg a^{-1} emitted into sewage; *solid bars*) versus German average (Alexy et al. 2006)

Data on hospital shares of antibiotic consumption are not available for most countries. De Wirth et al. (2004) found that on the basis of DDDs, the use of antibiotics in hospitals accounts for 5–20% of the total antibiotic use in European hospitals. Antibiotic use (expressed as DDD per day and capita) ranges from 8.6 to 36 in Europe (*http://www.esac.ua.ac.be/main.aspx?c=*ESAC2&n=1066l*).

Regional consumption also may be different within one country: 9.6–17.3 DDDs per capita and day in Germany (de Wirth et al. 2004). In another study, local differences were found within the nationwide average in Germany (Alexy et al. 2006): nationwide average use of antibiotics in 1998 was 4.95 g per capita and year whereas it was 2.9 g per capita and year in a small town (9 000 inhabitants, no hospital present but two elderly peoples' homes). If the share of the average use in hospitals is added, 3.85 g per capita and year would result. These data demonstrate that there should be a potential for the reduction of antibiotic use without negative health consequences. Most antibiotics are used outside hospitals (Fig. 6.3). In total, 412 t of antibiotics were used in Germany 1998. Taking the compound-specific metabolization rates into account, 305 t are emitted into wastewater, thereof were 92 t due to hospitals Kümmerer and Henninger 2003). In a Norwegian study, the authors found that only 10% of the antibiotics in the influent of the Oslo sewage treatment plant originated from hospitals (Thomas et al. 2007).

Only for the group of the cephalosporines are hospitals the major source (Fig. 6.4). However, these roughly calculated data are based on data from hospitals offering a maximum service spectrum. In these hospitals, 3rd and 4th generation antibiotics of which cephalosporines have a big share are overrepresented.

6.2.4
Veterinary Medicine and Animal Husbandry

Antimicrobials are amongst the most widely used pharmaceutical compounds for animals (Boxall et al. 2003a,b). These drugs are used in animal husbandry for veterinary purposes or as growth promoters (particularly in large-scale animal farming and intensive livestock treatment). Application of antibiotics in animal husbandry leads to the presence of these compounds in dust in stables (see Hamscher and Hartwig, this book). A review on veterinary use of pharmaceuticals including anti-

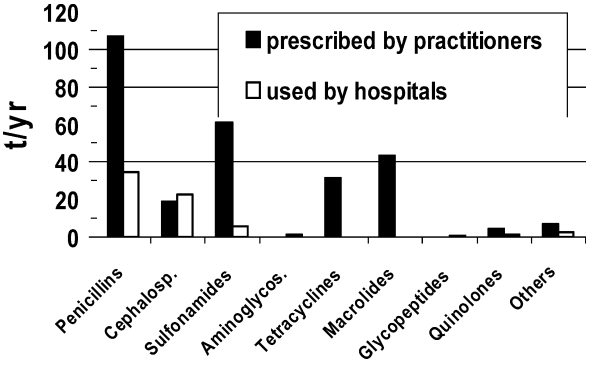

Fig. 6.3. Use of antibiotics in hospitals compared to prescription by general practitioners (Kümmerer and Henninger 2003)

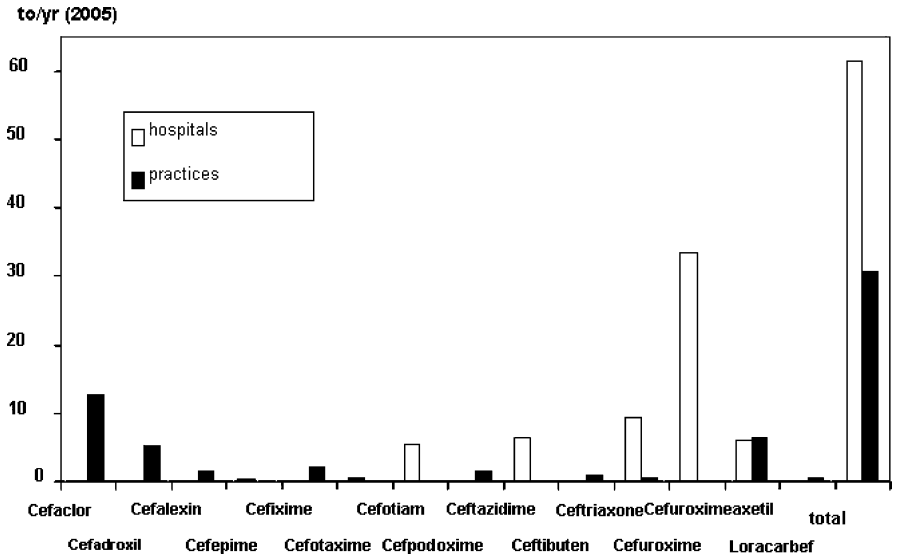

Fig. 6.4. Use of cephalosporins (cephalosporins, ATC-Code J01DB–E) in German hospitals with maximum service spectrum. Projections, calculated from: hospitals – University Medical Center Freiburg via hospital bed proportion; practices – Arzneiverordnungs-Report 2006: statutory health insurance – proprietary medicinal

biotics on a global scale was given only recently by Sarmah et al. (2006). Therefore, in this chapter only some rough information is given. In 1996, about 10 200 t of antibiotics were used in the EU, of which approximately 50% were applied in veterinary medicine and as growth promoters. In 1999, 29% were used in the veterinary field and 6% as growth promoters in the EU (FEDESA 2001). The ban on growth promoters within the EU has been in force since 2006. Therefore, the amounts used in livestock will decline as already shown by the examples of Sweden, Denmark and Switzerland. But usage in therapy may increase as has been learned in Switzerland. The World Health Organization also advises abandoning the use of antibiotics as growth promoters, as data show that there is no need to use growth promoters (Ferber 2003).

In the U.S., about 50–70% of all antibiotics consumed are given to healthy livestock. It is estimated that greater than 50% of the antibiotics used in the USA are given to feed animals (e.g., chickens, pigs and cattle) in the absence of disease (Union of Concerned Scientists 2001). According to data collected from a survey of members of the Animal Health Institute (AHI), the sales of antibiotics used to treat, prevent and control disease and maintain the health of animals rose 7.5% in the USA from 2003 to 2004 (*www.ahi.org/mediaCenter/documents/Antibioticuse2004.pdf*). In 2004, 9.8 million metric tons of antibiotics were sold for use in farm and companion animals, an increase from 9.16 million tons sold in 2002. Main groups are ionophores/arsenicals 4.28 million t, tetracyclines 2 million t, cephalosporins, macrolides, lincosamides,

polypeptides, streptogramins, and other minor classes of antibiotics 1.95 million t, sulfonamides and penicillins 0.54 million t. This group had the biggest increase (+50%) from 2003 to 2004.

6.2.5 Plant Agriculture

Antibiotics have been used since the 1950s to control certain bacterial diseases of high-value fruit, vegetable, and ornamental plants. Today, the antibiotic most commonly used on plants is streptomycin. Some oxytetracycline is used too. Primary uses are on apple, pear, and related ornamental trees for the control of fire blight caused by *Erwinia amylovora*. In the USA, antibiotics applied to plants account for less than 0.5% of total antibiotic use (McManus et al. 2002); 13.835 metric tons of streptomycin were applied in the USA (*http://www.apsnet.org/online/feature/Antibiotics/*). To be a viable candidate for disease control, the antibiotic needed to: (*i*) be active on or inside of the plant; (*ii*) tolerate oxidation, UV irradiation, rainfall, and high temperatures. These properties are just the ones cresting problems in the environment. However data on streptomycin concentrations in the soil from fruit crops are missing. Again due to different regulations, the situation varies from country to country. In Germany, for example, the use of streptomycin in fruit agriculture needs a special allowance that is available only on a case-by-case basis but not as a general one.

6.2.6 Aquaculture

The current definition of aquaculture, according to FAO, is "the farming of aquatic organisms including fish, mollusks, crustaceans, and aquatic plants." Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents. Antibiotics authorized for use in aquaculture are oxytetracycline, florfenicol, premix, sarafloxacin, erythromycin sulfonamides potentiated with trimethoprim, or ormethoprim (Serrano 2005).

6.3 Occurrence

After administration, human-use antibiotics or their metabolites are excreted into the effluent and reach the sewage treatment plant (STP). If they are not eliminated during the purification process, they pass through the sewage system and may end up in the environment, mainly in the water compartment. There are two main ways in which antibiotics reach the environment. Antibiotics for human use end up in the aquatic environment. Antibiotics used for veterinary purposes or as growth promoters are excreted by the animals and end up in manure. Since the publication of the second edition, numerous papers on the occurrence of antibiotics in the aquatic system in

various countries have been published. It has been found that the concentrations are mostly in the same range (see below). Therefore, not all literature published on the topic is covered in the following.

6.3.1 Wastewater, Surface Water, Groundwater, Drinking Water, and Seawater

Researchers have studied the presence of antibiotics in the environment quite extensively (Alexy and Kümmerer 2006). As for other pharmaceuticals besides antibiotics, it has been found that the concentrations measured for antibiotics in the different countries are in the same range as concentrations in the different compartments, respectively (see first and second editions of this book; this book; Batt et al. 2005; Botisi et al. 2007; Hernandez et al. 2007). The concentrations were in the higher µg/l range in hospital effluent, in the lower µg/l range in municipal wastewater, and in the higher and lower ng/l range in different surface waters, groundwater, and seawater in a harbor (Xu et al. 2007) – if found in the latter at all. The compounds that have been analyzed up to now cover a limited number of different important classes of antibiotics. They include mainly macrolides (e.g., chlarythromycin, erythromycin, roxithromycin), aminoglycosides (include for example amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, of which only gentmicin has been investigated), tetracyclines (naturally-occurring: tetracycline, chlortetracycline, oxytetracycline, demeclocycline (not analyzed); semi-synthetic: doxycycline), sulfonamides (many compounds among them sulfadimethoxine (not investigated)), sulfanilamides (only a few investigated), sulfamethoxazole, sulfasalazine), and quinolones (1st generation: nalidixic acid (rarely investigated); 2nd generation: e.g., ciprofloxacin, lomefloxacin (not investigated), norfloxacin, ofloxacin; 3rd generation: e.g., levofloxacin, sparfloxacin (not investigated), tosufloxacin (not investigated); 4th generation (not investigated): e.g., clinafloxacin, gemifloxacin, moxifloxacin, sitafloxacin)) to name just a few. Quinolones (ciprofloxacin most often analyzed) and other pharmaceuticals have been detected in the effluent of hospitals (Hartmann et al. 1998; Lindberg et al. 2004; Turiel et al. 2005a; Brown et al. 2006; Thomas et al. 2007; Martins et al. 2008) up to a low µg/l range. For most other antibiotics, no analytical methods have been described for detection in the environment up to now.

The occurrence of β-lactams (including penicillins, cephalosporins carbapenems, monobactams, β-lactamase inhibitors), has not been covered frequently, despite the fact that β-lactams account for by far the highest proportion of consumption (Färber 2002; Christian et al. 2003; Kümmerer and Henninger 2003; Goossens et al. 2005; *http://www.esac.ua.ac.be/main.aspx?c=*ESAC2&n=1066l*). In one study, β-lactams were detected in the lower µg/l range in hospital effluent and in the influent of a municipal STP (Christian et al. 2003). The concentrations found for β-lactams are low compared to the ones expected from the extensive use of β-lactams (some µg/l or less found instead 20–30 $\mu g\, l^{-1}$ expected). It is not clear whether they are not present in the aquatic environment because of the possible cleavage of the β-lactam ring or whether this finding is due to the fact that they have not been analyzed or whether it is due to possible analytical shortcomings and difficulties. Antibiotics have also been found in drinking water (Ye et al. 2007).

6.3.2
Sewage Sludge and Sediments

Human and veterinary antibiotics are present in sediments. Kim and Carlson (2007) detected tetracyclines, sulfonamides and macrolides. In intensive fish farming, infections are treated by feeding antimicrobial agents directly into the water. The substances used in fish farming can enter the sediments directly from the water without undergoing any kind of purification process. This results in high local concentrations in the water compartment and in the adjoining sediments. Some investigations have demonstrated the presence of antibiotics applied extensively in fish farming in sediments beneath fish farms (Jacobsen and Berglind 1988; Björklund et al. 1991; Coyne et al. 1994; Migliore et al. 1995).

6.3.3
Manure and Soil

Investigations have shown that tetracyclines and sulfonamides are present in liquid manure at concentrations of up to 20 and 40 mg l^{-1}, respectively (Hamscher et al. 2000; Thiele-Bruhn 2003). Antibiotics have been detected in soil in concentrations in the mg/kg range (Hamscher et al. 2002; Rabølle and Spliid 2000; Thiele Bruhn 2003; Jacobsen et al. 2004; Sarmah et al. 2006; Martinez Carballo et al. 2007; Schmidt and Römbke this book). Biodegradation in soils of compounds from different antibiotic groups such as virginiamycin, sarafloxacin, tetracycline, oxytetracycline, chlortetracycline, and cyclo-sporine A was slow. Tylosin disappeared soon after the application of manure. Hamscher et al. (2002) detected tetracycline and chlortetracycline in ten out of twelve soil samples. The highest average concentration of 86.2 µg kg^{-1} (0–10 cm), 198.7 µg kg^{-1} (10–20 cm), 171.7 µg kg^{-1} (20–30 cm) tetracycline, and 4.6-7.3 µg kg^{-1} (all three sublayers) chlortetracycline were found (also Hamscher et al., 2nd edition of this book). The authors conclude that tetracyclines enter the soil in significant concentrations in liquid manure, build persistent residues and accumulate in soil. Simon et al. (2003) also found a high persistence of tetracycline in the environment. Soil that has been fertilized with liquid manure also showed a sulfadimidine content even seven months after its application (Christian et al. 2003).

6.4
Elimination

Since it cannot be excluded that antimicrobials in the environment may have a severe impact on aquatic and terrestrial ecosystems, their elimination is of predominant importance. It has to be noted that the results of bio- or photodegradation studies depend on conditions, e.g., temperature, composition of matrix, etc.

6.4.1
Sorption

Binding to particles or the formation of complexes may cause a loss in detectability as well as a loss in antibacterial activity. The loss of antibacterial activity, for example, was demonstrated for an aquaculture antimicrobial in seawater driven by the formation of complexes with the magnesium and calcium naturally present in marine wa-

ter. Tetracyclines are able to form complexes with double cations, such as calcium or magnesium (Christian et al. 2003). Golet et al. (2002) and Giger et al. (2003) have confirmed the hypothesis that fluoroquinolones (FQs) become highly enriched in sewage sludge (concentrations ranging from 1.4 to 2.42 mg kg^{-1} of dry matter (also Alder et al., 2nd edition of this book). Quinolones have been found to sorb to sediment (Hektoen et al. 1995). Antibiotics applied in human medicine (e.g., FQs, macrolides) can reach the terrestrial environment with sewage sludge. The authors also demonstrated the persistence of trace amounts of FQs in sludge-treated soils several months after application. These results indicate the importance of sludge management strategies to determine whether human-excreted antibiotics enter the environment. In the case of sulfadiazine and other sulfonamides it has been found that elimination by sorption to soil particles is a significant process (Tolls 2001; Kreuzig and Höltge 2005; Heise et al. 2006; Schmidt et al. 2008). Due to the intense sorption character of tetracycline, the environmentally relevant concentration of this substance rules out any adverse effects against microbial biocenosis.

6.4.2 Photolysis, Hydrolysis, and Thermolysis

In general, data on instability of antibiotics can be found in the medical and pharmaceutical literature. Data from drug registration procedures may give guidance on compounds where degradation can be expected. However, the effectiveness of depletion processes is different under environmental conditions such as pH or water hardness (Werner et al. 2006) and may be impacted by matrix, location and season.

Some antibiotics are sensitive to light (e.g., quinolones, tetracyclines). This may be of significance for surface water as an additional elimination pathway. The significance and the extent of direct and indirect photolysis of antibiotics in the aquatic environment are widely unknown. It should be noted, however, that incomplete photo transformation and photodegradation may lead to toxic compounds, but not necessarily (Coqgor et al. 2006; Arslan-Alaton and Caglayan 2006; Gonzales et al. 2007; Iksender et al. 2007) The effectiveness of the processes depends on light intensity and frequency (Hu and Coats 2007). The latter relates to the absorption spectrum of a compound. Therefore, phototransformation may not occur when the compounds are present in turbid water or if the creek, river, or lake is shadowed by trees or covered in soil, sewage and sewage pipes,. It varies with seasons and the latitude (see Kallenborn et al., this book).

If a substance is sensitive towards light, photodecomposition may be of major significance in the elimination process, as reported by Lunestad et al. (1995). Photodecomposition takes place mainly in surface water since soil and sediment prevent a substance from undergoing photochemical degradation due to the lack of light in these matrices. Samuelsen (1989), for example, investigated the persistence of oxytetracycline sensitivity towards light in seawater as well as in sediments. The antibacterial substance proved to be stable in sediments rather than in seawater. As no mechanism of decomposition other than photodegradation is known for this antimicrobial (Oka et al. 1989), the substance remains in the sediment for a long period, as proved by Lunestad and Goksøyr (1990). Tetracyclines and aminoglycosides are susceptible to photodegradation. Fluorquinolones are insensitive to hydrolysis and increased tem-

peratures, but are degraded by UV light (Viola et al. 2004; Turiel et al. 2005b; Thiele-Bruhn 2003; Burhenne et al. 1997a). Other compounds are not photodegradable (Turiel et al. 2005b) In surface water, photolysis may play a role as has been shown by Burhenne et al. (1997b) and by Vasconcelos et al. (2008) for quinolones (see also Vasconcelos et al., this book; and Boree et al. (2004) for sulfonamides). Sulfanilic acid was found as a degradation product common to most of the sulfa drugs. In this study, photodegradation of these drugs in natural water samples (e.g., Lake Superior) was attributed solely to direct photolysis. Studies taking into account indirect photolysis and interaction with dissolved organic matter (DOM) such as humic and fulvic acids are rare (Sukul et al. 2008). Such data would be helpful to better understand the fate of antibiotics in surface waters. Photolysis of tylosin and photo deactivation in soil and surface water has been described (Hu and Coats 2007; Werner et al. 2007) as well as of nitrofuran antibiotics (Edlhund et al. 2006).

Another kind of abiotic elimination of substances is hydrolysis. Instability in water was demonstrated for some antibiotics (Halling-Sørensen 2000). However, sulfonamides and quinolones are stable against hydrolysis. In general, hydrolysis rates for oxytetracycline increase as pH deviates from pH 7 and as temperature increases. The same holds for β-lactams. In laboratory biodegradability testing with sewage sludge, it has been found that β-lactams are hydrolyzed. This leads to deactivation of antibiotic activity (Längin et al. 2008). A subsequent step is decarboxylation. Even if the compounds' structures are closely related, the degree of hydrolysis and decarboxylation, the share of microbial activity in these processes and the kinetics are different (Längin et al. 2008). This means that many of the most frequently applied penicillins can probaly not be detected in the environment at the expectated concentration level. The β-lactam ring from β-lactam antibiotics, e.g., penicillins, can be opened by β-lactamase, an enzyme in bacteria; Pouliquen et al. (1992) studied the elimination of an antibiotic in seawater. The half-lives of oxytetracycline under investigation varied due to differences in temperature, light intensity and flow rate from one test tank to another.

Li et al. (2008) reported thermal decomposition of penicillin G as treatment of the effluent of a production plant.

6.4.3 Biodegradation

Most antibiotics tested so far where not biodegraded (Hirsch et al. 1999; Richardson and Bowron 1985; Wiethan et al. 2000; Al-Ahmad et al. 1999; Kümmerer et al. 2000; Ingerslev et al. 2001; Ingerslev and Halling-Sørensen 2001; Thiele-Bruhn 2003; Alexy et al. 2003; Alexy et al. 2004; Gartiser et al. 2007a,b). Biodegradability has been poor for most of the compounds investigated so far in laboratory tests – even for some of the β-lactams. Of the sixteen antibiotics tested, only benzyl penicillin (penicillin G) was completely mineralized in a combination test (combination of two tests OECD 302B and OECD 301B) (Gartiser et al. 2007a). Trials with radio-labeled compounds revealed that approximately 25% of benzyl penicillin was mineralized within twenty-one days, whereas ceftriaxone and trimethoprim were not mineralized at all (Junker et al. 2006). Carucci et al. (2006) found that biodegradability of lincosamine in a sequence batch reactor was worse with municipal wastewater than with synthetic waste-

water. No evidence of biodegradation for tetracycline was observed during a biodegradability test (SBR), and sorption was found to be the principal removal mechanism of tetracycline in activated sludge (Kim et al. 2005).

Antibiotics occurring in soil and sediment proved to be quite persistent in laboratory testing and in field studies. They do not biodegrade well under anaerobic conditions (Alexy et al. 2006, 2nd edition of this book; Gartiser et al. 2007b; Schmidt and Römbke, this book). In a laboratory test, enrofloxacin was degraded by a white rot fungus, which may be present in soils but not in sewage or sewage sludge (Wettstein et al. 1997, 1999).

However, tylosin was biodegraded (Hu and Coats 2007). Substances extensively applied in fish farming had long half-lives in soil and sediment, as reported in several investigations (Marengo et al. 1997; Samuelsen et al. 1992, 1994; Hansen et al. 1992; Hektoen et al. 1995; Jacobsen and Berglind 1988; Capone et al. 1996). However, some substances were at least partly degradable (Gilbertson et al. 1990; Samuelsen et al. 1994, 1991; Donoho 1984; Capone et al. 1996; Thiele-Bruhn 2003). Gavalchin and Katz (1994) studied the degradability of seven fecal-borne antibiotics in soil and found that five antibiotics disappeared after incubation at 30 °C, while only two were eliminated when the samples were incubated at 4 °C. The influence of the soil composition on elimination and half-life was demonstrated by Weerasinghe and Towner (1997) when they were studying the aerobic biodegradability of virginiamycin in six different soils under laboratory conditions. The substance was found to degrade in each type of soil, but half-lives varied within a range of 87 to 173 days. Enrofloaxacin was transformed by a fungus (Wetzstein et al. 1997; Parshikov et al. 2000; Karl et al. 2006) as well as ciprofloxacin (Wetzstein et al. 1999).

Some antibiotics are taken up by plants such as vegetables and corn (Kumar et al. 2005; Grote et al. 2007). The test crops corn (*Zea mays* L.), green onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L., Capitata group) absorbed chlortetracycline but not tylosin (Kumar et al. 2005). The concentrations of chlortetracycline in plant tissues were small (2–17 $\mu g\ kg^{-1}$ fresh weight), but these concentrations increased with increasing amounts of antibiotics present in the manure. Results from the 45 d greenhouse experiment (Dolliver et al. 2007) showed that sulfamethazine was taken up by crops, with concentrations in plant tissue ranging from 0.1 to 1.2 $mg\ kg^{-1}$ dry weight. Sulfamethazine concentrations in plant tissue increased with a corresponding increase of sulfamethazine in manure. Highest plant tissue concentrations were found in corn and lettuce, followed by potato. Total accumulation of sulfamethazine in plant tissue after 45 d of growth was less than 0.1% of the amount applied to soil in manure. These results raise potential human health concerns of consuming low levels of antibiotics from produce grown on manure-amended soils.

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Veterinary Antibiotics in Dust: Sources, Environmental Concentrations, and Possible Health Hazards

G. Hamscher · J. Hartung

7.1 Introduction

Over the past decade, pharmaceuticals have been recognized as a new class of ubiquitously occurring persistent contaminants (Halling-Sørensen et al. 1998; Daughton and Ternes 1999; Kümmerer 2001; Boxall et al. 2003, 2004; Diaz-Cruz et al. 2003, Thiele-Bruhn 2003, Sarmah et al. 2006). A large number of drugs are used extensively in both human and veterinary medicine. Among hormonally active drugs, antibiotics are one of the most important substance classes with a possible environmental impact. Estimations of the European Federation of Animal Health (FEDESA) revealed that approximately 8 500 tons of antibiotics were used in human medicine and 4 700 tons in veterinary medicine in the European Union (plus Switzerland) in 1999 (Anonymous 2001).

For decades, liquid manure from livestock farming and sewage sludge from treatment plants have been applied to agricultural fields as a sustainable principle of nutrient recycling. With rapidly increasing knowledge about drugs entering the environment via these routes (Langhammer et al. 1990; Gavalchin and Katz 1994; Hamscher et al. 2000, 2002, 2005; Höper et al. 2002; De Liguoro et al. 2003; Schlüsener et al. 2003; Martinez-Carballo et al. 2007) and thus probably contaminating our feed, food and groundwater resources, there is increasing concern about the potential risks associated with this common practice.

Most antibiotics (e.g., amoxicillin, sulfamethazine, erythromycin, chlortetracycline and tetracycline) are only poorly metabolized after administration to humans or animals. It has been shown that tetracycline and sulfamethazine are present in liquid manure at concentrations of up to 66 mg l^{-1} and 40 mg l^{-1}, respectively, after application of these drugs in recommended dosages (Berger et al. 1986; Langhammer et al. 1988; Winckler and Grafe 2001).

Therefore, antibiotics and in some cases also their metabolites are excreted in substantial amounts and can enter the environment via several pathways. Today there are at least three well-established pathways for veterinary pharmaceuticals to enter the compartments sediment and soil (see Fig. 7.1): they reach sediments after direct application of antibiotics in aquaculture; they reach soil directly via urine and/or feces of animals kept outdoors or they reach soil via solid or liquid manure applied as fertilizer.

7.2 Sources and Environmental Concentrations of Antibiotics in Dust

Despite the worldwide availability of a vast body of knowledge about the concentrations of pharmaceuticals in the aquatic environment (Daughton and Ternes 1999;

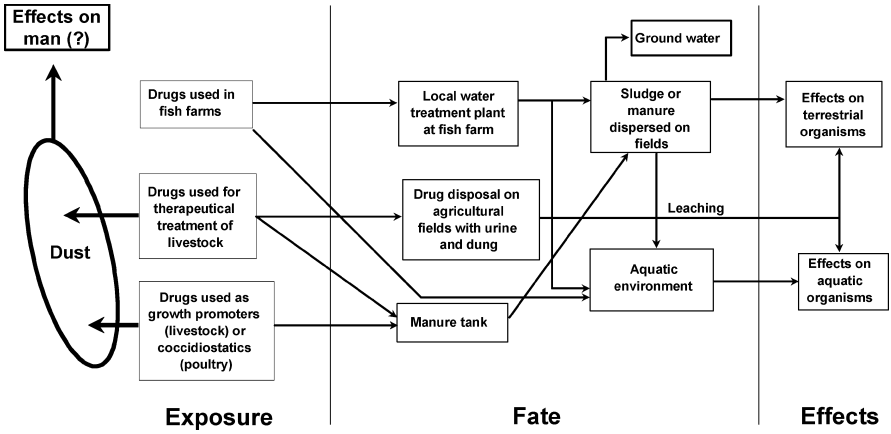

Fig. 7.1. Anticipated exposure routes of veterinary drugs into the environment (modified according to Jørgensen and Halling-Sørensen 2000; Hamscher et al. 2003)

Kümmerer 2001, 2004; Heberer 2002; Kolpin et al. 2002; Derksen et al. 2004; Jones et al. 2004; Sumpter and Johnson 2005; Hao et al. 2007), only one comprehensive report has been published on environmental concentrations of antibiotics in dust originating from a pig-fattening house (Hamscher et al. 2003).

In large-scale pig production, many antibiotics are commonly used, and this production system in particular represents a considerable source of dust (Hartung 1997, 1998; Pedersen et al. 2000). This results both in high dust exposure of farmers and farm workers in animal confinement buildings causing respiratory health hazards (Donham 1993; Platz et al. 1995; Nowak 1998; Iversen et al. 2000; Radon et al. 2002) and in permanent emission of dust particles into the environment by exhaust ventilation air (Hartung 1995; Seedorf and Hartung 2002). About 85% of the animal house dust consists of organic material including protein from pig skin, animal feed, endotoxins, fungi and bacteria in concentrations of up to 50 million colony-forming units per gram of dust (Hartung 1997). This cocktail of hazardous substances may be mainly responsible for the infectious, allergic and toxic risk of dust. Today there is no doubt as regards the health hazards of dust in animal confinement buildings, but there is still too little knowledge concerning the possible risk of specific substances in dust (Nowak 1998).

In a retrospective study, dust samples collected at a pig fattening farm from 1981 to 2000 were analyzed for various antibiotics including tetracyclines, sulfonamides, tylosin and chloramphenicol. In 90% of these samples, we detected up to five different antibiotics including tylosin, various tetracyclines, sulfamethazine and chloramphenicol in the mg/kg range (molecular structures of these compounds are shown in Fig. 7.2).

In particular, tylosin was present in sixteen out of twenty samples, three even containing very significant concentrations greater than 5 mg kg^{-1}. In thirteen samples, sulfamethazine was present at concentrations up to 2.9 mg kg^{-1}, and several tetracyclines were present in twelve samples (0.2–5.2 mg kg^{-1}). In another three samples we detected chloramphenicol – for which a prohibition on its use in animal husbandry

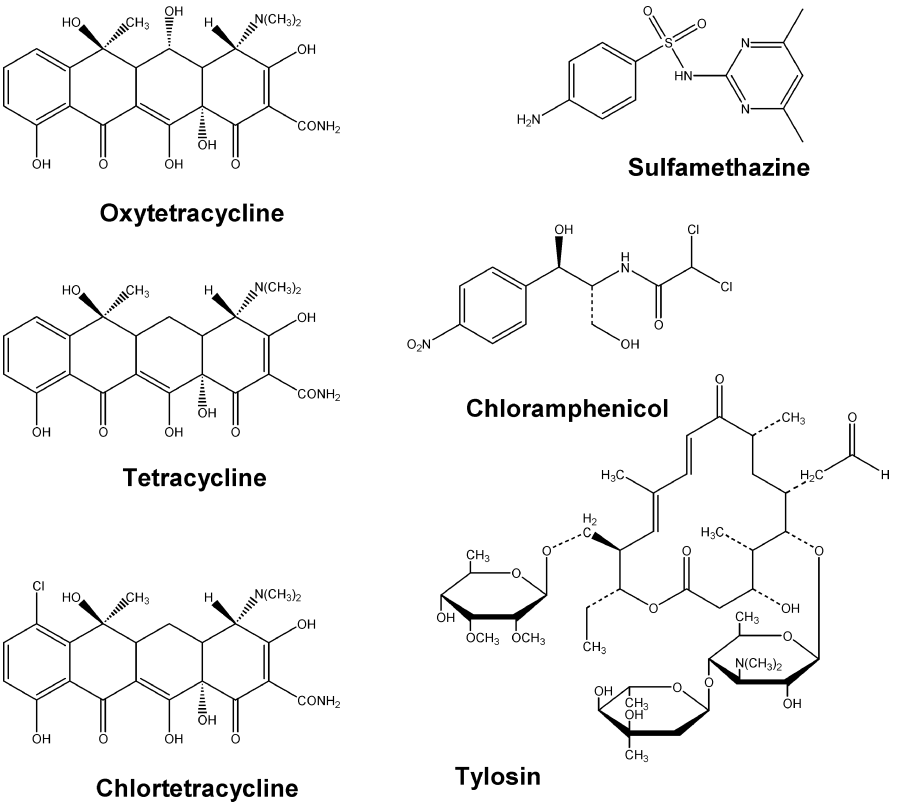

Fig. 7.2. Molecular structures of the antibiotics frequently found in dust samples originating from a pig fattening farm (Hamscher et al. 2003)

came into effect in 1994 in the EU and the USA – in concentration levels between 2.0 and 9.1 mg kg^{-1} (see Fig. 7.3).

7.3 Possible Health Effects of Antibiotics in Dust

Recently farmers' exposure (at least for up to 20 years) to various antibiotics via the contamination of dust was demonstrated (Hamscher et al. 2003). Consequently, allergic risks may arise from the occurrence of these compounds in the air. In particular, tylosin and sulfamethazine which occurred in 80% and 65% of the samples respectively are drugs with known allergic potential (Hjorth and Roed-Petersen 1980; Barbera and de la Cuadra 1989; Caraffini et al. 1994; Danese et al. 1994; Choquet-Kastylevsky et al. 2002).

In a preliminary study performed by Merchant et al. (2005), asthma and farm exposures in a cohort of rural Iowa children were investigated. The high prevalence of asthma health outcomes among children living on farms that raise pigs (44.1%) and farms that raise pigs and add antibiotics to feed (55.8%) may suggest a causal role for

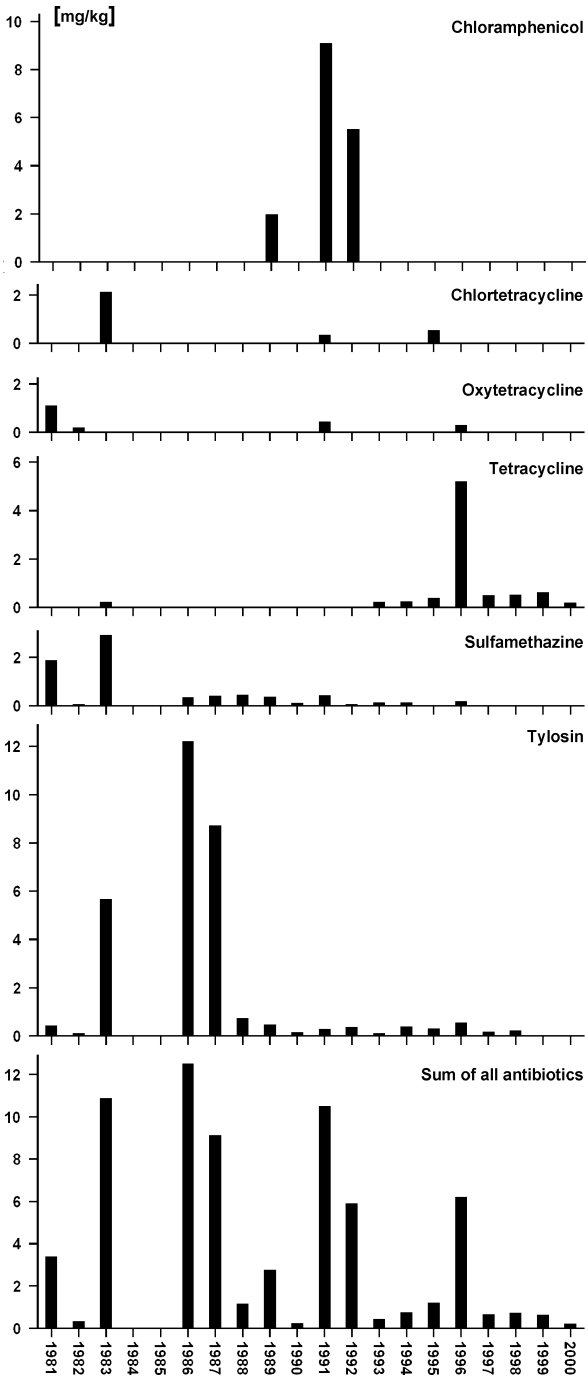

Fig. 7.3. Antibiotic residues in pig house dust (according to Hamscher et al. 2003)

antibiotics in this connection. However, the authors conclude that swine production in general contributes to the higher prevalence of asthma outcomes in this area and underline the need for more population-based studies assessing environmental or genetic determinants of asthma among farm children.

Nevertheless, there is the fact of farmers' exposure to chloramphenicol, an antibiotic with severe side effects including myelosuppression (Holt et al. 1993). Due to this reported severe side effect in humans and its genotoxic properties, it was totally banned for food-producing animals within the EU and the USA in 1994.

The development of antibiotic resistance is another risk which may also arise from the inhalation of dust contaminated with a cocktail of antibiotics. A recent survey on dust in pig fattening buildings in Europe revealed an average concentration of inhalable airborne dust of 2.2 mg m^{-3} (Takai et al. 1998). Hamscher et al. (2003) calculated the inhalation of about 5.8 mg dust contaminated with approximately 0.02 µg of various antibiotics for a farmer working eight hours a day in a confined pig building. In practice, the concentration of the antibiotics in dust can be three times higher than used in the calculation (refer to Fig. 7.3, sum of all antibiotics). Furthermore, the dust concentration in the air is usually higher in winter than in summer, and the breathing rate can also be distinctly higher during work, resulting in distinctly higher inhaled amounts of dust and antibiotics.

Although the resulting local concentration of antibiotics in the lung is far too low for any bacteriocide or bacteriostatic effect, permanent exposure to subtherapeutic concentrations of various antibiotics may represent optimal conditions for the development of antibiotic resistance.

In this regard, Zahn et al. (2001) demonstrated the transfer of tylosin and tylosin-resistant bacteria into the air of swine production facilities. In this production system, tylosin was added in subtherapeutic concentrations to the feed. Recently, Chapin et al. (2005) have found multidrug-resistant bacteria in the air of a concentrated swine feeding operation. They analyzed Enterococci, Staphylococci, and Streptococci for resistance to various antibiotics frequently used as growth promoters in the USA (clindamycin, erythromycin, virginiamycin, tetracycline and vancomycin). No vancomycin-resistant strains were found because this drug has never been approved for veterinary use in the USA. However, 98% of the bacteria showed resistance to two to four of the antibiotics under investigation and 37 of 124 isolates were resistant to clindamycin, erythromycin, virginiamycin, and tetracycline. Furthermore, the transmission of such multidrug-resistant pathogens from swine to humans has been demonstrated in several studies, and there is concern about the risk of more virulent or more resistant microorganisms emerging (Gilchrist et al. 2007).

7.4 Summary and Outlook

Dust represents a new entrance route for veterinary drugs into the environment. Substantial quantities of several antibiotics were found in dust from a pig finishing unit. Further research should include the investigation of dust from larger pig production systems and from hen houses.

As there may be adverse effects on animal and human health resulting from the exposure to dust contaminated with antibiotics, preliminary research has taken this type of exposure into consideration when assessing health risks to persons exposed to farm dust. These studies included monitoring of human health, genetic and epidemiologic aspects and the state of antibiotic resistance in farmers to antibiotics they are frequently exposed to.

In order to minimize the possible risks of antibiotics in dust, the use of antibiotics in livestock farming should be strictly reduced to therapeutic use.

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Fate of Veterinary Medicines Applied to Soils

A. B. A. Boxall

8.1 Introduction

Veterinary medicines are used widely to treat disease and protect the health of animals. Following administration, veterinary medicines may be metabolized and then released along with any metabolites either directly to the environment (as is the case with pasture animals) or indirectly during the application of manure or slurry.

Environmental assessments of veterinary medicines have been required in the USA since about 1980 and Europe since 1997. During these assessments, data may be generated on the effects of the veterinary medicine on fish, daphnids, algae, microbes, earthworms, plants and dung invertebrates. Results of the studies performed during these assessments are becoming increasingly accessible (e.g., many of the environmental assessments are now available on the USFDA website). Moreover, over the past few years, a large number of publications have been produced in this general area. Consequently a wealth of information is becoming available on the environmental fate and effects of veterinary medicines.

Following administration, veterinary medicinal products may be metabolized and the resulting metabolites excreted, along with any remaining parent compound in the urine and the feces. The resulting excreta may be released directly to land or stored and applied to land at a later stage. Once released to the environment, the medicines may be degraded transported and distributed between the different environmental compartments. Concentrations in the environment will therefore be determined by a range of factors including: the dosage of a compound; physicochemical properties of the substances; degradation in manure, slurry and soils; partitioning to soil and sediment; and the characteristics of the receiving environment (including soil type and climatic conditions).

In this chapter we review the current knowledge of those factors and processes affecting the fate of veterinary medicines that are applied to soils and provide recommendations on areas for future research.

8.2 Releases to the Environment

The amounts of veterinary medicines actually released to the environment will be primarily determined by the dosage of the substance used, the animal type treated, the method of application and the degree of metabolism. Substances used in aquaculture or as topically applied herd treatments are likely to have the highest potential to enter the environment, whereas highly metabolized herd treatments and companion

animal treatments are likely to have a low potential to reach the environment in large amounts (Boxall et al. 2004). In a recent study (Boxall et al. 2003), veterinary medicines in use in the UK were prioritized in terms of their potential to reach environmental media in large amounts. Three groups of compounds were identified as highest priority, namely, the antibiotics, antiparasitic agents and the coccidiostats.

A number of studies have investigated the metabolism of veterinary medicines by animals. The degree of metabolism varies across chemical classes as well as within classes (Table 8.1). For compounds that are administered by injection, some of the dose may remain at the injection site for sometime and may not be absorbed. For substances administered orally, the amount absorbed can range from a small proportion to 100%. Once absorbed, a product may undergo Phase I metabolism followed by Phase II metabolism. These reactions may produce polar metabolites that are excreted. If a compound is not metabolized, then it may be excreted unchanged.

Consequently, animal excreta may contain a mixture of the parent compound and metabolites. The degree of metabolism varies across substances and between species.

8.2.1 Persistence in Manure and Slurry

For animals kept at pasture and companion animal treatments, the veterinary medicines may be excreted directly to soils and surface waters. Aquaculture treatments will be released directly to surface waters.

In contrast, on livestock farms where animals are housed, large quantities of farmyard manure and or slurry will be produced and this will typically be collected and stored in manure pits for subsequent application or applied immediately to land. Slurry can be stored for many months. In the UK, the storage time for slurry varies from 0 to 50 months, with an average of 9 months and for manure from 0 to 48 months with an average of about 6 months (WRc-NSF 2000). Consequently, there is the potential for veterinary medicines to be degraded during a period of storage.

Table 8.1. Metabolism of major groups of veterinary medicines in animals (information taken from Boxall et al. 2004)

Chemical group	Metabolism
Tetracyclines	Minimal
Sulfonamides	High
Macrolides	Minimal
Aminoglycosides	Minimal to high
Azoles	Moderate
Macrolide endectins	Minimal to moderate
Lincosamides	Moderate
Fluoroquinolones	Moderate to high

Minimal: less than 20% metabolism; *Moderate*: 20 to 80% metabolism; *High*: more than 80% metabolism.

Data are available on the persistence in manure of a range of commonly used classes of veterinary medicines. The sulfonamides, aminoglycosides, β-lactams, and macrolides have half-lives of 30 d or lower (Table 8.2) and therefore may be significantly degraded during manure storage. In contrast, the macrolide endectin ivermectin and the tetracyclines have longer half-lives of many months and are therefore likely to persist during storage. There are differences in degradation rates in different manure/slurry types, which indicate that degradation in manure and slurry should be considered on a case-by-case basis.

8.3 Fate in Soil

Highest application rates of manure and slurry occur in spring and autumn. Once a veterinary medicine reaches the soil, it may partition to the soil particles, be transported to surface waters, leach to groundwater and/or be degraded. The behavior will be partly influenced by the climatic conditions at the time of application. Over the past few years, information has become available on the behavior of veterinary medicines in soils; this data is described in the following sections.

Table 8.2. Persistence of selected veterinary medicines in manure

Chemical group	Compound	Matrix	DT50 (d)	Reference
Macrolides	Tylosin	Pig slurry	<2	Loke et al. (2000)
	Eryhromycin	Liquid manure	41	Schlusener et al. (2006)
	Roxithromycin	Liquid manure	130	Schlusener et al. (2006)
Macrolide endectins	Ivermectin	Cattle dung	>45	Sommer et al. (1992)
Sulfonamides	Sulphachloro-pyridazine	Broiler faeces	<8	Van Dijk and Keukens (2000)
	Sulphachloro-pyridazine	Laying hen faeces	<90	Van Dijk and Keukens (2000)
	Sulphachloro-pyridazine	Pig slurry	>8	Boxall et al. (2003b)
Tetracyclines	Chlortetracycline	Chicken manure (+ soil)	>30	Gavalchin and Katz (1994)
	Oxytetracycline	Cattle manure	<30	Di Liguoro et al. (2004)
Others	Amprolium	Laying hen faeces	>8	Van Dijk and Keukens (2000)
	Amprolium	Broiler faeces	>90	Van Dijk and Keukens (2000)
	Nicarbazin	Broiler faeces	>8	Van Dijk and Keukens (2000)
	Salinomycin	Liquid manure	6	Schlusener et al. (2006)
	Tiamulin	Liquid manure	>180	Schlusener et al. (2006)

8.3.1 Sorption in Soil

The degree to which veterinary medicines may adsorb to soils varies widely. Consequently, the mobility of different veterinary medicinal products also varies widely. The variation in partitioning for a given compound in different soils can be significant and cannot be explained by variations in soil-organic carbon. For instance, the maximum reported organic-carbon normalized sorption coefficient for carbadox is approximately two orders of magnitude greater than the lowest reported value. These large differences in sorption behavior are explained by the fact that many veterinary medicines are ionizable with pK_a values in the pH range of natural soils. Medicines can therefore occur in the environment as negative, neutral, zwitterionic and positively charged species (e.g., Ter Laak et al. 2006a,b). Depending on the species, interactions with soil can occur through electrostatic attraction, surface bridging, hydrogen bonding or hydrophobic interactions (Ter Laak et al. 2006b). The sorption behavior is also influenced by the properties of the soil including pH, organic carbon content, metal oxide content, ionic strength and cationic exchange capacity (e.g., Ter Laak et al. 2006b; Strock et al. 2005; Sassman and Lee 2005; Jones et al. 2005).

The complexity of the sorbate-sorbent interactions means the unlike other organic substances (including many pesticides and industrial chemicals), organic-carbon normalized soil sorption coefficients are poorly correlated with octanol/water partition coefficients (a measure of hydrophobicity) (Tolls 2001; Fig. 8.1). Development of models for the prediction of the sorption behavior of veterinary medicines thus requires that predictor parameters other than logK_{OW} be considered.

Manure and slurry may also alter the behavior and transport of medicines. Recent studies have demonstrated that the addition of these matrices can affect the sorption behavior of veterinary medicines and that they may affect persistence (e.g., Thiele et al. 2004; Boxall et al. 2002). These effects have been attributed to changes in pH or the nature of dissolved organic carbon in the soil/manure system. For example, the impact of slurry amendment on sorption of the sulfonamide antibiotics has been assessed in the laboratory (Boxall et al. 2002b). Results of these investigations (e.g., Fig. 8.2) demonstrate that the addition of manure or slurry to soils does indeed affect the sorption of many veterinary medicines with sorption coefficients increasing as more slurry

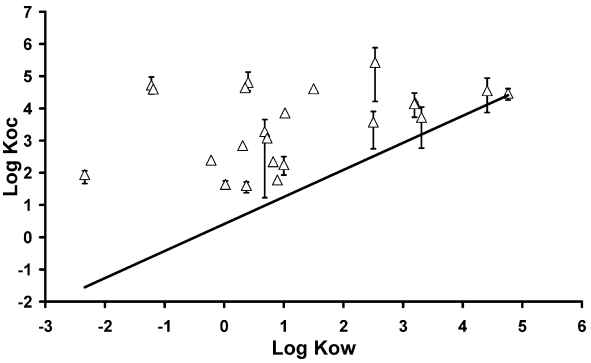

Fig. 8.1. Relationship between experimentally-derived organic-carbon normalized soil sorption coefficients and predicted coefficient for a range of veterinary medicines.

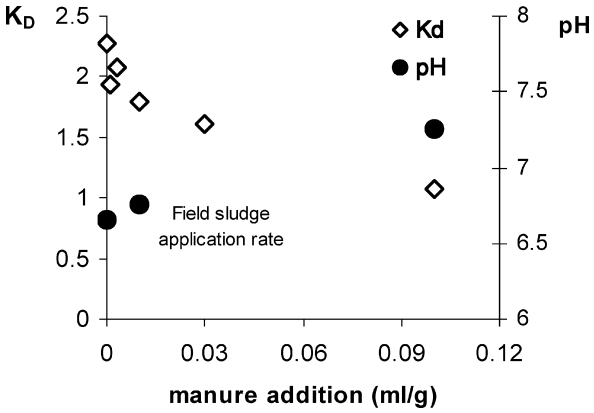

Fig. 8.2. Effect of manure amendment on the soil solution pH and K_d for the sulfonamide antibiotic sulfachloro-pyridazine in the clay loam (taken from Boxall et al. 2002b)

is added to the system. These observations could be explained by changes in the organic carbon quality and quantity caused by the manure amendment or due to changes in other characteristics of the system such as pH. Consequently, when assessing the mobility of veterinary medicines in soils, it may not only be necessary to understand other binding mechanisms but also the impacts of variables such the quality and quantity of applied slurry/manure on the behavior. Such an understanding is also likely to assist in assessing the behavior of pharmaceuticals associated with sewage sludge that is applied to land.

8.3.2 Persistence in Soil

Following application, the main route for degradation of veterinary medicines in soils is aerobic soil biodegradation. Degradation rates in soil vary across medicines with half-lives ranging from days to months (e.g., Table 8.3). The degradation of veterinary medicines is affected by environmental conditions such as temperature, pH, soil type, soil-organic carbon, nutrient conditions and the presence of specific degrading bacteria that have developed to degrade selected groups of medicine (Ingerslev and Halling-Sørensen 2001; Gilbertson et al. 1990). For example, the organophosphate diazinon has been shown to be rapidly degraded in flooded soils (DT_{50} = 1.7 d), whereas it is highly persistent in sandy soils (DT_{50} = 88–112 d). Studies into the effects of temperature on the degradation of ivermectin (Halley et al. 1993) demonstrated that under winter weather conditions the half-life ranges from 91–217 d whereas in the summer, the compound degrades much more readily with half-lives ranging from 7–14 d.

Depending on the nature of the chemical, other degradation and depletion mechanisms may also be important, including soil photolysis and hydrolysis. Photodegradation is only likely to occur in the top layer of the soil surface and hence the persistence of photodegradable substances will be dependent on farming practices such as the timing and depth of any ploughing. The degradation products of both photolytic and hydrolytic degradation processes may undergo aerobic biodegradation in the upper soil layers.

Table 8.3. Persistence of veterinary medicines in soils

Compound	$t_{1/2}$ (d)	Reference
Amoxicillin	<1	Boxall et al. (2006)
Ceftiofam	22.2–49	Boxall et al. (2004)
Chlorfenvinphos	<35–<120	Boxall et al. (2004)
Coumaphos	200–300	Boxall et al. (2004)
Danofloxacin	87–143	Boxall et al. (2004)
Deltamethrin	14–36	Boxall et al. (2004)
Enrofloxacin	>152	Boxall et al. (2006)
Erythromycin	20	Schlusener et al. (2006)
Florfenicol	<103	Boxall et al. (2006)
Ivermectin	14–56	Boxall et al. (2004)
Levamisole	<103	Boxall et al. (2006)
Metronidazole	9.7–26.9	Boxall et al. (2004)
Olaquindox	5.8–8.8	Boxall et al. (2004)
Oleandomycin	27	Schlusener et al. (2006)
Oxytetracycline	16–18	Blackwell et al. (2005)
Phenylbutazone	<103	Boxall et al. (2006)
Salinomycin	5	Schlusener et al. (2006)
Sarafloxicin	>65	Boxall et al. (2004)
Sulfachloropyridazine	2.8–3.5	Blackwell et al. (2005)
Sulfachloropyridine	21.3	Accinelli et al. (2007)
Sulfamethazine	18.6	Accinelli et al. (2007)
Tiamulin	16	Schlusener et al. (2006)
Trimethoprim	<103	Boxall et al. (2006)
Tylosin	4.1–4.2	Boxall et al. (2004)
Tylosin A	7	Hu and Coats (2007)
Tylosin D	8	Hu and Coats (2007
Virginamycin	>64	Boxall et al. (2004)

Studies have been performed to begin to characterize the transformation products that are formed as a result of degradation in soils (e.g., Ingerslev et al. 2001; Halling-Sørensen et al. 2002; Halling-Sørensen et al. 2003). For example in studies into the degradability of oxytetracycline, three transformation products were detected and four substances were tentatively identified (Halling-Sørensen et al. 2003). The degradation

products may have a similar toxicity to or even be more toxic to environmental organisms than the parent compound, and their sorption behavior may be very different. For example, anhydrotetracycline (ATC), a metabolite of tetracycline, had an EC_{50} value (i.e., 0.03 mg l^{-1} 95% CI 0.025–0.030) for sludge bacteria similar to the EC_{50} value (i.e., 0.08 mg l^{-1} 95% CI 0.02–0.25) for the parent compound Halling-Sørensen et al. (2002) (Fig. 8.3). The ATC is more mobile than the tetracycline, and hence water bodies are more likely to be exposed to the metabolite.

8.3.3 Dissipation in Field

Under field conditions, veterinary medicines may be removed from soils by a combination of degradation and leaching. Studies to date have focused on the dissipation of the sulfonamide, macrolide and tetracycline antibiotics and monensin (Blackwell et al. 2007; Kay et al. 2004, 2005; Burkhard et al. 2005; Hamscher et al. 2005; Aga et al. 2003; Halling Sørensen et al. 2005; Carlson and Mabury 2006).

Results from studies in the UK (Kay et al. 2004) demonstrate that tetracyclines (chlortetracycline, oxytetracycline and tetracycline) persist in field soils for some time (over six months) but are not generally detected at deeper layers in the soil profile. In contrast, studies using sulfonamides indicate that following application, these compounds dissipate much more rapidly with concentrations close to limits of detection being observed in soils three months after slurry application (Kay et al. 2004; Fig. 8.4).

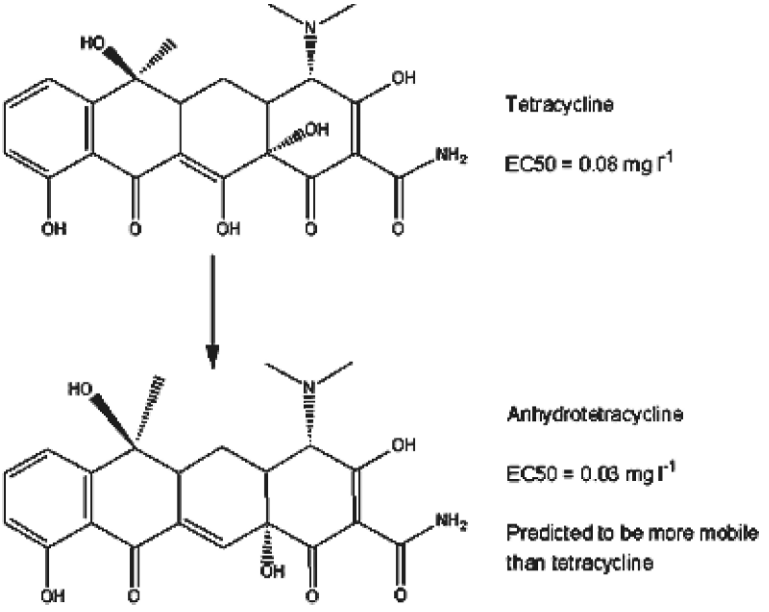

Fig. 8.3. Comparison of toxicity values to aerobic bacteria for tetracycline and its degradation product anhydrotetracycline. Data taken from Halling-Sørensen et al. (2002). Mobility was assessed using K_{oc} values which were predicted using the SRC software package PCKOC

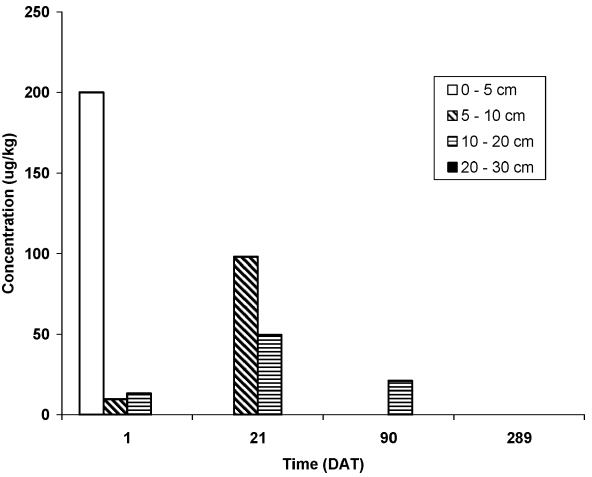

Fig. 8.4. Mean concentrations of the sulfonamide antibiotic sulfachloropyridazine following application of spiked slurry (*DAT* = days after treatment) (taken from Boxall et al. 2003b)

The differences in the observations at the field scale between classes probably reflect differences in the persistence and sorption potential of the two classes of substance. The tetracyclines are highly sorptive in soils and would therefore not be expected to leach; they are also moderately to highly persistent. In contrast, the sulfonamides are less sorptive and impersistent.

8.4 Transport from Soils to Water Bodies

Following application to soils, veterinary medicines may be transported to surface water via overland flow and drainflow and to groundwater via leaching. Recent studies have attempted to determine the movement of veterinary medicines to water bodies via each of the transport routes.

8.4.1 Overland Flow

Overland flow occurs either due to soil (or at least parts of it) becoming saturated or the intensity of rainfall being so great that the rate at which the soil can absorb it is exceeded. Runoff moving over the land surface therefore provides one of a number of hydrological pathways by which chemicals may be transported to surface waters. The transportation of antibiotics in overland flow may be particularly important, as precipitation can occur between slurry application and its incorporation into the soil (e.g., Kay 2005b; Burhard et al. 2005; Kreuzig et al. 2005; Davis et al. 2006).

In a recent study (Kay 2005b), the movement of sulfonamide and tetracycline antibiotics to surface waters in overland flow was assessed. Two contrasting scenarios were investigated: movement across undisturbed soil and movement across compacted soil. Following application of slurry, peak concentrations were measured in the first samples that were generated following rainfall; concentrations of the sulfonamide ranged from

around 0.5–1.0 mg l^{-1}, whereas the tetracycline ranged from around 0.05–0.1 mg l^{-1}. Highest concentrations were observed in runoff from the compacted soil.

Davis et al. (2006) investigated the runoff of tetracycline, chlortetracycline, sulfathiazole, sulfamethazine, erythromycin, tylosin and monensin. Highest concentrations in runoff were observed for monensin with the tetracyclines showing the lowest propensity to runoff. Sediment was shown to play an important role in the transport of erythromycin and tylosin.

Other studies with sulfonamides (Kreuzig et al. 2005) showed that the nature of the receiving environment is important in determining the potential for a medicine to runoff. In these studies, higher runoff was observed from grassland than from cultivated arable soils.

These results indicate that overland flow is indeed an important route of transport of veterinary medicines to surface waters, and the relatively high concentrations of medicines may be released to streams during runoff events.

8.4.2 Drainflow

Underdrainage in agricultural soils provides an additional route by which runoff may move and transport solutes and sediment associated pollutants to surface waters. Land drainage is employed in agriculture to remove excess water from the soil to increase yields, improve management flexibility, lower production costs and make farming more profitable. Thus, approximately 40% of agricultural land in the UK, excluding rough grazing, is drained. Tile drain systems form the primary drainage network and comprise either clay, or, in more contemporary systems, perforated plastic pipes of 70–100 mm diameter which are placed in trenches, usually 60–100 cm deep. The trenches are then back-filled to within 30 cm of the soil surface using hard crushed stone.

Movement to surface waters via drainflow has been shown to be an important route of transport for pesticides (e.g., Kladviko et al. 1991; Brown et al. 1995; Johnson et al. 1996; Novak et al. 2001), and field studies have therefore been performed to determine concentrations of veterinary medicines in drainflow from fields receiving slurry application (Kay et al. 2004; Kay et al. 2005a; Gupta et al. 2003). In studies by Kay et al. (2004), sulfonamide and tetracycline antibiotics were spiked into slurry obtained from pigs treated with tylosin, and this slurry was then applied to an underdrained field. Drainflow from the field was then monitored over time. High concentrations of the sulfonamide and tetracycline antibiotics were observed in samples obtained from drainflow events immediately following application (Fig. 8.5). Concentrations of both compounds then declined over a time although a sample containing oxytetracycline was obtained 196 days after slurry application.

Tylosin was not detected in any of the samples obtained during the study. These results are probably explained by the fact that tylosin was administered to the pigs rather than spiked into the slurry. Tylosin is very impersistent in pig slurry (Loke et al. 2000) so it is probable that the compound was removed during slurry storage.

Results from the studies indicate that drainflow is a significant transport route for veterinary medicines and that like pesticides, the transport of veterinary medicines in drainflow is related to the sorption behavior of the compounds. Non-sorptive substances will be transported to a greater extent than highly sorptive substances

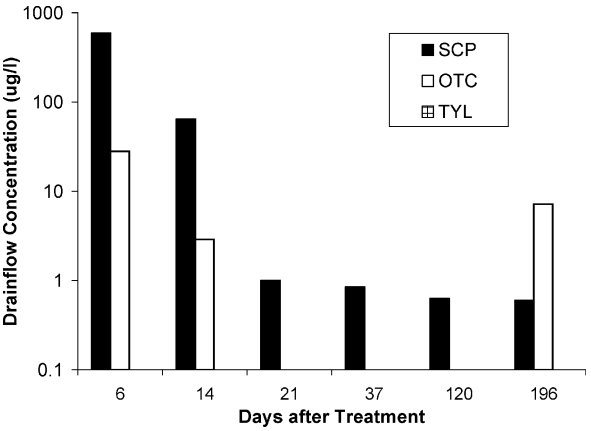

Fig. 8.5. Concentrations of sulfachloropyridazine (*SCP*), tylosin (*TYL*) and oxytetracycline (*OTC*) in drainflow obtained from a site treated with pig slurry (taken from Kay et al. 2004)

8.4.3 Leaching

A number of studies have assessed the potential for veterinary medicines to leach to groundwaters (Blackwell et al. 2007; Hamscher et al. 2000; Wehran et al. 2007). In investigations in Germany (Hamscher et al. 2000), soil water was collected and analyzed from four separate areas of agricultural land: two belonging to livestock farms and treated with animal slurry and two where no animal manure had been applied for approximately five years. Chlortetracycline, oxytetracycline, tetracycline and tylosin were all found at the limit of detection (0.1–0.3 $\mu g\, l^{-1}$) in water samples collected at 80 and 120 cm depth, independent of soil treatment. In addition, no biologically active residues could be detected with microbiological assays that had approximately fivefold higher detection limits.

Similar studies have been performed in the UK (Blackwell et al. 2007). In these studies, slurry from pigs treated with tylosin was spiked with oxytetracycline and sulfachloropyridazine and applied to field plots. Samples of soil water were then obtained over time from three depths (40, 80 and 120 cm). Concentrations of all three antibiotics in all of the water samples collected were below the limit of detection (around 0.2 $\mu g\, l^{-1}$).

Results from those studies performed to date indicate that the tetracyclines, macrolides and sulfonamides have a low potential to leach to groundwaters. Further studies are however required to determine whether other substances might pose a risk to groundwaters. Lysimeter and modeling studies suggest that leaching might be increased if veterinary medicines are frequently applied to soils at high concentrations (Wehran et al. 2007).

8.5 Uptake into Biota

Veterinary medicines may also be taken up from soil into biota (Migliore et al. 2003; Kumar et al. 2005; Boxall et al. 2006; Grote et al. 2007). Grote (2007) et al. demonstrated uptake of chlortetracycline and sulfadiazine into winter wheat, but trimethoprim was

not taken up. Studies with a range of veterinary medicines using lettuce and carrots (Boxall et al. 2006) showed that florfenicol, trimethoprim, levamisole, diazinon and enrofloxacin are taken up by these plants at environmentally-realistic concentrations. However, phenylbutazone, oxytetracyline, tylosin, sulfadiazine and amoxicillin were not detected in plant material. Kumar et al. (2005) showed uptake of chlortetracycline from manure-amended soils into corn (*Zea mays* L.), green onion (*Allium cepa* L.), and cabbage (*Brassica oleracea* L.). In this study, tylosin was not taken up by the three crops. The results of these studies appear to be contradictory, suggesting that differences in uptake may occur across plant species and test soils.

8.6 Fate in Surface Waters

Once a substance has entered surface waters, it may partition to sediment and/or undergo degradation.

A number of studies have investigated the persistence of veterinary medicines in surface waters and freshwater and marine sediment. Substances may be degraded abiotically via photodegradation and/or hydrolysis or biotically by aerobic or anaerobic organisms. The degree of photodegradation will depend on a range of factors including latitude, water depth, turbidity and the presence or absence of tree cover. Table 8.4

Table 8.4. Half-lives for primary degradation of major classes of veterinary medicines in waters (summary of data reported in Boxall et al. 2004)

Chemical group	Compound	Half life
Cephalosporin derivatives	Ceftiofur	4.2 – 100 d
2,4,-diaminopytimidines	Ormethoprim	>42 d
	Trimethoprim	>42 d
Fluoroquinolones/quinolones	Oxolinic acid	<9 d
	Sarafloxicin	<1 h
Macrolide endectins	Ivermectin	<0.5 h
Organophosphates	Chlorfenvinphos	<25 d
	Coumaphos	<7 d
	Dichlorvos	<1 d
	Propetamphos	5 d – 1 yr
Sulfonamides	Sulfadiazine	>21 d
	Sulfadimethoxine	>21 d
Synethetic pyrethroids	Cypermethrin	5 d
	Flumethrin	>3 months
Tetracyclines	Oxytetracycline	<9 d
	Tetracycline	3 h
Others	Furazolidone	<9 d

provides a summary of the persistence of major classes of veterinary medicines in water.

The quinolones, tetracyclines, ivermectin and furazolidone are all rapidly photodegraded with half-lives ranging from <1 h to 22 d (Lunestad et al. 1995; Halley et al. 1993; Davis et al. 1993; Oka et al. 1989). In contrast, trimethoprim, ormethoprim and the sulfonamides are not readily photodegradable (Lunestad et al. 1995).

Of the compounds studied in terms of the potential to hydrolyze, ceftiofur is the only compound to be rapidly hydrolyzed with a half-life of 8 d at pH 7 (Gilbertson et al. 1990). Whilst propetamphos was rapidly hydrolyzed at pH 3 (11 days), hydrolysis at pH 6 and 9 was slower (1 year and 41 days) (Lewis 1998).

Of the organophosphorous compounds that have previously been authorized for use in ectoparasitic sheep dip preparations, chlorfenvinphos, coumaphos and dichlorvos are all relatively impersistent in biologically active water with half-lives ranging from <1 to <25 days (Lewis 1998; Tomlin 1997; Lewis et al. 1993). Flumethrin, a synthetic pyrethroid also used as a sheep dip ectoparasiticide, was much more persistent in water, with a half-life greater than three months.

A large body of data exists on the degradability of veterinary medicines, used for aquaculture, in both marine and freshwater sediments (Chien et al. 1999; Marengo et al. 1997; Bohm 1996; Hektoen et al. 1995; Lunestad et al. 1995; Coyne et al. 1994; Samuelsen et al. 1994; Hansen et al. 1993; Pouliquen et al. 1992; Bjorklund et al. 1990; Samuelsen 1989; Jacobsen and Berglind 1988).

Of the compounds studied to date, florfenicol, chloramphenicol and furazolidone were the least persistent with half-lives of between 0.4 and 18.4 days. The other substances studied (flumequine, ormethoprim, oxytetracycline, oxolinic acid, sarafloxacin, sulfadiazine, sulfadimethoxine and trimethoprim) persisted in sediments with half-lives being generally greater than thirty days (Table 8.5).

Table 8.5. Half-lives for major classes of veterinary medicines in sediments (summary of data reported in Boxall et al. 2004)

Chemical group	Compound	Half life (d)
Phenicols	Chloramphenicol	0.4–18.4
	Florfenicol	1.7–7.3
2,4-diaminopyrimidines	Ormethoprim	<30
	Trimethoprim	<60–100
Fluoroquinolones/quinolones	Oxolinic acid	48–>300
	Sarafloxicin	>83–>300
Sulfonamides	Sulfadiazine	50–180
	Sulfadimethoxine	>180
Tetracyclines	Oxytetracycline	9–414
Others	Furazolidone	0.75

8.7 Summary and Recommendations

Over the past few years, a number of studies have been published that have investigated the fate of veterinary medicines in a range of media, including manure, slurry, water, soil and sediment. In this chapter, we have attempted to provide an overview of the available data and of our current understanding of the fate of veterinary medicines in the environment. In summary these studies indicate that

1. The amounts of veterinary medicines released to the environment are going to be determined by a range of factors including the amount used, usage pattern and the degree of metabolism. Three main groups are likely to be of highest concern, namely the antimicrobials, the antiparasitics and the coccidiostats;
2. Metabolism varies both across chemical class and with chemical class. There may also be differences in the degree of metabolism by different animal species. As a result of metabolism, it is likely that a mixture of parent compound and metabolites will be released to the environment;
3. For substances used to treat intensively-reared animals, the manure and slurry will typically be collected and stored before being applied to land. Many substances may be degraded during storage. The persistence of major groups of veterinary medicines in manure and slurry varies. Sulfonamides, beta-lactams, macrolides and aminoglycosides are all likely to be significantly degraded during typical UK manure/slurry storage regimes. In contrast, quinolones and tetracyclines are likely to persist. Persistence varies across manure/slurry types;
4. Sorption coefficients in soils range over four orders of magnitude and there is significant variation between coefficients for the same compound in different soil types. Unlike many industrial compounds and pesticides, this variation cannot be explained by hydrophobicity and soil organic carbon content. Other factors such as clay content and soil pH may be more important. Many of the models used for other substances may therefore be inappropriate for use with veterinary medicines;
5. Veterinary medicines can persist in soils for days to years and studies have demonstrated that half-lives are influenced by a range of factors including temperature, pH and the presence of manure. In sediments, the phenicols (chloramphenicol and florfenicol) as well as furazolidone have been shown to rapidly degrade whilst the 2,4-diaminopyrimidine, quinolone, tetracycline and sulfonamide classes all persist;
6. Generally, published tests have investigated the degradation of the parent compound. Information relating to the degradation of transformation products was unavailable. Whilst the fate of a range of veterinary products has been extensively investigated, few studies have assessed the fate of metabolites and transformation products;
7. Following application to soils, veterinary medicines may be transported to surface waters in overland flow and via drainflow. The degree of transport is related to the sorption behavior of the compound;
8. The leaching potential of selected classes of veterinary medicines has been assessed to determine risks to groundwater. The results indicate that the substances investigated are unlikely to be transported to groundwaters;

9. Once in surface waters, veterinary medicines may be degraded by both biotic and abiotic processes in the water column and the bed sediment.

There are, however, a number of gaps in the available data and in our current knowledge that need addressing before the fate of veterinary medicines can be fully characterized; many of these gaps are discussed in Boxall et al. (2003c). In the future, it is recommended that work focuses on some of the following areas:

1. Collation of better information on the amounts of veterinary medicines used in different countries and on how they are used. This will allow priorities to be set and should also assist in the interpretation of results from ongoing monitoring programs of soils and groundwaters;
2. Development of sensitive analytical methods to measure veterinary medicines and their degradation products that may be released into the environment in different media (i.e., water, soils, sediment and biota);
3. Investigations into the fate and behavior of major veterinary medicines that have not been extensively studied to date such as the coccidiostats and the benzimidazole antiparasiticides;
4. Improved modeling (i.e., structure-property relationships and structure-degradability relationships) approaches are required to assess the sorption and persistence of veterinary medicines in environmental media;
5. The development of a better understanding of the potential for releases to the environment for different treatment types (to include an assessment of aerial emissions, inputs from treatment of pasture and other 'novel' routes such as runoff from the farmyard);
6. Only a limited amount of information has been produced on the transfer of veterinary medicines into biota and up through food chains. Studies should be performed to determine those factors and processes affecting bioavailability and trophic transfer;
7. Efforts should be made to develop a better understanding of the formation of transformation products and the fate and behavior of these and metabolites arising from animals in soils and water bodies;
8. Results from studies into the transport of medicines from animals and between environmental media should be used as a basis to evaluate existing exposure assessment models that are used in the risk assessment process for other chemical classes.

These studies will require input from a wide range of disciplines, but the results should allow both industry and regulators to focus resources and to more accurately assess the risks of veterinary medicines to the environment.

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Pharmaceuticals as Environmental Contaminants: Modeling Distribution and Fate

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9.1 Introduction

Concern is growing over the environmental consequences of the use of drugs for human and animal health. Long term treatments for several illnesses are a common mass practice in human health care (e.g. diuretics, beta blockers, antibiotics), a number of females are taking daily hormones to prevent unwanted pregnancies, modern life stress is handled very frequently through sedatives and tranquillizers, moreover there is in animal farming a general trend towards the intensification of production methods and production gains based on greater reliance on pharmaceuticals, feed additives, hormones and potent parasiticides (Halling-Sørensen et al. 1998).

The extent of the problem has not yet been fully evaluated, and the information for risk management is far from adequate. Human and environmental implications resulting from the massive use of human and animal drugs should be quickly evaluated. These days an increasing number of research groups are involved on this subject. Most of the approaches utilized in these studies follow the classical risk assessment procedures: calculating or assessing mass balances on consumptions and uses and monitoring the presence of the most common chemical substances in different environmental compartments in order to evaluate potential exposure (Calamari 1993). Other groups produce original experimental data on ecotoxicology and degradation.

The difficulties, however, are huge, the lack of data for environmental assessment is bigger than expected, and testing methods are not appropriate for these types of substances. Therefore, even if strategies for the evaluation are available, there is a need for adaptation, reconsideration and possibly new approaches.

In this chapter, a methodological attempt to evaluate the environmental distribution and fate of pharmaceuticals is presented, starting from their physicochemical properties and modeling through a stepwise procedure from a generic scenario towards a specific site situation. It comprises 4 stages: data evaluation, the use of generic models, the use of regional models and the use site-specific models (Fig. 9.1). The main requirements and a simple illustration will be presented.

9.2 Data Evaluation

This is the first stage in the process of understanding the fate of the chemical of interest. It involves the collection and critical assessment of structural formulas and physicochemical data such as molecular weight, vapor pressure, solubility in water, K_{ow}, and pK_a. These data are necessary to characterize the chemicals and select the type of

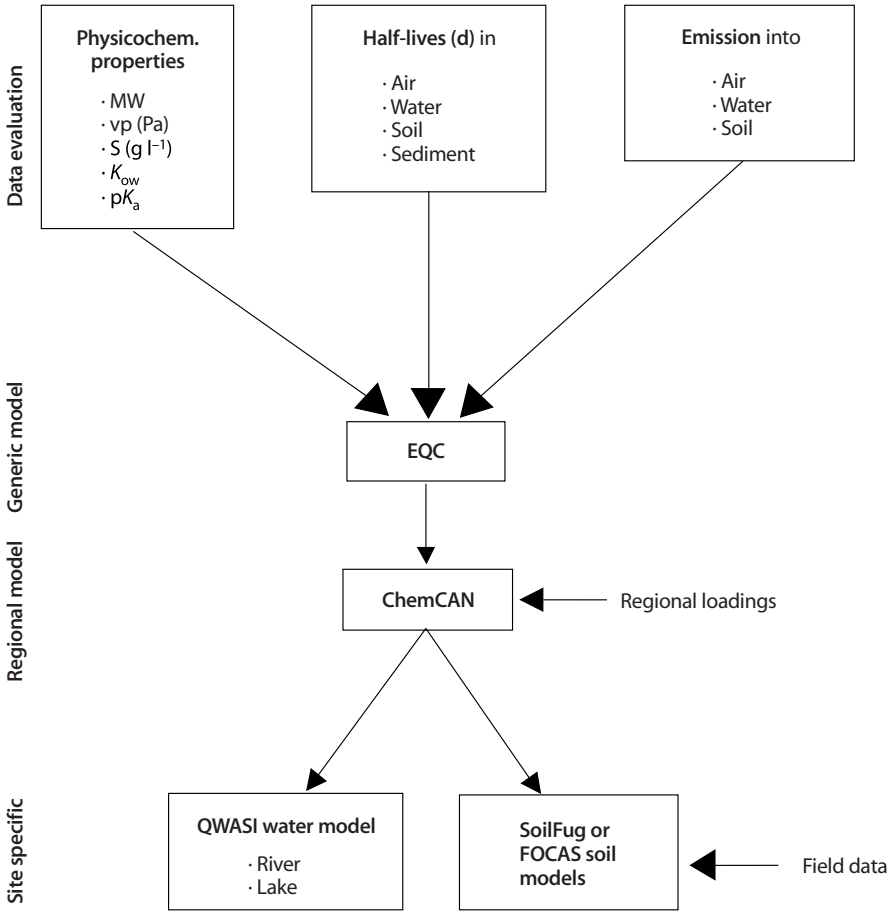


Fig. 9.1. Block diagram of the strategy for modeling pharmaceutical fate in the environment

model in order to run the simulations. (Mackay et al. 1996a). Chemicals can be classified as several "types", according to the scheme reported by Mackay et al. (1996a). The classification depends on the characteristics of the chemical and can be listed as follows:

- Type 1: Chemicals for which vapour pressure and water solubility are measurable and meaningful
- Type 2: Involatile chemicals
- Type 3: Insoluble chemicals
- Type 4: Chemicals that are both involatile and insoluble
- Type 5: Speciating chemicals (e.g. mercury)

According to the type of chemical the user can select the appropriate model "engine". For example, ionized chemicals cannot be modeled with a partitioning model

based on equations that are used to estimate soil-water partitioning from K_{ow} to K_{oc} and K_d. Among the desired parameters, pK_a values are also important to understand whether the chemical will dissociate (and to what extent) at environmental pH values. Half-lives have to be collected whenever possible for the main 4 phases (air, water, soil, sediment). Wastewater treatment plant half-lives are also invaluable.

Among the data needed, discharges and emission patterns in a defined area or drainage basin are to be sought. The emissions can generally be into air, water and soil. For pharmaceuticals, the water compartment can be reached directly or at a later stage, from leaching or runoff of chemicals from the soil compartment. This may happen when sewage sludge, resulting from municipal sewage treatment plants, is applied to soil.

For pharmaceuticals it might be necessary to measure all such properties. Some physicochemical data are available for parent compounds, while very often the properties for the excreted metabolites are not known. It might then be necessary to measure such properties with the methods available. Some problems may arise for environmental half-lives.

Data at this stage will be critically evaluated and selected. Gaps could be filled by QSPR (quantitative structure-property relationships) methods whenever possible.

9.3 Generic Model

This stage is necessary to understand the main environmental pathways of the chemicals in a generic regional environment with predefined emission scenarios (in water only, in air only, in soil only). This stage will give important information on the mobility and overall persistence of a chemical in the different phases.

As stated from the authors (Mackay et al. 1996a–c) in the fate assessment, the focus is on understanding how the diverse properties of the chemical control its distribution among compartments, how it is transported and transformed, and its general persistence. Only the parent compound is treated. None of the metabolites or degradation products are treated, because they will require separate evaluations.

The scenario adopted is that of a generic environment at 25 °C, the common temperature for data acquisition. Because environmental conditions are evaluative, validation is not normally possible. Mackay et al. (1996b) have suggested an evaluative area of 10^5 km^2 with about 10% of the area being covered with water. The reasons to conduct an evaluative fate assessment are that it reveals general features of chemical behaviour and focuses efforts on obtaining information on the most important characteristics of the chemical, whether it is of no concern, or it is of definite concern.

Key information obtained in this stage includes the tendency for intermedia transport (e.g. evaporation), for bioconcentration and bioaccumulation, and the persistence of the substance, which is a function of reaction and advection rate.

It should be noticed that similar systems are nowadays utilized both in Europe and the USA (Cowan et al. 1995).

A detailed description of the equilibrium criterion model (EQC) is given in Mackay et al. (1996b), as well as the data requirements, the environmental scenario and the equation used to calculate partitioning, transport and transformation.

The physicochemical data and half-lives used in the simulation are given in Table 9.1.

As shown in the table, not all the data were available in the literature, therefore some assumptions were made for some of the physicochemical properties. The least

Table 9.1. Physical chemical data and half-lives used in the simulation of cyclophosphamide, diazepam and ivermectin with the EQC model

Property	Chemical		
	Cyclophosphamide	Diazepam	Ivermectin
Molecular weight (g mol^{-1})	279.1	284	875
Melting point (°C)	49.5–53[a]	125–126[b]	155–157[c]
Vapour pressure (Pa)	1.00×10^{-6} [d]	5.00×10^{-5} [e]	1.00×10^{-6} [d]
Solubility in water (g m^{-3})	40 000[c]	41[f]	4[c]
Log K_{ow}	0.97[g]	2.99[h]	6.5[i]
Half-life in air (h)	200[j]	200[j]	200[j]
Half-life in water (h)	2 000[j]	2 000[j]	2 000[j]
Half-life in soil (h)	2 000[j]	2 000[j]	2 000[j]
Half-life in sediment (h)	20 000[j]	20 000[j]	20 000[j]

Note: Because of lack of property data, some values were assumed for comparison purposes.
[a] Parfltt (1999).
[b] Verschueren (1996).
[c] Budavari (1989).
[d] Assumed as low volatile.
[e] Assumed as slightly more volatile than cyclophosphamide and ivermectin.
[f] Newton et al. (1981).
[g] Taken from Syracuse Research Corporation Database (http://esc-plaza.syrres.com).
[h] Hansch et al. (1995).
[i] Estimated with PALLAS 2.0 (CompuDrug Chemistry Ltd.,1994–1995).
[j] Assumed half-lives.

frequently found data were vapour pressure together with half-lives in the main compartments. Considerable efforts should therefore be devoted to measurement and/or estimation of these properties for pharmaceuticals before reliable modeling exercises could be performed. The simulation shown later must therefore be considered as an attempt to show the probable behavior in the environment of three pharmaceuticals.

Basically, Level I represents an environmental situation in which a fixed quantity of chemicals is introduced in a closed system under steady state and equilibrium conditions. The model calculates their partitioning among compartments. This gives an idea of the potential for distribution or more generally the "affinity" towards one or more environmental phases. The results of the Level I simulations for three chemicals (cyclophosphamide, diazepam and ivermectin) are shown in Fig. 9.2.

The six main compartments (air, water, soil, sediment, suspended sediment and fish) reach a different equilibrium for the selected chemicals: cyclophosphamide (Fig. 9.2a) will partition mainly into water (>99%), with negligible amounts in the other compartments, while diazepam (Fig. 9.2b) will concentrate almost equally in water and soil. The last chemical, ivermectin (Fig. 9.2c), reaches equilibrium with about 97% in soil. Level II shows the same percent distribution as in the Level I, but in this case the chemical is continuously discharged at a constant rate and achieves a steady state and equilibrium condition where input and output rates are equal. Degradation rates in

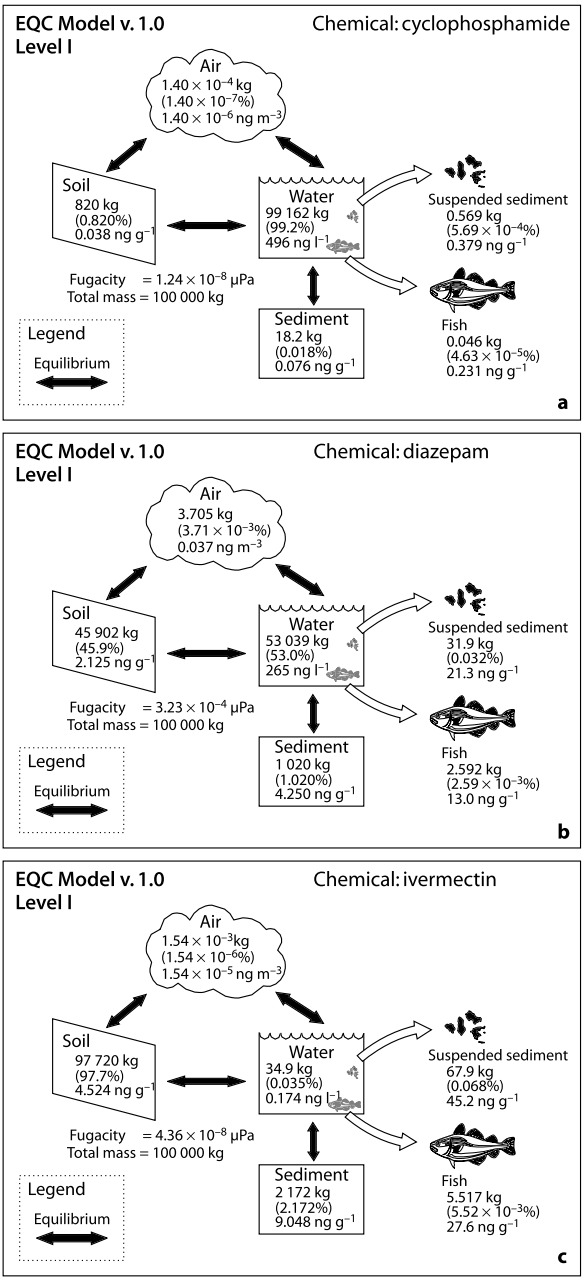


Fig. 9.2. Level I simulations for the selected pharmaceuticals; **a** cyclophosphamide; **b** diazepam; **c** ivermectin

the compartments are calculated from half-life and advection by calculating the output rates through a fixed advection flow of air and water in the unit of world. Figure 9.3 reports the results for the Level II simulation of the chemicals chosen for the illustra-

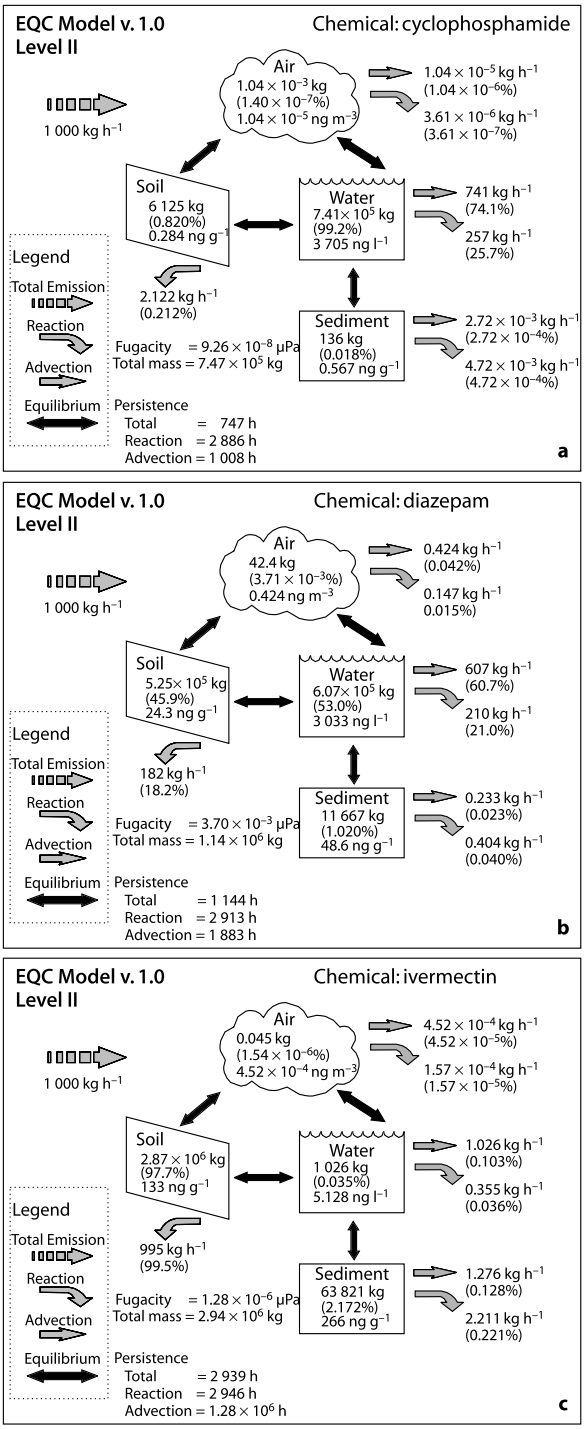


Fig. 9.3. Level II simulations for the selected pharmaceuticals; **a** cyclophosphamide; **b** diazepam, **c** ivermectin

tion. The amounts at equilibrium are as in Level I, while the information obtained at this stage regards the most relevant phenomenon for the disappearance of the chemical from the environment. In fact, the most relevant disappearance mechanism for cyclophosphamide (Fig. 9.3a) is advection in water (74%, which is flow of water out of the environment), while for diazepam (Fig. 9.3b) three phenomena are relevant: advection and degradation in water (61% and 21% respectively) and degradation is soil (18%). For ivermectin (Fig. 9.3c), the only important disappearance phenomenon is degradation in soil, where most of the chemical is present.

Figures 9.4, 9.5 and 9.6 show Level III simulations for the selected chemicals. Since in Level III the user can select the media in which emission takes place, two scenarios were adopted for each chemical: the first is emission into water only (1 000 kg h^{-1}), while the second is into soil only (1 000 kg h^{-1}), to allow for direct discharge into the surface water compartment or the possible addition of the chemical as included into sewage sludge applied to soil. This last scenario was done to evaluate the extent of water contamination resulting from soil distribution of the investigated chemicals.

Level III simulations depict a steady-state application of chemicals in a typical non-equilibrium situation among compartments, due to the resistance of chemical transfer from one medium to another. Among the Levels outlined, Level III is the one more reflecting realistic conditions. Figure 9.4 shows Level III results for cyclophosphamide.

When the emission is into water (Fig. 9.4a), most of the chemical will be present there, and the most important disappearance phenomenon is advection in water. When initially applied to soil (Fig. 9.4b), about 68% of the chemical partitions in soil, while a certain amount will move towards the water compartment (about 650 kg h^{-1}) and then advect out of the water compartment. Practically no cyclophosphamide will move to the air phase.

The picture is slightly different for diazepam (Fig. 9.5). When discharged to water only (Fig. 9.5a), diazepam will concentrate in it, at steady state. The main removal mechanism is still advection in water. When discharged to soil (Fig. 9.5b), the situation changes dramatically: because of the resistance to transfer to other compartments, most of the chemical will stay in the soil environment, with moderate transfer to the water compartment. This clearly shows how different patterns of discharge can result in profoundly diverse distributions.

Ivermectin, when discharged to water (Fig. 9.6a), will substantially "escape" from it and reach the sediment compartment, in which the removal mechanisms are predominant: degradation (about 400 kg h^{-1}) and burial (230 kg h^{-1}). When discharged to soil (Fig. 9.6b), it will basically "stick" to it, degradation (1 000 kg h^{-1}) being the only removal mechanism.

The results of these sequences of Level I, II and III can show the general trend of the distribution behaviour in terms of percent distribution and most important removal phenomena.

9.4 Regional Model

Following the diagram depicted in Fig. 9.1 and the results outlined above, regional modeling represents the following stage in the strategy (Mackay et al. 1996a,c). The use of a regional model (for example the regional model ChemCAN) implies the collection of regional environmental data and the simulations in these regional scenarios.

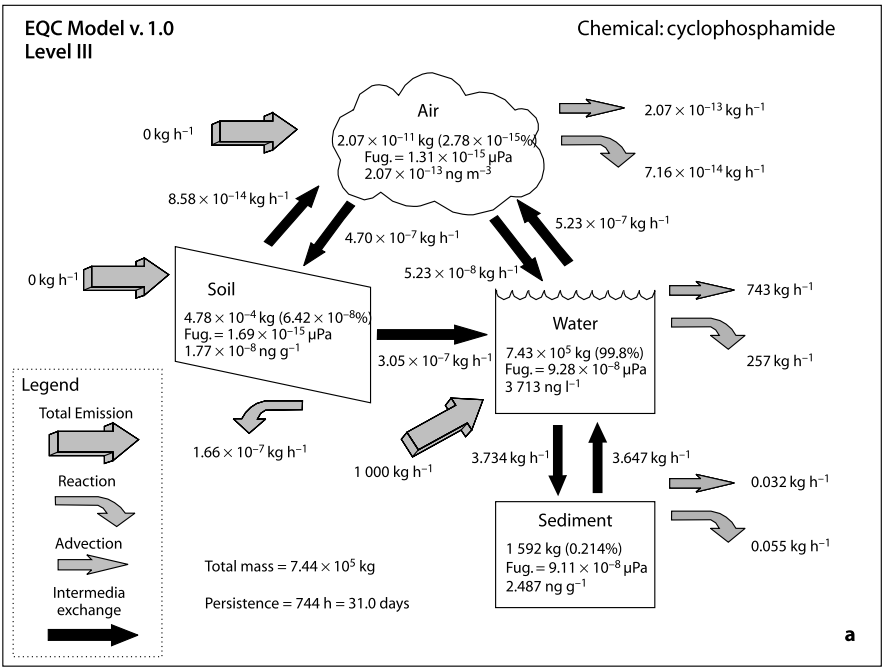


Fig. 9.4. Level III simulations for cyclophosphamide; **a** emission into water only; **b** emission into soil only

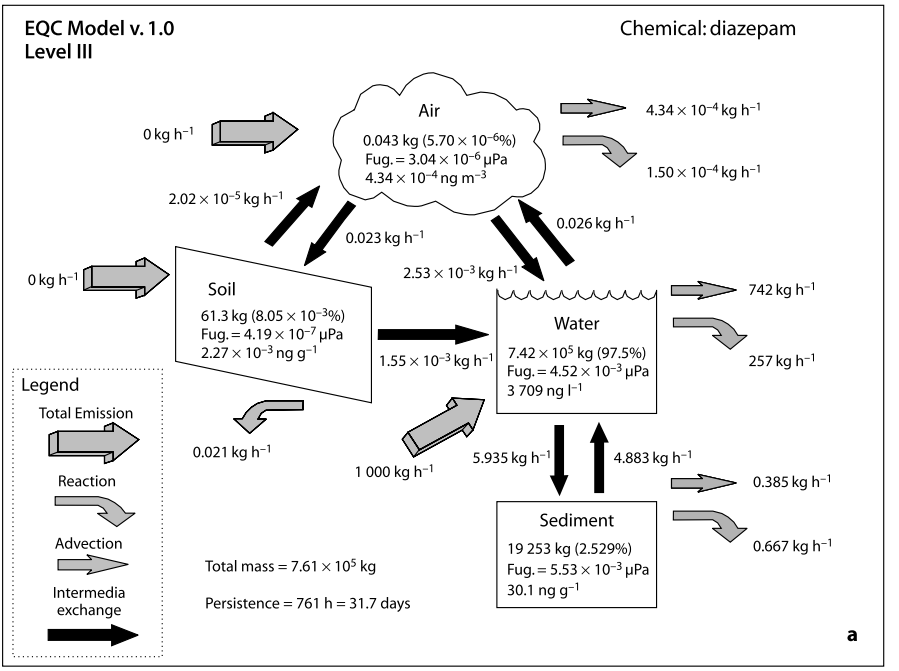
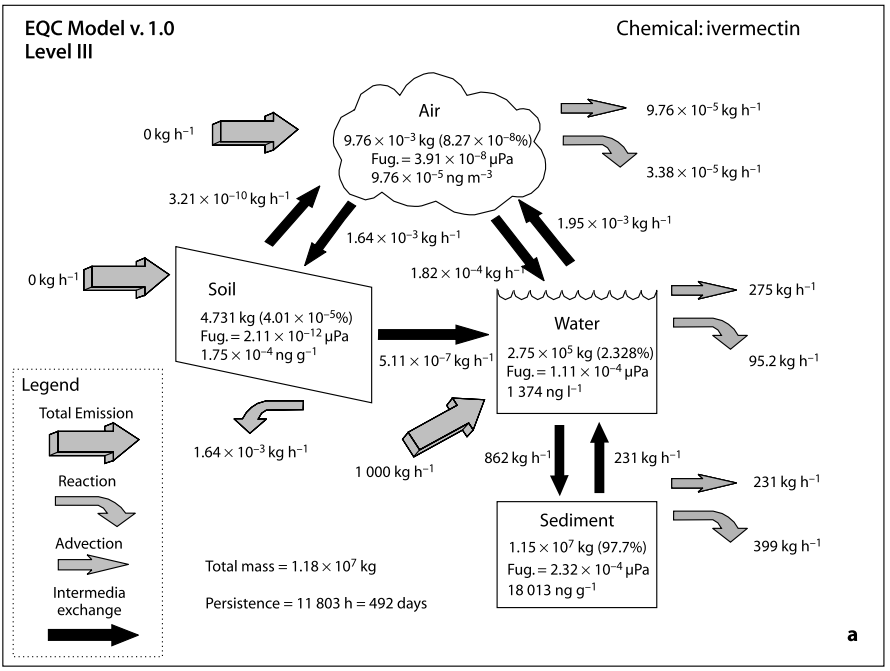


Fig. 9.5. Level III simulations for diazepam; **a** emission into water only; **b** emission into soil only



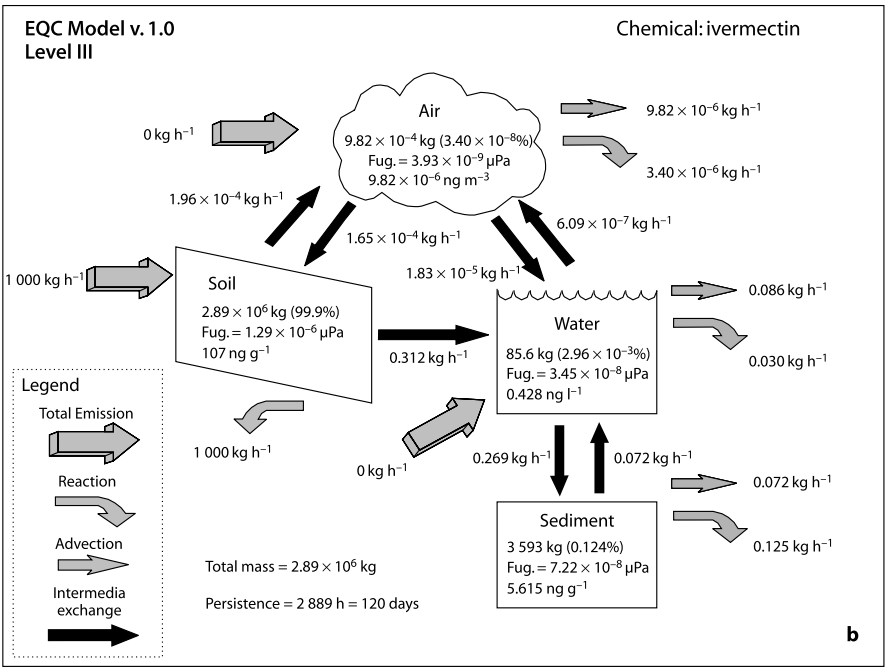


Fig. 9.6. Level III simulations for ivermectin; **a** emission into water only; **b** emission into soil only

The region in question could be, for example, Lombardy (Italy), for which approximate real use emission into the three main compartments can be employed. The results will be average estimates of concentrations in the different phases. The advantages of the use of a regional model are in the more realistic simulation of the area of interest, given the specific input data required (extent of soil and water compartments, depths, organic carbon composition of soil and sediment, average temperature etc.) and the pattern of distribution of the chemical in the environment, for example discharged to water or soil or both. A regional model gives realistic (order of magnitude) concentrations of the chemical in the main compartments. When better estimates of concentrations in a certain compartment are needed or when a regional evaluation shows that the major target for the distribution and fate of a chemical in the environment is a specific medium (e.g. soil or water), a site specific model can be employed, generally providing more accurate results.

9.5 Site Specific Models

When regional modeling exercises confirm that one medium (such as soil or water) is the environmental compartment that is relevant for the fate of a certain chemical, the use of a site specific model is required in order to predict its environmental concentrations with a satisfying level of accuracy. As an example we can cite the SoilFug (Di Guardo et al. 1994a) and FOCAS (Di Guardo et al. 2001) models, which can be used to evaluate chemical runoff from treated soil and the fate of amended- associated chemicals in soil respectively. Both models could theoretically be modified to be adapted to waste disposal sites or contaminated soils. The use of a such a local model requires that scenarios for simulations will comprise a detailed description of rain events, soil conditions etc.

Water compartment models, such as the QWASI models, can be used to calculate the fate, distribution and concentrations of chemicals in lakes and rivers (Mackay 1991). Again, these site specific models require a certain amount of additional environmental parameters in order to simulate the adopted scenario properly. In a number of cases the SoilFug model has been validated for research purposes (Di Guardo et al. 1994a,b; Barra et al. 1995) and in some cases utilised for risk assessment when analytical data were not available (Baldry et al. 1995; Calamari 1998).

9.6 Discussion and Conclusions

The general strategy outlined above can be utilized to assess the fate of pharmaceuticals in the environment. Some use-derived features may influence the fate of these compounds and in some cases disorient the assessor. For example, drugs emitted into water (through the sewer) will typically end up in sewage treatment plants, where they can be degraded to a larger extent and be transformed to metabolite products (with different chemical properties) or they can persist and largely reach the environment, still contained in water or in sewage sludge. An example of such a chemical is ibuprofen, which was measured in surface waters and wastewater treatment plant samples (Buser et al. 1999). When sludges are added to soil as amendments, they may release such

chemicals into the soil environment, therefore reaching a different compartment from the one of entry. Some other chemicals may be persistent and not very mobile and therefore they can build up in a compartment such as soil and sediment. In these compartments they may exert effects on non target organisms and even return to human beings through the food chain.

A strategy for the understanding the fate and distribution of pharmaceuticals in the environment by using modelling approaches has been described.

Models can be invaluable tools to formerly describe and capture the mass balance of a chemical in the environment. It is suggested here that a strategy should start with a proper data collection, continue with generic scenario modeling to "grasp" the typical behavior of a chemical in different discharge possibilities, proceed with a regional simulation that can provide information on the concentrations reached in the environment (given proper emissions), and eventually gain insight on the fate at a very detailed scale with a site specific modeling.

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Environmental Exposure Modeling: Application of P*h*ATE™ and GREAT-ER to Human Pharmaceuticals in the Environment

V. L. Cunningham

10.1 Introduction

The presence of human pharmaceuticals in the environment is receiving increased attention as improved analytical methods allow lower and lower limits of detection. Many active pharmaceutical ingredients (APIs) are not completely metabolized by patients and may be excreted into wastewater treatment plants (WWTP) unchanged. In the WWTPs, the APIs may undergo removal by a variety of mechanisms, including biodegradation and/or sorption to biosolids. However, some APIs may still be present in the effluent from the WWTP and enter the aquatic environment and/or enter the terrestrial environment from land application of the sludge. Both of these scenarios may lead to some exposure to these APIs by humans, animals and plants.

Understanding and evaluating the likely potential exposures and impacts of pharmaceuticals in the environment is a prerequisite for an assessment of risk. Risk assessments are needed to understand the implications of the exposures in the context of environmental effects. For this purpose, knowledge of the environmental concentrations of APIs is critical, and predictive tools (e.g., models) and monitoring data are important elements in this risk assessment process. Environmental exposure is defined by the concentrations of the APIs that occur in the environment. These concentrations can be estimated, the Predicted Environmental Concentration (PEC), with simple ones typically revealing results as an average in time and space, or measured, the Measured Environmental Concentration (MEC), with results typically associated with a specific location and a certain point in time. In order to put PECs and MECs of APIs into perspective, a conceptual understanding is required of how APIs may enter the environment, (e.g., WWTPs, septic systems, direct discharge), the form in which they are released (e.g., parent API, metabolite, hydrolysis product, etc.) and the various fate pathways and transport processes they undergo and which ultimately determine concentrations in different environmental compartments. Such a conceptual 'model' forms the basis of exposure assessment.

Modeling and monitoring play integral and complementary roles in exposure assessments. Models provide a simple and inexpensive approach to calculating environmental concentrations based on mathematical equations representing the various fate and transport processes; however, there may be considerable uncertainty in the results. On the other hand, monitoring data directly reflect actual environmental concentrations and should be considered for use when available. Uncertainties may limit the interpretation of monitoring data due to variability, which may reflect the complexity of the environment, and spatial and temporal scale limitations of monitoring programs. The uncertainties and limitations of models and monitoring can be reduced

when both are used to provide a better understanding of the fate of pharmaceuticals in the environment.

Environmental exposure models are used for a variety of purposes including:

- Initial prospective exposure assessments (when measured concentrations cannot be obtained); for new pharmaceuticals, the use of models is the only option available for exposure assessment;
- Conservative, screening exposure assessments (as a guide to environmental testing strategies);
- More realistic exposure estimates with uncertainty bounds and probabilistic distributions (e.g., 90th percentile concentrations);
- Designing monitoring studies.

In each case, the results of the modeling exercise are predicted environmental concentrations or PECs. The use of the prediction depends on the requirements of the assessor and the available data.

10.2 Pathway Analysis

There are several potential pathways by which human pharmaceuticals may enter the environment (Halling-Sørensen et al. 1998; Ternes 1998; Heberer 2002). These occur after manufacture and use by both humans and animals (Fig. 10.1). Use of pharmaceuticals by both humans and animals may result in metabolism of the APIs and excretion of the parent drug and/or drug metabolites in feces and urine. Drugs used in veterinary applications are typically disposed directly on land. Human wastes are typically treated by either an onsite wastewater treatment system such as a septic system or a private or municipal wastewater treatment plant (WWTP). The focus of this chapter is on exposure modeling of human pharmaceuticals excreted into WWTPs and discharged in effluent to surface waters.

During wastewater treatment, an API may be degraded via hydrolysis, oxidation, or biodegradation, or it may adsorb to solids during waste treatment and be isolated in WWTP sludge. Pharmaceutical concentrations in effluent and sludge depend on the nature of the API and the type and efficiency of these WWTP removal processes. Effluents and surface waters are normally considered the primary compartment of concern due to the water solubility of pharmaceuticals.

10.3 Environmental Exposure Modeling of Pharmaceutical Compounds

10.3.1 General Averaging Method for PECs

The simplest approaches to estimating surface water concentrations are those provided in guidance for environmental assessments for regulatory drug approvals, for example, the approaches recommended by the U.S. Food and Drug Administration (FDA 1998)

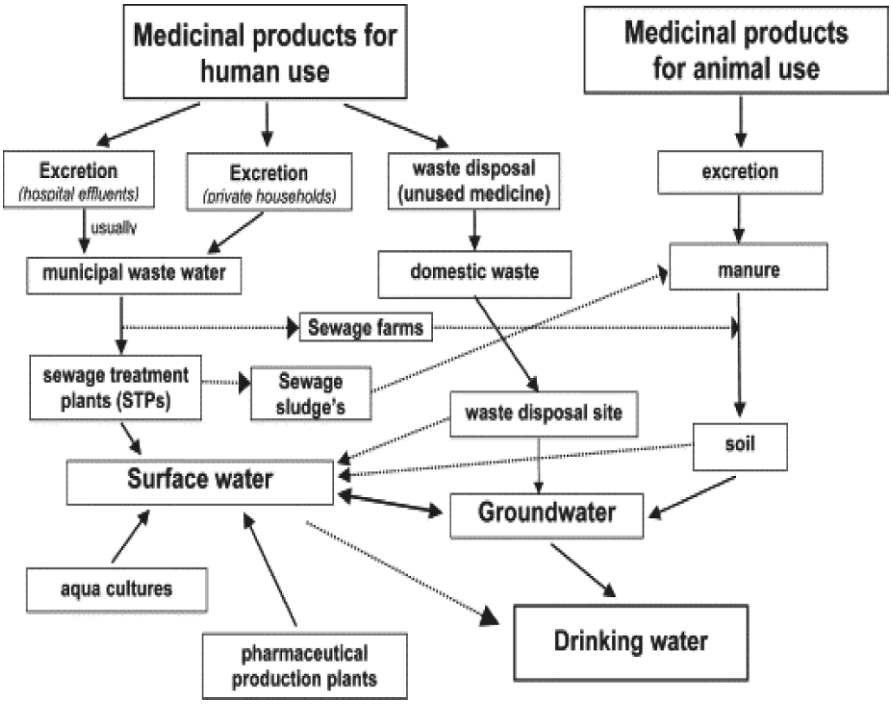

Fig. 10.1. Scheme showing possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. Reproduced with permission. Copyright © 2002 Elsevier Science Ireland Ltd. (Heberer 2002)

or the EU European Medicines Agency (EMEA 2006). The approaches are similar in concept.

10.3.1.1
FDA Approach

For the FDA approach, the expected introduction concentration (EIC) of an active moiety into the aquatic environment is calculated as follows:

$$\text{EIC-Aquatic (ppb)} = A \times B \times C \times D$$

where
A = kg/year produced for direct use (as active moiety)
B = 1/liters per day entering POTWs*
C = year/365 days
$D = 10^9$ µg/kg (conversion factor)
* 1.214 × 1 011 liters per day entering publicly owned WWTPs in the U.S. (EPA 1996).
This calculation assumes the following:

- All drug products produced in a year are used and enter the WWTP system;
- Drug product usage occurs throughout the United States in proportion to the population and amount of wastewater generated;
- There is no metabolism;
- There is no removal during wastewater treatment;
- There is no dilution.

Thus the EIC is essentially a measure of the maximum expected concentration in WWTP effluent. A refinement of this calculation is permitted if information on human metabolism is available, which may then be used to reduce the kilogram/year input. The estimate of the kilogram/year active moiety is based on or includes (1) the highest quantity of the active moiety expected to be produced in the U.S. for direct use in any of the first five years after product launch; (2) the quantity used in all dosage forms and strengths included in the application; and (3) the quantity used in an applicant's related applications. Related applications include those for other dosage forms using the same active moiety and for products using different forms of the active moiety (e.g., level of hydration, salt, free acid/base).

The expected environmental concentration (EEC), sometimes referred to as the *predicted environmental concentration* (PEC), is the concentration of the active moiety or other compound of interest that organisms would be exposed to in the environment (e.g., surface water) after consideration of, for example, spatial or temporal concentration or depletion factors such as dilution, degradation, sorption and/or bioaccumulation but independent of effects thresholds. Adjustments to the EIC are made to provide the EEC. In the majority of cases, the EEC for the aquatic environment would be expected to be significantly less than the EIC due to dilution. Based on dilution factors for WWTPs available from the EPA, applying a dilution factor of 10 to the $EIC_{aquatic}$ to estimate the $EEC_{aquatic}$ is normally appropriate.

10.3.1.2 *EMEA Approach*

For the EMEA Phase I PEC calculation, the following equation is used:

$$PEC_{surfacewater}\ (mg\ l^{-1}) = (DOSE_{ai} \times F_{pen})/(WASTEW_{inhab} \times DILUTION)$$

where $DOSE_{ai}$ = maximum daily dose consumed per inhabitant ($mg\ inh^{-1}\ d^{-1}$), F_{pen} = fraction of market penetration (default = 0.01), $WASTEW_{inhab}$ = amount of wastewater per inhabitant per day (default = 200 l), and DILUTION = dilution factor (default = 10).

This initial PEC is restricted to the aquatic compartment and assumes the following:

- A fraction of the overall market penetration (market penetration factor: F_{pen}) within the range of existing medicinal products. The default value may be used or the F_{pen} can be refined by providing reasonably justified market penetration data, e.g., based on published epidemiological data;
- The predicted amount used per year is evenly distributed over the year and throughout the geographic area;

- The sewage system is the main route of entry of the drug substance into the surface water;
- There is no biodegradation or retention of the drug substance in the WWTP;
- There is no human metabolism.

In higher tiers of the EMEA assessment, the $PEC_{surfacewater}$ may also be refined with information from WWTP modeling using the SimpleTreat model (Struijs et al. 1991) by incorporating adsorption of substances to sewage sludge in STPs, using the data from the estimation of the adsorption coefficient, and results of ready biodegradability in the WWTP.

Metabolism which breaks covalent bonds in the drug and which is not easily reversed by enzyme reactions should be factored into the estimation of the PEC. Conjugation reactions should be carefully studied to ensure that these reactions are not reversed during waste treatment to regenerate the parent pharmaceutical. For example, glucuronidation may occur on either oxygen or nitrogen; only the O-glucuronide is reported susceptible to hydrolysis by glucuronidases (Ternes 1999; Panter et al. 1999); the N-glucuronides are reported as resistant (Axelrod 1961). Once the mass of compound excreted as the parent drug is estimated, it is divided by the total waste flow of the region of interest to calculate the influent concentration. Use of the daily per capita water usage of a region ensures the drug is diluted in all the wastewater generated in a region. The daily per capita water use includes all industrial, commercial, and domestic water used and then released into a combined sewer system or disposed via on-site treatment. Commercial and industrial wastewaters, which do not mix with domestic wastewaters, are not included.

Wastewater treatment plant operational parameters (e.g., hydraulic retention time, sludge retention time, combined sewer overflow) lead to variability in the overall removal of drugs during waste treatment. For activated sludge plants, the effects of these operational parameters can be accounted for by wastewater simulation models such as WWTREAT (Cowan et al. 1993) or SimpleTreat (Struijs et al. 1991). However, there are different types of WWTPs including oxidation ditches, lagoons, rotating biological contactors, trickling filters, and primary treatment plants, and it is difficult to predict removal in all treatment types. If removal values differ among treatment types, it may be possible to use a weighting procedure to estimate a single average removal percent for a region (McAvoy 1993). Sorption removes pharmaceuticals from the liquid phase leading to levels of the drug substance in sludge. If this material is not degraded during treatment, it can lead to exposure of terrestrial organisms after sludge application to the terrestrial environment. Prediction of sludge concentrations depends on treatment plant operational parameters, and these are best calculated using wastewater treatment plant operational models (Struijs et al. 1991; Cowan et al. 1993).

10.4 Watershed/Catchment-Based Environmental Models

The increasing complexity of general averaging methods for calculating PECs that attempt to reflect the diversity of environments has led to the development of spatially explicit models based on watersheds or catchments (the term watershed is generally used in the US; catchment in the EU). A watershed or catchment, the drainage

basin of a receiving water body, is a geographic area in which water, sediments and dissolved materials drain to a common outlet. Use of this approach allows better understanding of the cumulative impact of human activities and is particularly useful with contaminants that enter the environment solely from human use, such as human pharmaceuticals. A Geographic Information System (GIS) is often used to manage watershed and hydrologic data, where a GIS is a computer system capable of assembling, storing, manipulating, and displaying geographically referenced information, i.e., data identified according to their locations. These refined exposure assessment tools should greatly enhance the accuracy of current local and regional exposure estimation methods and ultimately allow assessments on large area scales. However, they do require the availability of extensive GIS data, data quality assurance, and appropriate environmental fate input data. Currently there are two available GIS-based models being used for predicting PECs for pharmaceuticals in the environment: P*h*ATE™ and GREAT-ER.

10.4.1 P*h*ATE™

The P*h*ATE™ (Pharmaceutical Assessment and Transport Evaluation) model (Anderson et al. 2004) was developed as a tool to estimate concentrations of active pharmaceutical ingredients in U.S. surface waters that result from patient use. The model may be used in a screening mode using conservative input values or in a more realistic mode using available fate data. The model is based on eleven watersheds selected to be representative of most watersheds and hydrologic regions of the United States. The specific watersheds currently included in the model are Atlanta Headwaters, Georgia; Columbia River, Washington/Oregon/Idaho; Kansas River, Kansas; Lower Colorado River, Arizona; Merrimack River, Massachusetts/New Hampshire; Miami River, Ohio; Mississippi Headwaters, Minnesota; Sacramento River, California; Schuylkill River, Pennsylvania; Trinity River, Texas; and White River, Indiana. Additional watersheds in the U.S. are being developed, and the model is also being considered for use with watersheds in other areas of the world such as Canada, Korea and Japan. For use outside of the U.S., relevant watershed data need to be developed. The new watershed can then be incorporated into the P*h*ATE™ structure and used similarly to the existing watersheds.

P*h*ATE™ currently models specific U.S. watersheds, and the assumption is that taken together, these allow modeling of PECs in the U.S. as a whole. "Events" such as WWTP discharges, waterbody confluence, diversions such as reservoirs or lakes, or drinking water or other withdrawals are used to divide rivers within the watersheds into discrete river segments. The model uses the hydrologic cataloging unit system developed by the U.S. Geological Survey (USGS). Geographic data was obtained from the U.S. Environmental Protection Agency (USEPA) and the USGS. A mass balance approach is used to model PECs. Input parameters include fraction removals from metabolism, wastewater treatment and in-stream loss. There is also functionality to add drinking water removal and to predict drinking water concentrations. Concentrations are calculated from upstream contributions, WWTP effluent contributions, and losses via in-stream loss mechanisms or flow diversions (i.e., man-made withdrawals). The model predicts concentrations at low (7Q10 – the streamflow that occurs over seven

consecutive days and has a ten-year recurrence interval period, or a one in ten chance of occurring in any one year) and mean flows for WWTP effluents and surface waters. The current version of P*h*ATE™ does not consider veterinary pharmaceuticals or septic system discharges because these releases are through pathways other than a WWTP. PECs resulting from discharges into estuarine or marine environments are also not predicted.

P*h*ATE™ includes both river reaches, modeled as plug-flow segments, and reservoirs, modeled as mixed-flow segments. A bulk first order in-stream depletion rate constant is inputted as the sum of all relevant first-order loss rate constants that might be associated with factors such as biodegradation, hydrolysis, photolysis, evaporation, and sedimentation. While the model has the flexibility to provide output in several formats (single watersheds, combinations or totals), a useful depiction provides the PECs as a probability distribution over all eleven watersheds, plotting percent of segments (or frequency) versus predicted concentrations. The use of probability distributions is consistent with the intended purpose of the model as a tool for nationwide screening. Model PECs for the watershed segments included in P*h*ATE™ are plotted as two distributions, one for mean flow and one for low flow (Fig. 10.2).

The P*h*ATE™ model also allows output in the form of a watershed map, with concentrations depicted color-coded. An example for the White River Watershed is shown in Fig. 10.3.

Most assumptions in the model, such as assuming a constant WWTP removal efficiency, a constant in-stream loss rate for all segments, or a uniform usage per capita,

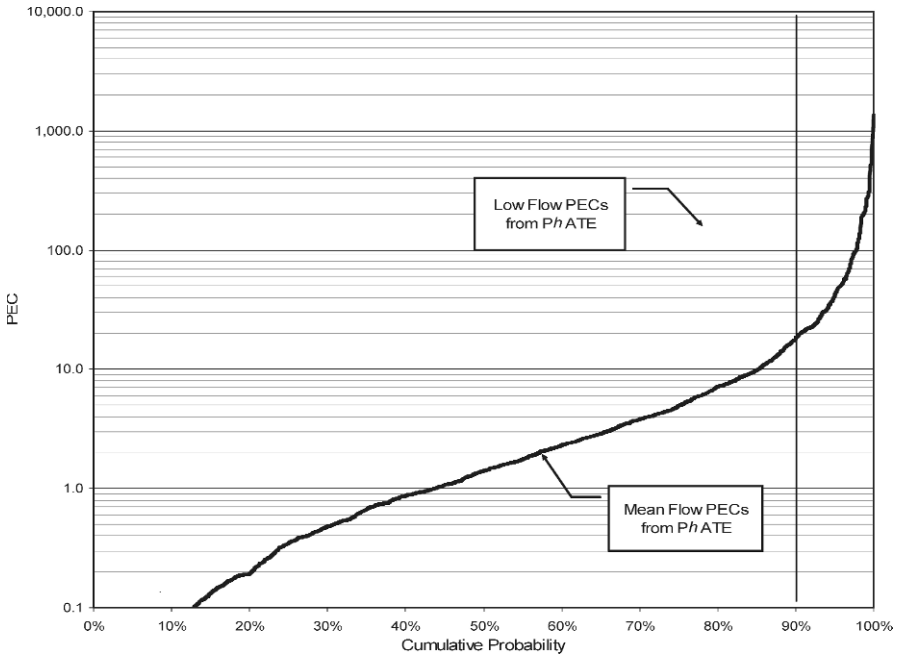

Fig. 10.2. Representative P*h*ATE™ graphical output

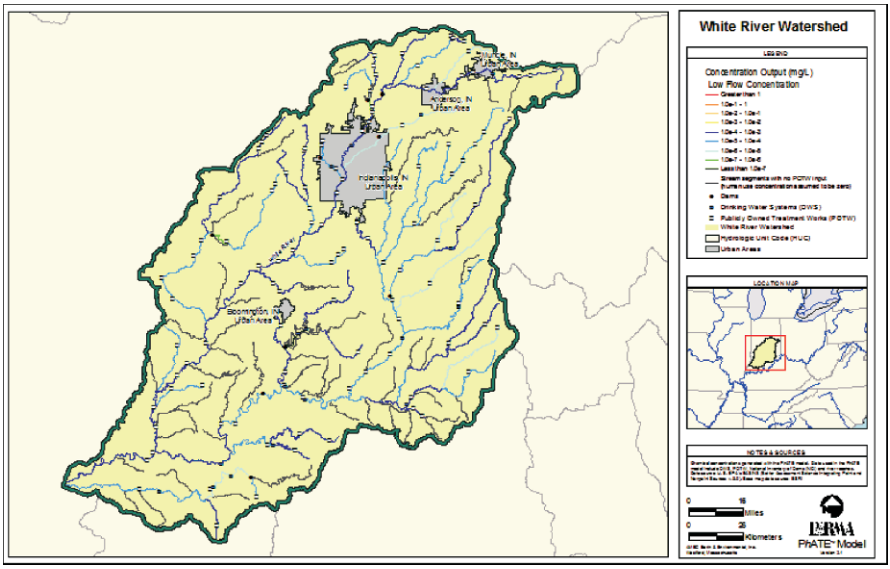

Fig. 10.3. Representative P*h*ATE™ map output

would not be expected to cause a systematic bias in PECs. Rather, P*h*ATE™ would overpredict PECs in some segments and underpredict PECs in others. Because PECs can be represented as a cumulative probability distribution, the model may provide a more realistic characterization of nationwide environmental concentrations than presenting only average or worst case conditions. This representation is well suited for assessing environmental exposure for the purpose of conducting environmental risk assessments. However, the flexibility of the P*h*ATE™ model also allows individual watersheds to be evaluated separately, and considerable variability in PECs would be expected. P*h*ATE™ is available upon consultation with P*h*RMA (Pharmaceutical Research and Manufacturers of America – *www.phrma.org*).

10.4.2 GREAT-ER

GREAT-ER (Geo-referenced Regional Exposure Assessment Tool for European Rivers) is a catchment scale model that was developed for predicting the distribution of concentrations of consumer products in surface waters (Feijtel et al. 1997) (Catchment is another term for watershed, frequently used in Europe). For each catchment in the model, the location of each WWTP is defined together with the population served, wastewater flow rates and treatment plant type (e.g., activated sludge or trickling filter). The hydrology module is based on measurements from flow gauging stations, combined with a hydrological module to provide statistical river flow distributions across the catchment. GREAT-ER uses multiple Monte Carlo simulations to generate a distribution of concentrations in each segment that reflects the variability of vari-

ous model parameters, e.g., WWTP removal efficiency, in-stream decay rate, flow, etc. Concentrations from the simulation, C_{sim}, collectively form a distribution from which various percentiles may be calculated. For example, the 90th percentile C_{sim} from GREAT-ER represents the 90th percentile value for a particular segment from the Monte Carlo simulations. For this analysis, model parameters such as WWTP removal efficiency and in-stream decay rate coefficient were input as constants; the only variable used for the Monte Carlo simulations was surface water flow. Therefore, the 90th percentile C_{sim} concentrations represent low flow conditions in each segment. Use of the 90th percentile value of the low flow predicted concentrations from P*h*ATE™ and GREAT-ER is considered to provide a conservative estimate of exposure for risk assessment purposes (Rapaport 1998; Fox et al. 2000; EU 2003). C_{sim} can be displayed as mean, 50th, 90th or any percentile values based on the spatial and temporal distribution of river flows across the catchment. Normal output is given as spatially distributed C_{sim} using color-coded river maps to identify areas of high and low concentrations (Fig. 10.4).

In addition, GREAT-ER provides output data in the form of tables that can be exported to EXCEL. The data in EXCEL can then be used to generate probability distribution plots similar to the output from P*h*ATE™ for either single catchments, combinations, or all combined. Figure 10.5 presents a graphical representation of the composite output from GREAT-ER as a probability distribution. A range of hydrological regimes across Europe has been modeled. Version 2.0 of GREAT-ER includes: Rupel, Belgium; Mayenne, France; Aire/Calder, Went, Don and Rother, UK; Itter, Rhine, and Elbe, Germany; Lambro, Italy; and the Rur, Germany, Belgium, and The Netherlands. Further catchments are being added as the hydrology and WWTP data are characterized. However, recent information (Ronse 2007) indicates that the original data devel-

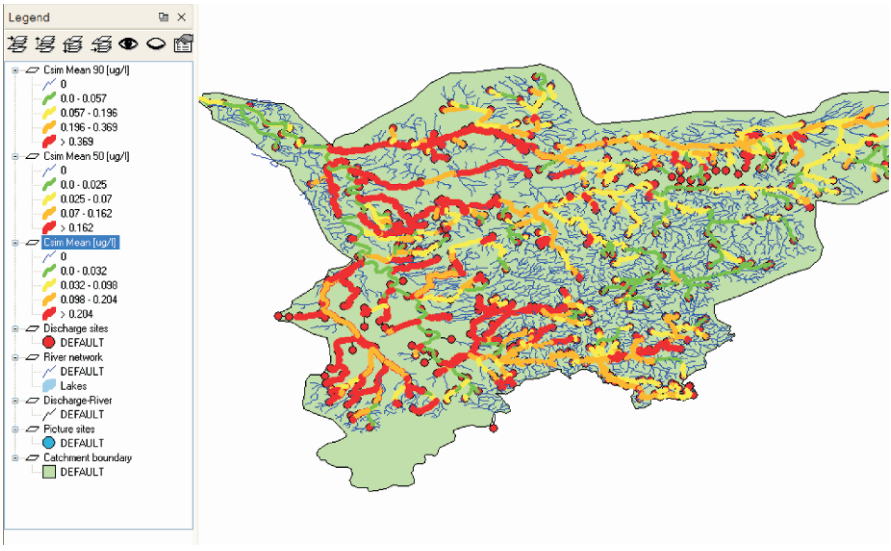

Fig. 10.4. Representative GREAT-ER map output

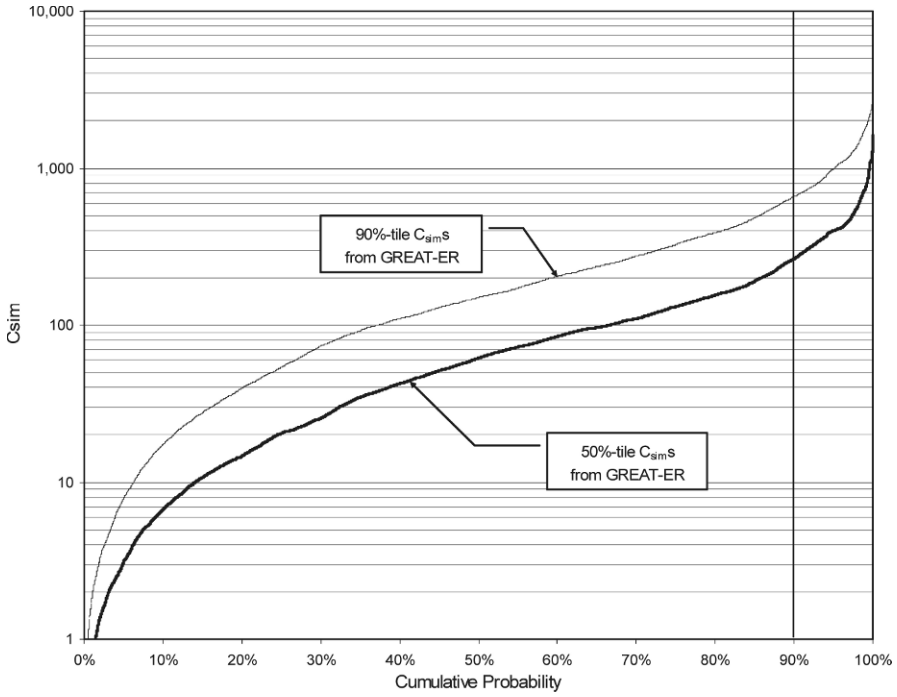

Fig. 10.5. Representative of Composite GREAT-ER output

oped for the Rupel catchment in GREAT-ER is no longer valid, so use of the Rupel for environmental exposure predictions should be suspended until a new catchment model is available.

GREAT-ER may be obtained through ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) via the Internet (*www.great-er.org*). GREAT-ER has been has been applied to pharmaceuticals for several catchments across the EU (Schowanek and Webb 2002; Schroeder et al. 2002; Johnson et al. 2007).

Watershed/catchment models are generally easy to use. At a screening level only the per capita use of product is required to estimate a PEC or a C_{sim}. In this case, when the user runs the model, the load is automatically distributed according to the populations served by each treatment plant, removal is assumed to be zero and the surface water dilution is calculated from the specific wastewater and river flows for each WWTP. All of the extensive hydrological and WWTP data is fixed internally for each catchment contained within the model, and the user cannot change these data. If a refined assessment is required, both P*h*ATE™ and GREAT-ER can include user-specified parameters such as biodegradation rates and WWTP removal rates, either as single values or as ranges of values for use within an uncertainty (i.e., Monte Carlo) approach (for GREAT-ER).

10.5 Parameterization, Sensitivity Analysis and Uncertainty

Parameter selection is critical to the generation of relevant model output. In the fate models discussed here, parameters include per capita wastewater flow, sorption coefficients, dilution, removal in different treatment types, and suspended solids concentrations among others. In many cases, default input values are selected for these parameters and often represent 'bounding' or 'worst-case' conditions. While boundary or worst case PECs have their use, selection of biased input values can cause difficulties:

- Subsequent users of the models may select the same 'default' input values and be unaware of the criteria used to select the input and hence perhaps misinterpret the results;
- Use of multiple, biased input values may result in conservative output; however, the final level of bias is unknown and may be far greater than desired or required.

For risk assessment purposes, it is often desirable to estimate 'worst-case' conditions. However, to avoid compounding the level of conservatism, the use of probabilistic approaches as illustrated in Figs. 10.6 and 10.7 should be used to account for the uncertainty in each parameter and understand the overall level of conservatism. Output distributions reflect the accumulated uncertainty in input variables (model uncertainty is another source of uncertainty but is not addressed here). Interpretation of these distributions reflects the increased amount of information in the analysis. From a typical output distribution, one can select, for example, the 50th, 90th, or 99th percentile concentration where the 90th percentile concentration is defined as the concentration which is exceeded at only 10% of the sites (assuming random sampling over time and space). The spatially explicit models account for a degree of uncertainty by using site-specific data. However, parameter uncertainty remains an issue (e.g., use of a single K_d value or removal estimate).

Stochastic, episodic events are another source of uncertainty and can help explain differences between field-measured and predicted concentrations. For example, release of untreated wastewater during and following heavy rainfall events, i.e., combined sewer overflows (CSOs), will cause models (simple algorithms and spatially explicit models) to underestimate environmental concentrations unless the model specifically incorporates a scenario to estimate PECs in these situations. Such discrepancies highlight the need for modeling and monitoring studies to be closely aligned so that the outliers may be understood and taken into account in data interpretation.

10.6 Comparisons of PECs and MECs

Figures 10.6 and 10.7 represent examples of comparison of PECs and MECs for P*h*ATE™ and of C_{sims} and MECs for GREAT-ER. For these examples, data for carbamazepine (CBZ) were used, since CBZ is frequently detected in the environment and a consider-

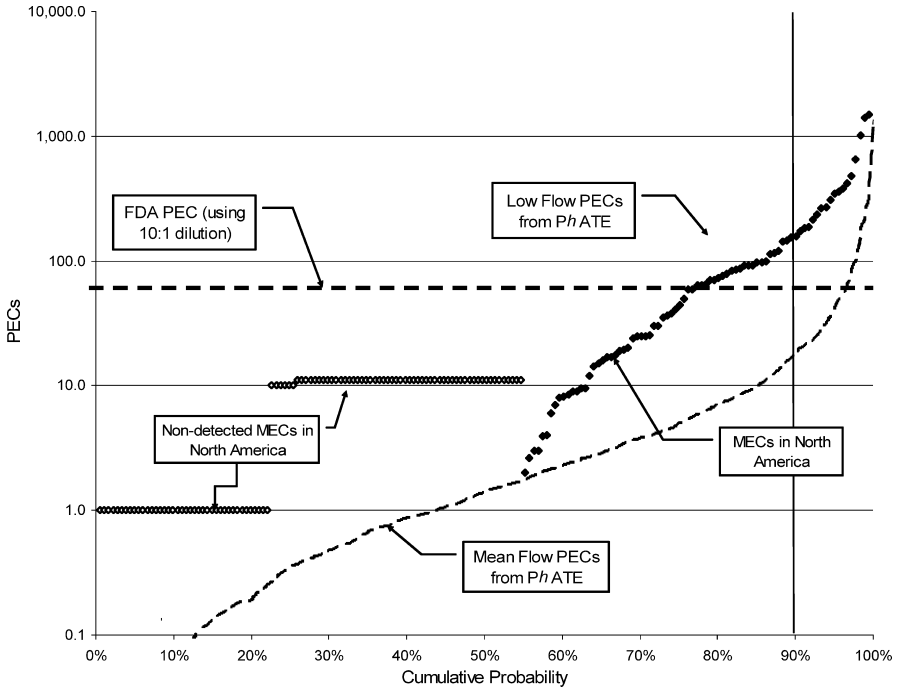

Fig. 10.6. The cumulative probability distribution of measured CBZ concentrations ($n = 180$) in North American surface waters and PECs generated by P*h*ATE™ for model segments ($n = 2710$) in eleven watersheds at mean and low flow conditions

able dataset of MECs was available. For the P*h*ATE™ output shown in Fig. 10.6, the MECs fall between the PECs for mean and low flow.

For the GREAT-ER model output shown in Fig. 10.7, the C_{sim} exceed the MECs by about a factor of 3 at the 90th percentile but are very close to the MECs at the 50th percentile.

Both P*h*ATE™ and GREAT-ER show very good agreement between modeled and monitored data for carbamazepine. One reason for such good agreement is that carbamazepine appears to behave conservatively in the environment and to remain predominantly in the aqueous compartment.

10.7 Integration of Modeling and Monitoring

Models have the following advantages: ease of use; lower cost (once the model is constructed); ability to evaluate "what if" scenarios (e.g., climate, soil, season); greater coverage spatially and temporally; the ability to generate PECs before the compound is used in commerce; the ability to explore alternative volume impacts; to compare exposure assessments; and to simulate concentrations below analytical limits of quantitation. On the negative side, model predictions have many uncertainties, both

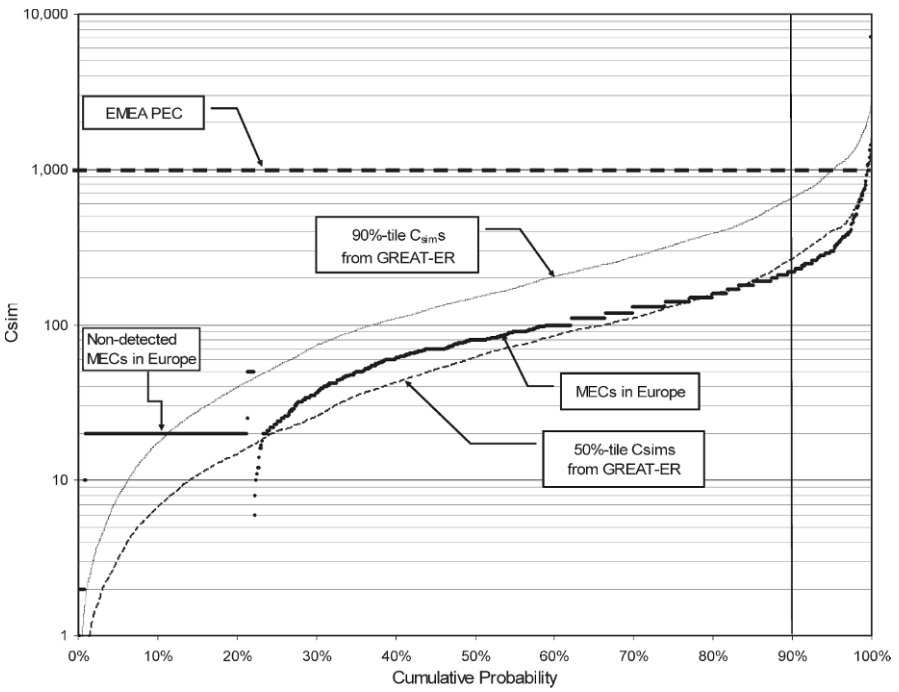

Fig. 10.7. The cumulative probability distribution of measured CBZ concentrations ($n = 1241$) in European surface waters and 50th and 90th percentile C_{sim} generated by GREAT-ER for model segments ($n = 20346$) in ten watersheds

with regard to model parameters and input values; simplifications of complex processes and calibration and corroboration are required; and there is some general public reluctance to accept predicted data. Monitoring has the following advantages: actual measurement of compounds of concern; fewer assumptions and lower uncertainty at the location and time the samples are collected; accounts for inherent heterogeneity of the system; and greater acceptance of confidence in measured data. However, monitoring studies' drawbacks include the following: cost; time – often take weeks to years; are difficult to design to be both cost effective and technically viable; and present difficulties in handling analytical non-detects. However, both modeling and monitoring are essential elements of quantitative risk assessment, and these approaches are best used in combination as they provide complementary information. Model results are built from a series of assumptions concerning the environment and a chemical's behavior in that environment. Monitoring data are used to actually measure concentrations, to verify model assumptions and to support or refute model predictions. While monitoring data have fewer assumptions, they are more site and time specific and require models to expand the scope of the measurements across time and space. Depending on the scope of the assessment, the amount of data on the API, and the relative risk posed by the pharmaceutical of interest, either approach or a combination can be used to establish the PEC. In general, modeling efforts are less expensive than

monitoring once appropriate models are built and predictions can be made prior to the introduction of a pharmaceutical into the market.

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Exposure Assessment Methods for Veterinary and Human-Use Medicines in the Environment: PEC vs. MEC Comparisons

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11.1 Introduction

For the environmental risk assessment of veterinary medicines and human-use pharmaceuticals, it is necessary to assess the potential for exposure of terrestrial and aquatic organisms to the active pharmaceutical ingredient in the product. The European Union (EU) has developed procedures for conducting environmental risk assessments of pharmaceuticals prior to product approval. According to the EU guidance documents for risk assessment of veterinary medicines (VICH 2004; CVMP 2006) and human-use pharmaceuticals (CHMP 2006), Phase I of the assessment involves making rough calculations of predicted exposure concentrations (PEC) in relevant environmental compartments (e.g., soil, surface water, groundwater). If Phase I of the risk assessment indicates that there is potential for exposure to the chemical at concentrations hazardous to organisms, Phase II is necessary to refine the PECs for the various environmental compartments.

Assessments of exposure must take into account the many different pathways and scenarios that influence the transport of medicines into the environment. In some cases, we have a good understanding of how these exposure scenarios should be evaluated, but in other cases, there is insufficient knowledge to guide the exposure assessment. In this chapter, we evaluate the exposure assessments described in the EU guidance documents, compare PEC values generated using Phase I and Phase II exposure assessment tools to the measured environmental concentrations (MECs) for model compounds, and make recommendations on uncertainties in the exposure assessment framework that may inform the regulatory community.

11.2 Veterinary Medicines Released into the Environment

The EU guidelines provide information on how to assess exposure to veterinary medicines in aquatic and terrestrial systems (VICH 2004; CVMP 2006). During Phase I of the risk assessment, simple algorithms are used that provide a conservative estimate of exposure in soils. If an environmental risk is shown at this stage, more sophisticated models are used. An outline of a number of the different algorithms is provided below. We have evaluated the results of these calculations by comparing them to data on the measured concentrations of veterinary medicines.

11.2.1 PEC Calculations

Guidance is provided for estimating exposure concentrations in soil. For intensively reared animals, concentrations in soil are estimated using a standard equation and a database containing information on the number of animals raised per location and year, animal body weights, nitrogen produced per year and the time spent indoors for all major agricultural species (CVMP 2006). For pasture animals that excrete dung onto pasture land, there is a similar equation that is based on stocking density to estimate the amount of active substance reaching the land. The output from the model is a PEC_{soil}. Concentrations in soil pore water ($PEC_{porewater}$), in groundwater ($PEC_{groundwater}$) and in surface water ($PEC_{surfacewater}$) are then estimated from the predicted concentrations in the soil.

Maximum concentrations in groundwater are approximated by pore water concentrations (i.e., $PEC_{groundwater} = PEC_{porewater}$), which can be derived according to equations provided in the guidelines for evaluating exposures to new and existing substances (CVMP 2006). Based on these pore water concentrations, surface water concentrations are approximated by assuming that runoff and drain flow concentrations are equal to the pore water concentrations, and subsequently applying a dilution factor of 10 to simulate the dilution of these concentrations in a small surface water body ($PEC_{surfacewater} = PEC_{porewater} / 10$). If these highly conservative approximations indicate a risk to the environment, more advanced models are recommended for calculating refined PECs in groundwater and surface water (i.e., Phase II). Two modeling approaches have been recommended for use with veterinary medicines, namely VetCalc and FOCUS (CVMP 2006). The models used in the FOCUS suite (FOCUS 2000) were originally developed for risk assessment of agricultural pesticides whereas VetCalc (available at *http://www.vmd.gov.uk/downloads.htm*) was specifically developed to predict exposure concentrations of veterinary medicines. However, to date, there have been few studies to evaluate whether these models provide realistic estimates of concentrations of veterinary medicines in environmental matrices.

VetCalc was developed to predict the concentrations of veterinary pharmaceuticals in groundwater and in surface water using twelve predefined scenarios in Europe. The scenarios were chosen to cover a wide variety of agricultural and environmental situations over three different European climate zones (Mediterranean, Central and Continental Scandinavian), including characteristics of the major livestock animals, associated manure characteristics, local agricultural practices, characteristics of the receiving environment (e.g., soil, water), and fate and behavior of chemicals within three critical compartments (soil, surface water, groundwater). VetCalc first calculates initial predicted concentrations in manure and soil, and these PECs are then used to simulate transport to surface water and groundwater using algorithms that were originally developed for pesticide assessment and account for potential for runoff, leaching and degradation (Mackay et al. 2005). The outputs from the leaching and runoff models are then integrated into a fugacity-based model that simulates the subsequent fate in surface water and considers potential for dilution/advection, degradation and partitioning in order to estimate concentrations in the water column (Mackay et al. 2005).

A working group in Europe known as FOCUS (i.e., Forum for the Coordination of Pesticide Fate Models and Their Use) has developed another suite of mechanistic en-

vironmental models to simulate the fate and transport of pesticides in the environment. Groundwater calculations developed by FOCUS involve the simulation of the leaching behavior of contaminants using a set of three models (PEARL, PELMO and MACRO) in a series of up to ten geographic settings, varying in crops, soils and climate. PEARL is an acronym of Pesticide Emission Assessment at Regional and Local scales. It is a one-dimensional numerical model of pesticide behavior in a soil-plant ecosystem which has been developed through close cooperation between two institutes in the Netherlands (FOCUS 2000). PELMO, which was first released in 1991 (Klein 1991) is a one-dimensional model that simulates the vertical movement of pesticides in soil by chromatographic leaching. PELMO is based on the PRZM 1.0 model developed by the U.S. Environmental Protection Agency (Carsel et al. 1984) with improvements that met the requirements of German regulatory agencies responsible for the registration of pesticides. The actual version used for the FOCUS simulations is PELMO 3.2. MACRO is a physically-based one-dimensional numerical model of water flow and reactive solute transport in field soils (Jarvis 1994). The surface water and sediment calculations are performed using an overall calculation shell called SWASH, which controls four models that simulate runoff and erosion (PRZM), leaching to field drains (MACRO), spray drift (internal to SWASH) and finally, aquatic fate in ditches, ponds and streams (TOXSWA). These simulations provide detailed assessments of potential aquatic concentrations in a range of water bodies. The EU guidance document (CVMP 2006) provides some recommendations for manipulating the FOCUS model for applications to veterinary medicines, but much more model validation is needed to assess its performance for this application.

11.2.2
PEC vs. MEC Comparisons in Soil and Water

11.2.2.1
Veterinary Antibiotics

The relatively simple algorithms suggested by CVMP (2006) for predictions of PECs in groundwater and in surface water would be expected to yield conservative estimates of levels in the environment. To test this assumption, we compared measured environmental concentrations (MECs) that have been determined for soil, leachate, runoff, drain flow and groundwater in semi-field and field studies to the PECs estimated for soil, porewater and surface water that were predicted according to the algorithms reviewed earlier. Comparisons between PEC and MEC data were conducted for a group of veterinary antibiotics for which there are analytical data. These data were taken from a number of field studies (Aga et al. 2003; Kay et al. 2004; Boxall et al. 2005; Burkhard et al. 2005; Halling-Sørensen et al. 2005; Stoob et al. 2007), which are reported in detail by Metcalfe et al. (in press).

Wherever possible, actual measured or spiked manure concentrations were used as the starting point for the calculation of soil concentrations. Where data were available, the actual depths of incorporation were used instead of the default value of 5 cm. In all other cases, default concentrations in manure for a given animal type and veterinary medicine had to be predicted from a knowledge of the treatment dosage and regime (Spaepen et al. 1997). The measured concentrations in soil in field studies (Aga

et al. 2003; Kay et al. 2004; Boxall et al. 2005; Burkhard et al. 2005; Halling-Sørensen et al. 2005; Stoob et al. 2007) were either close to or significantly lower than the predicted concentrations of the medicines in soil, indicating that the models are indeed conservative. In cases where manure loadings had to be estimated, the predicted soil concentrations were much higher than the actual measured concentrations, perhaps indicating that predicted loadings of veterinary medicines in manure are overestimated. In cases where concentrations of veterinary medicines in manure were either measured or spiked, there was better agreement between predicted and measured concentrations in soil.

To see whether the algorithms for predicting concentrations in the aquatic environment (CVMP 2006) were also conservative, the $PEC_{porewater}$ values for each veterinary antibiotic were estimated using minimum and median reported organic carbon-water partition coefficients (i.e., K_{oc}) and then compared to measured concentrations of the antibiotics in leachate, groundwater, drain flow and runoff reported for eight of the studies. As shown in Fig. 11.1a, the results show that the pore water PECs are usually conservative estimates of the measured concentrations. However, when measured concentrations in receiving waters are compared to $PEC_{surfacewater}$, which are predictions derived from the $PEC_{porewater}$ estimates, there were three instances where measured concentrations exceeded predicted concentrations (Fig. 11.1b). In all three cases, the antibiotics concerned belonged to the group of tetracyclines which are known for their strong adsorption (Tolls 2001). This is in agreement with the observation by Kay et al. (2004) that strongly sorbing compounds such as tetracyclines can be transported bound to colloidal organic matter. This mode of transport is currently not considered in the simple algorithms suggested by CVMP (2006). Thus, in the case of strongly sorbing compounds, the algorithms may not provide a conservative estimate of the PEC.

The predictions provided by VetCalc were also evaluated against measured concentrations from the field studies referenced above. The persistence data and the K_{oc} values used in this evaluation are reported by Metcalfe et al. (in press). VetCalc estimates of PEC_{soil} were generally higher than the measured soil concentrations under field application conditions (Fig. 11.2a), probably because the animal husbandry scenarios and manure application scenarios are likely to overestimate actual inputs to soils and for most of the study compounds. In addition, degradation during manure/slurry storage was not considered, as experimental data on degradation rates are not available. The only exception was tylosin, where the predicted soil concentration was several orders of magnitude lower than the measured soil concentration, which was 0.03 mg kg^{-1}. In contrast to many of the other medicines studied, the assessment for tylosin did consider degradation during storage under a default manure storage scenario, but it is possible that the field storage duration was significantly lower than the default value, explaining the high measured concentrations.

For the concentrations of antibiotics in surface water, with the exception of oxytetracycline, there was always at least one VetCalc scenario where the model predicted higher concentrations than the measured maximum concentrations (Fig. 11.2b). Generally, highest concentrations were obtained for the Italian scenario. This is perhaps not surprising, as field studies are generally performed at sites that are known to be vulnerable to transport of chemicals to water, whereas VetCalc predicts the fate of

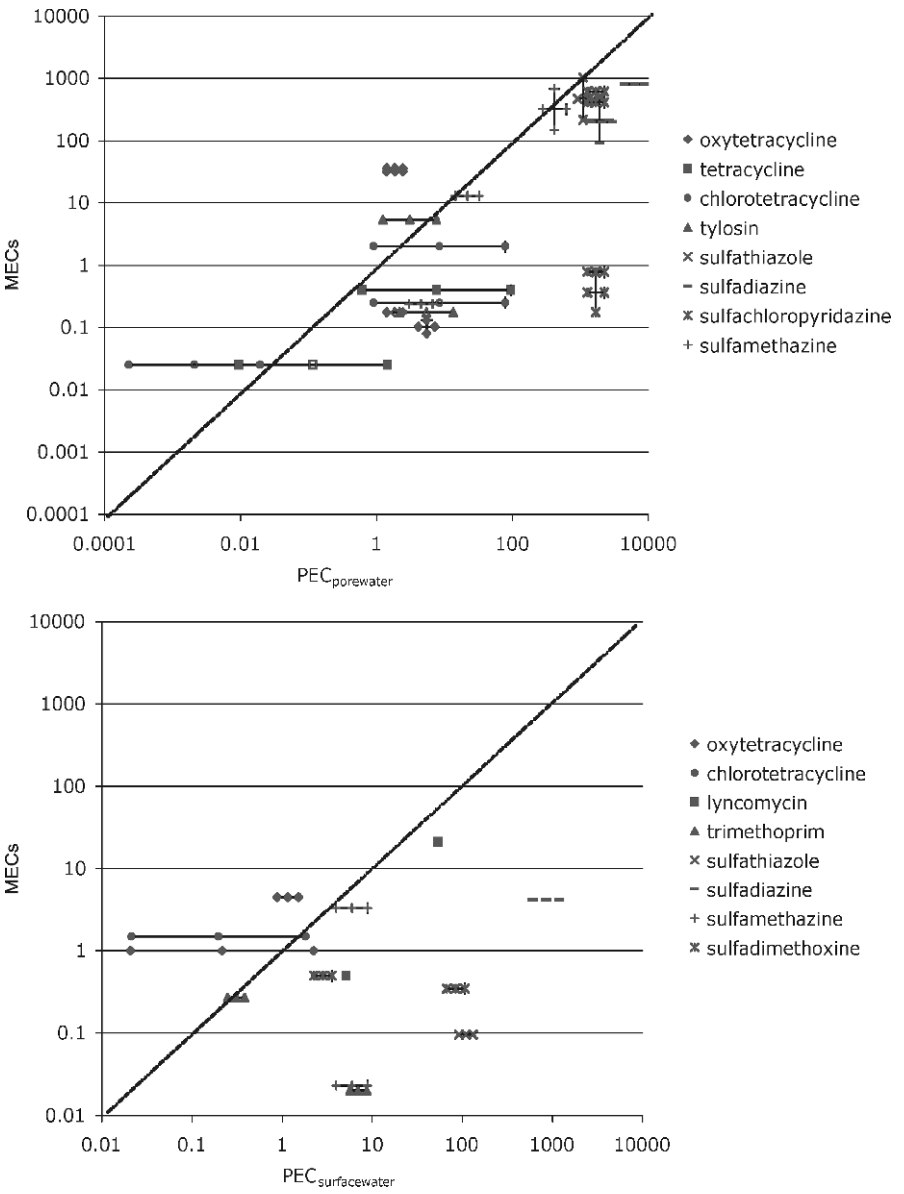

Fig. 11.1. PECs (ng l^{-1}) predicted using Phase I assessments (CVMP 2006) and MECs (ng l^{-1}) in **a** pore water and **b** surface water, for eight veterinary antibiotics. MECs for pore water were the maximum concentrations reported for leachate, groundwater, drain flow and runoff. *Empty symbols* indicate measured concentrations below the limit of detection (LOD), plotted as LOD/2 (i.e., between LOD and zero). Ranges for MEC (*y-axis*) are the minimum and maximum values measured in a given study. For PEC (*x-axis*), ranges were calculated from a median K_{oc} value based on all reported K_{oc} values and the minimum reported K_{oc} value. Figure from Metcalfe et al. (in press)

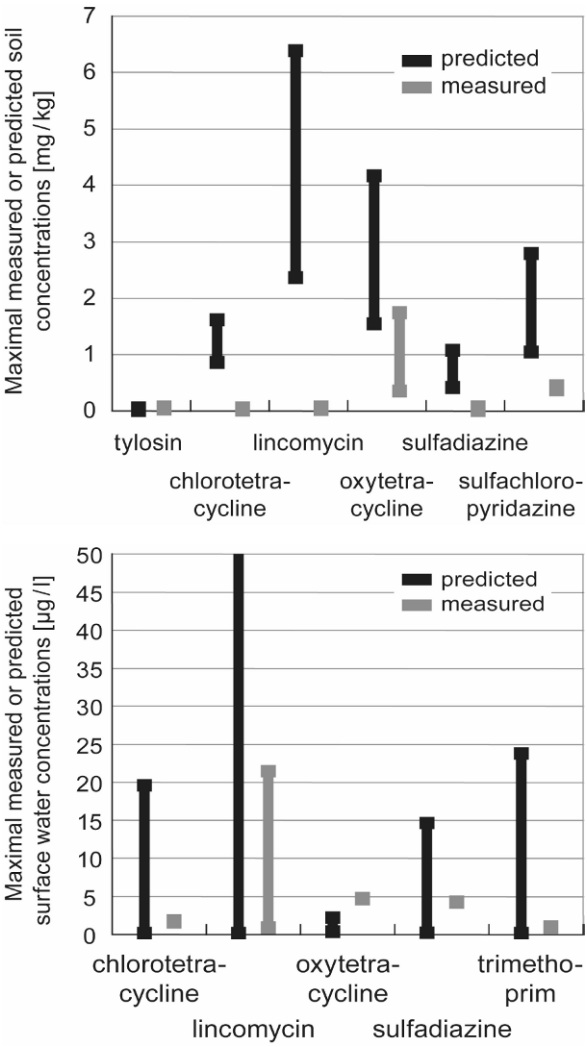

Fig. 11.2. Comparison of VetCalc predictions of PECs for veterinary antibiotics under twelve scenarios with data on measured concentrations (MECs) for **a** soils ($\mu g\,kg^{-1}$), and **b** surface water ($\mu g\,l^{-1}$). The average and maximal predicted concentrations of lyncomycin in surface water are 184 $\mu g\,l^{-1}$ and 550 $\mu g\,l^{-1}$ (not shown in graph *b*). Figure from Metcalfe et al. (in press)

substances across a range of European scenarios. Since the maximum predictions from VetCalc appear to be conservative (i.e., they overestimate actual concentrations), this modeling package is probably suitable for risk assessment of veterinary medicines. For our case study compounds, the VetCalc scenarios for Belgium, Denmark, Finland, France, Germany and the UK tended to give estimates of $PEC_{surfacewater}$ that were below the measured concentrations reported in the few studies on veterinary medicines in European surface waters. As with the simple algorithms, surface water concentrations of oxytetracycline were under-predicted; probably because colloidal or particle-bound transport is not currently considered by VetCalc.

11.2.2.2
Veterinary Anti-parasitic, Ivermectin

Ivermectin may enter the terrestrial and aquatic environments as a result of its use as an anti-parasitic treatment in medicated feeds or for short-term treatment of domestic animals. Ivermectin may contaminate soils when manure from treated animals is spread on agricultural soils or when dung from treated animals is deposited directly on pasture land. Ivermectin may then be transported to the aquatic environment via overland transport (runoff) from agricultural soils amended with manure or from pasture land, or by direct excretion to surface water from treated animals (e.g., cattle) that are standing in streams and rivers. Once in surface waters, ivermectin may partition into sediments. Ivermectin may also leach from contaminated agricultural soils into groundwater. Therefore, at least five PECs have to be determined for ivermectin in various environmental compartments, including soils (PEC_{soil}), dung (PEC_{dung}), surface water ($PEC_{surfacewater}$), groundwater ($PEC_{groundwater}$) and sediments ($PEC_{sediments}$). The calculations for soil summarized in Table 11.1 show a range of PECs under different scenarios.

The variations in PECs are dependent on the animal, the form and frequency of administration and the depth of soil for which the PEC was estimated. Assuming a housing factor (i.e., H) of 0.5, and depths in soil of 20 cm and 5 cm, minimum PEC_{soil} of 0.3 and 1.2 μg kg^{-1}, respectively were estimated for dairy cattle, while maximum PEC_{soil} of 2.7 and 10.8 μg kg^{-1}, respectively were estimated for beef bullocks. The highest concentrations of ivermectin in soil were predicted for application of manure from intensively reared calves that were kept inside for the whole year, reflecting the continuous dosages for the animals and year-round collection of manure. This worst case PEC_{soil} was estimated at 8.3 and 33.3 μg kg^{-1} at the 20 cm and 5 cm soil depths, respectively.

For estimates of PEC_{dung}, if one assumes that the total dose administered during one treatment is excreted within one day, the highest concentration of ivermectin in dung is predicted for sheep injected at 32 μg kg^{-1} (Table 11.1). For pigs treated with ivermectin as a feed additive, the PEC_{dung} is even higher, but this scenario is unrealistic, since it assumes treatment with ivermectin over one week, but total excretion within one day. For horses and slaughter calves, the PEC_{dung} values are 10.9 and 10.8 μg kg^{-1}, respectively (Table 11.1). The lowest PEC_{dung} was predicted for dairy cows after injection of ivermectin at 1.9 μg kg^{-1}.

The concentrations of ivermectin in surface water, pore water and groundwater were estimated from the values for $PECs_{soil}$ (CVMP 2006). To calculate $PEC_{pore\ water}$, it was assumed that the sorption equilibrium for ivermectin between soil and pore water can be estimated from the soil-water adsorption coefficient (i.e., K_d), or the soil organic matter-water adsorption coefficient (i.e., K_{om}). The latter value differs from the K_d by normalizing the adsorption coefficient to the organic matter content of the soil. K_{oc} normalizes the adsorption to the organic carbon content of the soil. The predicted pore water concentrations, and hence the $PEC_{groundwater}$ varied from 25 pg l^{-1} to 130 ng l^{-1} (Table 11.1), depending on the PEC_{soil} and the sorption assumptions. The soil concentrations for these calculations were selected according to the calculated minimum and maximum values for PEC_{soil}. According to the EU guidelines (CVMP 2006), the con-

Table 11.1. Overview of calculated PECs and reported MECs for ivermectin in different environmental compartments

Compartment	Unit	PEC value	MEC value	Remark PEC	Remark MEC
Soil	µg kg^{-1} dw	2.7/10.8[a]		Beef bullock, 5 treatments a^{-1}, pour on, $H = 0.5$	
		0.3/1.2[a]		Dairy cow, 2 treatments a^{-1}, injection, $H = 0.5$	
		8.3/33.3[a]		Slaughter calf, 5 treatments a^{-1}, pour on, $H = 1$	
			5, 9–46[b]		Outdoor pigs (feed mixt 0.6% W/W Ivm)[b]
		0.13/0.5[a]		Sow, 1 treatment a^{-1}, injection, $H = 1$	
			0.096 [±0.078][c]		Soil (5 cm) under dung (intial dung conc. 609 µg / kg dw) after 6 month[c]
			0.124 [±0.062][c]		Dung-amended soil (5 cm) under dung (1 kg dung fresh weight / m^2) after 6 month[c]
Dung	µg kg^{-1} fw	1.9	0.8 [±0.18][c]	Dairy cow, injection	After 3 days post treatment
			1.6[d]		
			0.3–1.3[b]		
		10.8	25.9[d]	Slaughter calf, pour on	2–18 days after inject
			1.8[b]		
		10.9		Horse and foal, paste	2 days after pour on treatment
		32		Sheep, injection	4–7 days after treatment
Surface water (Fresh water)	pg l^{-1}	2.5	200[b]	PEC_{sw} = one tenth of PEC_{gw}	
	ng l^{-1}	13	6–88[c]		
Ground water	pg l^{-1}	25	ND	$PEC_{gw} = PEC_{pore\ water}$;	
	ng l^{-1}	130		based on best and worst case assumptions for PEC_{soil}	
Sediment	µg kg^{-1} dw	0.8–38.1	0.8–4.9[b]	FOCUS Step 1	Post cattle pour-on treatment
			1.4–6.8[e]		
		0.4–19.8		FOCUS Step 2	

[a] $PECs_{soil}$ at 20 or 5 cm depth; H = housing factor (fraction of the year in which the animals are kept in house). [b] Boxall et al. (2006). [c] Alvaro Alonso, personal communication; Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria, Madrid, Spain. [d] Herd et al. (1996). [e] Cannavan et al. (2000).

centrations in surface water are assumed to be one tenth of the $PEC_{groundwater}$. Hence, the $PEC_{surfacewater}$ values ranged between 2.5 pg l^{-1} and 13 ng l^{-1} (Table 11.1). The PEC values in the sediment of receiving waters (i.e., $PECs_{sediment}$) were calculated using FOCUS software. The calculated values (Table 11.1) were based on typical or extreme application scenarios in the range of 0.5–25 g ha^{-1}, resulting in minimum and maximum $PEC_{sediment}$ estimates.

Table 11.1 includes MEC data for ivermectin reported in the literature. No data on measured concentrations were found for ivermectin in groundwater. This may be expected, as the predicted concentrations are in the pg/l to ng/l range, lower than the detection limit for ivermectin in water. As illustrated in Fig. 11.3 for the four environmental compartments for which there were MEC data, there was reasonable agreement between MECs and PECs. However, measured concentrations in soil and surface water sometimes exceeded the PECs (Fig. 11.3), indicating that the concentrations of veterinary medicines predicted using the EU guidelines are not always conservative estimates.

11.3 Human-Use Medicines Released into the Environment

11.3.1 PEC Calculations

The EU guidance document for risk assessment of human-use pharmaceuticals (CHMP 2006) describes various methods for assessing exposures in the environment. Phase I exposure assessment methods include a simple algorithm that can be used to provide

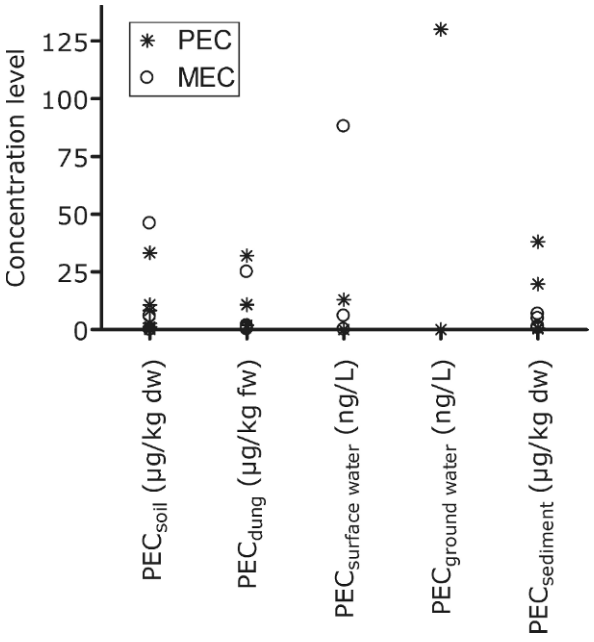

Fig. 11.3. Comparison of PEC and MEC data for ivermectin in various environmental compartments. Note that no MEC data are available in the literature for ivermectin in groundwater

conservative estimates of exposure. If Phase I indicates that there is a potential ecological risk if the compound is released into the environment, more sophisticated approaches are recommended in Phase II to refine the risk estimates. Table 11.2 provides a summary of the different algorithms that are recommended to estimate PEC values in both Phase I and Phase II assessments.

The exposure scenario included in all PEC calculations is the release of pharmaceuticals into receiving waters as a result of discharges of treated sewage from wastewater treatment plants (WWTPs) into surface waters. The $PEC_{surfacewater}$ calculation recommended for Phase I assessments (Table 11.2) utilizes data on the maximum daily dose (mg) recommended by the pharmaceutical company requesting the drug approval, the predicted market penetration (i.e., the fraction of the population predicted to be taking the drug if approved), which has a default value of 1% (0.01) for the Phase I assessments, the daily per capita wastewater output, which has a default value of 200 l, and a dilution factor, which is assumed to be 1:10 for wastewater discharged into receiving waters.

This calculation assumes that all of the daily dose of the drug is excreted unchanged in the urine and feces, and further assumes that there is no loss of the compound within the WWTP. If the Phase I risk assessment indicates that there is a need to advance to a Phase II assessment, there are more complex algorithms suggested in the guidance documents (Table 11.2) to refine the initial estimates for concentrations in surface water (i.e., $PEC_{surfacewater}$). These Phase II calculations allow for data on more realistic daily doses, human excretion rates and removal rates in the WWTPs to be included in the $PEC_{surfacewater}$ calculation. Finally, in Phase IIb, information on the fate of the drug in surface waters needs to be integrated in the PEC calculation (Table 11.2), although there is no specific guidance provided on how fate data can be applied in this case.

Table 11.2. Algorithms used to predict the concentrations of human-use pharmaceuticals in surface water (i.e., $PEC_{surfacewater}$) immediately downstream of discharges from a wastewater treatment plant (WWTP)

Phase I PEC	= (max daily dose, mg) · 0.01[a] ÷ (200 l cap^{-1} d^{-1}[b] · 10[c])
Local PEC	
Ph II A PEC	= (realistic DD, mg)[d] ÷ (200 l cap^{-1} d^{-1} · 10)
Local PEC	
Ph II B PEC	= (realistic DD) · excretion rate · WWTP removal[e] ÷ (200 L cap^{-1} d^{-1} · 10)
Local PEC	
Ph II B PEC	= (realistic DD) · excretion rate · WWTP removal · partitioning · fate ÷ (200 l cap^{-1} d^{-1} · 10)
Continental PEC; however, there is no guidance on how to include partitioning and fate data	

[a] The default penetration factor assumes that 1% of population will take the new drug (CHMP, 2006).
[b] Default wastewater production per capita (European Commission, 2003).
[c] Default surface water dilution factor (European Commission, 2003).
[d] Realistic DD, penetration factor revised using health statistics/epidemiology.
[e] Estimated from "SimpleTreat" model.

11.3.2
Comparisons of Predicted and Measured Concentrations

11.3.2.1
PEC vs. MEC in Untreated Municipal Sewage

Pharmaceuticals enter WWTPs as a result of excretion in urine and feces after drug dosing. The PEC calculations for Phase I and for Phase IIA make the conservative assumption that there is no removal of pharmaceuticals in the WWTPs. Thus, the measured concentrations of pharmaceuticals in untreated (raw) sewage should provide a reasonable point of comparison to PEC values where there has been an assumption of no removal in the WWTP. For the purposes of calculating the PECs for comparison to MECs in untreated wastewater, a value should be included that represents the percent of the parent compound excreted by humans after dosing.

Equation 11.1 was used to predict the PECs for raw sewage, using data on annual drug consumption, the pharmacokinetics of the drug in humans and per capita wastewater production (European Commission 2003; Alder et al. 2006). The calculation assumes that the estimated consumption is evenly distributed over the year and throughout the geographic area:

$$\mathrm{PEC}_{\mathrm{WWTPin}} = \frac{F_{\mathrm{API}} \cdot 10^{12} \cdot E}{365 \cdot Pop \cdot AWW} \tag{11.1}$$

where $\mathrm{PEC}_{\mathrm{WWTPin}}$ = predicted concentration in the raw sewage (ng l^{-1}), F_{API} = consumption of a active pharmaceutical ingredient per year in the area (kg yr^{-1}), E = fraction excreted without metabolism in urine and feces (–), Pop = population within the geographic region, and AWW = amount of wastewater produced per capita per day (300 to 500 l cap^{-1} d^{-1}).

For compounds that are readily degraded in wastewater, such as penicillin (Al-Ahmad et al. 1999), transformation may occur during transport in the sewer collection system, and this is not accounted for in Eq. 11.1. However, due to the significantly more intense contact with the degrading biomass inside the WWTP, it is reasonable to assume that the loss of most pharmaceuticals will be negligible during transport of the sewage, compared to degradation within the activated sludge reactor (Jones et al. 2005). In Table 11.3a, data are presented on the predicted (PEC) and measured (MEC) concentrations of four β-blocker drugs in untreated wastewater in the WWTP (i.e., raw sewage). Note that the estimates of *AWW* used in this case account for the volume of human wastewater produced, which is usually in the range of 200 l cap^{-1} d^{-1} (European Commission 2003), plus the per capita production of industrial wastewater that passes through municipal WWTPs. The estimates of 375 l cap^{-1} d^{-1} and 300 l cap^{-1} d^{-1}, respectively were calculated for Germany and Denmark from national data on the total volumes of municipal wastewater and the size of the population. For Switzerland, the *AWW* estimate of 400 l cap^{-1} d^{-1} was taken from Gujer (1999). For Canada, the estimate of 500 l cap^{-1} d^{-1} was made from data on the average wastewater flows and the municipal populations served by fourteen wastewater treatment plants (Metcalfe et al.

Table 11.3. Comparisons between predicted and median measured concentrations (ng l^{-1}) of selected pharmaceuticals in untreated wastewater (**a**) and treated wastewater (**b**) in WWTPs in Switzerland, Germany, Canada and Denmark. MEC data are from Maurer et al. (2007) for Switzerland; Ternes et al. (2000) and BLAC (2003) for Germany; Metcalfe (unpublished) for Canada; and Halling-Sørensen (unpublished) for Denmark. Drug consumption data for each country were provided by IMS Health, except for Germany. *n.a.*: data not available

a Untreated wastewater

Compound	Estimated excretion[a]	Switzerland (7.3 Mio cap., 400 l·cap^{-1}·d^{-1})			Germany (85 Mio cap., 375 l·cap^{-1}·d^{-1})			Canada (30 Mio cap., 500 l·cap^{-1}·d^{-1})			Denmark (5.4 Mio cap., 300 l·cap^{-1}·d^{-1})		
	%	Consumption (2004) kg·yr^{-1}	PEC WWTP in ng·l^{-1}	MEC[c] WWTP in (median) ng·l^{-1}	Consumption (2001)[b] kg·yr^{-1}	PEC WWTP in ng·l^{-1}	MEC[c] WWTP in (median) ng·l^{-1}	Consumption (2001) kg·yr^{-1}	PEC WWTP in ng·l^{-1}	MEC[c] WWTP in (median) ng·l^{-1}	Consumption (2005) kg·yr^{-1}	PEC WWTP in ng·l^{-1}	MEC[c] WWTP in (median) ng·l^{-1}
Atenolol	85	3 200	2 550	2 160	13 600	1 490	405	6 700	1 040	n.a.	936	1 350	3 800
Sotalol	70	800	525	317	26 650	2 405	800	1 120	144	n.a.	380	450	n.a.
Metoprolol	10	3 200	300	245	92 970	1 200	425	11 800	216	n.a	5 021	850	2 300
Propranolol	20	800	150	49	3 400	90	<LOQ	2 500	88	n.a	570	190	n.a.

b Treated wastewater

Compound	Estimated excretion[a]	Switzerland (7.3 Mio cap., 400 l·cap^{-1}·d^{-1})			Germany (85 Mio cap., 375 l·cap^{-1}·d^{-1})			Canada (30 Mio cap., 500 l·cap^{-1}·d^{-1})			Denmark (5.4 Mio cap., 300 l·cap^{-1}·d^{-1})		
	%	Consumption (2004) kg·yr^{-1}	PEC WWTP out ng·l^{-1}	MEC[c] WWTP out (median) ng·l^{-1}	Consumption (2001)[b] kg·yr^{-1}	PEC WWTP out ng·l^{-1}	MEC[c] WWTP out[b] (median) ng·l^{-1}	Consumption (2001) kg·yr^{-1}	PEC WWTP out ng·l^{-1}	MEC[c] WWTP out[c] (median) ng·l^{-1}	Consumption (2005) kg·yr^{-1}	PEC WWTP out ng·l^{-1}	MEC[c] WWTP out (median) ng·l^{-1}
Atenolol	85	3 200	1 250	511	13 600	730	250	6 700	512	484	936	660	140–480
Sotalol	70	800	365	249	26 650	1 660	630	1 120	100	146	380	310	n.a.
Metoprolol	10	3 200	160	194	92 970	640	615	11 800	136	447	5 021	540	590–1260
Propranolo	20	800	90	59	3 400	50	37	2 500	56	27	570	110	n.a

[a] Human excretion data from: Bourne (1981) and Frishman et al. (2002), and estimates from Lienert et al. (2007).
[b] BLAC (2003).
[c] 24-h composite samples for Switzerland, Germany, and Canada; Grab samples for Denmark.

2003). The data presented in Table 11.3a show that the MECs for the four β-blockers in raw sewage calculated for WWTPs in Switzerland, Germany and Denmark were predicted reasonably well by the Phase I PEC calculation.

11.3.2.2 *PEC vs. MEC in Treated Wastewater*

PEC calculations for Phase IIB exposure assessments can include estimates of removal rates within the WWTP. One of the most important routes for removal of human pharmaceuticals from sewage is partitioning into sludge. The tendency of a pharmaceutical to partition into sludge can be estimated from experimentally determined sludge partition coefficients (K_d). Removal could also occur by biological degradation and stripping into air. It must be noted that the parent compound could be reintroduced back into the sewage in the free form by de-conjugation processes (Alder et al. 2006). If empirical data on partition coefficients and biological degradation rates are available, PECs for treated wastewater (i.e., effluent) can be calculated according to the Eq. 11.2 (Alder et al. 2006):

$$\mathrm{PEC}_{\mathrm{WWTPout}} = \frac{\mathrm{PEC}_{\mathrm{WWTPin}} + Conj_{\mathrm{cleavage}}}{(1 + K_{\mathrm{d,prim}} SP_{\mathrm{prim}})(1 + K_{\mathrm{d,sec}} SP_{\mathrm{sec}} + k_{\mathrm{biol}} X_{\mathrm{SS}} \theta + K_{\mathrm{H}} Q_{\mathrm{air}})} \tag{11.2}$$

where $\mathrm{PEC}_{\mathrm{WWTPout}}$ = predicted concentration in the treated WWTP effluent (ng l^{-1}), $\mathrm{PEC}_{\mathrm{WWTPin}}$ = predicted concentration in the raw sewage (ng l^{-1}), $Conj_{\mathrm{cleavage}}$ = concentration of conjugated compounds in the WWTP influent that can be retransformed into the original active pharmaceutical ingredient during treatment (e.g., by cleavage) (ng l^{-1}), K_d = primary or secondary solids partition coefficient at ambient pH (can be assumed equal for primary and secondary sludge in most cases; see below) (l g SS^{-1}), SP = specific primary or secondary sludge production per amount of wastewater treated, including primary and secondary sludge (g SS l^{-1}), k_{biol} = degradation rate constant (l g SS^{-1} d^{-1}), X_{SS} = suspended solids concentration in the reactor (g SS l^{-1}), θ = hydraulic retention time of the wastewater in the biological reactor (d), K_{H} = Henry Law coefficient (dimensionless gas water partitioning coefficient) (–), Q_{air} = specific air consumption for aeration (m^3_{air} $m^{-3}_{\mathrm{wastewater}}$).

The amount of conjugated compound in the influent ($Conj_{\mathrm{cleavage}}$) can be estimated from human excretion data, recognizing that excretion data that are not peer-reviewed may vary in quality. The term $K_d SP$ represents the sorbed amount removed with the sludge withdrawal, and $k_{\mathrm{biol}} X_{\mathrm{SS}} \theta$ represents the biological degradation during wastewater treatment. $K_{\mathrm{H}} Q_{air}$ represents the amount stripped into the air during the aeration of the biological reactor. Equation 11.2 assumes that the particulate matter in the sewage is completely removed during the treatment. However, during the regular operation of most municipal WWTPs, 5 to 20% of the particulates are retained in the effluent, depending on the technology used for wastewater treatment. The sorption coefficient K_d describes the ratio of the concentration of sorbed substance (ng g^{-1} SS) to the concentration of dissolved substances in wastewater (ng l^{-1}) and must be experimentally determined for each compound. This coefficient is significantly dependent on ambient pH in the case of acidic and basic compounds. The degradation rate constant (k_{biol}) must be taken from the literature or determined experimentally.

Table 11.3b provides a comparison between PECs and MECs for β-blocker drugs in treated wastewater (i.e., effluents) in WWTPs from Switzerland, Germany, Canada and Denmark. The PEC values for treated wastewater were calculated using Eq. 11.2. The experimentally determined sorption coefficients (K_d) and the degradation rate constants (k_{biol}) for the β-blockers in sludge were determined in bench-scale experiments, as reported by Maurer et al. (2007). The degradation rates for the β-blockers varied between 0.29 and 0.69 $l\,d^{-1}\,g^{-1}$.

For atenolol, sotalol and metoprolol, sorption to sludge was shown to be minimal ($K_d < 0.04\ l\ g\ SS^{-1}$). Although the sorption coefficient of propranolol is higher ($K_d = 0.29\ l\ g\ SS^{-1}$), the total amount eliminated via partitioning to sludge is less than 5%, and thus, the quantities lost in biosolids are not significant. A low K_d value indicates that the differences in concentrations in the influent and effluent in a WWTP can be attributed primarily to biological degradation. The data presented in Table 11.3b indicate that the concentrations of the four β-blockers in untreated and treated wastewater can be predicted reasonably well when there are sufficient empirical data to support the PEC calculation.

It is possible that pharmaceuticals can enter raw sewage via the disposal of out-of-date or unwanted drugs that are flushed down the toilet. There is anecdotal evidence that 15–20% of the pharmaceuticals distributed for human use are flushed unused into the sewage system, although definitive data are difficult to obtain (Versteeg et al. 2005). This could contribute to MEC values being higher than the PEC values.

11.3.2.3
PEC vs. MEC in Surface Waters

Phase II Calculations

For estimating the PEC in surface water, the dilution of the WWTP effluent in the receiving water, as well as the background concentration of the pharmaceutical in the receiving waters must be considered (Alder et al. 2006). Equation 11.3 includes these factors in the PEC calculation for surface water:

$$PEC_{SurfaceWater} = PEC_{WWTPout} R_{dilution} + C_{background} \tag{11.3}$$

where $PEC_{SurfaceWater}$ = predicted concentration in the receiving water body ($ng\ l^{-1}$), $R_{dilution}$ = ratio between wastewater flow and receiving water flow (–), $C_{background}$ = background concentration in the receiving water body prior to wastewater discharge ($ng\ l^{-1}$).

The dilution ($R_{dilution}$) in the receiving waters depends on local hydrological conditions. Dilution factors may vary widely, depending on the site and the season (Kolpin et al. 2004; Ashton et al. 2004). In order to predict environmental concentrations, it is crucial to obtain data on dilution factors. In addition to dilution, significant amounts of the pharmaceuticals released into surface waters may be removed by partitioning into suspended or bottom sediments, or by hydrolytic, photolytic or biological degradation. More advanced PEC calculations used in Phase II assessments could include coefficients that empirically describe these processes.

Under the current EU guidelines for environmental risk assessment of human pharmaceuticals (CHMP 2006), the PECs derived in Phase I and Phase IIA are based on a total residue approach, where the entire administered dose of a pharmaceutical is as-

sumed to reach surface waters without human metabolism or removal by wastewater treatment, or without partitioning or elimination in the receiving waters. However, these removal processes are important routes for the elimination of pharmaceuticals. Removal by adsorption onto sewage sludge is currently considered in the guidelines for Phase IIA assessments of pharmaceuticals with a high adsorption constant (i.e., $K_{oc} > 10\,000$); requiring a specific soil risk assessment in Phase IIB, because the pharmaceutical may be spread with biosolids onto agricultural soils. Also in Phase IIB calculations, data on the fractions of parent compound and major metabolites excreted by humans can be used to estimate PECs that are based on excretion patterns.

Both Phase I and IIA PECs constitute "local PECs" in the terminology of the EU Technical Guidance Document (TGD) on risk assessment (European Commission 2003). This means that they represent predicted concentrations in a WWTP effluent that is diluted by a standard factor in surface waters but without consideration of any further environmental fate processes. According to the TGD, the PECs for new substances can be estimated based on estimates of amounts to be sold, metabolism, entry pathways into the environment, degradation and distribution characteristics, as well as dilution, fate and advection in the medium. The EU guideline for human pharmaceuticals (CHMP 2006) also acknowledges that further fate processes can be introduced into refined PEC calculations. However, little direction is given in the guidelines on how to integrate these data into the risk assessment.

For pharmaceuticals that have been on the market for some time, MECs can be compared to PECs derived using different levels of refinement. However, the MECs reported in the literature differ in reporting format, including median values and/or ranges, and may be with or without information on the number of determinations, the median and 90th percentile values, maxima, numbers below limits of detection, etc. It is mathematically impossible to reconstruct an original single data point from a distribution. As an approximation, however, averaged back-distributions are possible (Straub, this volume). Such back-distributions can be weighted according to the known number of original data points, and further, several such weighted back-distributions can be integrated into one single distribution (Fig. 11.4). The calculated regression line for MECs can then be used to generate graphical estimates of overall 50th, 90th or 95th percentile values, which can in turn be used for comparisons with PECs.

A few such MEC vs PEC comparisons have been conducted by Straub (2006), Straub and Stewart (2007), and Straub (this volume), based on actual drug use data for Europe compiled by the medical data provider, IMS Health. These comparisons can be illustrated with the data for ibuprofen, which is shown in Fig. 11.4. In this analysis, Phase I, IIA and IIB PECs were calculated according to EU guidelines (CHMP 2006) graphed at the 90th percentile, or as a TGD refined continental surface water PEC (European Commission 2003) graphed at the 50th percentile. The refined PECs are based on recorded data (i.e., IMS Health) on the total use of the ibuprofen in Europe. The Phase I PEC calculated according to the EU guidelines is always very conservative relative to the MEC data. As shown in Fig. 11.4, the EU Phase IIA and IIB PECs are typically closer to the measured concentrations, and the TGD average PEC is generally within a factor of less than 5 from the 50th percentile MEC. The Phase I PEC that is based on a default penetration factor (i.e., 1%) and the maximum daily dose, without considering human metabolism and WWTP removal may have the intended property of providing a protective base calculation for risk assessment, but it does not provide a close prediction

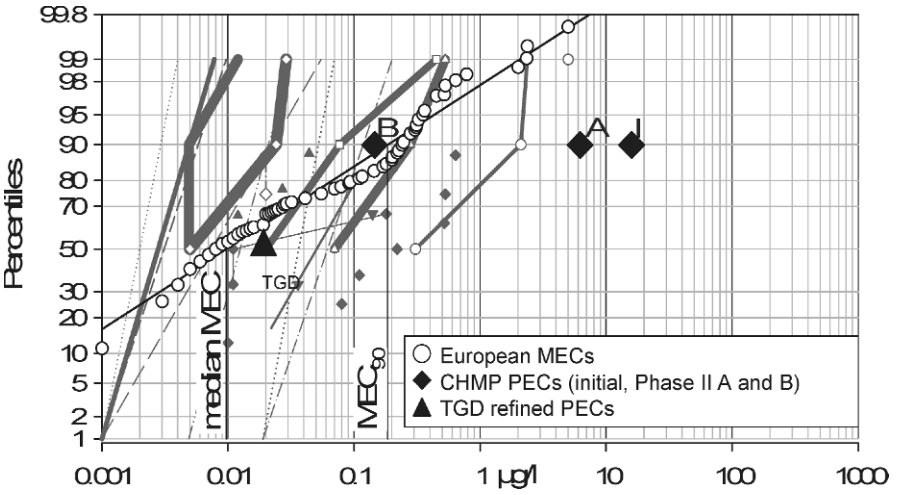

Fig. 11.4. Weighted back-distribution and combination of MEC values ($n = 311$) for ibuprofen in surface water in Europe. The *grey line* thickness is proportional to the number of MECs, and *symbols* show single values or 50th and 90th percentile MECs. The 99th percentile values are maximum reported values. The *open symbols* are the compiled data points of the integrated overall distribution. The *large diamonds* are the Phase I (*I*), IIA (*A*) and IIB (*B*) PECs, calculated according to the CHMP (2006). The *large triangle* is the TGD surface water PEC (TGD) calculated according to the European Commission (2003). Figure adapted from Straub (2006)

of the MEC. Only if the individual properties of the pharmaceutical regarding metabolism, WWTP removal and fate in receiving waters have been reasonably well characterized, will these PECs approximate the MECs. The TGD method for estimating a PEC shows the best correlations with available MEC data for ibuprofen, which is consistent with similar analyses for other drugs (Straub and Stewart 2007; Straub, this volume). These results show the value of further refining PEC estimates in Phase II risk assessments.

GREAT-ER Model

More sophisticated models can be used to refine Phase II PECs for human-use pharmaceuticals in surface waters downstream of WWTP discharges. GREAT-ER is a GIS-assisted model that can be used to predict geo-referenced patterns of water pollution in a set of important European catchments. Currently, the GREAT-ER model is only applicable to pollutants whose main emission pathway into the aquatic environment is through point sources, such as WWTPs. To evaluate the capacity of the GREAT-ER model to predict the concentrations of human-use pharmaceuticals in surface water, this model was used to predict the concentrations of the β-blocker drug, atenolol at various locations in the Glatt River in Switzerland. The watershed of the Glatt River covers an area of about 180 km^2, with 17 WWTPs that were in operation in 2006. There are two lakes in the upper part of the watershed: Lake Pfäffikon with an area of about 3.0 km^2 and average residence times of about 770 days, and Lake Greifensee with an area of about 8.5 km^2 and residence times of 100 and 430 days in winter and summer, respectively.

The publicly available version of GREAT-ER (*http://www.great-er.org/pages/home.cfm*) was utilized for this exercise. However, since implementation of the Glatt River watershed in GREAT-ER is not yet publicly available, this spatial analysis module was provided by Dr Jörg Klasmeier of the University of Osnabrück in Switzerland. The temporal variability of flow conditions was entered into the model as the mean discharge (Q_{mean}) and the discharge that is only exceeded during 5% of the days in a given year (Q_{347}), based on measurements from sixteen gauging stations in the watershed. Flow conditions for the stretches between the gauging stations were interpolated. To represent temporal variability in flow conditions, the model can be run repeatedly using a Monte Carlo approach, where the flow conditions for each run are sampled randomly from the probability distribution of possible flow conditions. The model was run in Mode 1, as defined in the GREAT-ER modeling framework; that is, removal in WWTPs and degradation or other loss processes in river stretches are represented by a lumped removal efficiency or loss rate constant, respectively. The characteristics of the modeling exercise are described in detail by Alder et al. (in preparation). The input data on the chemical and physical characteristics of β-blocker drugs were gathered from various literature sources. The concentrations of the β-blocker drugs were measured analytically in samples collected at three points along the River Glatt, at the outflow of Lake Greifensee, 12 km further downstream, and 35 km further downstream, where the Glatt enters the Rhine River. Data were available for seven samples (24-hr composite) that were taken over a period of seven consecutive days from August 15–August 21, 2006, and for one-week composite samples collected over that same time period. During the sampling week, flow conditions were in the lower 22nd-37th percentile of the year-round flows. No data were collected at other times of the year, when flows are greater in the watershed.

In Fig. 11.5, data are plotted for the measured (i.e., MEC) and modeled (i.e., PEC) concentrations for atenolol in the river Glatt watershed over the study period. The comparison indicates good agreement between MECs and PECs for atenolol, as the averages of the measured concentrations of atenolol in the 24-hr samples collected at two sampling points in the lower reaches of the Glatt River lie close to the median PECs.

However, the model over-predicted the concentration of atenolol at the outflow of Lake Greifensee, where atenolol was not detected (Fig. 11.5; arrow). The most likely explanation for this finding is that there are additional degradation processes in surface water for these compounds, including biodegradation or photolytic transformation. Given the fact that flow conditions during the sampling period in August were low, the predicted and measured concentrations might be considered high relative to the yearly median.

Overall, these data show that geo-referenced models like GREAT-ER, or an equivalent model developed in the USA, PhATE (Cunningham, this volume; Cunningham et al. 2004; Robinson et al. 2007), which are calibrated using actual discharge data, produce reasonably good predictions of the concentrations of highly water-soluble pharmaceuticals, such as β-blockers. Errors in PECs can be expected to lie within a factor of two or less. Note, however, that in order to achieve a high prediction accuracy, good experimental data for the removal rates in WWTPs and the half-lives of loss processes in receiving waters are essential. Half-lives usually only become important if they are on the same order of magnitude as the hydraulic residence time of the watershed considered. Indirect and direct photolytic processes can be rather fast processes and are therefore of primary importance for an accurate prediction of aquatic fate.

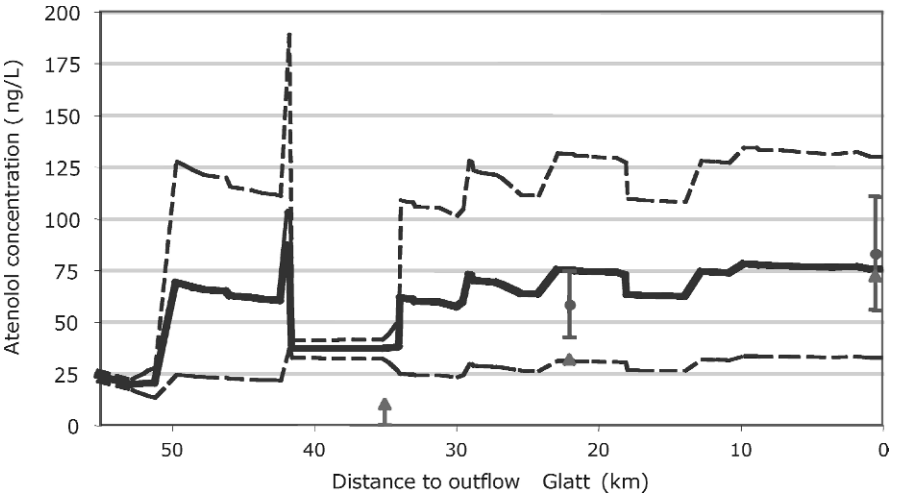

Fig. 11.5. GREAT-ER simulations of the concentrations (ng l^{-1}) of atenolol in the Glatt River watershed compared to MECs from three sampling points (circles: 24 h composite samples with standard deviations from a seven-day study period; *triangles*: one-week composite samples). The non-detected data at the outflow from the Lake Greifensee is shown by the *arrow*. Simulated concentrations cover the entire watershed (*solid line*: median concentration, *broken lines*: 10th and 90th percentile of temporal concentration variability)

11.3.3
Batch Test Systems for Predicting Degradation and Sorption

Accurate estimates of removal rates from WWTPs are essential for making Phase II predictions of concentrations in treated wastewater and surface water. The main elimination processes for the removal of pharmaceuticals in municipal wastewater treatment plants (WWTPs) are sorption onto particulate matter and biological transformation. Biodegradation depends on the concentration of a compound, the redox conditions (i.e., aerobic, anoxic and anaerobic) and the solids' retention time (SRT) of the sludge.

Trace pollutants in WWTPs are estimated to make up only a minor part of the total carbon used for microbial growth (usually less than 1%). They are believed to be degraded as secondary substrates (i.e., co-metabolism) concurrently with bulk organic matter and are therefore not growth dependent. Additionally, microorganisms are able to utilize mixed substrates, and additional substrates may influence the biodegradation of the target chemical occurring at low concentrations. The diversity of bacteria present and their number are also of importance.

Biodegradation tests are used increasingly to make predictions of the fate of chemicals in the environment and to support risk assessment decisions. As reviewed by Jones et al. (2005), many pharmaceuticals do not meet the criteria for ready biodegradability using these screening tests. However, it is important to note that the currently available screening tests (e.g., OECD 301, 302, 303, 308, 310) do not simulate conditions in WWTPs, and the translation of results from these tests to exposure assessments for

pharmaceuticals in wastewater is limited. This conclusion was recently supported by an evaluation of the OECD 308 biodegradation test for application to pharmaceuticals (Ericson 2007). These tests are not designed to provide kinetic values at the low residual concentrations of pharmaceuticals observed in WWTPs (Hales et al. 1997; Jones et al. 2005). Relative to conditions in a WWTP, the screening tests use relatively high substance concentrations (i.e., 100–1 000 times higher) and low biomass concentrations (i.e., 100 times lower). Many of the screening tests use non-specific endpoints such as DOC removal, CO_2 evolution and oxygen demand, which provide little information on the kinetics of degradation.

It is possible to evaluate the degradation of pharmaceuticals in WWTPs using pilot-scale systems (Zwiener and Frimmel 2003), but these are fairly complex systems to construct and operate. As an intermediate approach, relatively simple batch experiments can be run with sludge and wastewater from a WWTP at concentrations of pharmaceuticals that are representative of untreated wastewater. A batch system consists of a simple vessel equipped with an aeration system, a stirring unit and some sensor devices. The amount of sludge is assumed to be constant (i.e., no sludge formation or sludge withdrawal) over the experimental period. It is recognized that each WWTP is different, and that degradation rates will vary with the treatment technologies, and even temporally as input concentrations change. Microbial populations may also evolve in their capacity to eliminate pharmaceuticals (Zwiener and Frimmel 2003). Examples of pharmaceutical removal under aerobic conditions in batch experiments have been reported by Joss et al. (2004, 2006a,b), Perez et al. (2005) and Maurer et al. (2007). In addition, Ternes et al. (2004) used a batch assay to determine the sorption coefficient onto sewage sludge of several pharmaceuticals.

To assess the interactions with the sludge, control experiments can be run without activated sludge (Joss et al. 2004) or by inactivating the biomass with microbial toxins, such as $HgCl_2$ (Maurer et al. 2007) or sodium azide (Perez et al. 2005). When control experiments are run with inactivated sludge, removal is entirely due to sorption. Pseudo first-order kinetics is assumed for the modeled processes, which are represented by the rate constant, k_{bio}. Joss et al. (2006a) determined the biological elimination of twenty-five pharmaceuticals, hormones and fragrances in batch experiments using sewage sludge at concentrations found in municipal WWTPs and suggested a simple classification scheme to characterize the biological degradation during wastewater treatment. Depending on kinetic degradation data, the compounds were divided into different classes according to their persistence; that is, removal <20%, partial removal and transformation >90%.

Using a similar approach, Maurer et al. (2007) investigated in batch experiments the sorption coefficients and biodegradation rates for four β-blockers(i.e., atenolol, sotalol, metoprolol, propranolol). Sludge from a membrane bioreactor pilot plant was diluted with wastewater, and the experiments were run at spiked concentrations that were three times the background concentration. There was good agreement between the measured and estimated eliminations in WWTPs for atenolol, sotalol and propronalol (Table 11.4). The elimination estimated for metoprolol was slightly overpredicted and for one WWTP there was poor agreement between measured and predicted removals. Overall, these studies illustrate that batch experiments may be more realistic lab-scale systems than the OECD screening tests for determining sorption coefficients and biodegradation rates for pharmaceuticals at low concentrations.

Table 11.4. Measured rates of elimination of β-blockers (percent ±standard deviation) in two WWTPs measured over a three-day period, compared with values estimated with batch experiments with sludge material from the WWTPs (data from Maurer et al. 2007)

	WWTP-1		WWTP-2	
	Measured	Estimated	Measured	Estimated
Atenolol	79 ±17	53 ±9	73 ±9	71 ±6
Sotalol	26 ±7	27 ±14	27 ±2	41 ±11
Metoprolol	31 ±11	47 ±10	29 ±5	64 ±7
Propranolol	28 ±2	39 ±15	35 ±3	54 ±12

11.4 Assessing the Risks of Human-Use Pharmaceuticals in Biosolids

Sewage sludge is an inevitable byproduct of the treatment of municipal wastewater. Failure to regularly remove the sludge from sewage treatment units in wastewater treatment plants (WWTP) would soon result in excessive pollution entering surface waters at WWTP discharges. When treated, sludge is referred to as "biosolids", which have to be used or disposed of by methods that are safe, acceptable to stakeholders and economically feasible. In the UK, about 20 kg per capita of dry biosolids is produced each year (Water UK 2004). In the USA, it was estimated that the average WWTP produces 240 kg dry weight of biosolids per million liters of wastewater treated (Kinney et al. 2006).

In many EU countries and in North America, recycling of biosolids to agricultural land is the preferred disposal route. The per capita production of treated sludge in EU countries is estimated to vary between 9 and 38 kg dry weight per annum (European Commission 2001), and Ireland, Finland and the United Kingdom reuse the highest percentage of their sludges in agriculture (i.e., >70%). The fraction of biosolids currently used in agriculture in Germany is 30% (Agricultural Board of North Rhine Westphalia, personal communication). The fraction of biosolids used as fertilizer in Denmark declined from 70% to 60% over 1995 to 2001 because of increasing quality requirements (Jensen and Jepsen 2005). In the province of Ontario in Canada, about 40% of the biosolids generated from domestic waste, or about 16 000 tons dry weight are applied annually to approximately 2 000 hectares of agricultural land (Topp et al. 2008).

The guidance document for exposure assessment for human-use pharmaceuticals (CHMP 2006) only addresses the risks associated with the release of drugs into the terrestrial environment in cases where the K_{oc} value for adsorption of the drug to sludge exceeds a value of 10 000. In these cases, it is necessary to estimate a PEC_{soil} and evaluate the potential for effects on soil organisms. However, given the amounts of biosolids that are produced in WWTPs and subsequently applied to agricultural land, it may be prudent to develop guidelines for PEC_{soil} calculations that apply to all pharmaceuticals; even those with K_{oc} values <10 000. Data presented by Golet et al. (2002), Xia et al. (2005), Miao et al. (2005), Gobel et al. (2005) and Kinney et al. (2006) have shown that

pharmaceuticals can be present in biosolids at μg/kg or mg/kg concentrations, which are comparable or greater than the measured concentrations of veterinary medicines in dung. Pharmaceuticals in land applied biosolids have potential to contaminate water resources via preferential and overland flow. However, the degree of mass loading to adjacent water sources can be significantly reduced by using best management practices for land application. In a recent semi-field study reported by Topp et al. (in press), pharmaceuticals were not detected in overland flow when liquid municipal biosolids were applied to agricultural land below the soil surface, but the pharmaceuticals were present in runoff following surface application plus soil incorporation.

In an experiment conducted in Ontario, Canada with liquid municipal biosolids applied to agricultural land, model pharmaceuticals were transported through soil into tile water in amounts that were reasonably well predicted by concentrations of a fluorescent dye tracer, rhodamine WT (Lapen et al., in press). Two regression models were required to predict the movement of pharmaceuticals to tile drains: (*i*) a regression model developed exclusively from data collected during the application induced tile drain hydrograph, and (*ii*) a regression model developed from data collected after this event, which included rainfall induced tile flow. At the time of land application, biosolids applied to the soil surface moved rapidly into tile drains via large soil macropores, thereby contaminating tiles within minutes of application. However, subsequent to biosolids application, less extensive tile drain contamination occurred during modest rain storm events. These responses were a result of analyte retention within a more complex soil hydrologcal environment.

The dual permeability model, MACRO (Larsbo and Jarvis 2003) was used to simulate the flow of pharmaceuticals into tile drains for comparison to the data reported by Lapen et al. (in press). Larsbo et al. (in preparation) observed that most successful MACRO modeling strategies involved minimizing the influence of pharmaceutical sorption during land application and/or when rainfall intensities were significant enough to promote contaminant flow through the largest soil macropores. One approach would be to define a threshold water flow velocity in the macropores, above which sorption in the macropores is minimized. As soon as the flow velocity decreases below the threshold velocity, sorption is reset to the original value. Another modeling strategy consisted of having two separate Koc values for an analyte within the modeling framework; one for extreme preferential flow events (including land application), and one for other soil hydrological conditions. Simulation results of this second modeling strategy are shown in Fig. 11.6. The high flow leaching events created by land application of liquid biosolids and large rain events warrants further investigation. However, determination of the persistence of pharmaceuticals within soil environments is also crucial for a reliable assessment of losses over time.

11.5 Conclusions

The information presented above demonstrates that the methods described in the EU guidelines for risk assessment of both veterinary and human-use pharmaceuticals provide Phase I PECs that are conservative relative to the actual concentrations in the environment, and provide a level of protection in requiring that risk assessments advance to Phase II. However, refined PEC calculations in Phase II must include realistic

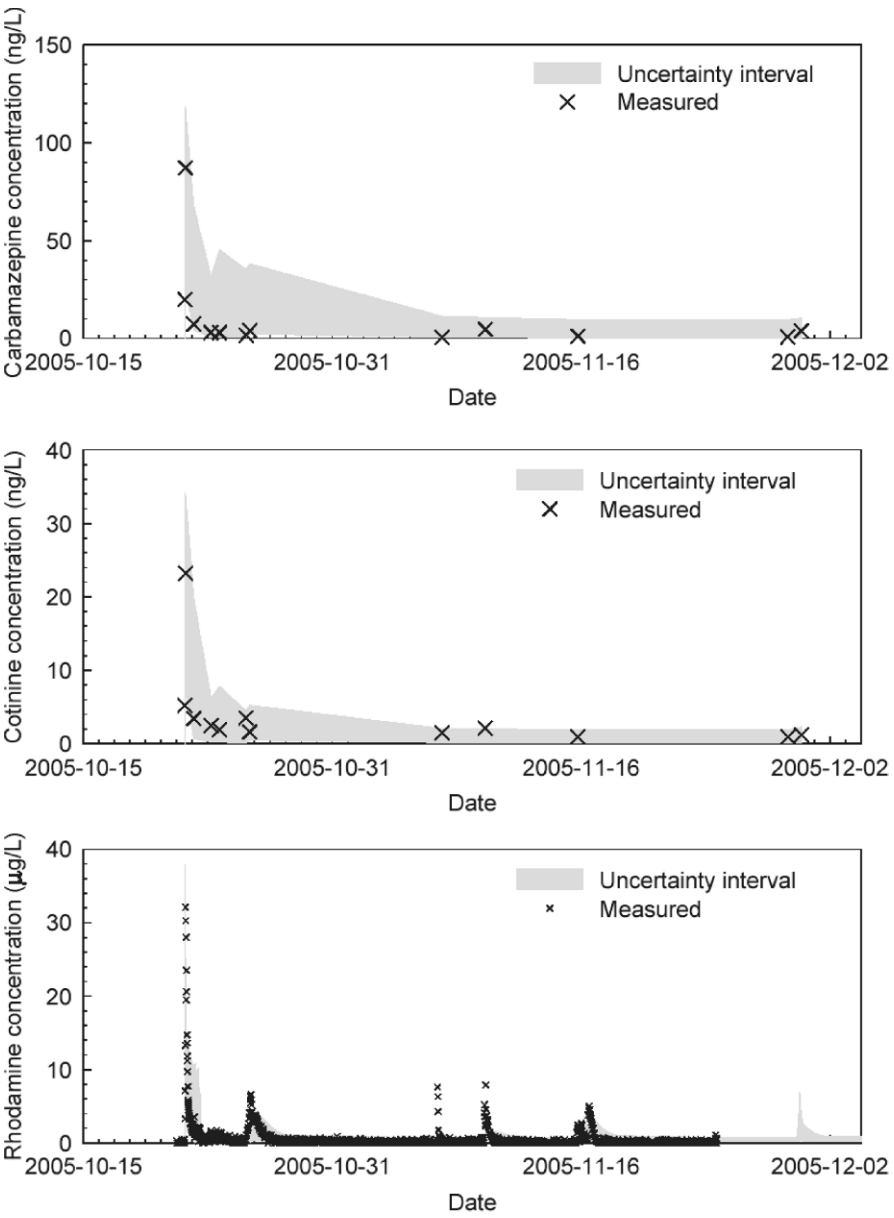

Fig. 11.6. Measured and simulated data for carbamazepine, cotinine and rhodamine WT in tile drain discharge over time in an experimental agricultural field in Ontario, Canada that was applied with liquid municipal biosolids. Simulations (*grey area*) are from the MACRO model, and the modeling strategy employed two different sorption (K_{oc}) values for: (*i*) the application induced tile drain hydrograph event, and (*ii*) the following rain induced events. Uncertainty intervals were calculated using the GLUE approach (Beven and Binley 1992)

fate parameters in order to generate reasonable estimates of pharmaceuticals in the environment. More guidance is required to develop the standardized approaches to refining the PECs; especially for Phase IIB assessments. The data provided here indicate that modeling approaches show promise as tools for estimating PECs in Phase II assessments, but more work is required to validate and refine existing models.

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Part III
Effects

Chapter 12

Effects of Pharmaceuticals on Aquatic Organisms

K. Fent

12.1 Introduction

In the last decade, residues of many different pharmaceuticals have been detected in wastewater and in surface water. Pharmaceuticals are excreted after application in their native form or as metabolites. Municipal wastewater is the main route by which these compounds reach the aquatic environment. Many compounds are not readily degraded in sewage treatment plants (STP). In addition, disposal of unused medicines into the (aquatic) environment sum up to the environmental load (Bound and Voulvoulis 2004). Another source is wastewater from manufacturers and hospitals, as well as landfill leachates (Holm et al. 1995). Contamination of soil, run-off into surface water, but also drainage may occur from the application of sewage sludge in agriculture. Veterinary pharmaceuticals may enter aquatic systems via manure application and subsequent runoff, but also via direct application in aquaculture (fish farming). Overall, the environmental persistence and biological activity of pharmaceuticals, particularly on biological key functions such as development and reproduction, are of environmental concern.

Monitoring studies demonstrate the ubiquitous presence of drug residues in aquatic systems (Ternes 1998a; Ternes et al. 2001; Kolpin et al. 2002; Kümmerer 2004) Up to about one hundred pharmaceuticals from many classes of drugs and some of their metabolites were determined in treated sewage, rivers and creeks, seawater, groundwater and drinking water (Ternes 1998a; Ternes et al. 2004; Hummel et al. 2006). Figure 12.1 gives the ranges of concentrations found for different pharmaceuticals. In STP effluents, concentrations are in the range of ng/l to µg/l, and in rivers, lakes and seawaters, they are in the range of ng/l (Buser et al. 1998; Kolpin et al. 2002; Weigel et al. 2002; Ashton et al. 2004; Thomas and Hilton 2004). Data on environmental concentrations have been summarized (e.g., Halling-Sørensen et al. 1998; Daughton and Ternes 1999; Kümmerer 2001; Heberer 2002; Kümmerer 2004).

The rather persistent antiepileptic carbamazepine and its degradation product (Hummel et al. 2006) and clofibric acid, a metabolite of some lipid lowering agents, have regularly been detected in STP effluents, freshwater (rivers and lakes) and even in seawater (Buser et al. 1998; Weigel et al. 2002). In surface water, clofibric acid was detected up to 0.55 $\mu g\, l^{-1}$ (Ternes 1998b), and carbamazepine up to 1.2 $\mu g\, l^{-1}$ (Wiegel et al. 2004). In forty-four rivers across the USA, average levels were 60 $ng\, l^{-1}$ in water and 4.2 $ng\, mg^{-1}$ in the sediment (Thaker 2005). Frequently, the analgesic ibuprofen and its metabolites were detected in STP effluents (Ternes 1998b; Buser et al. 1999; Boyd et al. 2003; Weigel et al. 2004). They are found in surface water up to 1 $\mu g\, l^{-1}$ (Kolpin et al. 2002), but also

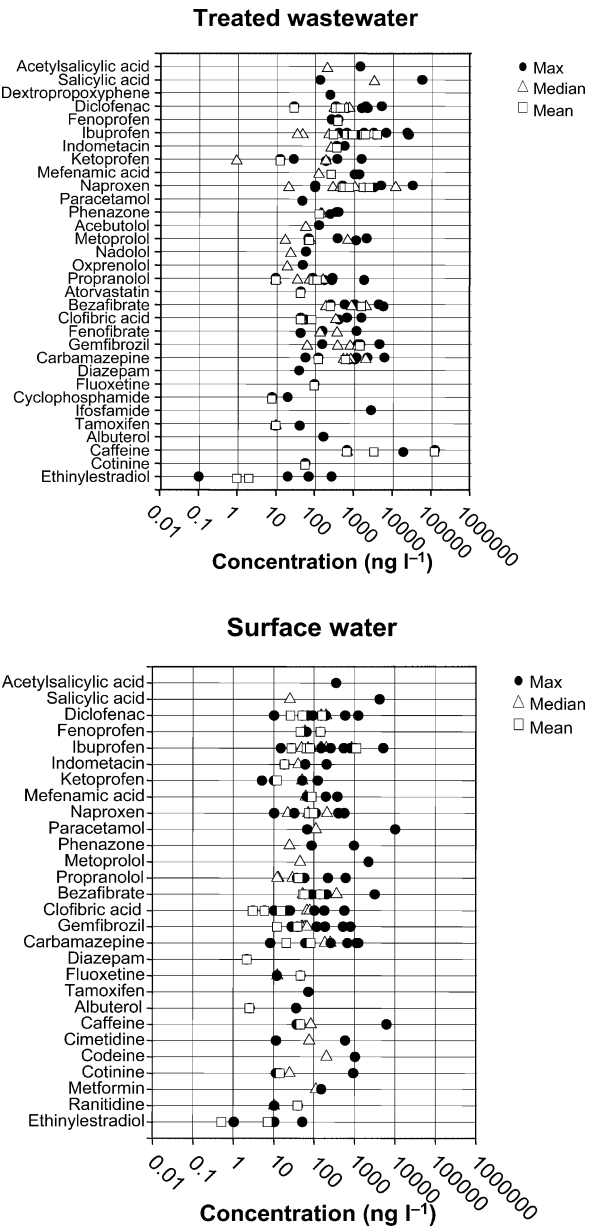

Fig. 12.1. Ranges of concentrations of pharmaceutical residues reported in treated wastewaters (*above*) and surface waters (*below*)

in seawater (Thomas and Hilton 2004; Weigel et al. 2004). In the UK, the β-blocker propranolol (median level 76 ng l^{-1}) was usually found in STP effluents, whereas diclofenac (median 424 ng l^{-1}) was found in 86%, ibuprofen (median 3 086 ng l^{-1}) in 84%, mefenamic acid (median 133 ng l^{-1}) in 81%, dextropropoxyphene (median 195 ng l^{-1}) in 74%, and trimethoprim (median 70 ng l^{-1}) in 65% of the samples (Ashton

et al. 2004). Synthetic steroidal hormones such as 17α-ethinylestradiol (EE2) have been reported in Europe, Canada, the USA, Japan, Brazil and many other countries. Maximal and median EE2 concentrations in STP effluents were as high as 831 and 73 ng l^{-1} in the USA, respectively (Kolpin et al. 2002), but in general, concentrations are typically at 0.5–1 ng l^{-1} (Baronti et al. 2000). These concentrations must be regarded in the context of their high biological activity, accounting for potential estrogenic effects in fish (Stumpf et al. 1999; Desbrow et al. 1998).

Distribution of pharmaceuticals in the environment will occur primarily through aqueous transport, but also via transfer to soil in the agricultural context (manure), and to some extent via bioaccumulation. In wastewater treatment, two elimination processes are of importance: adsorption to suspended solids (sewage sludge) and biodegradation, which mainly takes place in the aerobic treatment. Sorption of acidic pharmaceuticals such as the nonsteroidal anti-inflammatory drugs (NSAIDs) to sludge doesn't seem very important (Kümmerer et al. 1997; Buser et al. 1998; Ternes et al. 2004); however, basic pharmaceuticals and zwitterions can significantly adsorb to sludge (Golet et al. 2002) and be transferred to the terrestrial environment via sludge application. For pharmaceuticals occurring mainly in the dissolved phase, biodegradation is the most important elimination process. Elimination rates during treatment vary according to construction and treatment technology, hydraulic retention time, season, and performance of the STP. Some studies (Ternes 1998b; Stumpf et al. 1999; Carballa et al. 2004) indicate elimination efficiencies to span a large range from 7–8% for carbamazepine (Ternes 1998b; Heberer 2002; Clara et al. 2004) up to 99% for salicylic acid (Ternes 1998b; Ternes et al. 1999; Heberer 2002). In surface waters, biodegradation is important as well as photolysis (Buser et al. 1998) (Andreozzi et al. 2003).

There is no information about the bioaccumulation potential of pharmaceuticals in biota or food webs with the exception of diclofenac accumulating in the prey of vultures (Oaks et al. 2004) and in fish where experimental bioconcentration factors of 10–2 700 were determined (Schwaiger et al. 2004). However, residues of several pharmaceuticals (e.g., fluoxetine) were detected in fish (Brooks et al. 2005), and the lipid-lowering drug gemfibrozil occurred at 113-times higher levels in blood as compared to water (Mimeault et al. 2005). It should be borne in mind, however, that the bioavailable concentration of a pharmaceutical is the important determinant for toxicity, not the concentration per se (Fent 2003).

Only in the last few years have regulatory agencies issued guidelines on how pharmaceuticals should be assessed for possible environmental effects. The European Commission released a guideline (Directive 2001/83/EC) specifying that an authorization for a medicinal product for human use must be accompanied by an environmental risk assessment. In 2006, a guideline was released by the European Medicines Agency (EMEA 2006) for the assessment of potential environmental risks. It recommends a step-wise procedure using mainly acute toxicity tests for effect assessments. Among them are OECD tests such as growth inhibition in algae, *Daphnia* reproduction and fish early life stage toxicity. In an extended phase, a terrestrial plant growth test, earthworm acute toxicity and Collembola reproduction test should be conducted. The adoption of this guideline is an important step forward.

According to the U.S. FDA guidelines for the assessments of human drugs, applicants are required to provide an environmental assessment report when the expected introduction concentration of the active ingredient in the aquatic environment is

$\geq 1\ \mu g\ l^{-1}$ (FDA-CDER 1998), which corresponds to about 40 tons as a trigger level. Environmental assessment of veterinary pharmaceuticals has been required by the U.S. FDA since 1980 (Boxall et al. 2003).

In contrast to the knowledge about environmental concentrations, only little is known about ecotoxicological effects of pharmaceuticals on aquatic and terrestrial organisms, both in laboratory experiments and in the ecosystem itself. Standard acute ecotoxicity data exist for a number of pharmaceuticals; however, such data alone are not sufficient for a comprehensive hazard and risk assessment (Fent 2003; Fent et al. 2006b). Being exposed over their whole life at contaminated sites, aquatic organisms are particularly important targets, and the current lack of knowledge on chronic effects hampers ecotoxicological risk assessment.

In this chapter, the present knowledge about acute and chronic effects of human pharmaceuticals (some of which are also applied in veterinary medicine) on aquatic organisms is critically reviewed. The focus lies on environmentally relevant pharmaceuticals as reflected by consumption volumes, toxicity and persistence in the environment. The effects of steroidal hormones are discussed only briefly, as other reviews have dealt with them (Damstra et al. 2002). Special veterinary pharmaceuticals (Montforts et al. 1999; Boxall et al. 2003) and antibiotics are addressed elsewhere (Halling-Sørensen et al. 1998; Daughton and Ternes 1999; Hirsch et al. 1999).

12.2 Modes of Actions in Humans and Mammals and Targets in Lower Vertebrates and Invertebrates

For understanding the effects of pharmaceuticals, it is important to know their modes of action and to know whether similar targets exist in lower vertebrates and invertebrates. Knowledge about evolutionary similarities of receptors and molecular targets of pharmaceuticals exists primarily for fish, but only little is known in invertebrates. Moreover, known side effects in humans may give hints to possible adverse effects in lower animals. In the following, some pharmaceutical classes are regarded.

NSAIDs act by inhibiting either reversibly or irreversibly one or both of the two isoforms of the cyclooxygenase enzyme (COX-1 and COX-2), which catalyze the synthesis of different prostaglandins from arachidonic acid (Vane and Botting 1998). Classical NSAID inhibit both COX-1 and COX-2 at different degrees, whereas new NSAID act more selectively on COX-2; the inducible form is responsible for the inflammatory reactions. Differences in binding site size are responsible for the selectivity of these drugs (Kurumbail et al. 1997; Penning et al. 1997; Gierse et al. 1999). NSAID are commonly used to treat inflammation and pain and to relieve fever, and sometimes they are used for long-term treatment of rheumatic diseases.

Prostaglandins play a variety of physiological roles according to their cells' source and target molecules, and they are involved in inflammation and pain, regulation of blood flow in kidney, coagulation processes and synthesis of protective gastric mucosa. Since NSAIDs non-specifically inhibit prostaglandin synthesis, most side effects (at least after long-term treatment) are related to the physiological function of prostaglandins. Renal damage and renal failure after chronic NSAID treatment seems to be triggered by the lack of prostaglandins in vasodilatation-induction. Gastric damages are thought to be caused by inhibition of both COX isoforms (Wallace 1997;

Wallace et al. 2000). In contrast, liver damages are apparently due to the formation of reactive metabolites (e.g., acyl glucuronides) rather than inhibition of prostaglandin synthesis (Bjorkman 1998).

In fish, an inducible COX-2 homologue has been found to be expressed in macrophages in rainbow trout (*Oncorhynchus mykiss*). The translation product of the COX gene has a high homology of 83–84% and 77% to its human counterpart COX-2 and COX-1, respectively (Zou et al. 1999). Macrophages express a COX enzyme, which is an equivalent to mammalian COX-2 (Zou et al. 1999). A COX-1 and COX-2 homologue was cloned from brook trout ovary (Roberts et al. 2000), and recently, a shark COX was cloned showing 68% and 64% homology to mammalian COX-1 and COX-2, respectively (Yang and Carlson 2004). Prostaglandins are formed by vertebrates and invertebrates, but in corals, their synthesis is independent of COX (Song and Brash 1991). In arthropods and mollusks, COX-like activity is apparently responsible for the formation of prostaglandins, but these enzymes have not been characterized (Pedibhotla et al. 1995). In birds, prostaglandins play a role in the biosynthesis of egg shells and treatment with the COX-inhibitor indometacine resulted in egg shell thinning (Lundholm 1997). Diclofenac was shown to hinder the stimulation of prostaglandin E2 synthesis in head kidney in vitro, an indication of COX inhibition (Hoeger et al. 2005).

β-blockers act by competitive inhibiting *β*-adrenergic receptors and they are used in the treatment of high blood pressure (hypertension), and to treat patients after heart attack to prevent further attacks. The adrenergic system is involved in many physiological functions such as regulation of heartbeats and oxygen supply to the heart,, vasodilatation mechanisms of blood vessels, and bronchodilation. Furthermore, the adrenergic system is also known to interact with carbohydrate and lipid metabolisms, mainly in response to stress such as starvation (Jacob et al. 1998).

β-adrenoceptors are 7-transmembrane receptor proteins coupled with different G-proteins that ultimately enhance the synthesis of the second messenger signaling molecule cAMP (Rang et al. 2003). According to medical needs, *β*-blockers may selectively inhibit one or more *β*-receptors types. Side effects of this therapeutic class are mainly bronchoconstriction and disturbed peripheral circulations (Hoffman and Lefkowitz 1998; Scholze 1999). Due to their lipophilicity, they are supposed to pass the blood-brain barrier and act in the central nervous system (Soyka 1984; Soyka 1985).

β-adrenoceptors were found in fish (*O. mykiss*) liver and red and white muscle with a high degree of sequence conservation with other vertebrate homologues. They are also supposed to play similar roles in humans (Nickerson et al. 2001). The presence of a β_2-adrenoceptor subtype was also suggested in liver membranes of other fish and amphibians. β_2-adrenoceptors of rainbow trout (Nickerson et al. 2001) show a high degree of amino-acid sequence conservation with other vertebrate β_2-adrenoceptors. The β_2-adrenoceptor gene is highly expressed in the liver, as well as red and white muscle, with lower expression in gills, heart, kidney and spleen (Nickerson et al. 2001).

Whereas mammals have three α_2-adrenoceptors, five distinct α_2-adrenoceptor genes are expressed in zebrafish (Ruuskanen et al. 2005). Localization of the α-adrenoceptors in zebrafish shows marked conservation when compared with mammals, and the α_2-adrenergic system is functional in zebrafish.

Blood lipid lowering agents. There are basically two types of anti-lipidemic drugs, namely statins and fibrates. Both are used to decrease the concentration of cholesterol, and fibrates also decrease the concentration of triglycerides in the blood plasma. Statins

as inhibitors of cholesterol synthesis act by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is responsible for the limiting step in the cholesterol synthesis, namely the conversion of HMG-CoA to mevalonate (Laufs and Liao 1998). As a consequence of the intracellular cholesterol depletion, the expression of LDL receptors in hepatocyte membranes is increased and therefore, the resorption of LDL-cholesterol from blood plasma. Due to interactions of statins with mevalonate metabolism, multiple additional effects occur (anti-inflammatory, antioxidative).

There is evidence that statins do not only affect mammals, but also interfere with juvenile hormone synthesis in insects (Debernard et al. 1994) and adversely affect algae and plants. It is interesting that the herbicidal effect of statins is based on the same mode of action, the inhibition of the enzyme HMG-CoA reductase, which in plants, regulates cytosolic isoprenoid biosynthesis, and in turn leads to phytotoxicity. The EC_{10} value for atorvastatin and lovastatin in duckweed was 26 and 33 $\mu g\, l^{-1}$ (Brain et al. 2006).

In contrast, effects of fibrates are mediated, at least in part, through alterations in transcription of genes encoding for proteins controlling lipoprotein metabolism. Fibrates probably act by activating the lipoprotein lipase enzyme, which is mainly responsible for the conversion of very low density lipoprotein (VLDL) to high density lipoproteins (HDL), therefore decreasing plasma triglycerides concentration (Staels et al. 1998). Binding of fibrates to peroxisome proliferator-activated receptors (PPARs), nuclear hormone receptors known to be activated during different cellular pathways, stimulates the expression of several lipid regulatory proteins such as, for example, the lipoprotein lipase (Staels et al. 1998). To date, three subtypes of PPAR have been described, and they are involved in a number of different processes by modulation of target gene expression in response to endogenous and exogenous ligands. Activators of PPARα include a variety of endogenously present fatty acids, leukotrienes and hydroxyeicosatetraenoic acids and drugs, such as fibrates (Cajaraville et al. 2003), phthalate monoesters, metabolites of trichloroethylene, perfluorooctanoic acid and perfluoroctansulfonic acid. PPARβ activators include fatty acids, prostaglandin A_2 and prostacyclin. PPARγ is the most selective receptor, and prostaglandin J_2 has been described to be a specific ligand (Ibabe et al. 2005). PPARγ activators also include the anti-diabetic thiazolidinediones (Peraza et al. 2006). In isolated zebrafish hepatocytes, mRNA of both PPARα and PPARγ was induced by clofibrate at 0.5–2 mM, but to a low extent (Ibabe et al. 2005).

Heterodimerization of PPARs with the retinoid X receptor and their binding to response elements in the promoter regions of genes leads to their activation. Numerous PPAR target genes have been identified in the last fifteen years (Desvergne and Wahli 1999), and they play an important role for a variety of biological processes. PPARα is involved in peroxisome proliferation and plays a pivotal role in controlling hepatic lipid metabolism (Schoonjans et al. 1996), whereas PPARβ has diverse roles in basic lipid metabolism, and PPARγ plays a key role in the differentiation of adipocytes (Kersten et al. 2000).

Fibrates stimulate cellular fatty acid uptake, conversion to acetyl-CoA derivatives, and catabolism by the beta-oxidation pathways, which (combined with a reduction in fatty acid and triglyceride synthesis) results in a decrease in VLDL production (Staels

et al. 1998). Hepatic damage may occur after chronic exposure to fibrates in rats (Qu et al. 2001). In rodents, fibrates lead to a massive proliferation of peroxisomes (Hess et al. 1965). Strong correlation between exposure to fibrates and hepatocarcinogenicity in rodents was found, while this was not observed in humans. These findings increase the interest for ecotoxicological impact of this therapeutic class of drugs (Cajaraville et al. 2003).

PPAR genes have been found in fish such as plaice (Leaver et al. 1998), Atlantic salmon (Ruyter et al. 1997) and zebrafish (Ibabe et al. 2002). Fish PPARs display an amino acid sequence identity of 43–48% to the human and amphibian PPARγ (Andersen et al. 2000). All PPAR forms have been found in zebrafish. PPARα was mainly expressed in hepatocytes and tissues that catabolize high amounts of fatty acids (Ibabe et al. 2002). Furthermore, PPAR$\gamma$was shown to be induced in response to clofibrate and bezafibrate in salmon hepatocytes (Ruyter et al. 1997), although it was less responsive than PPAR$\gamma$of rodents (Andersen et al. 2000). All three PPAR receptors are already expressed in the larval stage, with a similar tissue distribution pattern to that in adult zebrafish (Ibabe et al. 2005). The physiological and toxicological roles of PPARs in fish have yet to be investigated, and their involvement in potential effects of lipid lowering drugs is not yet known. With regard to invertebrates, no information is currently available on the existence of PPARs, although investigations were performed in cnidarians and platyhelminths (Escriva et al. 1997).

Antiepileptic drugs act on the CNS by decreasing the overall neuronal activity. This can be achieved either by blocking voltage-dependent sodium channels of excitatory neurons (e.g., carbamazepine), or by enhancing inhibitory effects of the GABA neurotransmitter by binding on a specific site in the gamma subunit of the corresponding receptor (e.g., diazepam, a member of benzodiazepine family) (Study and Barker 1981; MacDonald and Olsen 1994; Rogers et al. 1994). Evidence of the occurrence of the GABA system in fish *O. mykiss* (Cole et al. 1984; Meissl and Ekstrom 1991) was found.

Fluoxetine is a widely used *antidepressant*, which acts by inhibiting the reuptake of serotonin. A pump directs serotonin from the synapse space back to the presynapse, and serotonin reuptake inhibitors (SSRI) inhibit this pump, thus increasing the serotonin level in the synapse space. Serotonin as a neurotransmitter occurs in lower vertebrates and invertebrates (Fong 1998); however, the effects associated with this transmitter are different, and possibly so are the effects of SSRI. Serotonin mediates, among others, endocrine functions in aquatic organisms such as fingernail clams, *Sphaerium striatinum*, (Fong et al. 1998), and Japanese medaka, *Oryzias latipes*, (Fong et al. 1998; Foran et al. 2004). Fluoxetine and sertraline and the SSRI metabolites norfluoxetine and desmethylsertraline have been detected in fish sampled from the wild in the U.S. (Brooks et al. 2005).

Another important class are *cytostatic* cancer therapeutics interacting with cell proliferation by different modes of actions. Methotrexate acts as a potent inhibitor of the folate dehydroreductase enzyme, which is responsible for the purine and pyrimidine synthesis (Schalhorn 1995; Rang et al. 2003). Doxorubicin is an intercalating substance inducing DNA-strand brakes. The anti-estrogenic tamoxifen acts by competitive inhibition of the estrogen receptor (Rang et al. 2003).

12.3 Ecotoxicological Effects

Pharmaceuticals are designed to target rather specific metabolic and molecular pathways in humans, but often they also have important side effects, too. When introduced into the environment, they may affect the same pathways in animals having identical or similar targets, or alternatively, dissimilar modes of actions leading to unexpected effects. For many drugs, specific modes of actions are not well understood and often, different mechanisms occur. This makes specific toxicity analysis, particularly in lower animals, difficult to perform. Currently, there is a need for more specific toxicological investigations focusing on specific targets of the pharmaceutical in lower vertebrates and invertebrates. This is based on the hypothesis that the modes of action are similar as in humans. Unfortunately, current toxicity testing is not designed along these lines, rather general and established test systems and traditional organisms according to guidelines are being used and traditional endpoints such as mortality assessed.

Thus far, ecotoxicity testing merely provides data on acute effects in organisms of different trophic levels after short-term exposure. Aquatic organisms are often exposed over their entire life cycle to these compounds, but this is very rarely investigated. Chronic toxicity is only marginally known, and therefore, ecological risk assessment is mainly or solely based on acute toxicity with its inherent limitations. Beyond laboratory investigations, some mathematical models have been developed to estimate ecotoxicological effects. The most often applied program is the ECOSAR (*http://www.epa.gov/oppt/newchems/sarman.pdf*) (Sanderson et al. 2004b), based on the quantitative structure activity relationship (QSAR). Despite considerable drawbacks (e.g., inadequate structure coverage for pharmaceuticals), the program has been repeatedly applied to estimate pharmaceutical baseline toxicities (Jones et al. 2002; Sanderson et al. 2004b; Cleuvers 2005), but it cannot replace experimental data.

The influence of environmental parameters such as pH on the bioavailability and toxicity has only rarely been investigated. This is important for acidic or basic pharmaceuticals, where the speciation influences toxicity. Moreover, effects of drug metabolites have only rarely been regarded. As an example, phototransformation products of naproxen showed higher toxicities than the parent compound (Isidori et al. 2005). In the following, the current ecotoxicological data are summarized and subsequently related to environmental levels in order to assess the potential hazard for the different classes of pharmaceuticals.

12.3.1 Acute Effects

In 2004, a high death rate among three species of vultures in India and Pakistan leading to severe population declines was reported to be caused by diclofenac (Oaks et al. 2004). High mortality is associated with renal failure and visceral gout in exposed vultures as well as the accumulation of uric acid throughout the body cavity following kidney malfunction. A direct correlation between residues of diclofenac and renal failure exists, both by experimental oral exposure and through feeding vultures diclofenac-treated livestock (Oaks et al. 2004). The drug has come into widespread use in these countries as a veterinary medicine, but it has also been widely used in human

medicine since the 1970s. Vultures are natural scavengers feeding on carrion of wildlife and domestic livestock and cattle contaminated with diclofenac. The three vulture species continue to decline in Pakistan, India, Bangladesh and southern Nepal. In 2006, India banned diclofenac for non-human use. Apart from this severe case, which had never been anticipated, potential ecotoxicological effects of drug residues in the environment on wildlife are largely unknown.

Pharmaceuticals are assessed for their acute toxicity by means of traditional standard tests according to established guidelines (e.g., OECD, U.S. EPA, ISO), using established laboratory organisms such as algae, zooplankton and other invertebrates, and fish. Acute toxicity data of pharmaceuticals are compiled by Halling-Sørensen et al. (1998), Webb (2004) and Fent et al. (2006b). The data given in the following cover different classes of pharmaceuticals and originate from different sources including Webb (2004). Studies were performed under different quality criteria (i.e., nominal versus measured exposure concentrations), making comparisons difficult, however.

Analgesics and NSAID. In general, toxicity data vary for each pharmaceutical. Diclofenac seems to be the compound having the highest acute toxicity within the class of NSAID, since for all the tests the effect concentrations were below 100 mg l^{-1} (Fig. 12.2). Short-term acute toxicity was analyzed in algae and invertebrates (Webb 2001; Cleuvers 2003; Webb 2004); phytoplankton was more sensitive (lowest EC_{50}(96h) = 14.5 mg l^{-1}, Ferrari et al. (2004)) than zooplankton (lowest EC_{50}(96h) = 22.43 mg l^{-1}, Ferrari et al. (2004)).

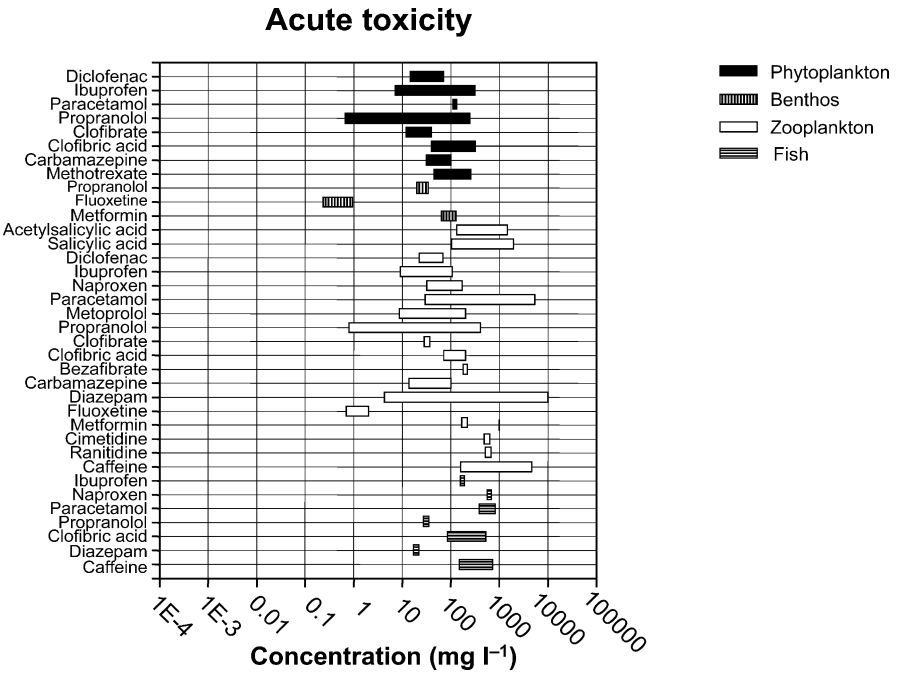

Fig. 12.2. Acute toxicity of human pharmaceuticals in different aquatic organisms of different trophic levels. Given are ranges of toxicity data from different organisms and/or experiments and they span up to several orders of magnitude for a specific pharmaceutical

In general, not much is known about the acute toxicity to fish. Acute toxicity data of naproxen in different organisms vary from 12.3 mg l^{-1} (cyanobacteria) to 690 mg l^{-1} (*O. mykiss*) (Straub and Stewart 2008). Recently, salicylate, ibuprofen and acetaminophen were found to inhibit interrenal corticosteroidogenesis in rainbow trout in vitro. Salicylate at 100 mg kg^{-1} led to depression of mRNA levels of key proteins involved in corticosteroidogenesis of cortisol in the interrenal tissue, such as the steroidogenic acute regulatory protein, peripheral-type benzodiazepine receptor, cytochrome P450 cholesterol side-chain cleavage enzyme and 11β-hydroxylase (Gravel and Vijayan 2006). The in vitro depression of the steroidogenic cells to synthesize cortisol has also been found in vivo. The disruption of steroidogenesis in fish interrenal tissue, an organ homologous to the adrenal gland of mammals, seems relevant.

β-blockers. As shown in Fig. 12.2, the acute toxicity of β-blockers is not extensively studied, with the exception of propranolol. This compound shows highest acute toxicity and highest logK_{ow} as compared to other β-blockers (Fig. 12.2). This and the fact that it is a strong membrane stabilizer, whereas other investigated β-blockers are not, may in part explain its higher toxicity (Doggrell 1990; Huggett et al. 2002). Comparison of toxicity is difficult in this case, since other β-blockers, except metoprolol, were only analyzed in *D. magna* (Hernando et al. 2004). Metoprolol and verapamil caused the acceleration of heart rate at a low concentration, but lowered it at high concentrations in *D. magna* (Villegas-Navarro et al. 2003). It should be noted that such alterations may have consequences in the ecological context, but this is unknown, unless predator-prey relationships are regarded or model ecosystem experiments performed. For propranolol it seems that phyto- and zooplankton are more sensitive than fish. *Ceriodaphnia dubia* (EC_{50}(48h) = 0.8 mg l^{-1}, Ferrari et al. (2004)) displayed higher sensitivity than *D. magna* (EC_{50}(48h) = 1.6 mg l^{-1}, Huggett et al. (2002)) or other zooplankton organisms. Within phytoplankton, the microorganism *Synechococcus leopolensis* reacted most sensitively (EC_{50}(96h) = 0.668 mg l^{-1}, Ferrari et al. 2004)).

Blood lipid lowering agents. The acute toxicity of lipid lowering agents is not extensively studied. Clofibrate showed LC_{50} values in the range of 7.7 to 39.7 mg l^{-1} and clofibric acid at 87.22–526.5 mg l^{-1} in different organisms. The fish *Gambusia holbrooki* (LC_{50}(96h) = 7.7 mg l^{-1}, Nunes et al. (2004)) seems to be the organism most sensitive to acute clofibrate concentrations that has been studied so far. The known rodent peroxisome proliferator gemfibrozil injected into rainbow trout led to significant increases in fatty acyl-CoA oxidase (FOA) activity at doses of 46–152 mg kg^{-1} day^{-1} (Scarano et al. 1994), and in *D. magna*, the LC_{50} was 100 mg l^{-1}. Significant dose-related increases in peroxisomal FOA were observed after exposure of rainbow trout primary hepatocytes to clofibric acid and ciprofibrate, but not to gemfibrocil (Donohue et al. 1993). The in vitro activity is weak, however. Statins inhibit the enzyme HMG-CoA reductase in mammals. Interestingly, this also occurs in plants, where it regulates cytosolic isoprenoid biosynthesis. Atorvastatin and lovastatin had herbicidal activity in duckweed (*Lemna gibba*) with EC_{10} values of 26 and 33 μg l^{-1}, respectively (Brain et al. 2006).

Antidepressants and antiepileptics. The serotonin reuptake inhibitor fluoxetine is – besides antibiotics – apparently among the most acutely toxic human pharmaceuticals reported so far with acute toxicity ranging from EC_{50}(48h, alga) = 0.024 mg l^{-1} (Brooks et al. 2003) to LC_{50}(48h) = 2 mg l^{-1} (Kümmerer 2004). For benthic organisms, acute toxicity is in the range of 15–43 mg kg^{-1} sediment (*Chironomus tentans*

LC_{50}(10d) = 15.2 mg kg^{-1}, *Hyalella azteca* LC_{50}(10d) = 43 mg kg^{-1}, Brooks et al. (2003)). Fluoxetine seems to affect phytoplankton stronger than other aquatic organisms.

Diazepam and carbamazepine, both antiepileptics, can be classified as potentially harmful to aquatic organisms, because most of the acute toxicity data are below 100 mg l^{-1}. For both compounds it seems that *D. magna* is affected more than other species, but the reasons for the higher susceptibility is not known. Acute toxicity of carbamazepine was found at 17.2 mg l^{-1} in *Daphnia* and at 34.4 mg l^{-1} in midges, but growth was inhibited at 12.7 mg l^{-1} in *Daphnia* and at 9.2 mg l^{-1} in midges (Thaker 2005).

Cytostatic compounds and cancer therapeutics. Acute toxicity of methotrexate on a highly proliferative species, namely the ciliate *Tetrahymena pyriformis*, indicated acute effects (EC_{50}(48h) = 45 mg l^{-1}, Henschel et al. (1997)). Teratogenicity in fish embryos was observed at even higher concentrations (EC_{50}(48h) = 85 mg l^{-1}, Henschel et al. (1997)).

The acute toxicity data summarized in Fig. 12.2 show that 38% of the pharmaceuticals such as acetylsalicylic acid, betaxolol, sotalol, bezafibrate, gemfibrozil, cimetidine and ranitidine displayed LC_{50} values higher than 100 mg l^{-1}, which, according to EU-Directive 93/67/EEC, are classified as not being harmful for aquatic organisms. 17% of the pharmaceuticals displayed an acute toxicity below 100 mg l^{-1}, and for fluoxetine, all toxicity values were below 10 mg l^{-1}. The vast majority of pharmaceuticals have EC_{50} values of >1 mg l^{-1}. The other 45% of pharmaceuticals displayed a considerable variability of acute toxicity values, spreading over a wide range, thus making a classification difficult. When antibiotics are also included, there are only about 1% of the compounds having lowest acute EC_{50} values of <0.01 mg l^{-1} (Cunningham et al. 2006).

The variability of data both within the same and between different species is obvious. Different actual exposure concentrations (only nominal concentrations were used), different sensitivities of clones and laboratory performances are among the reasons for variability within the same species (for example, toxicity of clofibric acid in *D. magna* varies between 72 and 200 mg l^{-1}; the LC_{50}(48h) of acetyl salicylic acid varies between 168 mg l^{-1} (Calleja et al. 1994) and 1 468 mg l^{-1} (Lilius et al. 1994); the LC_{50}(24h) of diazepam varies between 9.6 mg l^{-1} (Calleja et al. 1993) and 10 000 mg l^{-1} (Calleja et al. 1994). Depending on the quantity and quality of data, ranges of acute toxicity values span one to two orders of magnitude; in some cases such as propranolol, the species differences are quite large, spanning three to four orders of magnitude. When different categories are compared, a tendency of lower LC_{50} (EC_{50}) values is found for β-blockers and neuroactive drugs as compared to anti-inflammatory drugs or various other compounds.

Often, acute toxicity is related to non-specific mode of actions and not to mechanisms involving specific targets. The compounds are thought to interact with cellular membranes leading to unspecific membrane toxicity (Caminada et al. 2006). This general mechanism may be only one possibility; additional ones (e.g., oxidative stress) come into play with particular pharmaceuticals. We evaluated whether the acute toxicity data of the different classes of pharmaceuticals correlate with the $\log K_{ow}$ of the compound. Lipophilicity determined by $\log K_{ow}$ is an important parameter for membrane toxicity. However, no correlation was found between acute toxicity either of a certain species, a group of organisms, or all of them (Fent et al. 2006b). This is prob-

ably based on laboratory differences, nominal concentration differences, clone susceptibility differences, but also on the fact that logK_{ow} may not be the best model for lipophilicity, which particularly holds true for ionizable compounds with pH-dependent speciation (Fent 2007). In contrast, a good correlation was found between the cytotoxicity of pharmaceuticals and their log$D$ in fish cells under controlled conditions (Caminada et al. 2006).

In conclusion, acute toxicity to aquatic organisms is unlikely to occur at measured environmental concentrations, as acute effects concentrations are 100 to 1 000 times higher than residues found in the aquatic environment. For example, the lowest acute effect concentration of fluoxetine was 20 μg l^{-1}, whereas the highest estimated environmental concentration was 0.01 μg l^{-1}, and the lowest acute effect of salicylic acid was 37 mg l^{-1}, whereas the highest environmental concentration was ~60 μg l^{-1}. Therefore, acute toxicity of an individual compound seems only relevant in case of spills.

12.3.2 In Vitro Studies

Pharmaceuticals have also been investigated in in vitro systems such as fish cell lines, which are suited for the first screening of the acute toxicity of pharmaceuticals. Hepatoma cells derived from topminnow (PLHC-1) and rainbow trout gonadal cells (RTG-2) (Caminada et al. 2006), fibroblasts derived from bluegill sunfish (BF-2) (Henschel et al. 1997), and primary fish hepatocytes (Laville et al. 2004) were used. In a series of compounds fenofibrate (EC_{50}(24h) = 3.25 mg l^{-1}) and clofibrate (EC_{50}(24h) = 0.46 mg l^{-1}) were the most active compounds (Laville et al. 2004). In PLHC-1 and RTG-2 cells, cytotoxicity was found in twenty-one out of thrity-four common pharmaceuticals from different classes with EC_{50} values ranging from 2.1 μM (1.14 mg l^{-1}) for doxorubicin to 8.66 mM (1 200 mg l^{-1}) for salicylic acid (Caminada et al. 2006). We found that the PLHC-1 cells were slightly more sensitive than RTG-2 cells or trout hepatocytes (Laville et al. 2004). The cytotoxicity showed a correlation with in vivo data in *Daphnia*, but not in fish due to insufficient and heterogeneous data (Caminada et al. 2006). Three out of four compounds showed higher toxicity in vitro as compared to in vivo in ciliates, and methotrexate (EC_{50}(48h) = 3 mg l^{-1}) may negatively interact with cell proliferation and therefore survival (Henschel et al. 1997). Sensitivity of cell lines in culture to toxicants may vary within species (Rau et al. 2004). Some of the differences may be based on the difference in the ability of the cells to metabolize toxicants.

The potential for screening and first evaluation of potential toxicity is an advantage of in vitro systems (cell cultures or reporter gene systems) (Fent 2001). Cytotoxicity and oxidative effects (formation of lipid peroxidation) of pharmaceuticals were tested in rainbow trout primary hepatocytes (Gagne et al. 2006). Most compounds including carbamazepine increased lipid peroxidation, and many decreased cell viability and increased CYP3A-related activity. Surprisingly, six out of thirty-seven pharmaceuticals belonging to different classes and found in the aquatic environment are estrogenic in vitro (Fent et al. 2006a). Tamoxifen, furosemide, paracetamol, phenazone, fenofibrate and cimetidine exhibited weak estrogenicity. Furosemide showed full activity, whereas the other compounds had low efficacy. The estrogenic activity of binary mixtures of furosemide and phenazone with estradiol followed the model of

concentration addition; mixtures of other pharmaceuticals often deviated from this model and indicated synergistic interactions.

12.3.3 Chronic Effects

At sewage-influenced and other contaminated sites, aquatic species are continuously exposed over long periods of time, or even their entire life. However, data on chronic toxicity are scarce, and where available, only marginal, because important key targets are not regarded. Toxicity experiments are usually performed according to established guidelines. Targeted investigations, or studies over different life stages, are lacking or have only rarely been performed. Life cycle analyses are reported only for EE2 (Länge et al. 2001; Parrott and Blunt 2005), and the toxicity to benthic and soil organisms has only rarely been evaluated. In hazard and risk assessment, the ratio between acute and chronic toxicity is often taken as a parameter for risk evaluation. However, chronic toxicity cannot be derived from acute toxicity by simple calculations. In this chapter and in Fig. 12.3, chronic data are summarized.

The best knowledge exists for the synthetic steroid EE2 contained in contraceptive pills. It exhibits estrogenicity and adversely inhibits fertility and reproduction at ex-

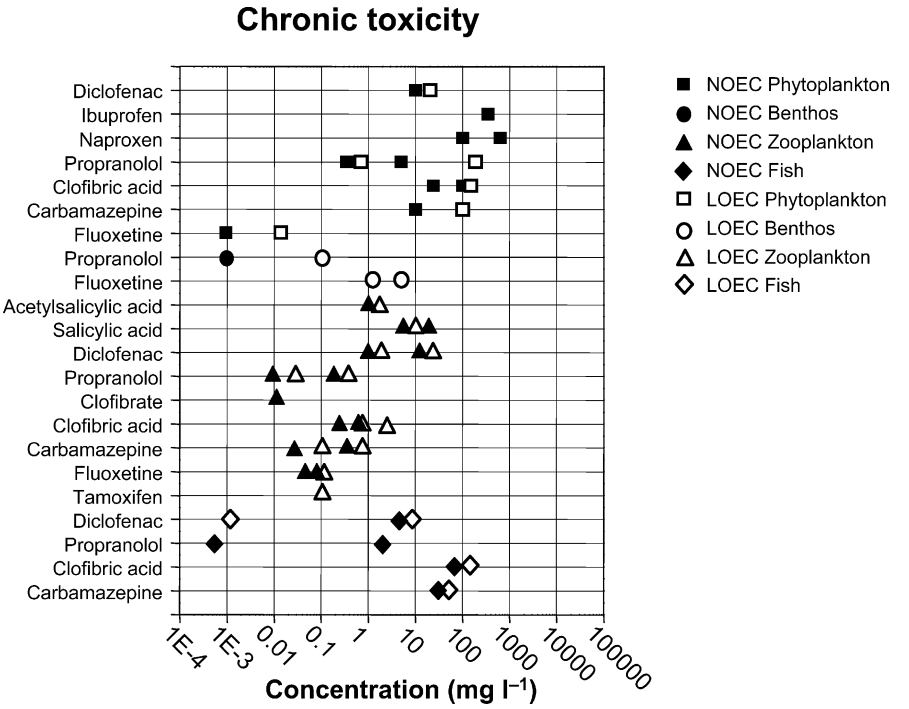

Fig. 12.3. Chronic toxicity of pharmaceuticals in different aquatic organisms of different trophic levels. Shown are lowest observed effect concentrations (LOEC) and no observed effect concentrations (NOEC). Each data point refers to one species and/or experiment

tremely low and environmentally relevant concentrations in fish (Länge et al. 2001). The NOEC values were ≥ 1 ng l^{-1} in the F_0 generation, and F_1 embryo hatching success and larval survival. Male fish exposed to EE2 at 4 ng l^{-1} failed to develop normal secondary sexual characteristics, the sex ratio was altered, and no testicular tissue was observed in any fish. A recent study shows vitellogenin (VTG) induction in fathead minnows with an EC_{50} value as low as 1 ng l^{-1}. EE2 was 25–30 times more potent than estradiol (Brian et al. 2005), confirming previous reports on VTG induction at concentrations between 0.1 and 1 ng l^{-1} (Pawlowski et al. 2004). Further, decreased egg fertilization and sex ratio changes (skewed toward females) were observed, both of which were at extremely low concentrations of 0.3 ng l^{-1} EE2 (Parrott and Blunt 2005). The next most sensitive parameter was demasculinization (decreased male secondary sex characteristic index) of males exposed to 1 ng l^{-1}. Full life cycle exposure of zebrafish to 3 ng l^{-1} EE2 lead to elevation of VTG and caused gonadal feminization in all exposed fish, and thus inhibited reproduction (Fenske et al. 2005). Life-long exposure of zebrafish to 5 ng l^{-1} in the F_1 generation caused a 56% reduction in fecundity and complete population failure with no fertilization. Infertility in the F_1 generation was due to disturbed sexual differentiation with males having no functional testes and intersex gonads (Nash et al. 2004).

Among other pharmaceuticals, most data exist on *NSAIDs.* Acetyl salicylic acid affected reproduction in *D. magna* and *D. longispina* at 1.8 mg l^{-1} (Marques et al. 2004). Diclofenac, commonly found in wastewater at about 0.81 µg l^{-1} (Ternes 1998b), reaches maximal levels in wastewater and surface water of up to 2 µg l^{-1} (Stumpf et al. 1996; Ternes 1998b; Schwaiger et al. 2004). Chronic toxicity of diclofenac was reported in invertebrates (Ferrari et al. 2003; Ferrari et al. 2004). Histopathological effects were observed in rainbow trout; at the LOEC of 5 µg l^{-1} renal lesions (degeneration of tubular epithelia, interstitial nephritis) and alterations of the gills (Schwaiger et al. 2004) occurred. Subtle subcellular effects were observed even at 1 µg l^{-1} (Triebskorn et al. 2004). Impairment of renal and gill functions occurred after long-term exposure. The kidney was also the target in vultures; acute renal failure was the reason for the visceral gout (Oaks et al. 2004), as well as the occurrence of extensive deposits of uric acid on and within internal organs (Gilbert et al. 2002). In zebrafish embryos, delayed hatching occurred at 1 and 2 mg l^{-1} (Hallare et al. 2004). Additional side effects of diclofenac have been observed in humans in the liver with degenerative and inflammatory alterations (Banks et al. 1995), in the lower gastrointestinal tract and in the esophagus (Bjorkman 1998), but not in fish. Brown trout showed a decrease of hematocrit levels at 0.5–50 µg l^{-1}, and signs of inflammation in gills and trunk kidney were observed after twenty-one days at 50 µg l^{-1}, and in liver at 5 µg l^{-1}, showing no dose-response relationships, however (Hoeger et al. 2005).

β-blockers. As fish contain β_2-receptors in the heart and liver (Gamprel et al. 1994), and probably in reproductive tissues (Haider and Baqri 2000), unspecific antagonists such as propranolol may be active in fish. In fact, propranolol indicated chronic toxicity not only on the cardiovascular system, but also on reproduction; the NOEC and LOEC affecting reproduction in *C. dubia* were 125 and 250 µg l^{-1}, and reproduction was affected after twenty-seven days in *Hyalella azteca* at 100 µg l^{-1} (Huggett et al. 2002). In medaka *O. latipes,* significant changes in plasma steroid levels occurred after fourteen days. The number of eggs released by fish was reduced at 0.5 µg l^{-1} after a four-week exposure to 0.5 and 1 µg l^{-1}, but not at 50 and 100 µg l^{-1} (Huggett et al. 2002). No

alteration in vitellogenin levels was observed. It was suggested that alteration in sex steroids led to decreased oxytocin excretion, which could decrease the number of eggs released. Propranolol was also analyzed in invertebrates. LOEC and NOEC for different organisms span several orders of magnitude (Fig. 12.3), due to species and laboratory differences. Propranolol, metoprolol and nadolol were identified in U.S. wastewaters up to 1.9, 1.2 and 0.36 $\mu g\, l^{-1}$, respectively (Huggett et al. 2002).

Data on *blood lipid lowering agents* are rare. Fibrates have been evaluated by traditional toxicity tests. Clofibrate showed a NOEC for reproduction in *Daphnia* at 10 $\mu g\, l^{-1}$. NOECs for clofibric acid in *C. dubia* (NOEC(7d) = 640 $\mu g\, l^{-1}$), the rotifer *B. calyciflorus* (NOEC(2d) = 246 $\mu g\, l^{-1}$), and early life stages of zebrafish (NOEC(10d) = 70 $mg\, l^{-1}$) were reported (Ferrari et al. 2003). Gemfibrozil became concentrated 113 times in blood plasma of goldfish, as compared to water levels. Plasma testosterone was reduced by over 50% after exposure to 1.5 and 10 $mg\, l^{-1}$, as well as levels of steroid acute regulatory protein transcript in goldfish testes (Mimeault et al. 2005). In fish exposed for fourteen and twenty-eight days to 1.5 $mg\, l^{-1}$ hepatic PPARβ mRNA levels were reduced, and antioxidant defense enzymes were significantly induced, but not acyl-CoA oxidase (Mimeault et al. 2006). At 10 $\mu g\, l^{-1}$, no effects occurred on fish survival and lipid contents after seventeen days (Emblidge and DeLorenzo 2006).

For *neuroactive compounds*, most data were reported for the antiepileptic carbamazepine and on serotonin reuptake inhibitors (SSRI) (Fig. 12.3). Chronic toxicity of carbamazepine was reported in *C. dubia* (NOEC(7d) = 25 $\mu g\, l^{-1}$), in the rotifer *B. calyciflorus* (NOEC(2d) = 377 $\mu g\, l^{-1}$), and in early life stages of zebrafish (NOEC(10d) = 25 $mg\, l^{-1}$) (Ferrari et al. 2003). Sublethal effects occurred in *Daphnia* at 92 $mg\, l^{-1}$ and the lethal concentration in zebra fish was 43 $mg\, l^{-1}$ (Thaker 2005). In a study with the cnidarian *Hydra vulgaris*, diazepam was shown to inhibit polyp regeneration at 10 $\mu g\, l^{-1}$ (Pascoe et al. 2003).

Serotonin is a neurotransmitter found in lower vertebrates and invertebrates, and it may directly act on the immune system, alter appetite, influence behavior and modulate sexual function. The role of serotonin in reproduction varies between different phyla and so do the effects of SSRI, which also may adversely interact with the function of the nervous and associated hormonal system. Exposure to 10-100 $ng\, l^{-1}$ of fluoxetine and ibuprofen resulted in a significant decrease in behavioral activity of *Gammarus pulex*, whereas at higher levels no difference was observed (De Lange et al. 2006). The antidepressant drug mianserin was studied in zebrafish for gene expression using a brain-specific microarray and the impact on egg production, fertilization and hatching. After two days, microarray analysis showed that this compound can modulate neuroendocrine processes. Adverse effects on egg viability were found after fourteen days at 250 $\mu g\, l^{-1}$, indicating adverse effects on the brain-gonad axis (van der Ven et al. 2006).

Fong (1998) found that SSRI (fluvoxamine, paroxetine) led to induction (at 10 nM–100 μM) and fluoxetine to potentiation (at 5 μM, and if co-applied with 7–100 μM serotonin, but not at other concentrations) of parturition in fingernail clams. Induction of spawning in zebra mussels by fluvoxamine was observed at levels as low as 0.032 $\mu g\, l^{-1}$. This points to an interference with serotonin action, since serotonin in invertebrates may stimulate ecdysteroids, ecdysone and juvenile hormone, responsible for controlling oogenesis and vitellogenesis (Nation 2002). A reproductive stimulation was also found in *D. magna* exposed to 36 $\mu g\, l^{-1}$ fluoxetine for thirty days, and in *C. dubia* fecundity was increased at 56 $\mu g\, l^{-1}$ (Flaherty et al. 2001), but reduced in another study (Brooks et al.

2003). An evaluation of five SSRI (fluoxetine, fluvoxamine, paroxetine, citalopram, sertraline) showed negative effects on *C. dubia* reproduction. For the most active compound, sertraline, the LOEC was 45 $\mu g\, l^{-1}$ and the NOEC 9 $\mu g\, l^{-1}$ (Henry et al. 2004). When medaka were exposed for four weeks to 0.1–5 $\mu g\, l^{-1}$ fluoxetine, vitellogenin plasma content, plasma steroids, fecundity, egg fertilization and hatching rate were not affected (Foran et al. 2004), indicating no reproduction impairment. Taken together, the chronic effects of SSRI on reproduction of fish and invertebrates are not yet clear; interference with reproduction occurred at much higher concentrations than 12 $ng\, l^{-1}$ (Kolpin et al. 2002) and 99 $ng\, l^{-1}$ (Metcalfe et al. 2003) found in stream water and treated sewage, respectively.

The anti-diuretic furosemide, found in surface water in Italy of up to 0.3 $\mu g\, l^{-1}$ (Calamari et al. 2003), induced population growth inhibition in *C. dubia* and *B. calyciflorus* at a LOEC of 0.3 and 1.25 $mg\, l^{-1}$. Its photoproduct had a much higher activity with LOEC of 0.02 and 0.31 $mg\, l^{-1}$ in *C. dubia* and *B. calyciflorus*, respectively (Isidori et al. 2006). Chronic data on numerous other compounds are lacking, although they have been shown to occur in considerable concentrations in surface waters (Fig. 12.2). This holds particularly true for fish.

For the antiestrogen tamoxifen, chronic data are found for *Acartia tonsa* (EC_{50} = 49 $\mu g\, l^{-1}$, Andersen et al. (2001)). Various morphological and developmental effects (early embryonic mortality) were induced in sea urchin embryos after exposure to 10^{-8} to 10^{-5} M, which is caused by oxidative stress (Pagano et al. 2001). The antiandrogenic compound flutamide and the aromatase inhibitor fadrozole were analyzed for effects in fish, mainly as positive controls in experiments evaluating hormonally active compounds. Flutamide significantly reduced male sex characteristics in fathead minnows at 0.9 $mg\, l^{-1}$, reduced fecundity at 0.5 $mg\, l^{-1}$, and altered gonadal histology (Jensen et al. 2004). Ovaries from females indicated a decrease in mature oocytes and males exhibited spermatocyte degeneration and necrosis. Concentration-dependent VTG and testosterone increase were observed in females. Embryo hatching was also reduced. Flutamide had an antiandrogenic effect and reduced fecundity, yet at rather high concentrations. Moreover, in adult male guppies, reduction in ejaculated sperm, reduced sex coloration and smaller testes occurred. The male courtship behavior was also disrupted at doses of 1 and 10 $mg\, kg^{-1}$ in feed (Baatrup and Junge 2001). The aromatase inhibitor fadrozole reduced fecundity after twenty-one days at water concentrations of 10 and 50 $\mu g\, l^{-1}$ and inhibited brain aromatase activity (Ankley et al. 2002). In females a concentration-dependent reduction in plasma estradiol and VTG was observed. In males, androgens in plasma were significantly increased and resulted in a marked accumulation of sperm in the testes. Fadrozole significantly inhibited ovarian and induced testis growth at 0.05 and 0.96 $mg\, l^{-1}$ after twenty-one days, and led to inhibition of VTG in females and induction in males (Panter et al. 2004).

12.3.4 Toxicity of Pharmaceutical Mixtures and Community Effects

There are only a few studies dealing with mixture effects and interactions of pharmaceuticals in mixtures, although this actually represents the actual situation in the environment. The ecological potential of anti-inflammatory drugs and of diverse act-

ing pharmaceuticals in different sets of biotests with aquatic organisms was evaluated (Cleuvers 2003, 2004, 2005; Brain et al. 2004a). A mixture of NSAIDs (diclofenac, ibuprofen, naproxen, acetylsalicylic acid) has been tested in acute *Daphnia* and algal tests. Toxicity of the mixture was found at concentrations at which the single compound showed no or only minimal effects. The mixture toxicity followed the concept of concentration addition, which means that the concentration of each compound contributes to the overall effect in an additive fashion. A mixture containing seven pharmaceuticals (acetaminophen, diclofenac, gemfibrozil, ibuprofen, naproxen, salicylic acid and the bactericide triclosan) at 100 ng l^{-1} each, which is representative for the upper concentration range in Canadian waters, did not affect the amphipod *H. azteca* over three generations, except with a 17% increase in percent males (Borgmann et al. 2006).

Cleuvers (2003, 2004) performed acute toxicity tests using *D. magna*, the alga *D. subspicatus* and the macrophyte *Lemna minor* to analyze the acute toxicity of nine diverse drugs (clofibric acid, carbamazepine, ibuprofen, propranolol, metoprolol, diclofenac, naproxen, captopril, and metformin). The combined effects of two substances, clofibric acid and carbamazepine, followed the concept of concentration addition in the *Daphnia* test, whereas in the algal tests, the concept of independent action was adequate. When a combination of NSAIDs, ibuprofen and diclofenac was analyzed, the effect on algae followed the concentration addition concept, whereas for *Daphnia*, the combination effect was stronger. For the acute toxicity of these pharmaceuticals, concentration addition can be assumed, which means that the concentration of each individual pharmaceutical has to be added for the combination effects. This implies that compounds occurring at concentrations below their individual NOEC can nevertheless contribute to the total effect of the mixture.

Only few pharmaceuticals have been analyzed in ecologically more realistic model ecosystems such as microcosms and mesocosms. In two recent studies, outdoor aquatic microcosms of a total volume of 12 000 l containing water and sediment were used to analyze the effects of combinations of pharmaceuticals. Brain et al. (2004a) evaluated the effects of combinations of eight pharmaceuticals at three concentration levels on the macrophytes *L. gibba* and *M. sibiricum* over thirty-five days. Antibiotics and the blood lipid regulator atorvastatin induced phytotoxicity. Using similar microcosms, effects on phyto- and zooplankton were assessed after exposure for thirty-five days at three concentrations to two pharmaceuticals (ibuprofen, fluoxetine) and the antibiotic ciprofloxacin (Richards et al. 2004). The microcosms contained periphyton, phytoplankton, zooplankton, algae and benthic communities, and juvenile sunfish in mesh cages. Species abundance and number of phyto- and zooplankton were affected at the medium (60–100 µg l^{-1} each compound), and high treatment level (600–1000 µg l^{-1} each), whereas at the low treatment (6–10 µg l^{-1} each), only trends were visible. Unexpected high lethality occurred in fish at the high and medium treatments, and lethality was observed in plants in addition to decreased growth. Decreased diversity of both phyto- and zooplankton communities and increased abundance of both communities may have important ecological implications. However, it was unclear whether the effects were caused directly or indirectly, and by which pharmaceutical. Maximal concentrations of ibuprofen, fluoxetine and ciprofloxacin in the USA were 1.0, 0.012 and 0.03 µg l^{-1}, respectively (Kolpin et al. 2002). Richards et al. (2004) concluded that a low probability exists that these three pharmaceuticals are currently present in surface waters

at concentrations negatively affecting aquatic communities. By comparing calculated whole-body therapeutic doses (and not human and fish plasma levels) the authors note that all responses occurred at levels well below the equivalent pharmacologically active concentrations in mammals. Concentrations of pharmaceuticals in fish can reach significantly higher concentrations in plasma than in the ambient water (Mimeault et al. 2005).

12.4 Comparison of Effect Concentrations with Environmental Concentrations

The potential risk of a substance to the environment is often characterized by comparing the Predicted Environmental Concentration (PEC) with the Predicted No Effect Concentration (PNEC) (Fent 2007). PEC of pharmaceuticals are often estimated using calculations, which include usage or sales figures, population density, wastewater volume, and dilution in watersheds to generate likely concentrations in surface waters (Halling-Sørensen et al. 1998; Jones et al. 2002; Straub 2002; Sanderson et al. 2003; Bound and Voulvoulis 2004). Due to the lack of experimental, in particular chronic data in the public domain on the ecotoxicity of pharmaceuticals, the estimation of PNEC, and therefore a hazard and risk assessment, is difficult or even impossible to perform. In the open literature or databases, data are available for less than 1% of pharmaceuticals, and only a small number of new pharmaceuticals have undergone risk assessment using ecotoxicological tests (Halling-Sørensen et al. 1998; Jones et al. 2002; Sanderson et al. 2003). In the absence of experimental data, information is often derived from Quantitative Structure-Activity Relationships (QSAR) predictions, for example by applying the EPA's ECOSAR program (Jones et al. 2002; Sanderson et al. 2004a). While this concept is a pragmatic approach for identifying hazards or prioritizing critical substances, it is not sufficiently precise for accurate hazard and risk assessments of pharmaceuticals.

The empirical data currently available in the open literature on maximal STP effluent concentrations are compared with chronic LOEC and NOEC levels of individual pharmaceuticals (Fig. 12.4). This approach is based on experimental data allowing to prioritize pharmaceuticals according to their ecotoxicological potential and to gain knowledge about worst case situations. LOEC and NOEC values of pharmaceuticals for different aquatic organisms are about one to two orders of magnitude higher than maximal concentrations in STP effluents. For diclofenac, the LOEC for fish toxicity was in the range of wastewater concentrations, whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured STP effluent concentrations. This shows that for diclofenac, propranolol and fluoxetine the margin of safety is narrow, and chronic effects at highly contaminated sites can not be ruled out, in particular, when the combined effects of pharmaceutical mixtures are taken into account. Median sewage effluent concentrations are lower, and dilution in receiving waters results in lower levels in surface waters reducing the environmental risk. It should be noted, however, that more experimental data on chronic toxicity and on the bioaccumulation potential is needed to fully evaluate the environmental risk posed by individual pharmaceuticals.

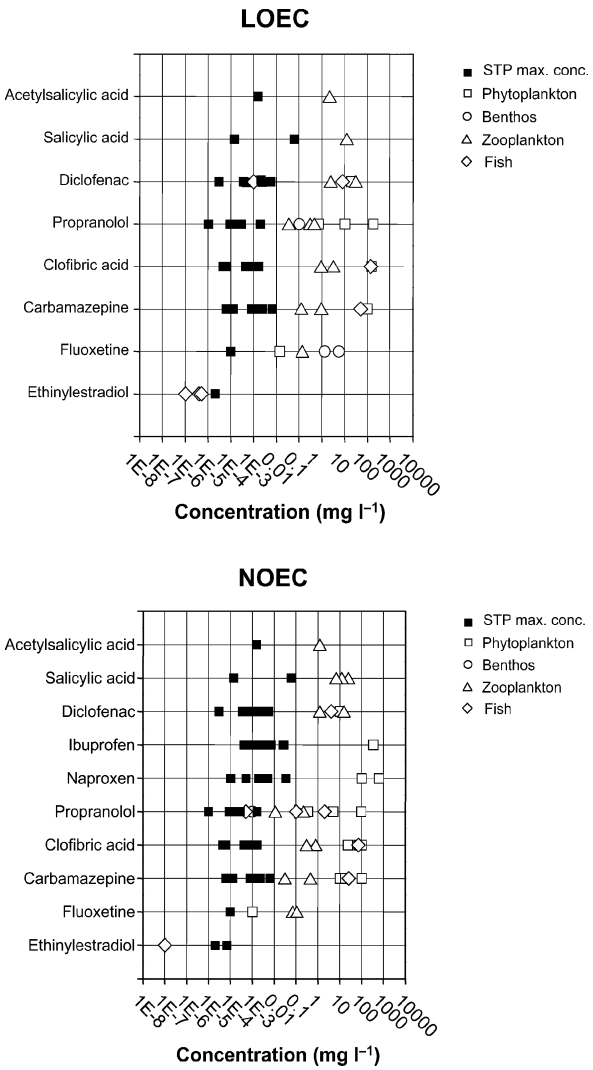

Fig. 12.4. Comparison of maximal concentrations of pharmaceuticals in wastewater treatment plant effluents and chronic toxicity in different aquatic organisms. Shown are lowest observed effect concentrations (*above*) and no observed effect concentrations (*below*).

12.5 Conclusions and Future Directions

Our present knowledge about residues of pharmaceuticals in aquatic systems indicates that they are unlikely to pose a risk for acute toxicity. Environmental concentrations are in the range of 10^3–10^7times lower than known LC_{50} or EC_{50} values (ratio of 10^3 between lowest acute effect of fluoxetine and highest environmental concentration; difference of 10^7 between lowest LC_{50} of diazepam and highest environmental

concentration). However, as the collapse of vulture populations in the Indian subcontinent indicates, important and surprising adverse effects can occur under certain circumstances.

There is a general lack of chronic toxicity data on pharmaceuticals, in particular in fish. Many pharmaceuticals need more investigation about potential long-term ecotoxicological effects, particularly with respect to potential disturbances in hormonal homeostasis (endocrine disruption), immunological status, or physiological and histological alterations after long-term exposure. For better understanding of possible effects, a mechanism-based approach focused on target molecules, tissues and organs should be followed, which yields more meaningful results and insights than traditional acute toxicity testing. Current data on acute and chronic toxicity of pharmaceuticals support the conclusion that more target- or bio-molecule-oriented, or mode-of-action-based investigations, will allow more relevant insights into the effects on survival, growth and reproduction than traditional standard ecotoxicity testing. Often, similar target biomolecules are present in non-mammalian organisms and so are the adverse effects. Unless more is known about possible chronic effects of individual pharmaceuticals and mixtures thereof, conclusions concerning hazards or risks of pharmaceuticals to the aquatic ecosystem are premature.

In general, only a minor part of pharmaceuticals has been tested for potential ecotoxicological effects. Mainly classical acute toxicity tests are performed using traditional species including algae, zooplankton and (rarely) fish, and only a small set of pharmaceuticals has been analyzed for chronic toxicity. Based on such classical OECD studies, the current population decline of three species of vultures due to diclofenac exposure would not have been anticipated. Furthermore, these tests alone are not sufficient for the accurate profile of possible hazards and risks of the pharmaceutical in question. Current tests cover only a small set of laboratory organisms, which are often not sensitive enough and often not able to unravel adverse effects of pharmaceuticals, particularly in the ecological context. Disturbances of the reproductive and hormonal systems, immune depression, neurobehavioral changes, to name some key effects, may have far reaching effects on the population level. Consequently, more specific chronic tests are needed.

12.5.1 Future Strategy

Future ecotoxicological investigations should take the following into account:

1. Pharmaceuticals may have similar (chronic) effects in non-mammalian species, because targets may be similar and conserved during evolution;
2. Due to biological differences in pharmacodynamics, pharmacokinetics and physiology, some pharmaceuticals may have unexpected (chronic) effects in lower organisms, however.

Future chronic toxicity studies should be directed to the pharmaceutical's known physiological targets and they should include histopathological investigations. Existing knowledge about possible side effects should be considered when pharmaceuticals are investigated in aquatic organisms. Furthermore, known drug-drug interac-

tions in humans may be relevant for compound mixtures in the environment. The hypothesis that targets and side effects of a given pharmaceutical may be identical or similar in lower organisms, due to evolutionarily conserved receptors, biochemical pathways and enzymes, should be further tested. Nuclear steroid receptors (Wilson et al. 2004), nuclear peroxisome-proliferator activated receptors (PPARs) (Escriva et al. 1997), adrenoceptors such as β_1- and β_2-receptors (Nickerson et al. 2001), insulin receptors, insulin-like growth factor and glucagon receptors are present in lower vertebrates and invertebrates (Navarro et al. 1999). Basic mechanisms such as signal transduction, cell division, and key metabolizing enzymes (cytochrome P450s) are also similar in a large variety of organisms (Nelson et al. 1996). Effects in mammals may have their counterparts in lower animals, as shown for diclofenac (Schwaiger et al. 2004; Hoeger et al. 2005). Consequently, analysis of pharmaceuticals should specifically address

1. *Target specificity*: specific targets (bio-molecules, tissues, organs);
2. *Side effect specificity*: known adverse side effects in mammals;
3. *Species specificity*: general chronic effects accounting for physiological differences.

The strategy is illustrated in the following examples. When the ecotoxicity of NSAID is studied, effects on inhibition of prostaglandin synthesis and COX inhibition should be addressed in lower organisms, while at the same time focus on side effects already known in mammals. Known side effects of diclofenac in mammals on the kidney (and other organs such as the liver) subsequently were found in vultures (Oaks et al. 2004) and fish (Schwaiger et al. 2004). Inhibition of COX was also found in head kidney macrophages of brown trout (Hoeger et al. 2005). Cardiovascular pharmaceuticals act on the cardiovascular system, and therefore, lower vertebrates should be investigated in this respect. Lipid lowering agents such as fibrates act on lipid metabolism and PPARs (Kliewer et al. 1997). These nuclear receptors play a key role in the catabolism and storage of fatty acids and are important for blood lipid regulation. Indeed, PPARs are affected in amphibians (Kliewer et al. 1997) and fish by clofibrate, bezafibrate and fenofibrate (Ruyter et al. 1997). The target enzyme of statins, HMGR, is also inhibited in duckweed (*Lemna gibba*) resulting in herbicidal activity (Brain et al. 2004b), or in insects, resulting in interference with juvenile hormone synthesis (Debernard et al. 1994).

β-blockers bind to the β-adrenergic receptors and block its activation by physiological agonists. These receptors are located in mammals in many tissues including the heart, and its blockade causes a decrease in heart rate and contraction. β-blockers differ in specificity to the different receptor subtypes, some are non-specifically acting on β_1- and β_2-receptors (e.g., propranolol), while others are specific for the β_1-receptor subtype (e.g., atenolol). In *D. magna*, heart rate, fecundity and biomass were reduced after chronic exposure to 0.11 mg l^{-1} of β-blockers (Dzialowski et al. 2003), although it is not known whether β_2-receptors occur. Long-term exposure to propranolol reduced reproduction in *C. dubia* at 250 µg l^{-1} and in *H. azteca* at 100 µg l^{-1} (Huggett et al. 2002).

Many antineoplastic drugs used in cancer therapy have a high mutagenic and cancerogenic potential, also expected in exposed aquatic organisms. However, such studies are lacking for hospital wastewater, in which the genotoxic potential was found to be induced by antibiotics such as ciprofloxacin (Hartmann et al. 1998).

12.5.2 Unexpected Effects

Although the pharmaceutical's target is the same, the effect might be different in different organisms. A case in point are the statins, which inhibit the enzyme HMGR. In mammals, they have a lipid lowering effect, in insects they interfere with the hormone system (Debernard et al. 1994), and in plants they induce herbicidal effects (Brain et al. 2006). It should be noted, however, that in general additional or other targets may be affected alternatively or in addition to known targets of a given pharmaceutical resulting in unexpected effects. Examples are effects on sex hormones in blood plasma of fish and reduced reproduction in *C. dubia* and *H. azteca* induced by the β-blocker propranolol after long-term exposure (Huggett et al. 2002) or the effects of serotonin-reuptake inhibitors on reproduction of mollusks (Fong 1998; Fong et al. 1998). As the effects of the antiestrogen tamoxifen indicate, pharmaceuticals may have not only one, but multiple modes of action, for instance oxidative damage (Pagano et al. 2001). This fact complicates the strategy of analyzing for chronic effects. However, many of these unexpected chronic responses will be elucidated in the context of careful chronic toxicity analyses, which includes histology, reproduction and development endpoints. More specific toxicity analyses are needed in forthcoming studies, taking full advantage of the available knowledge that is generated during the pharmaceutical drug development process (e.g., mechanisms of action, pharmacokinetic behavior and metabolism, target organs and side effects in mammals) and in in vitro studies (Fent 2001; Caminada et al. 2006).

12.5.3 Mixtures and Comparisons of Environmental and Effect Levels

Only rarely are pharmaceuticals analyzed for possible effects when occurring as mixtures (Cleuvers 2003; Borgmann et al. 2006), the normal situation in the environment. Effects of mixtures most probably follow the concept of concentration addition; hence, the overall toxicity is the result of the sum of the individual concentrations of each compound. Therefore effects may occur even at the NOEC of individual compounds. It should also be recognized that even subtle changes of normal homeostasis including behavioral alterations may have direct and indirect effects, even if only minor ones, that eventually result in significant deteriorating effects on a species or population in the ecological context.

One important approach to easing the load of pharmaceutical residues in wastewater and surface water is to optimize STP processes. There is a need to increase the knowledge about the fate of pharmaceuticals during sewage treatment for implementation of better removal techniques. Future work on STP treatment optimization will show to what extent pharmaceuticals can be removed from wastewater and to what extent the implementation of an improved technology is feasible, while taking into account other macro- and micropollutants as well as the broad variety of complex wastewater matrices.

Comparison of available chronic toxicity data with environmental concentrations indicates that for most investigated pharmaceuticals the concentrations in aquatic systems are too low to induce chronic effects on traditional laboratory organisms such

as inhibition of algal growth and reproduction in *Daphnia*. For diclofenac, the LOEC for fish toxicity on an organ level was in the range of wastewater concentrations, however (Schwaiger et al. 2004), whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were close to the maximal measured STP effluent concentrations. Whether the margin of safety is narrow for additional human pharmaceuticals should be investigated in future studies. The future requirement of testing algae (chronic) and early life stages of fish, as well as chronic tests in *Daphnia* is an important step forward (EMEA 2006). Moreover, the potential of combined effects of pharmaceutical mixtures should be addressed. In the ecological context, subtle changes and disturbances may negatively affect the organism's fitness. Therefore, much more should be known about potentially chronic effects of pharmaceuticals in the aquatic system.

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Another Example of Effects of Pharmaceuticals on Aquatic Invertebrates: Fluoxetine and Ciprofloxacin

G. Nentwig

13.1 Introduction

Pharmaceuticals and their metabolites are more and more recognized as environmental pollutants. While first publications on this topic only reported detection of pharmaceuticals (Hignite and Azarnoff 1977; Richardson and Bowron 1985), newer research aims at possible environmental effects (Oetken et al. 2005; Brooks et al. 2003a). Though pharmaceuticals occur only in traces (µg/l and ng/l), effects on non-target organisms, especially aquatic invertebrates, are possible as these substances are designed to be effective at low concentration levels and to be resistant against degradation. Considering the safety regulations for xenobiotics as stated in the Technical Guidance Document on Risk Assessment (ECB 2003), effects are already possible at concentrations measured in rivers and streams. As shown by Stroben et al. (1992), Triebskorn et al. (1994a,b), Oehlmann et al. (1996), Bauer et al. (1997) and Routledge et al. (1998), environmental hormones are already effective in these low concentration ranges. A first example of effects of non-hormone pharmaceuticals was described by Oetken et al. (2005). One reason for this is the increasing use of long-term ecotoxicity tests using a broader variety of test organisms and monitoring more refined endpoints. The acute tests used before only aimed at the mortality of test organisms. Algae, daphnids and fish were used as test organisms (the "aquatic trias"). These tests did not show possible effects on development and reproduction of the test organisms. Another difference is the mesocosm structure of the long-term tests that allows the implication of several environmental compartments such as the sediment phase. Many pharmaceuticals are lipophilic (e.g., simvastatin, Zhi et al. 2003) and have thus the potential to accumulate in sediments, as they are hardly soluble in water (Prorsi and Müller 1987; Fiedler and Rösler 1993). They also can be remobilized when the sediment is churned up (Kram et al. 1989). Thus, sediments also can be a reservoir for potentially harmful xenobiotics. The implication of this compartment and the use of sediment-dwelling test organisms provide new insights in the modes of action of pharmaceuticals in the environment and leads to a better judgement of their role in the environment.

In this chapter, results of the exposure of the non-biting midge *Chironomus riparius*, the freshwater mudsnail *Potamopyrgus antipodarum* and the aquatic oligochaete *Lumbriculus variegatus* to fluoxetine and ciprofloxacin are reported.

All three test organisms play an important role in the food web. The larvae of *C. riparius* serve as food for many fish and other aquatic organisms. Chironomids are frequently the most abundant insect species in freshwater ecosystems (15 000 species are estimated worldwide, 1 000 species in Europe). The larvae of *C. riparius* live within the sediment. *Lumbriculus variegatus* also serves as food for predatory invertebrates and fish. The

worms inhabit the sediment; feeding is done via ingestion of the complete sediment and extraction of nutrients from the organic matter. The mudsnail *Potamopyrgus antipodarum* was introduced as a neozoon to Europe 150 years ago. Mudsnails inhabit hard substrates but also soft sediments. The snails serve as food for ducks and other water birds as well as for fish. All these organisms are suitable for ecotoxicity testing and are well established, *C. riparius* and *L. variegatus* are standardized by OECD (Phipps et al. 1993; Schulte-Oehlmann 1997; West and Ankley 1998; OECD 2004; Egeler et al. 2005). Their behavior and living properties make them very useful for displaying possible effects of substances diluted in water and accumulated in sediments.

The two pharmaceuticals tested, the antidepressant fluoxetine (Fig. 13.1) and the antibiotic ciprofloxacin, belong to the most prescribed pharmaceuticals in their particular substance group. Especially fluoxetine (Prozac) is widely used and has almost become a lifestyle drug (Olfson et al. 1998, Medawar 1994). The substance belongs to the selective serotonin reuptake inhibitors (SSRI). It inhibits the 5-HT-receptors (Aktories et al. 2005). As it was one of the first mood-lifting antidepressants with great efficacy, it soon became advertised in the lay press and was prescribed in high amounts. Olfson (1994) reports that many psychiatrists prescribed fluoxetine to outpatients, which lead to high prescription amounts. In a book (*Listening to Prozac®*, P. Kramer), the drug was advertised as a "wonder drug" in a very lurid manner. The use also increased due to medical reasons, especially as diagnostic methods were refined and more people were diagnosed with depression that formerly was treated in other ways (Schwabe and Paffrath 2003). Since the substance became available as a generic product, the prescribed amounts have increased once again. Fluoxetine is mainly excreted as glucuronide; thus, it could be set free again in sewage treatment plants. Kolpin et al. (2001) measured 0.012 $\mu g\ l^{-1}$ in rivers, Metcalfe et al. (2003) found 0.099 $\mu g\ l^{-1}$. According to Brooks et al. (2003a,b), information about the environmental fate, especially about the occurrence of residues in sediments are lacking, as well as data about potential effects on aquatic invertebrates. An accumulation of fluoxetine in fish tissue was detected in 2004 (Brooks 2004, pers. comm.).

Ciprofloxacin (Fig. 13.2) belongs to the group of fluorochinolone antibiotics; it acts as a gyrase inhibitor by inhibiting the supercoiling of DNA (Mutschler 1991). By this, division and reproduction of bacteria is inhibited. Higher organisms are not affected

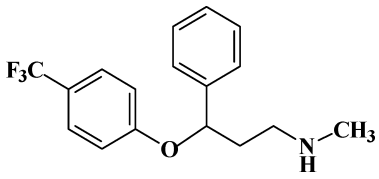

Fig. 13.1. Molecule formula of fluoxetine

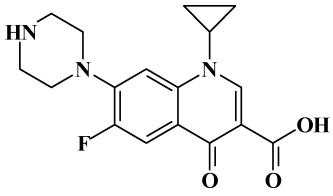

Fig. 13.2. Molecule formula of ciprofloxacin

because of differences in the cell structure. Ciprofloxacin belongs to the systemic active fluorochinolones with a wide range of medical indications. These fluorochinolones can be used against gram-positive and gram-negative bacteria. The use has increased in the last years, especially in hospitals (Schwabe and Paffrath 2004). Compared with 2002, the prescribed amount was 20% more in 2003.

Ciprofloxacin is excreted to 32% via feces and to 44% via urine, partially glucuronized. An additional 11–12% is excreted as inactive metabolites (Forth et al. 1990). As glucuronides can be reactivated in the environment and in sewage treatment plants (Möhle et al. 1999; Wegener et al. 1999; Ternes 2000), it is possible that ciprofloxacin metabolites can be reactivated.

Detection of ciprofloxacin occurred mainly in the effluents of hospitals and sewage treatment plants. Hartmann et al. (1999) detected 125 ng l^{-1} in hospital effluent, Golet et al. (2001) found up to 331 ng l^{-1} in sewage effluents. Calamari et al. (2003) found 26.2 ng l^{-1} in surface waters.

Physicochemical parameters of both pharmaceuticals are listed in Table 13.1.

The aim of the present study was to detect potential harmful effects of ciprofloxacin and fluoxetine on *Chironomus riparius*, *Lumbriculus variegatus* and *Potamopyrgus antipodarum* and to evaluate whether these effects indicate an environmental relevance of these pharmaceuticals.

13.2 Materials and Methods

Fluoxetine was purchased from Alltech Applied Science Labs, State College, USA; ciprofloxacin (as Ciprofloxacin-HCL) was purchased from Fährhaus Pharma, Hamburg, Germany. All test organisms (*C. riparius, P. antipodarum and L. variegatus*) were bred in our laboratory cultures.

Acute toxicity tests were only performed with ciprofloxacin; *C. riparius* and *L. variegatus* served as test organisms. The tests were performed using 96-well-microtiter plates. One worm or one larvae (1st instar) were used per well, eight worms or larvae per treatment. The test concentrations were 0.3, 0.6, 1.2, 2.4 and 4.8 mg l^{-1}.

Table 13.1. Physicochemical parameters of the tested pharmaceuticals

	Fluoxetine	Ciprofloxacin
CAS-number	54910-89-3	85721-33-1
Formula	$C_{10}H_{11}ClO_3$	$C_{17}H_{18}FN_3O_3$
Molecular weight (g mol^{-1})	214.65	331.35
Water solubility (mg l^{-1})	583	1.15×10^{4}
Vapor pressure (mm Hg)	0.000113	2.85×10^{-13}
Melting point (°C)	118–119	258–260
pKa	3.2	6.18 ±0,05
Henry-constant (atm-m^3 mol^{-1})	2.19×10^{-8}	5.09×10^{-19}
Log P_{OW}	2.57	0.28

The first concentration was chosen to be ten times higher than the environmentally detected concentration and raised by factor 2 in the following treatments. The tests with *L. variegatus* lasted for 96 h and were evaluated daily. The tests with *C. riparius* could only be performed within 24 hours as the larvae starved after two days. Lethal endpoints were lack of blood circulation and lysis for *L. variegatus* and lysis, lack of reaction and immobility for *C. riparius*.

In the acute tests as well as in the chronic assays, reconstituted water was used to exclude any influences from tap water. The reconstituted water was prepared using deionized water and adjusting it to a salinity of 540 µS cm^{-1} and a pH of 7.9–8.4. This water was used as test medium for the tests with *P. antipodarum*. For the chronic sediment tests, artificial sediment was prepared according to OECD (2004). Kaolin was not added as well as *Sphagnum* moss peat. The moss does not occur in the rivers in which the worms and the midges typically live in. The use of kaolin could limitate the bioavailability of the test compound because the fine particle content would be much higher as in normal sediments, leading to adsorption and probably covalent binding of the test compound. As the main part of the sediment, quarry sand was used. The particle size distribution was as follows: 90–125 µm: 1%, 125–180 µm: 57%, 250–355 µm: 14% and 355–500 µm: 1%. Additionally, 1.6% by weight of ground leaves of alder was added to the sediment for the *L. variegatus* assay and 1% of a mixture of ground alder and stinging-nettle leaves for the *C. riparius* assay. Stinging-nettle and alder leaves grow on the banks of many rivers and streams; their use as a carbon source created similar conditions to those present in natural sediments. Due to the addition of organic carbon, feeding was not needed during the test.

The test concentrations for fluoxetine in the sediment tests were as follows: 0.15, 0.38, 0.94, 2.34 and 5.86 mg kg^{-1} sediment on a dry weight basis. Ciprofloxacin was tested in the following concentrations: 0.25, 0.5, 1.0, 2.0 and 4.0 µg kg^{-1}. The first concentration of each substance was estimated as possible environmentally relevant sediment concentration; the others resulted from raising the first by a factor of 2.5 and 2, respectively. The sediment was spiked by preparing a stock solution of the test substance, diluting the needed amount of it with water and mixing it with the sediment in the test beaker. After overnight drying of the sediment, water was added to the beakers. As both substances were sufficiently soluble in water, no organic solvent was needed. For the *C. riparius* test, 100 g sediment was used for each replicate and covered with 400 ml water; for the *L. variegatus* assay the respective amounts were 40 g sediment and 200 ml water. The vessels were aerated via glass pipettes, and the sediment was aged two weeks before test organisms were added to ensure equilibration of the test compound between water and sediment. In the case of ciprofloxacin, the test substance was added only three days before insertion of the test organisms as the substance has a low photostability (Phillips et al. 1990, Tiefenbacher et al. 1994). A longer time may have lead to a very high reduction of the ciprofloxacin concentrations.

To ensure that all worms used in the *L. variegatus* test were at the same developmental stage, the worms were cut into half two weeks before the beginning of the tests. *L. variegatus* reproduces asexually by morphallaxis, so natural reproduction can be imitated by cutting the worms. New heads and tails are regenerated within two weeks (Brust et al. 2001). Only the former tail fragments were used for the test. The regeneration of the head was just completed when the worms were inserted into the test vessels. Ten worms were added to every test vessel (day 0), four replicates were used for every test

concentration and for the controls. Effects on *L. variegatus* were assessed by counting the worms at the end of the test (day 28) and by measuring the biomass of the worms.

In the chronic 28 d sediment toxicity test with *C. riparius*, which is often also referred to as a life cycle test, larvae were used immediately after hatching (first-instar larvae). Twenty larvae were used per vessel. As in the tests with *L. variegatus*, four replicates were used per concentration and for the controls. The emergence was controlled daily to assess effects on the midges. Each test was conducted once. The laboratory temperature was kept at 20 ±1 °C. A light-dark regime of 16:8 hours was used, according to the OECD-recommended conditions.

Another reproduction test was conducted with *P. antipodarum.* As the snails can also live on hard substrate, no sediment was used in the test. For each replicate, eighty snails were held in a 1-liter-Erlenmeyer flask that was aerated via glass pipettes. Three replicates were used for each test concentration and for the controls. The test medium was renewed twice a week and the snails were fed at each change of test medium. The test concentrations of fluoxetine were 0.64, 3.2, 16, 80 and 400 µg l^{-1}; ciprofloxacin was tested in concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 µg l^{-1}. The first of the concentrations always roughly corresponded to the measured environmental concentrations (Calamari et al. 2003; Kolpin et al. 2001). The concentrations were then raised for each new treatment by a factor of 5 and 2, respectively. As effects on environmentally relevant concentrations should be detected, the concentrations were lower than those in the acute test. As no direct toxic effects were to be assessed in these tests, except for the effects affecting the reproduction of the test organisms, a lower concentration range could be used. Potential effects on *P. antipodarum* were determined by counting the embryos in the brood pouch. The tests lasted for fifty-six days. To determine potential effects on reproduction of *P. antipodarum*, the embryos in the brood pouch were counted. Therefore, two, four and eight weeks after the beginning of the experiment, twenty snails were taken out from the beakers and narcotized in a 2.5%-solution of $MgCl_2$. The shell was broken and the embryos in the brood pouch were counted (for details cf. Duft et al. 2003). In the fluoxetine assay, an additional evaluation was performed after three weeks.

Statistical analysis was performed using GraphPad Prism 4.03. For normality, data were analyzed using the Kolmogorov-Smirnov-Test and the d'Agostino and Pearson (omnibus normality) test. The homogeneity of variances was estimated with the Bartlett-Test. NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values were calculated via one-way-ANOVA followed by a Dunnett or Bonferroni Test as post hoc comparison. In case of not meeting the requirement for parametric tests, data were analyzed using the Kruskal-Wallis Test with the Dunn Test as post hoc comparison. Linear regression was used to determine EC_x and LC_x values. Therefore, the program EXELSTAT was used.

13.3 Results and Discussion

13.3.1 Chemical Analysis

Analyses were carried out by Kümmerer and coworkers in the Institute of Environmental Medicine and Hospital Epidemiology, Freiburg, Germany. The sediment was

extracted with 1 N HCl in an ultrasonic bath; extracts were centrifuged and filtrated via cellulose filters. Only traces of ciprofloxacin could be detected, which were too low to be quantified. This can either be due to massive degradation and photolysis or covalent binding of ciprofloxacin to the sediment. As sorption of ciprofloxacin is a known phenomenon, the extraction was not exhaustive. Bioavailability and effectiveness under test conditions therefore remain unclear. The analytical results were thus only used for a rough estimation of the concentration.

Fluoxetine residues were analyzed by Medizinisches Labor Bremen GmbH via HPLC. Table 13.2 displays the results of a three-day renewal cycle in the *P. antipodarum* assay. Water samples were analyzed after 24, 48 and 72 h. The concentrations were nearly constant; degradation didn't take place in remarkable ranges.

Table 13.3 shows the sediment concentrations of fluoxetine in the assay with *C. riparius* at day 30. Analyses for the assay with *L. variegatus* were not performed, as

Table 13.2. Fifty-six-day reproduction test with *Potamopyrgus antipodarum*. Analytically determined fluoxetine concentrations at 0, 24, 48 and 72 h after changing the test medium (limit of detection: 1 µg l^{-1})

Nominal concentration (µg l^{-1})	Concentrations 72 h after change of test medium					
	Determined concentration (µg l^{-1})				Recovery rate at t_{72} (%)	Norfluoxetine, t_{72} (µg l^{-1}) (% of nominal concentration, related to fluoxetine)
	t_0	t_{24}	t_{48}	t_{72}		
Control	2	0	0	0	–	0
3.2	3	2	2	2	66.0	0
16	12	12	11	11	68.8	0
80	65	66	33	58	72.5	0
400	363	317	341	345	86.3	13

Table 13.3. Life-cycle test with *Chironomus riparius*. Analytically determined concentrations of fluoxetine and norfluoxetine in water and sediment at day 30 (limit of detection: 1 µg l^{-1} in water and sediment extract)

Nominal sediment concentration (mg kg^{-1})	Determined concentration (µg l^{-1})		Recovery rate (% of nominal concentration)
	Fluoxetine	Norfluoxetine	Fluoxetine
Control	--	--	--
0.15	13.0	--	8.70
0.38	45.0	3.00	11.8
0.94	158	9.00	16.8
2.34	278	17.0	11.9
5.86	734	63.0	12.5

the proportions were the same. The recovery rates were quite low, which may be due to the centrifugation of the sediment. The pore water was therefore not analyzed.

The concentrations are rather low but increase constantly, rising approximately for the same factor from treatment to treatment as the nominal concentrations do. The results therefore can be considered realistic.

13.3.2 Acute Toxicity

Aqueous exposure of *Lumbriculus variegatus* and *Chironomus riparius* to ciprofloxacin during the acute test did not show an increased mortality for any of the tested concentrations. Therefore, the acute tests determined a LC_{50} of >4.8 mg l^{-1} for ciprofloxacin in both species (Table 13.4). No toxic effects occurred at these concentrations.

The lowest acute toxicity that has been detected for ciprofloxacin is a LC_{50} for bacteria of 9.3 µg l^{-1} (Hanisch et al. 2002). These authors also found an environmental concentration of 0.22 µg l^{-1} and therefore estimated ciprofloxacin to be of high environmental relevance. As the substance is designed to be toxic to bacteria, this value cannot be seen as representative for the complete environment. As the acute toxicity for *C. riparius* and *L. variegatus* is beyond environmental concentrations, the substance should not be considered as generally environmentally relevant, based on the outcome of these acute tests.

13.3.3 Life-Cycle Test and Reproduction Test

13.3.3.1 *Fluoxetine*

The sediment concentrations were roughly estimated, as no measured environmental concentrations were available. Experiments were carried out by Hecker (2004) as a diploma thesis.

Lumbriculus variegatus

Fluoxetine concentrations are given as nominal concentrations. The numbers of worms were not significantly reduced by fluoxetine, compared to the controls. But, as Figs. 13.3–13.5 show, a significantly enhanced number of worms occur in the treatments 0.94 mg kg^{-1} and 2.34 mg kg^{-1}.

The graphs tend to show an inverted u-shape. Though the results do not match the validity criteria postulated by Egeler et al. (2005), it seems that fluoxetine causes a hormetic effect among the worms. This may either be due to an unspecific reaction to

Table 13.4. *Lumbriculus variegatus* and *Chironomus riparius*. Acute toxicity (LC_{50}) of ciprofloxacin

Species	Exposure time (h)	LC_{50} (mg l^{-1})
C. riparius	24	>4.8
L. variegatus	96	>4.8

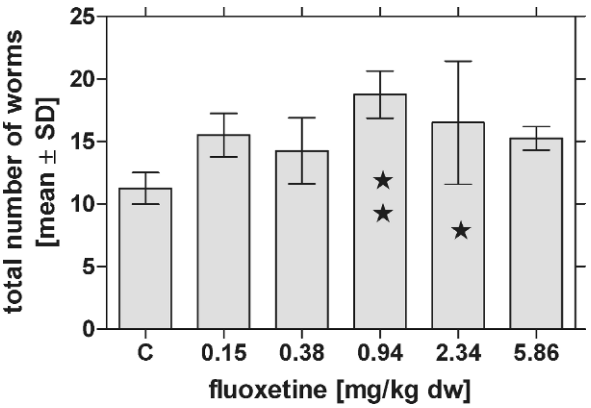

Fig. 13.3. *Lumbriculus variegatus*. Chronic 28 d sediment toxicity test. Test substance: fluoxetine. Mean total number of individuals at the end of the test (±*SD*). *C*, control, ★ = $p < 0.05$, ★★ = $p < 0.01$

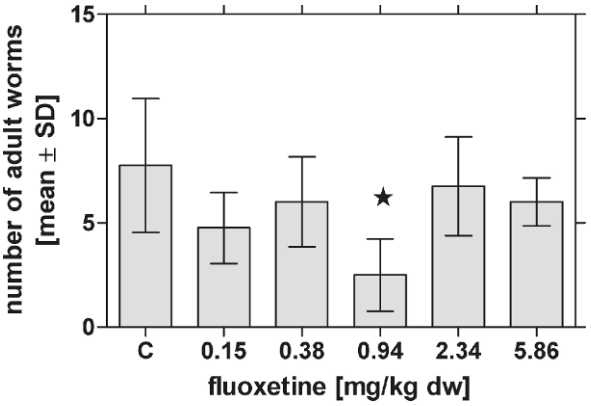

Fig. 13.4. *Lumbriculus variegatus*. Chronic 28 d sediment toxicity test. Test substance: fluoxetine. Mean number of adult worms at the end of the test (±*SD*). *C*, control, ★ = $p < 0.05$

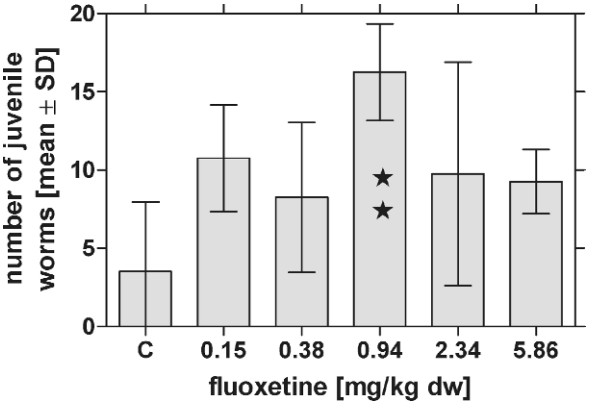

Fig. 13.5. *Lumbriculus variegatus*. Chronic 28 d sediment toxicity test. Test substance: fluoxetine. Mean number of juvenile individuals at the end of the test (±*SD*). *C*, control, ★★ = $p < 0.01$

fluoxetine as a stressor. The worms may increase reproduction so that the population survives until the environmental situation ameliorates again (r-strategy). The lowering worm numbers could then be due to the toxicity of fluoxetine.

The second possibility is a specific reaction to fluoxetine. According to Hessling et al. (1999), *L. variegatus* has serotonergic neurons, so, fluoxetine could cause effects via a reuptake inhibition. Williams and Herrup (1988) report a mitogenic effect of serotonin. Enhanced proliferation could cause an accelerated reproduction, as the worms reproduce asexually. Fong (1998) reports an induction of egg-laying in snails at low fluoxetine levels, Uhler et al. (2000) found an induction of rotation of embryos in *Physa elliptica* at low fluoxetine levels. Both effects disappear at high fluoxetine levels, so it could be typical for the unspecific effects of fluoxetine to vanish at high substance concentrations.

Chironomus riparius

The validity criteria according to the OECD guideline 218 (OECD 2001) were fulfilled: Emergence started at day 15, the mortality in the controls did not exceed 30% (Figs. 13.6 and 13.7). Oxygen content, pH and water temperature also stayed in the prescribed range. Fluoxetine concentrations are given as nominal concentrations. The midges did not show any effect that might be due to fluoxetine. As Fig. 13.6 shows, 100% emergence occurs in the controls, in all other treatment except from the highest one, 5.86 mg kg^{-1} dw, emergence rates over 90% are detected. The emergence rate in the last treatment (87.5% in mean) is statistically significantly lower than the control, but this may be an artifact, as all other values are that high. The low value in the highest treatment results from one of the replicates where the emergence rates were only 70%.

These results are contradictory to those of Brooks et al. (2003 b) who found an increased larval mortality among *Chironomus tentans* (LC_{50}: 15.2 mg kg^{-1} sediment). Obviously, *C. riparius* is less sensitive to fluoxetine.

Potamopyrgus antipodarum

The mud snails were significantly affected by fluoxetine. As Figs. 13.7–13.9 show, embryo numbers declined significantly, compared to the control.

A significant reduction of the embryo numbers could be found from the first evaluation at day 14. In both the highest treatments, embryo numbers were significantly reduced, compared to the control. This reduction was found when the total number

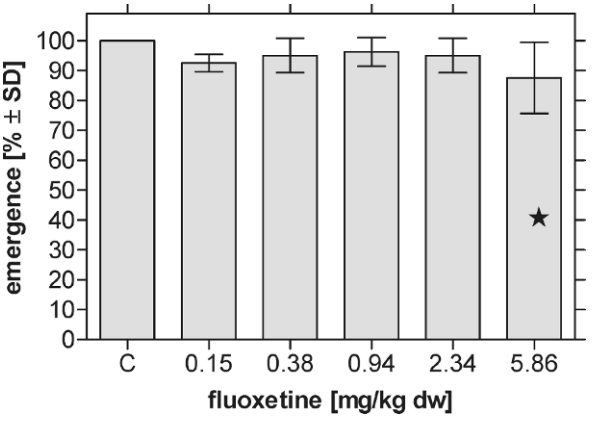

Fig. 13.6. *Chironomus riparius*. Chronic 28 d sediment toxicity test at 20 °C. Test substance: fluoxetine. Mean emergence (%; ±*SD*). *C*, control, ★ = $p < 0.05$

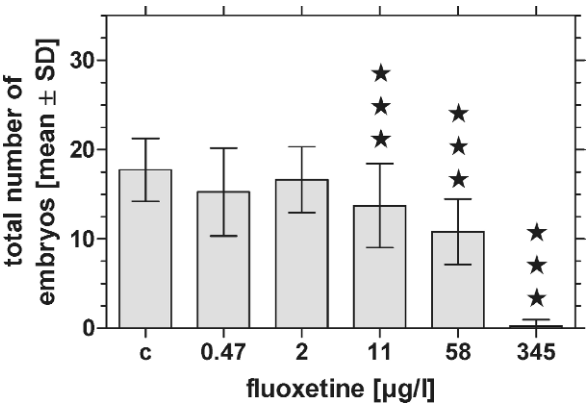

Fig. 13.7. *Potamopyrgus antipodarum*. Chronic 56 d reproduction test. Test substance: fluoxetine. Mean total numbers of embryos at day 56 (±*SD*). ★★★ = $p < 0.001$

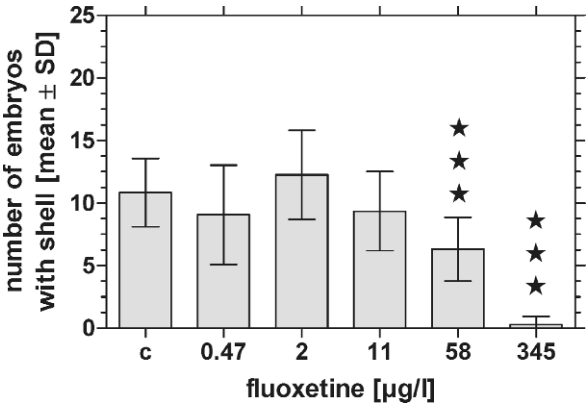

Fig. 13.8. *Potamopyrgus antipodarum*. Chronic 56 d reproduction test. Test substance: fluoxetine. Mean numbers of embryos with shell at day 56 (±*SD*). ★★★ = $p < 0.001$

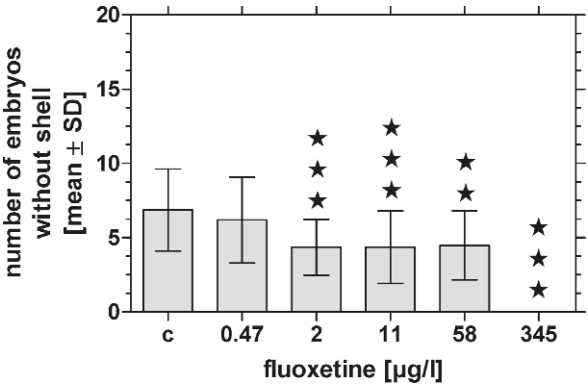

Fig. 13.9. *Potamopyrgus antipodarum*. Chronic 56 d reproduction test. Test substance: fluoxetine. Mean numbers of embryos without shell at day 56 (±*SD*). ★★ = $p < 0.01$, ★★★ = $p < 0.001$

of embryos was evaluated and also when it was distinguished between embryos with and without shells. Furthermore, the snails in the highest treatment were paralyzed and unable to feed. They stayed alive up to the last week of the experiment. As it can

be seen in the graphs, a significant reduction occurred already at a fluoxetine concentration of 11 µg l^{-1}. This could not be found in the separate evaluation of the embryos with shells, where only a reduction at the treatments 58 and 345 µg l^{-1} was visible. The embryos without shells proved to be a more sensitive parameter. A significant reduction occurred already at a concentration of 2 µg l^{-1}. During the experiment, the embryos without shells always showed significant reductions at concentrations lower than the total number of embryos, indicating that fluoxetine inhibits the production of new embryos. For this reason, EC_{10} and EC_{50} were calculated based on the numbers of embryos without shells at day 56. The EC_{10} was 0.81 µg l^{-1} and the EC_{50} was 36.1 µg l^{-1}.

The results correspond with data of Fong (1998), where effects on the reproduction of *Dreissena polymorpha* were described. The drug induced earlier spawning, which may be fatal under environmental concentrations as larvae could hatch in adverse environmental conditions. Additionally, the author reports that the animals looked unhealthy at higher concentrations, thus corresponding with the paralysis of the snails in this experiment. Here, fluoxetine exerts a clearly negative effect, inhibiting the production of new embryos.

Several authors report effects of serotonin and fluoxetine on aquatic invertebrates. Avila et al. (1996) found an increased metamorphosis success in laboratory cultures of the nudibranch *Hermissenda crassicornis,* Honkoop et al. (1999) found an induction of spawning results for a marine bivalve species, *Macoma balthica,* at a fluoxetine concentration of 1 mg l^{-1}. These results and present data indicate that fluoxetine has a high potential to affect freshwater and marine mollusks.

For this reasons, a risk assessment for fluoxetine was conducted according to EMEA (2006) and ECB (2003). The value of 0.012 µg fluoxetine l^{-1} (Kolpin et al. 2001) was used as the measured environmental concentration (MEC). A predicted no-effect concentration (PNEC) was calculated using the EC_{10} of 0.81 µg l^{-1} and applying an assessment factor of 100. This factor has to be used according to EMEA (2006) when chronic data from organisms of one trophic level are available, obtained in a chronic assay under aqueous exposure. This leads to a PNEC of 0.0081 µg l^{-1} and a MEC/PNEC-ration of 1.48. This value indicates environmental relevance of fluoxetine; a risk management for this substance is necessary. The calculation is to be considered as conservative. The MEC of 0.099 µg l^{-1} determined by Melcalfe et al. (2003) would give a MEC/PNEC-ratio of 12.2. Environmental relevance of fluoxetine was also found by Sebastine and Wakeman (2003) with a PEC/PNEC-ratio of 14.2, one order of magnitude higher than in this study. This supports the assumption that effects of fluoxetine at field-relevant concentrations are possible. As requested by EMEA (2006), a risk management for this substance is necessary.

13.3.3.2 *Ciprofloxacin*

The sediment concentrations were roughly estimated, as no measured environmental concentrations were available. Ciprofloxacin concentrations are given as nominal concentrations.

Lumbriculus variegatus

The experiment was repeated once, as the first assay showed a tendency towards decreasing worm numbers at the two highest ciprofloxacin concentrations. However, in

both assays, the differences were not continuously statistically significant, so a substance-related effect could not be determined safely.

Series 1 showed no significant results except from the lower number of adult worms in the treatment 1 µg kg^{-1} dw, compared to the control (Fig. 13.10). Especially in the total number of worms, a tendency of decreasing numbers in higher treatments can be seen, but this is not statistically significant. The assay was repeated due to the high standard deviations. The results are displayed in Fig. 13.11.

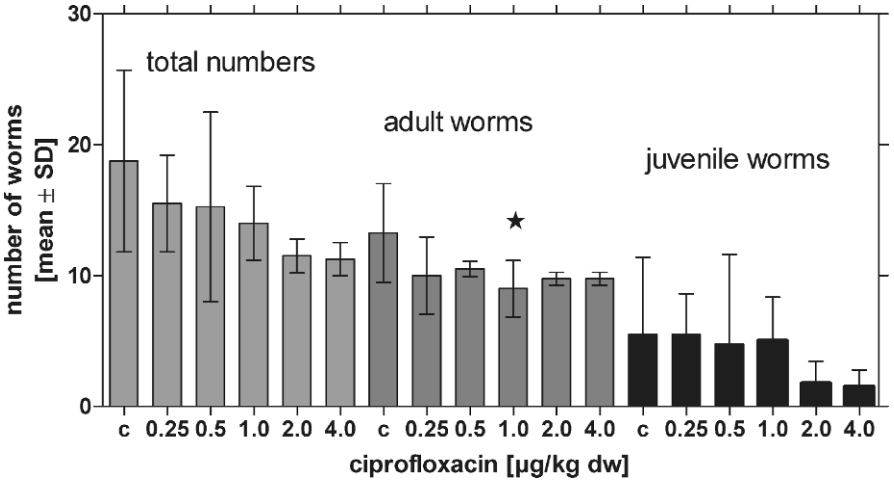

Fig. 13.10. *Lumbriculus variegatus*. Chronic 28 d sediment toxicity test, series 1. Test substance: ciprofloxacin. Mean total number of individuals at the end of the test (±*SD*). *C*, control. ★ = $p < 0.05$

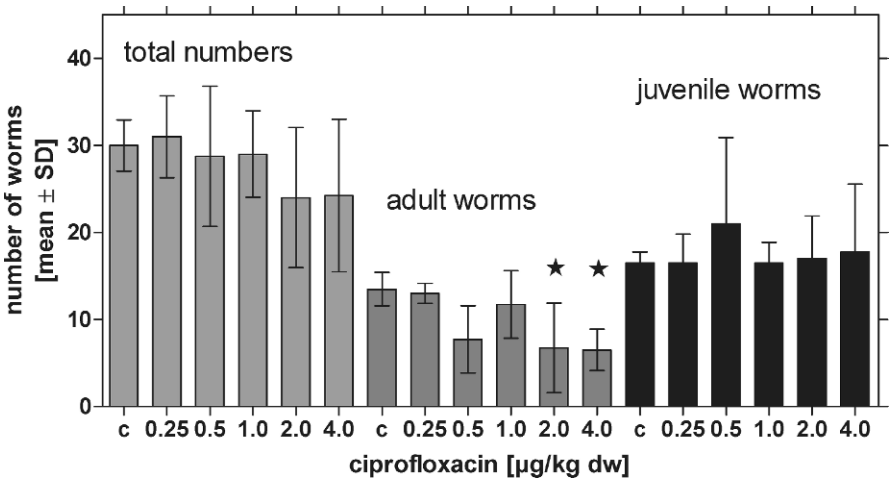

Fig. 13.11. *Lumbriculus variegatus*. Chronic 28 d sediment toxicity test, series 2. Test substance: ciprofloxacin. Mean total number of individuals at the end of the test (±*SD*). *C*, control. ★ = $p < 0.05$

The number of worms generally has increased, compared to series 1. Again, a tendency towards decreasing worm numbers in the highest treatments can be observed, but it is not statistically significant, so again a possible substance-related effect could not be stated with certainty. The only significant differences occur at 2 and 4 µg ciprofloxacin kg^{-1} in the adult worms, but, conversely to series 1, the numbers of juvenile worms are higher than the numbers of adult worms. In series 1, more adult worms than juveniles were found. The high number of juvenile worms in the treatment 0.5 µg kg^{-1} is remarkable. In the treatments 2 and 4 µg kg^{-1}, slightly more worms are counted than in the control, but none of the differences is significant. As ciprofloxacin can induce apoptosis (Herold et al. 2002), this could be an explanation for the higher numbers of juvenile worms, but then, a similar pattern should have occurred in series 1. As this is not the case, an induction of reproduction by apoptosis is unlikely. Also, the results do not indicate an r-strategy. The high numbers of juvenile worms could indicate reproduction as an answer to chemical stress, but then, this should also be visible in series one. Thus, no clear effect on reproduction can be observed. Nevertheless, the possible effects of ciprofloxacin on *L. variegatus* need to be further investigated. Further, environmental data are needed to reveal measured environmental sediment concentrations. As the results here may indicate significant effects at concentrations beyond 4 µg kg^{-1}, it needs to be investigated whether such values would occur in the environment. Estimations of the possible sediment concentrations based on K_{oc} values published after the experiments were done (Cardoza et al. 2004) resulted in a value of 7.88 µg kg^{-1}. This value needs to be confirmed with measured data. Should it prove to be correct, ciprofloxacin could be classified as environmentally relevant.

Chironomus riparius

The validity criteria as described above were fulfilled. As it can be seen in Fig. 13.12, no significant effects occurred. The only treatment that shows a very low emergence rate is 2.0 µg kg^{-1}, but this is due to the very low emergence in one single replicate. The other parameters evaluated (number of clutches, egg per clutch, EmT_{50}, time of hatching) also didn't show any effects. Thus, it can be stated that ciprofloxacin has no effect on *C. riparius*.

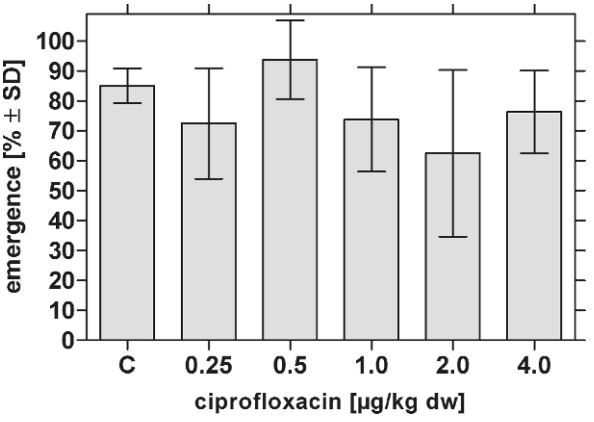

Fig. 13.12. *Chironomus riparius.* Chronic 28 d sediment toxicity test at 20 °C. Test substance: ciprofloxacin. Mean emergence (%; ±*SD*). *C*, control

Potamopyrgus antipodarum

The displayed results for day 56 (Figs. 13.13–13.15) are the only ones in which significant differences to the control occurred. At the other evaluation times, no embryo numbers that differed significantly from the control were observed. The differences remarked here are not sufficient to state any substance-related effect. Evaluating the total numbers of embryos, a significant difference occurred in the highest treatment, 0.8 µg ciprofloxacin l^{-1}, where significantly fewer embryos than in the control were observed. In all other treatments, slightly more embryos than in the control were found. The difference in the highest treatment cannot be found, when embryos with and without shells are evaluated separately. Though the highest treatment always has fewer embryos than the control, the difference is not significant. Looking separately at the embryos without shells, all treatments except for 0.8 µg l^{-1} have nearly the same number of embryos. No significant differences occur. The embryos without shells show a different pattern: In the treatment 0.05 µg l^{-1}, significantly more embryos than in the control occur ($p < 0.05$); the embryo numbers decrease continuously in the following, but no differences to the control can be observed.

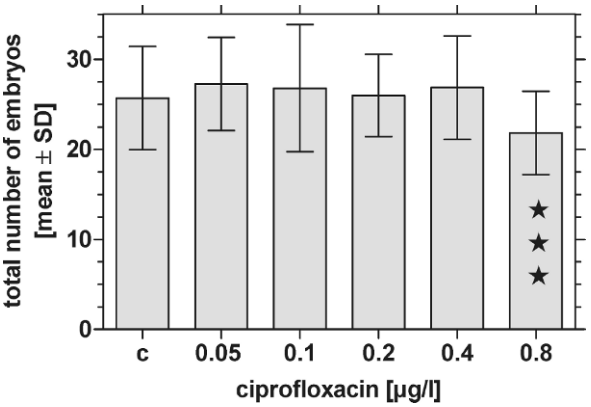

Fig. 13.13. *Potamopyrgus antipodarum*. Chronic 56 d reproduction test. Test substance: ciprofloxacin. Mean total numbers of embryos at day 56 (±*SD*). ★★★ = $p < 0.01$

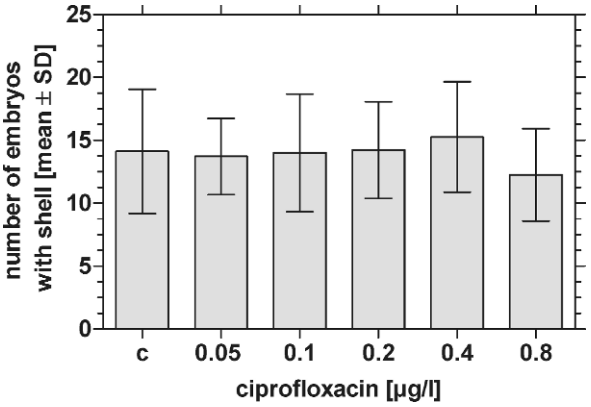

Fig. 13.14. *Potamopyrgus antipodarum*. Chronic 56 d reproduction test. Test substance: ciprofloxacin. Mean numbers of embryos with shell at day 56 (±*SD*)

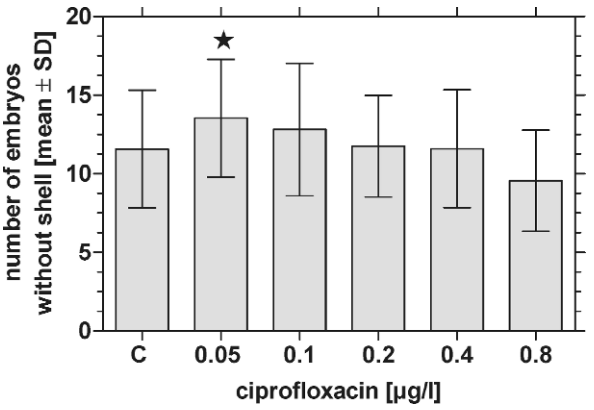

Fig. 13.15. *Potamopyrgus antipodarum.* Chronic 56 d reproduction test. Test substance: ciprofloxacin. Mean numbers of embryos without shell at day 56 (±*SD*). ★ = $p < 0.05$

As these patterns give no overall tendency and especially no real hints of an inhibition of the formation of new embryos, a substance-related effect cannot be detected.

13.4 Outlook

The presented data show that pharmaceuticals in the environment may be of environmental relevance despite their low environmental concentrations. They especially indicate the necessity for testing with a broad variety of testing organisms. While ciprofloxacin was considered environmentally relevant by Hanisch et al. (2002), present data do not indicate any environmental relevance for the substance, at least according to the present regulatory rules. Thus, more data on effects are necessary. The decreasing worm numbers indicate a possible effect; therefore, measurements of sediment concentrations are necessary for a valid evaluation of the ecotoxicological potential of ciprofloxacin. McDermott et al. (2002) describe a spreading of ciprofloxacin resistance in humans after fluorochinolones have been inducted in poultry elevation, indicating that there is high toxicological potential of antibiotics even in low concentrations. As these resistances are transferred between different species (Witte 2000), the release of ciprofloxacin in the environment may endanger the efficacy of antibiotics in therapeutic use in the future.

The toxicity could multiply due to mixture toxicity. Cleuvers (2003) showed that the toxicity of carbamazepine and clofibric acid on *Daphnia* doubled when both pharmaceuticals were mixed together into the test medium. Richards et al. (2004) showed similar effects; Foran et al. (2004) showed a disturbed development in *Oryzias latipes* at very low fluoxetine concentrations. Thus, the testing of pharmaceuticals for ecotoxicity must include not only testing for toxicity, but also effects on reproduction and possible effects in the next generations. The present results support this strongly. They also prove that efforts are needed to reduce the intake of pharmaceuticals in sewage and surface waters. More efficient methods of sewage treatment must be developed and more sensibility in the use of pharmaceuticals, considering their environmental potential is necessary to avoid every superfluous intake.

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Effects of Antibiotics and Virustatics in the Environment

K. Kümmerer

14.1 Introduction

If a substance is not eliminated in any way, it can reach the environment with the potential to adversely affect aquatic and terrestrial organisms. Bacteria, fungi and micro algae are the organisms primarily affected, because antibiotics are designed to affect microorganisms. Antibiotics are of particular interest, because we do not currently know whether their presence in natural waters contributes to the spread of antibiotic resistance of microorganisms (Kümmerer 2004, Kümmerer 2nd edition of this book).

In general, the effects of antibacterial agents on bacteria and micro algae are found to be two to three orders of magnitude below the toxic values for higher trophic levels (Wollenberger et al. 2000). Effectiveness may be modulated by environmental conditions, e.g., if bioavailability is reduced by sorption.

Since experimental parameters influence the results of toxicity investigations, sometimes by several orders of magnitude (Koller et al. 2000), the exact conditions of testing (e.g., temperature, pH value, time scale etc.) have to be stated in order to be able to assess the impacts on the environment. Therefore, the effects outlined below should merely give an approximate indication of what may happen if an antibacterial is present in the environment.

14.2 Wastewater and Sewage System

Antibiotics have the potential to affect the microbial community in sewage systems. The inhibition of wastewater bacteria may seriously affect organic matter degradation; therefore, the effects of antibacterial agents on the microbial population are of great interest.

A reduction in the number of bacteria together with alterations in microbial populations were observed in a model sewage purification system when different commonly applied antibiotics were added in concentrations that may occur in hospital wastewater (Stanislawska 1979; Kümmerer et al. 2000; Al-Ahmad et al. 1999; Kümmerer et al. 2008). As inhibitory concentrations in laboratory testing for a variety of antibiotics were found to be of the same order of magnitude as the concentrations expected for hospital wastewater, the possibility of these substances affecting the microbial populations of hospitals' sewage systems could not be excluded. Nitrification is an important step in wastewater purification, eliminating toxic ammonia. Several antibiotics

proved to have low toxicity in relation to nitrifying bacteria in acute tests. These substances showed no effects upon nitrification in concentrations even higher than what might be environmentally expected (Tomlinson et al. 1966; Gomez et al. 1996). However, the time period of the test significantly influences the results (Halling-Sørensen 2000; Kümmerer et al. 2004). An antimicrobial was found to require high concentrations to inhibit the nitrification process in a short-term test (two to four hours), but a prolonged test period over five days showed effects one order of magnitude below the inhibitory concentrations of the acute test (Tomlinson et al. 1966). Lincomycin showed significant inhibition of nitrification activity in a SBR test, which is consistent with its antibiotic activity spectrum (Carucci et al. 2006).

Acute tests seem to be inappropriate as a means of determining the effects of antibiotics on bacteria. Antibiotics possess specific modes of operation. Impacts frequently become evident by extending the incubation period (Alexy 2003; Alexy et al. 2003; Backhaus and Grimme 1999; Backhaus and Grimme 2000; Froehner et al. 2000; Kümmerer et al. 2004). Christensen and coworkers found synergistic mixture effects of antibiotics against sewage sludge bacteria (Christensen et al. 2006)

14.3 Surface Water

Substances which are not or are only partly degradable in the sewage treatment plant will reach surface water where they may affect organisms of different trophic levels. Ciprofloxacin was active against *Vibrio fisheri* in a high concentration (5 mg l^{-1}, Hernando et al. 2007). However, toxicity tests with bacteria have shown that chronic exposure to antibiotics is critical rather than acute (Backhaus and Grimme 1999; Backhaus and Grimme 2000; Froehner et al. 2000; Kümmerer et al. 2004). Thomulka and Mc Gee (1993) determined the toxicity of different antibiotics (e.g., novobiocin, tetracycline, chloramphenicol, ampicillin, and streptomycin) in relation to *Vibrio harveyi* in two bioassay methods. Almost no toxic effects were found after short incubation times when luminescence was used as an endpoint. But in a long-term assay using reproduction as an endpoint, a toxic effect in environmentally relevant concentrations could be detected for almost all the substances. These results are in accordance with the observations of Froehner et al. (2000). The same effect was found by Kümmerer et al. (2004) in tests with sewage sludge bacteria. Comparison of the results of short- and long-term bioassays with *Vibrio fischeri* demonstrates the risk of underestimating the severe effects of substances with delayed toxicity in acute tests. Similar findings concerning toxicity values were reported by Backhaus and Grimme (1999). In a long-term bioluminescence inhibition test with *Vibrio fischeri,* toxic effect values (EC_{10}) were found for two antibiotics in the range of concentrations expected in the environment.

In a model aquatic system using synthetic freshwater, nitrifying bacteria were significantly affected by an aquaculture antibiotic. The disruption of the nitrification process already occurred in concentrations likely to be found in fish treatment tanks and sediments (Klaver and Matthews 1994). The results of the toxicity tests with bacteria indicate that adverse toxic effects on natural bacterial communities cannot be excluded.

The sensitivity of algae towards antibiotics varied widely. In an algal toxicity test, *Selenastrum capricornutum* was found to be two to three orders of magnitude less sensitive to most antibiotics than micro algae *Microcystis aeruginosa*. The growth of *Microcystis aeruginosa* was inhibited at concentrations of less than 0.1 mg l^{-1} (Halling-Sørensen 2000). Similar observations were documented by Holten-Lützhøft et al. (1999). Blue-green algae (cyanobacteria) seem to be sensitive to many antibiotics, for example amoxicillin, benzylpenicillin, sarafloxacin, spiramycin, tetracycline, and tiamulin (Boxall et al. 2003). The potential ecotoxicological effect of then antibacterial substance metronidazol on Chlorella sp. and *Selenastrum capricornutum* in an acute toxicity test was outlined by Lanzky and Halling-Sørensen (1997). The results indicated that potential adverse effects of antibiotics on algae could not be excluded. As algae are the basis of the food chain, even slight decreases in the algal population may affect the balance in an aquatic system.

Common receptors have been identified in plants for a number of antibiotics affecting chloroplast replication (fluoroquinolones), transcription, and translation (tetracyclines macrolides, lincosamides, P-aminoglycosides, and pleuromutilins), metabolic pathways such as folate biosynthesis (sulfonamides) and fatty acid biosynthesis (triclosan) (Brain et al. 2008). Toxicological investigations into the potency of these compounds indicates susceptibility across multiple plant species, although sensitivity to these compounds varies widely between blue-green algae, green algae, and higher plants in a rather inconsistent manner, except that cyanobacteria are largely the most sensitive to antibiotic compounds (Brain et al. 2008). Effects on macrophytes have been investigated; effect thresholds were high (McGregor et al. 2007). Single compound seven-day daily static renewal toxicity tests with *L. gibba*, sulfamethoxazole and levofloxacin were found to elicit phytotoxic effects in the concentration range utilized (100–1 000 µg l^{-1}) (Brain et al. 2004). Organisms of higher trophic levels such as crustaceae are less seriously affected by antimicrobials. Adverse impacts on these organisms are reported but in most cases in environmentally irrelevant concentrations. Effect concentrations are often within a range of 10 to 100 mg l^{-1} or even more as summarized by Holten-Lützhøft et al. (1999). However, secondary effects due to changes in the natural balance are not negligible.

Exposure to antibiotics in the environment may have adverse reproductive effects in the early life stages of different organisms. This in turn may affect populations dramatically. A significantly depressed hatching rate of cysts of artemia sp. and a high mortality rate of nauplii as well as toxic effects on reproduction of *Daphnia magna* demonstrates how serious the impacts of antibiotics on these organisms are (Migliore et al. 1993, 1997; Brambilla et al. 1994; Wollenberger et al. 2000). The capability of altering the pigmentation of *Artemia salina* nauplii, thus resulting in a loss of fitness of these individuals, was demonstrated for the antibiotic flumequine. This underlines the toxic potential of antimicrobial agents (Brambilla et al. 1994). LC_{50} values below 1 mg l^{-1} for the antibacterial agent furazolidone demonstrated a significant toxicity on *Culex pipiens molestus* larvae, *Daphnia magna* and *Artemia salina* (Macrì et al. 1988). Based on the results, the authors outlined the possibility of considerable damage to the natural equilibrium, since the organisms under investigation constitute the nourishment of other aquatic animals and therefore their disappearance affects other organisms as well. Beside the impacts on the populations outlined above, antibiotics in the envi-

ronment can also affect the behavior of aquatic organisms. For instance, it has been demonstrated that antibiotics influence the phototaxis of *Daphnia magna* (Dojmi di Delupis et al. 1992; Brambilla et al. 1994).

Antimicrobial agents are not likely to affect fish adversely. In all the studies, effects are either found only in high, environmentally ineffectual concentrations, or no toxic effects are observed at all. Toxicity tests using different fish species (*Acartia tonsa*, *Brachydanio rerio, Lebistes reticulatus*, *Salmo gairdneri, Salvelinus namayeush)* showed no toxicity of antibiotics against the species tested (Lanzky and Halling-Sørensen 1997; Canton and van Esch 1976; Marking et al. 1988). An antimicrobial extensively applied in aquaculture has been reported to cause skeleton deformations; however, the substance is applied in concentrations higher than those expected to be found in the environment (Lunestad 1992).

14.4 Soil and Sediments

Several papers have addressed the impacts of antibiotics on soil-dwelling organisms. Antimicrobials may have qualitative and quantitative effects upon a sediment's resident microbial community, and this can affect the degradation of organic matter (e.g., Sengelov et al. 2001; Hamscher et al., 2nd edition of this book; Schmidt and Römbke, this book). Furthermore, direct toxic effects upon the resident organisms cannot be excluded (Nygaard et al. 1992; Kong et al. 2006).

The composition of the soil-dwelling community has been found to be affected by antimicrobial substances (Boleas et al. 2005). Strong inhibitory effects on several bacteria and reduction in the length of the hyphe of active moulds in forest soil have been observed when antibiotics are added in concentrations of 10 mg kg^{-1} soil (Colinas et al. 1994). Hossain and Alexander (1984) demonstrated that antimicrobials in environmentally irrelevant concentrations have an influence on soil microbial composition. Antibiotics in the soil seem to favor fungal growth (Patten et al. 1980). The situation in sediments beneath fish farms is critical because of the high local antimicrobial concentrations. Some antimicrobial agents have been seen to reduce the number of bacteria in concentrations that are relevant to fish farm sediments. Organism activity is also affected. A temporary effect on sulfate reduction was observed when antibiotics were added to sediment, either due to the growth of sulfate-reducing bacteria or of the fermenting and acetogenic bacteria supplying them with the substrate being inhibited (Hansen et al. 1992). Antibiotics present in soil and sediment can lose their antimicrobial activity as a result of their binding to sediment particles or their forming complexes with ions. This ability has already been demonstrated for a few substances. However, contradictory results concerning the loss of antibacterial activity due to binding or complex formation have been found for one and the same substance (Lunestad and Goksøyr 1990; Björklund et al. 1991; Hansen et al. 1992; Hektoen et al. 1995). The reason could be the differences in sediment composition, which seem to play a key role in the effects of substances upon the resident populations, because the composition of the sediment or soil determines the degree and strength of sorption. The magnitude of the effect of ciprofloxacin on microbial salt marsh communities was inversely correlated to the degree of sorption to the sediments. Despite the fact that ciprofloxacin is a wide-spectrum antibiotic, its impact on sediment microbial com-

munities was selective and appeared to favor sulfate-reducing bacteria and Gram-negative bacteria (Córdova-Kreylos and Scow 2007).

Non-target organisms living in soil were not found to be affected by antibiotics. The effects of two antibiotics oxytetracycline and tylosin in environmentally relevant concentrations on earthworms, springtails and enchytraeids have been investigated. Neither antibiotic showed any toxicity against the organisms under investigation. Nevertheless, indirect effects due to changes in the microbial community could not be excluded (Baguer et al. 2000). The potential of a pollutant to accumulate in organisms has to be considered as critical. Antibiotics that are poorly water soluble, especially if the bioconcentration factor is between 500 and 1 000 or the octanol/water distribution coefficient exceeds the value of 1 000, tend to accumulate in organisms. The enrichment of substances in organisms has been proved for some antibiotics, e.g., sulphadimethoxine (Migliore et al. 1993, Lunestad 1992).

14.5 Resistance

Antibiotics are designed to be effective against bacteria. The unwanted effects of microbial growth have long been controlled through the use of antimicrobials such as antibiotics. A vast amount of literature is available on the emergence of resistance and the use of antimicrobials in medicine, veterinary medicine and animal husbandry. The history of resistance due to the use of antibiotics as growth promoters and the lessons to be learned from it has been described only recently (Edquist and Pedersen 2001). In general, the emergence of resistance is a highly complex process, which is not yet fully understood in respect to the significance of the interaction of bacterial populations and antibiotics, even in a medicinal environment (Martinez et al. 2000; Björkman et al. 2000). It is known, for example, that antibiotics in sub-inhibitory concentrations can have an impact on cell functions and change the genetic expression of virulence factors or the transfer of antibiotic resistance (Ohlsen et al. 1998; Salyers et al. 2002). In vitro experiments have shown that gentamicin in a concentration of 100 µg per liter increased the transfer rate of resistance in staphylococci but did not select resistant bacteria. Other substances, such as macrolides, quinolones or vancomycin did not have such an impact (Ohlsen et al. 2003).

Antibiotic resistance as a phenomenon is, in itself, not surprising. Nor is it new. It is, however, recently of concern because it is accumulating and accelerating, while the world's tools for combating it are decreasing in power and number (Harrison and Lederberg 1998). The most prominent medical examples are vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and multiresistant pseudomonads. The transfer of resistant bacteria to humans may occur via water or food if plants are watered with surface water or sewage sludge, if manure is used as a fertilizer or if resistant bacteria are present in meat (Khachatourians 1998; Salyers 2002; Perretin et al. 1997).

Many bacterial species multiply rapidly enough to double their numbers every twenty to thirty minutes, so their ability to adapt to changes in the environment and survive unfavorable conditions often results in the development of mutations that enable the species to survive changing external conditions. Another factor contributing to their adaptability is that individual cells do not rely on their own genetic re-

sources. Many, if not all, have access to a large pool of itinerant genes that move from one bacteria cell (horizontal and vertical transfer) to another and spread through bacterial populations through a variety of mobile genetic elements (Schlüter et al. 2007), of which plasmids and transposable elements are two examples. Genetic material can also be taken up from viruses. The capacity of bacteria to adapt to changes in their environment and thus survive is called resistance. In other words, wherever there is a change in susceptibility that renders an agent ineffective against a certain organism, this organism is referred to as resistant. Some organisms have always been resistant to a particular agent by the nature of their physiology or biochemistry (inherent or intrinsic resistance); others have acquired resistance as a result of the application of antibiotics by humans (acquired resistance).

Antibiotics exhibit different activity spectra and mechanisms of action. It has been recognized for some time that susceptibility to antibiotics varies markedly among different groups of organisms and within these groups. From medical experience it has been learned that transfer of resistance genes as well as the already resistant bacteria themselves is caused by the application of antibiotics and favored particularly by the presence of antibiotics over a long period and in subtherapeutic concentrations, i.e., in concentrations that inhibit or kill not all bacteria sensitive to the antibiotic. Results from research into the use of antibiotics in aquaculture show similar results as experience with the medical use of antibiotics (Westen 1996). Important findings are the following: (1) The use of one antibacterial agent can increase levels of resistance not only to that specific drug, but to many others, even those using very different modes of antibacterial action (cross resistance); (2) Antibacterial resistance does not always respond in a predictable fashion that correlates with the amount of drugs used or with the concentrations of residues in the environment; (3) Therefore, care must be taken in interpreting the literature, and the reader is advised to refer to the literature for details.

Disinfectants are widely used in the food and glue industries, in medicine and in livestock farming. Whether or not they contribute to the emergence of resistance is under discussion (Gilbert and McBain 2003; Kümmerer 2004; Kim and Aga 2007). Schlüter et al. (2007) and Hingst at al. (1995) found resistance against benzalkonium chloride in wastewater.

For antibiotics, resistance is usually quantified as the minimum concentration required to assert a definable effect (e.g., growth inhibition) on a population of cells. The different mechanisms of action and the methods used to evaluate susceptibility are crucial for the results of susceptibility testing and the evaluation of resistance. Resistance is a description of the relative insusceptibility of a microorganism to a particular treatment under a particular set of conditions. Therefore, it should be noted that resistance or at least the resistance level depends strongly of the test type and test conditions, as well as the type of compound and its mode of action.

14.5.1 Antibiotics and Resistance in Test Systems

An understanding of the interaction of antibiotic compounds and bacteria in the environment – also in relation to test systems – is crucial for a sound risk assessment. In the study of the fate of antibiotics in the environment, generally applied test methods

for the assessment of persistence and toxicity against environmental organisms as for instance laid down by OECD and ISO have been used. These methods have been applied successfully in the environmental assessment of chemicals for many decades. For reasons of efficiency, legislation, and risk management they have also been applied to antibiotics and other pharmaceuticals. It has not yet been fully established as to whether they are valid for testing of antibiotics.

Some tests for the assessment of effects of antibiotics on bacteria in the environment are inadequate in terms of the microorganisms used, e.g., only Gram-negative bacteria are employed in the growth inhibition test with *Pseudomonas putida.* Such tests are not suitable for antibiotics that are active against Gram-positive bacteria. Other tests are inadequate in terms of the testing period as was only recently demonstrated for the respiration inhibition test (OECD 209) (Kümmerer et al. 2004) and the Microtox test (luminescent test with *Vibrio fisheri*) (Froehner et al. 2000). Some tests, such as the nitrification inhibition test give inadequate results (Halling-Sørensen 2001).

Furthermore, due to the differing activity spectrum of the different compounds in some tests, microbial population dynamics may overrule effects on some populations. They may thereby mask effects. Environmental compartments differ not only with respect to their physicochemical conditions such as pH, temperature, light and nutrients, but also with respect to the quality and quantity of the bacteria and other microorganisms that are present. The diversity and density of bacteria is high in sewage, activated sludge, and in sediments, but low in surface water. There are generalists that are able to degrade a broad range of chemicals, mostly easily biodegradable chemicals in high concentrations. These bacteria often grow quickly and may quickly adapt to test conditions and test compounds. There are also specialists that grow in a narrow niche (e.g., temperature, pH, oxygen concentration, type of substrate and its concentration). They often grow slowly: generation times of up to several days or more are found. They are most frequently present in nutrient-poor environments such as surface water or in environments representing special conditions (e.g., core of sewage sludge flocs). They form a minority and may be out-competed if fast-growing bacteria are present and if these are favored by test conditions such as high nutrient concentration.

A vast number of the bacteria in the environment are unknown in respect to species, metabolism and function as well as their habitat requirements. We do not know which bacteria with special capabilities are affected or even lost due to test conditions (Hiraishi 1998). Because of differing nutrient needs and sensitivity to antibiotics, the variation among bacteria in the environment as well as in test systems may be high. It is often not accounted for. Total diversity of the organisms present, i.e., the biodiversity, has two major components (*i*) the number of organisms of the same species present and (*ii*) the number of different species present. The higher both numbers, the faster adaptation may occur and the fewer may be the effects exerted by a chemical on the total mix of populations. Both components of bacterial diversity are of importance. High diversity is assumed to be one reason for the high elasticity and resilience of microbial consortia with respect to their function such as biodegradation of organic compounds, as well as their reaction to toxic effects. The adaptation period is shorter if the total diversity is high, because there is a strong probability that fast-adapting organisms and/or organisms specialized in a certain compound are present. Bacteria differ in their enzymatic inventory and their ability to biodegrade organic compounds.

According to the different activity spectra of antibiotics, bacteria may be affected in different ways and to a different extent by antibiotics according to their activity spectrum. Will functions of affected bacteria be overtaken by others?

In surface water and in the free water phase the total number of bacteria is much lower than in areas with high bacterial density such as biofilms on surfaces, in sewage sludge flocks, or in sediments. High biodiversity may not only lead to better biodegradability results, but may also mask the toxic effects of a test compound against some bacteria or groups of bacteria. Bacterial density is therefore an important parameter. Lower functional diversity leads to a narrower spectrum of compounds that are biodegraded in such an environment. Another point is that some bacteria utilize the biodegradation products of other groups of bacteria. If total diversity is higher, the probability of biodegradation is higher as well; this is well known. It is of outstanding importance for the testing of antibacterial compounds and the evaluation of test results.

The importance of the factors described above has not yet been investigated in the context of testing antibiotics in the environment. Most of the studies performed until now used a single compound approach. It is known that antibiotics from the same group or sometimes also from different groups may result in at least an additive effect. For β-lactams, it has been shown that their potency is much higher in the presence of a 5-fluorouracil, a cytotoxic compound also present in sewage in concentrations in the mg/l to µg/l range (Alexy 2003). Will this also happen in STPs or the aquatic environment? If the answer is yes, then the effects of antibiotics in the aquatic environment may be underestimated. The effects of (active) human metabolites as well as the significance of the cleavage of glucuronides during sewage treatment, in surface water or in sediments resulting in the free active compound has not been studied intensively for pharmaceuticals in general or for antibiotics. Effects on bacteria can often not be assessed properly due to the limitations of tests used in the routine assessment of chemicals. There may be a selection for resistant bacteria and effects against other sensitive bacteria and other microorganisms. For example, the reason for applying antibiotics as growth promoters in subtherapeutic doses is that they change microbial populations in the gut of the animals, thus affecting the uptake of nutrients. Will the same happen in the environment when the compounds are enriched in certain compartments, e.g., by sorption? Information in this field is still scarce. When a complex mixture of bacteria is exposed to antibiotics, increased activity can be observed in some cases; that is, some antibiotics seem to intensify nitrification in mixed populations but not in single populations (Halling-Sørensen 2000; Alexy et al. 2001). Should this increased activity be called a toxic effect?

The population dynamics monitored using laboratory scale batch testing applying chemotaxonomic methods showed that the populations were affected by benzalkonium chloride (Kümmerer et al. 2002). However, in a test with a laboratory-scale flow-through system, no effects of antibiotics were found (Kümmerer et al. 2008). But what does "affected" mean? Does it mean all the bacteria are present? And if so, are all the organisms affected in the same way? Does it mean that resistant bacteria are selected or that others or the same ones produce enzymes to degrade a compound which is not toxic to them but may be so to others?

It is known that antibiotics in subinhibitory concentrations can have an impact on cell functions and that they can change the genetic expression of virulence factors or the transfer of antibiotic resistance (Ohlsen et al. 2003). But is this relevant for bio-

degradation tests for antimicrobial compounds? And if it is the case, should it be obtained for all of them? In other words, one has to take microbial ecology into account. In the case of biodegradability testing resistance is an important issue, since in the presence of resistant bacteria, compounds may perhaps be biodegraded that would not be degraded without these resistant bacteria, for example penicillins by bacteria that exprime β-lactamases.

14.5.2 Resistance in the Environment

In the treatment of an infection, bacteriostasis is often effective because the killing and elimination of the pathogen are mediated through host immune defenses. Such augmentation is typically absent in the environment. In this respect, there is little knowledge on environments such as wastewater, sludge, surface water, and soil compared to medical use and effectiveness of antibiotics. However, the amount of literature available has steadily increased throughout the past years.

Bacteria resistant against antibiotics have been found in the aquatic environment (Watkinson et al. 2007; Caplin et al. 2007; reviews: Kümmerer in the 2nd edition; Kümmerer 2004; Kim and Aga 2007; Schlüter et al. 2007) and in soil (see below and Schmitt and Römke, this book).

Whether resistance may develop in the sewage treatment plant or in the soil itself is presently under discussion. An important source of the resistance in hospital effluents, municipal sewage and sewage treatment plants is the input of bacteria which have already become resistant through use of antibiotics in medical treatment. There have been reports that the widespread use of biocides such as triclosan and quaternary ammonium compounds used in hospitals and homes could select for antibiotic-resistant bacteria (Russel 2000). Triclosan, for example, has been shown to select for low-level antibiotic resistance in *E. coli* (McMurray et al. 1998) and high-level ciprofloxacin resistance in triclosan-sensitive *P. aeruginosa* mutants (Chuanchen et al. 2001). In general, resistant bacteria and genetic material correlated with resistance do not match with the concentrations and activity spectrum of compounds found in the environment. For example, β-lactams have not been detected in the environment, whereas resistant bacteria and genetic material encoding resistance against certain β-lactams have been found in sewage treatment plants. Additionally, resistance against vancomycin, of which in Europe only small quantities are used, has been found in European sewage and waters. These findings indicate that the input of bacteria that are already resistant into the environment may be more important for the presence of resistant bacteria in the environment than the active compounds themselves.

Often, existing data used to assess the environmental effects of antibiotics are not adequate to establish how long bacteria maintain antibacterial resistance in the absence of continued selective pressure for that resistance. On the one hand, knowledge of subinhibitory concentrations of antimicrobials and their effects on *environmental* bacteria is scarce and contradictory, especially with respect to resistance. On the other hand, there is a huge volume of evidence that antibiotic resistance is already present in natural environments and that it is exchanged between bacteria for at least a decade (Davison 1999). Schlüter et al. (2007) concluded that animal, human and plant pathogens and other bacteria isolated from different habitats (among them wastewa-

ter treatment plants) share a common pool of resistance determinants that can easily be exchanged. The transfer as well as the new combination of resistance genes will preferably happen in compartments with high bacterial density, i.e., biofilms. Such biofilms are not only found in a medical context. Biofilms are not a taxonomic barrier to horizontal transfer of genetic material. They are important in sewage pipes, aerobic and anaerobic sludge tanks, in wastewater treatment plants, as well as in sediments and soils. A prerequisite for a direct transfer of resistance is that the bacteria are able to survive, or at least the genetic material is stable enough for transfer to the new environment, e.g., from the human body to surface water, which is colder and much poorer in nutrients, or the transfer from plants to animals. Therefore, the question is whether the input of antibiotics into the environment is an important source for the emergence of resistant bacteria in the environment, i.e., is the concentration of the antibiotic and the bacterial density high enough, and is the exposure long enough to promote resistance or to select resistant bacteria? Or is the transfer of resistance from already resistant bacteria following improper use of antibiotics much more important than the input of the antibiotic compounds themselves? The link between the presence of antimicrobials and the favoring of resistant bacteria as well as the transfer of resistance at concentrations as low as those found for the antimicrobials in the environment is not yet established. Some results indicate that the transfer of resistance and the selection of resistant bacteria are not favored at antibiotic concentrations as high as found in hospital effluents or the aquatic environment (Ohlsen et al. 1998, 2003). Results of Kümmerer et al. (2008) and Wiethan et al. (2000) suggest that bacteria that have already become resistant through application of antibiotics will not have necessarily a selection advantage in sewage treatment.

Concentrations of antibiotics and disinfectants are normally some orders of magnitude lower in the free water phase in the environment than for therapeutic use (Lorian 1999). The concentration of antibiotics may be much higher if the active compounds are persistent and accumulate, e.g., by sorption to solid surfaces in certain environmental compartments such as sewage sludge, sediments or soil. In these cases, the role of antimicrobial concentration could differ to that in water. It is not known how strong the antibiotics are sorbed and under what circumstances they are still (bio)available and active after sorption.

14.5.3 Role of Hospitals

It is often assumed that hospitals are the most important source for the input of antibiotics and resistant bacteria into municipal wastewater. Resistant bacteria may be selected or favored by antibiotic substances in hospital effluent. For single compounds, concentrations calculated and measured in hospital effluents (see Chap. 6) are beyond MIC_{50}-values. They may reach this range or even exceed MIC_{50}-values in hospital effluent if not only single compounds but groups of compounds acting via the same mechanism are used (Kümmerer and Henninger 2003). Results have been published which indicate that the transfer of resistance and the selection of resistant bacteria are not favored even at the high antibiotic concentrations found in hospital effluents

(Ohlsen et al. 1998, 2003; Wiethan et al. 2000) or at the lower levels found in the aquatic environment.

Several bacterial strains carrying different resistance genes were found in hospital effluent (Kümmerer 2004). Concentrations of the quaternary ammonium compound benzalkonium chloride found in the effluent of European hospitals was as high as 5 mg l^{-1}. Effect concentrations IC_{50} (growth inhibition) of nitrifying bacteria have been found in the order of 1–2 mg l^{-1}. Resistance found in the environment was high in hospital effluents and sewage treatment plants (Kümmerer 2004; Wiethan et al. 2001). The number of resistant bacteria found in the effluent of an intensive care unit (ICU) of a hospital with maximum medical service spectrum was in the same range as those found in the influent of municipal STPs, and no difference was detectable between municipal sewage that contained hospital effluents and municipal sewage that did not.

Guardabassi et al. (1998) showed that hospital effluent only had a low effect on the prevalence of single or multiple resistances concerning Acinetobacter species, unlike the effluent of a pharmaceutical plant, which had prepared antibiotic containing products. Taking into consideration that hospital effluents contribute to less than 1% of the total amount of municipal sewage, it is plausible that hospitals are not the main source for resistant bacteria in municipal sewage. Resistant bacteria are also present in municipal sewage without hospital effluent. Because of the use of antibiotics in the home, the conclusion is that it is probably the general community that is responsible for the main input of resistant bacteria into STPs. This correlates with the use of antibiotics.

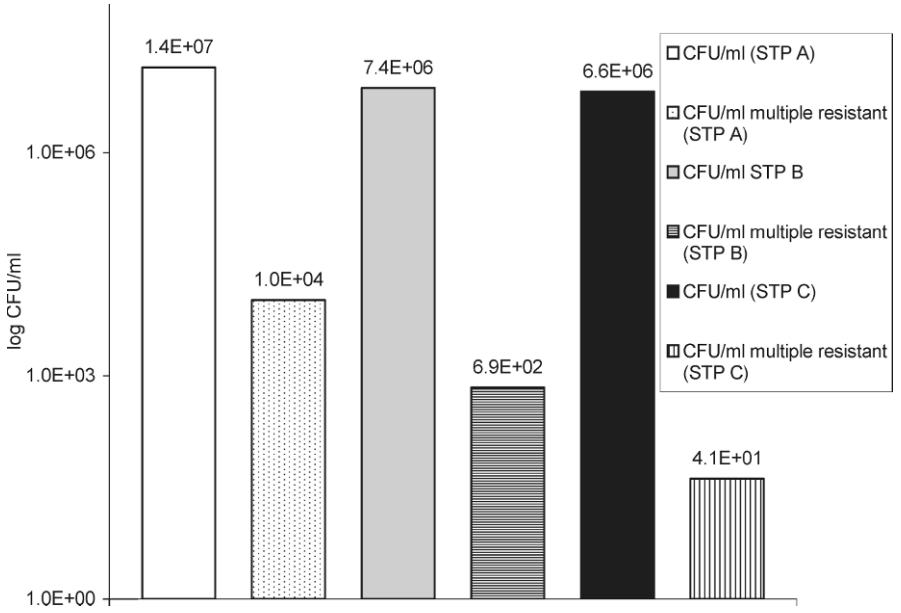

Fig. 14.1. Total colony forming units (CFUs) in the influent of different STPs, only *STP A* received municipal sewage with effluents from hospitals, *STP B* municipal sewage with effluents from nursing homes, *STP C* only municipal sewage without effluents from hospitals or nursing homes

In Germany, for example, only one quarter of the total consumption of antibiotics can be attributed to hospitals. The situation might be different for multi-resistant bacteria. Multi-resistant bacteria, it is assumed, are selected mainly in hospitals and passed into wastewater. The number of multi-resistant bacteria in sewage correlated with the size and the number of hospitals connected to an STP (Fig. 14.1, Wiethan et al. 2001). The numbers and types of resistant bacteria found in the effluent of the intensive care unit (ICU) of a hospital offering maximum medical service showed that the number in the ICU effluent and in the influent of the STP were in the same range in some studies and different in others (Kümmerer 2004). Separate treatment of hospital effluent to reduce the input of resistant bacteria into the aquatic environment is therefore not recommendable, based on the present state of knowledge. Instead proper use of antibiotics is advised.

14.5.4 Wastewater, Municipal Sewage and Sewage Treatment Plants

Peak et al. (2007) determined the abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. The abundance of six tetracycline resistance genes tet(O), tet(Q), tet(W), tet(M), tet(B) and tet(L), were quantified over time in wastewater lagoons at concentrated animal feeding operations (CAFO) to assess how feedlot operation affects resistance genes in downstream surface waters. Resistance gene levels were highly seasonal with abundances being 10–100 times greater in the autumn versus the summer. Results show that antibiotic use strategy strongly affects both the abundance and seasonal distribution of resistance genes in associated lagoons, which has implications on water quality and feedlot management practices.

Concentrations of antibiotics in municipal sewage and in sewage treatment plants are much lower than in hospital effluent. Resistant and multi resistant bacteria such as *E. coli*, *P. aeruginosa, Acinetobacter*, *Pseudomonas*, and Enterobacteriaceae and in phylogenetically distant bacteria, such as members of alpha and beta-proteobacteria are present in municipal sewage as well as in the aeration tanks and the anaerobic digestion process of STPs. Resistance against β-lactams, quinolones, tetracycline and sulfamethoxazole/trimethoprim and other sulfonamides have been found in wastewater and sewage sludge all over the world using classical means, i.e., cultivation and resistance testing as well as detection of resistance encoding genes (Kümmerer 2004; Schlüter et al. 2007). It has not yet been proven that permanent exposure to antibacterials in sewage systems promotes the development of antibiotic resistance and selective effects on bacterial communities. In a study using ciprofloxacin and ceftazidime it was concluded that the average concentrations of ciprofloxacin and ceftazidime actually found in surface water will be clearly below concentrations able to change bacterial populations. This was monitored by classical microbiological methods such as Gram-staining, aminopeptidase and katalase tests as well as employment of metabolic fingerprints using the Biolog system (Wiethan et al. 2001). However, some methodical restrictions have to be taken into account in this study.

A multi-resistant acinetobacter strain which is known to be able to survive in sewage was introduced to a laboratory-scale sewage treatment plant containing a mix of antibiotics at a concentration up to 100 fold above that expected in the aquatic environment in Germany and reflecting German-wide antibiotic use (Kümmerer et al.

2008). The strain was resistant to seven of the antibiotics present. Despite the antibiotics present and the resistance pattern, this bacterial strain was neither detectable in the laboratory-scale sewage treatment plant by classical microbiological methods, nor by chemotaxonomy. Furthermore, two weeks after introduction of the bacterium into the treatment plant, the genetic material responsible for the multi-resistance could no longer be detected. These results suggest that the continuous input of resistant bacteria due to the application of antibiotics is by far much more important than the input of antibiotics. However, this topic requires further consideration and investigation.

Resistant bacteria are eliminated quite well from sewage in STPs. Up to 99% of Campylobacter spp were eliminated from sewage water through treatment in an STP. During the wastewater cleaning process in different municipal STPs, the number of selected resistant pathogens has been reduced up to 93–100%, which seems to be a sufficient elimination. Similar results have been described by other authors, e.g., concerning *Camphylobacter*, 79–100% (Jones 2001) or coliforms, more than 90% (Stelzer and Ziegert 1988). A similar elimination rate was found for imipenem-resistant *P. aeruginosa*, ciprofloxacin-resistant *E. coli* and vancomycin-resistant enterococci (VRE). A seasonal pattern was found in the elimination rate of campylobacter (Jones 2001).

The prevalence of bacteria with reduced sensitivity against benzalkonium chloride was elevated in the effluent of a municipal STP (Hingst et al. 1995; Schlüter et al. (2007) detected ARGs of benzalkonium. A strong selecting effect of benzalkonium chloride was found in biodegradability in a batch test (Kümmerer et al. 2002). In such tests, the benzalkonium chloride concentration was at least 100-fold higher than in hospital effluents or municipal sewage.

In a study, the input of resistant bacteria into three different STPs (one municipal and two located in the countryside) and their elimination was monitored (Wiethan et al. 2001) (Fig. 14.1). The STPs were different in process engineering, size and with regard to antibiotic administration (Table 14.1).

Elimination rates were 95–99% for *E. coli, Pseudomonas* spp. and *Enterococcus* spp. For resistant bacteria, elimination was 93.5–100%. There was no difference between resistant and non-resistant bacteria. These results apply in winter and spring. A correlation between input, i.e., size and number of hospitals passing wastewater and the STPs was not found (Wiethan et al. 2001, Fig. 14.2). It is open to discussion whether to demand wastewater disinfection.

Table 14.1. Investigated sewage treatment plants

	STP A	STP B	STP C
Population equivalent	600 000	14 300	7 500
Process engineering	Activated-sludge process with nitrification, denitrification, phosphate precipitation, sand filter	Activated-sludge process	Activated sludge process
Emitter discharge	One big university hospital, several other hospitals, industry and households	Households, no hospital	Households, three geriatric care centers, no hospital

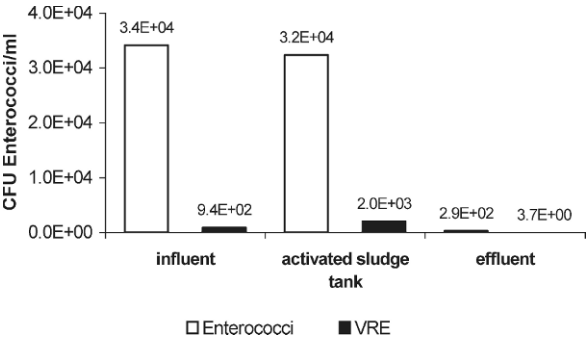

Fig. 14.2. Reduction of enterococci and vancomycin-resistant enterococci (VRE) by wastewater treatment in STP A (see Fig. 14.4, Table 14.1)

Other factors like dilution effects, survival of ARGs, additional regrowth caused by agriculture or natural background load, which can be higher than human input (Edge and Hill 2005), also have to be taken into account. Another question is how much of wastewater disinfection is part of an illusion of hygiene. Moreover, a spread of bacteria and resistances doesn't seem to be expected in the course of the drinking water generation from surface water because of corresponding reduction potentials caused by preparation techniques (Feuerpfeil et al. 1999).

14.5.5 Surface Water

Bacteria that are resistant to antibiotics are present in surface water. A correlation between resistant bacteria in rivers and urban water input has been found, as have resistance genes (Watkinson et al. 2007; Salmore et al. 2006; Edge and Hill 2005; Kümmerer 2004). Antimicrobial resistance has also been found in marine bacteria (Neela et al. 2007) and bacteria living in estuaries or coastal waters polluted with sewage water (Kümmerer 2004; Córdova-Kreylos and Scow 2007; Kimiran-Erdem et al. 2007). But even in remote places such as the Arctic Sea, *Escherichia coli* isolates originating from Arctic birds carry antimicrobial drug resistance. These results show that resistance genes can be found even in a region where no selection pressure exists (Sjölund et al. 2008).

Leaking swine waste storage pits and the land application of swine manure can result in the dispersion of resistant bacteria to water sources. In a study of Sapkota et al. (2007) median concentrations of enterococci, fecal coliforms, and *Escherichia coli* were four- to thirty-three-fold higher in surface water and groundwater samples collected up and down gradient from a swine facility from 2002 to 2004 in down-gradient surface water and groundwater. Fecal bacteria counts were also elevated indicating the animals, i.e., antibiotic application as the source. Higher minimal inhibitory concentrations for four antibiotics in enterococci isolated from down-gradient versus up-gradient surface water and groundwater were observed. The following trend was observed with respect to the concentrations of antibiotic resistance genes (ARGs) for sulfonamides and tetracyclines by Pruden et al. (2006): dairy lagoon water > irrigation ditch water > urban/agriculturally impacted river sediments, except for sul II, a sulfonamide ARG, which was absent in ditch water. It was noted that tetracycline ARGs tet(W) and tet(O)

were also present in treated drinking water and recycled wastewater, suggesting that these are potential pathways for the spread of ARGs to and from humans. The antibiotic resistance (AR) patterns of 462 *Escherichia coli* isolates from wastewater, surface waters, and oysters were determined by Watkinson et al. (2007). Rates of AR and multiple-AR among isolates from surface water sites adjacent to wastewater treatment plant (WWTP) discharge sites were significantly higher ($p < 0.05$) than those among other isolates, whereas the rate of AR among isolates from oysters exposed to STP discharges was low (<10%).

14.5.6 Groundwater

Antibiotics are rarely found in groundwater and if at all they are usually only occurring in concentrations far below the µg per liter range. Leaching from fields fertilized with animal slurry or passing through sediments into the groundwater might be a source of antibiotics in groundwater (Sapkota et al. 2007; Pruden et al. 2006). However, the volume load of antibacterial agents in groundwater in rural areas with high concentrations of livestock has proved to be small. Antibiotic resistant *E. coli* have been found with a surprisingly high incidence in rural groundwater (Kümmerer 2004). Runoff from farms and leakage from septic tanks are clear possibilities for the input of resistant bacteria into groundwater as well as broken sewage pipes.

14.5.7 Drinking Water

Antibiotic-resistant bacteria were detected in drinking water as early as the 1980s and later in the 1990s. These authors found that resistant bacteria identified using classical microbiological methods, i.e., standard plate counting, occurred within the distribution network of drinking water supply systems. They concluded that the treatment of raw water and its subsequent distribution selects for antibiotic-resistant bacteria. In agreement with these data, increased phenotypic resistance rates were also detected at the drinking water sampling points (Scoaris et al. 2007; Kümmerer 2004). Most often, *Aeromonas* spp. were investigated.

14.5.8 Sediments

High loads of antibiotics in sediments in concentrations potent enough to inhibit the growth of bacteria have been reported for aquaculture. Resistant bacteria may be present in sediments because of the application of antibiotics in fish farming or because of the selection by the antibiotics present in the sediments. High antibiotic load in sediments and in concentrations potent enough to inhibit the growth of bacteria have been reported for aquaculture. The fact that the exposure is highly locally concentrated has to be considered critical. The substances used in fish farming can enter sediments directly from the water without undergoing any kind of purification process. Some investigations have demonstrated the presence and persistence of antibiotics applied extensively in fish farming in sediments beneath fish farms (Kümmerer

2004). Quinolones, sulfonamides and tetracyclines are strongly sorbed by organic matter. Therefore, they can accumulate in sediments. It is not yet known to what degree and under what circumstances the compounds are effective after sorption or whether they are released and may contribute to resistance. Antimicrobials may have qualitative and quantitative effects upon the resident microbial community of the sediments. In the fish farming (aquaculture, mariculture, etc.) sector, the widespread use of antibiotics for treating bacterial diseases has been associated with the development of antibiotic resistance in *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda, E.icttaluri*, *Vibrio anguillarum*, *V. salmonicida*, *Pasteurella piscida*, and *Yersinia ruckeri* (Serrano 2005). Bacteria resistant against these compounds have been detected in sediments. An increased antibacterial resistance in sedimentary bacteria is often the most sensitive environmental indicator of past antibacterial use (Kümmerer 2004). Various patterns of resistance among strains were isolated from very close geographical areas during the same year, suggesting diverse patterns of drug resistance in environmental bacteria from this area. In addition, the cross-resistance patterns suggested that the resistance determinants among *Vibrio* spp. are acquired differently within the sediment and seawater environments (Neela et al. 2007).

14.5.9 Soil

Antibiotics occur naturally in soils. Resistance against these antibiotics plays an important role in the population dynamics of soils (see Schmidt and Römbke, this book). Antibiotics are a natural mechanism that has been used by microbes in their natural ecology for millions of years. The abundance of natural antibiotics seems to be low on average and seems to be restricted to the nearest surroundings, i.e., the micro-environment of the bacteria. Tetracycline, for example, is produced by bacteria occurring naturally in soils. To the authors' best knowledge, tetracycline is not found in soils that have not been fertilized with manure containing tetracycline. The tetracycline concentration was always below the detection limit in soils used as a control when studying the input and fate of tetracycline in soils (see Hamscher, 2nd edition of this book). This situation may be different in tropical soils as the bacteria producing tetracycline naturally in such soils occur in a higher density. In soil, the dynamics of bacterial populations is controlled by naturally occurring antibiotics including bacteria and fungi, amongst other things. Most of the compounds used nowadays are synthetic or at least semi-synthetic. The speed of their depletion will probably be lower than that of naturally occurring compounds, but to the best of the author's knowledge no information is available on this.

Antibiotic use in food animal production has been associated with the emergence of antibiotic-resistant strains of bacteria including *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Enterococcus* spp. Evidence from some U.S. and European studies suggest that these resistant bacteria cause infections in humans that do not respond to commonly prescribed antibiotics. In response to these practices and attendant problems, several organizations (e.g., The American Society for Microbiology, ASM), have called for restrictions on antibiotic use in food animal production and an end to all non-therapeutic uses. Thus, the antibiotics may accumulate and reach con-

centrations in the MIC range. If they are still effective after sorption, resistant bacteria may be selected by antibiotic substances due to their application in animals, their use as growth promoters, and in soil. A laboratory test proved the acquisition of Gram-positive tetracycline resistance genes in *Mycobacterium* and *Streptomyces* species. This is one of the few reports of possible gene transfer between soil bacteria and human intestinal bacteria. Also, the exchange of genetic material between soil bacteria was reported. A high incidence of bacteria resistant against fluoroquinolones was found in soil isolates. The origin of this resistance is not clear as enrofloxacin is widely used in agriculture. Resistance could be a result of the input of already resistant bacteria into soils following the application of the antibiotic's pressure (Kümmerer 2004). So far the spread of resistant bacteria and resistance genes by manure and sewage sludge used as fertilizer in agriculture or for land amendment has not been sufficiently investigated. It was found that some soil microbial populations are affected by applying manure containing antibiotics. Some weeks after application of the manure, the bacterial composition returned to its original state. However, whether or not the change was due to antibiotics or to other constituents of the manure such as resistant bacteria was not investigated (Sengelov et al. 2001).

14.5.10 Plant Agriculture

Resistance of plant pathogens to oxytetracycline is rare, but the emergence of streptomycin-resistant strains of *Erwinia amylovora*, *Pseudomonas* spp., and *Xanthomonas campestris* has impeded the control of several important diseases (McManus et al. 2002). A fraction of streptomycin-resistance genes in plant-associated bacteria is similar to those found in bacteria isolated from humans, animals, and soil, and are associated with transfer-proficient elements. However, the most common vehicles of streptomycin-resistance genes in human and plant pathogens are genetically distinct (McManus et al. 2002). Tetracycline resistance genes have been identified in nonpathogenic bacteria in apple orchards (Palmer and Jones 1999). However, the role of antibiotic use on plants in the antibiotic-resistance in human medicine is the subject of debate.

14.5.11 Antiviral Resistance

Oseltamivir is the main antiviral for treatment and prevention of pandemic influenza. Only recently it has been found that the active moiety of oseltamivir is not removed in normal sewage water treatments and is not degraded substantially by UV light radiation, and that the active substance is released in wastewater leaving the plant (Fick et al. 2007). The increase in oseltamivir resistance reported recently has therefore sparked a debate on how to use oseltamivir in non pandemic influenza and the risks associated with widespread use during a pandemic (Singer et al. 2007). It has been concluded by Fick et al. (2007) that a ubiquitous use of oseltamivir may result in selection pressures in the environment that favor development of antiviral drug-resistance. The same is expected for tamiflu (Singer et al. 2007).

14.6 Conclusion

At present, there is insufficient information available to reach a final conclusion on the impact of antibiotics on the structural and functional changes of bacterial populations in the environment, which would allow for the assessment of the potential risks related, for instance, to human health and soil fertility. According to present knowledge, the input of antibiotics in general and specifically from hospitals seems to be of minor importance. Their impact on the frequency of resistance transfer by antibiotics present in the environment is questionable. The available knowledge suggests that the input of resistant bacteria into the environment from the different sources seems to be the most important source of resistance in the environment. The possible impact of resistant bacteria on the environment is not yet known. However, studies show that application of antibiotics and close contact with animals may probably be the most important route for the transfer of antibiotic resistance to humans: not only the application in human medicine but also in pig fattening and treatment of pets. This is demonstrated by the following example: A new type of MRSA recently emerged in the Netherlands. The first isolate was found in 2003, and since then it has been found with increasing frequency (van Loo et al. 2007). The geographic origin of NT-MRSA correlated with the density of pig populations. This association was confirmed by the results from this case-control study, which show that NT-MRSA is significantly related to contact with pigs. In addition, a significant association was found with cattle (van Loo et al. 2007). Transmission of MRSA between animals and humans has previously been described, e.g., associated with colonized companion animals, horses, and persons who take care of them (Duquette and Nuttall 2004; Cefai et al. 1994; Loeffler et al. 2005).

What has been learned so far is that it is critical to prevent the selection of resistant strains in the first place, both in human and veterinary medicine and animal rearing etc. The opportunities and the routes whereby this may be achieved differ in both fields. Therefore, prudent use of antibiotics and disinfectants in human medicine and livestock farming will significantly reduce the risk for the general public and for the environment. This not only includes limiting the duration of the selective pressure by reducing the treatment period and the continuous use of subtherapeutic concentrations. It also includes controlling the dissemination of antibiotics being used and a prudent monitoring of resistance.

An environmental risk assessment cannot be performed on the basis of data available. There is still a lack of fundamental data on the fate and effects of ARGs in the environment, and the availability of such data is a prerequisite if proper risk assessments and risk management programs both for humans and the environment are to be undertaken. Results of a study conducted by Engemann et al. (2006) revealed that light exposure should be maximized in receiving waters in order to maximize resistance gene tet(O), tet(W), tet(M), tet(Q) loss rate after release. Results for other ARGs are missing. As ARGs are similar in chemical nature it can be expected that all ARGs are sensitive to light.

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Realizing the Potential Benefits of Small Animal Models for the Aquatic Hazard Assessment of Human Pharmaceuticals: A Conceptual Approach

T. H. Hutchinson

15.1 Introduction

The protection of aquatic life from potential impacts of pharmaceuticals is an important element within recent environmental assessment regulation by the European Medicines Evaluation Agency (EMEA) and other regulators. Where a compound's physicochemical properties predict exposure in water, an aquatic hazard (effects) assessment is usually needed. Typically, Predicted No-Effect Concentrations (PNECs) for freshwater life are based on chronic testing with plants (seventy-two-hour algal growth test), crustaceans (21d daphnid lifecycle test) and fish (30d embryo-larval development test). From these data, the most sensitive experimental No-Observed Effect Concentration (NOEC) is used as a basis for calculating the $PNEC_{freshwater}$. If a given pharmaceutical has a therapeutic mode of action (MOA) that involves the mammalian reproductive endocrine system, this may warrant the inclusion of a freshwater fish full lifecycle test NOEC to support a robust $PNEC_{freshwater}$. Current European regulatory guidance advises that dividing the $PNEC_{freshwater}$ by a factor of 10 can derive the $PNEC_{marine}$, unless marine ecotoxicology data exist. While there is clearly a need to provide aquatic life hazard data on pharmaceuticals, this should be done as efficiently as possible through the use of small-scale methods and ideally avoiding unnecessary in vivo routine testing with fish. In the future, this goal may be realized by linking ecotoxicology with molecular evolutionary biology to build a 'knowledge bridge' between target proteins found in fish and closely related invertebrates (e.g., Chordates such as ascidians). Pending future validation, such a conceptual approach could have three major benefits: (1) provide major savings on the amount of drug substances used by using small static-renewal invertebrate tests instead of large-scale flow-through fish toxicity tests; (2) provide both freshwater and marine data at reduced cost so that adding safety factors to derive $PNEC_{marine}$ values would be unnecessary; and (3) eliminate the need for routine fish toxicity testing of human pharmaceuticals as per the current EMEA and FDA approaches (while recognizing the likely need for fish data on a case-by-case basis). Focusing on chronic effects testing, this chapter will review the possibilities of and potential limitations to this conceptual approach with an emphasis on the current data gaps.

15.2 Proteins as Therapeutic Targets for Pharmaceuticals

Due to the increasing power of molecular pharmacology, active pharmaceutical ingredients (APIs) are increasingly being developed to have a clear therapeutic mode-

of-action (MOA), which seeks to target specific proteins in human patients. While there are exceptions of chemistry-based (so called 'small molecule') APIs targeting the genetic machinery of cells (e.g., cellular immunophilins or tubulin), Rang et al. (2003) describe a quartet of protein targets including receptors, ion channels, enzymes and transporters. A notable example is the estrogen receptor agonist, 17α-ethinylestradiol, a synthetic analogue of the steroidal estrogens widely detected in freshwater ecosystems across many regions (Vos et al. 2000). The estrogen receptor is also the therapeutic MOA target for the widely used anti-cancer drug tamoxifen. This example, however, also serves as an alert to the fact that certain drugs may also have a toxic MOA at high doses (in the case of tamoxifen, this relates to induction of oxidative damage in mammalian non-target tissues and in sea urchin embryos (Pagano et al. 2001; Roepke et al. 2005). Table 15.1 summarizes other examples of human drugs that have receptors as their therapeutic targets, together with examples for other major classes of protein receptors (see Rang et al. 2003 for more information).

15.3 Genes and Proteins are Often Conserved During Evolution

As described by Schubert et al. (2006), the discovery that molecular mechanisms controlling key features of animal development are remarkably conserved among organisms as different as fruit flies and mice has led to a fundamental change in the use of model organisms as study systems. Today, it is clear that information obtained in one model animal can be applicable to research in other animal species, as well as being useful for understanding the evolution of animals as a whole. This not only relates to preclinical mammalian models (e.g., mice and rats) but also to zebrafish (Zon and Peterson 2005) and to invertebrates (de Wildt et al. 1999; Iguchi et al. 2006; Nebert and Dieter 2000). For example, in 2002 the Nobel Prize in Physiology or Medicine was awarded to Sidney Brenner, H. Robert Horvitz and John E. Sulston based on their work on the developmental and molecular biology of the nematode *Caenorhabditis elegans* (Sulston and Ferry 2002). As the invertebrate "molecular toolbox" continually grows to include aquatic invertebrates such as the ascidian tunicate *Ciona intestinalis* (Corbo et al. 2001; Holland and Gibson-Brown 2003; Davidson 2007), this underscores the potential scientific rewards of harnessing the practical utility of small organisms for the aquatic hazard assessment of pharmaceuticals and related chemicals (Hutchinson 2007).

For example, in the context of mammalian steroid hormone receptors and the evolutionary aspect, there is recent evidence of an ancestral estrogen receptor from the mollusk *Aplysia californica* (Thornton et al. 2003; Baker 2004). Their observations suggest that steroid receptors are ancient and widespread, having diversified from a primordial gene before the origin of bilaterally symmetric animals, and that this ancient receptor had estrogen receptor-like functionality. This gene was, however, apparently lost in the lineage leading to arthropods and nematodes (members of the phyla Ecdysozoa). In support of this view, Dinan et al. (2001) used an in vitro ecdysteroid screening assay and observed no significant activity for a wide range of mammalian steroids and related pharmaceuticals. Fig. 15.1 illustrates the animal groups discussed in an evolutionary context.

Table 15.1. Proteins as therapeutic targets (after Rang et al. 2003)

Protein class	Drug targets	Effectors	
Receptor	Examples	Agonists	Antagonists
	Androgen receptor	Testosterone	Flutamide
	Beta-adrenoreceptor	Noradrenaline (norepinephrine)	Propranolol
	Histamine (H_1 receptor)	Impromidine	Ranitidine
	Oestrogen receptor	17α-ethinylestradiol	Tamoxifen
	Progesterone receptor	Norethisterone	Danazol
Ion channel	Examples	Blockers	Modulators
	ATP-sensitive potassium channels	ATP	Cromokalim
	Renal tubule sodium channels	Amiloride	Aldosterone
	Voltage-gated calcium channels	Divalent cations (e.g., Cd^{2+})	Beta-adrenoreceptor agonists
	Voltage-gated sodium channels	Tetrodotoxin	Veratridine
Enzymes	Examples	Inhibitors	False substrates
	Cyclooxygenase	Aspirin	–
	HMG-CoA reductase	Atorvastatin	–
	Thymidine kinase	Aciclovir	–
Carriers	Examples	Inhibitors	False substrates
	Na^+ / K^+ pump	Cardiac glycosides	–
	Noradrenaline uptake 1	Tricyclic antidepressants	–
	Proton pump	Omeprazole	–
Others	Examples	Inhibitors	False substrates
	Immunophilins	Ciclosporin	–
	Tubulin	Colchicine	–

15.4 Crustaceans and Insects (Ecdysozoans) as Test Species

Globally, this phylum has immense economic and ecological importance, representing almost 95% of animal species known today (Wilson 1992). The Ecdysozoans include the crustaceans, insects and nematodes, with *Daphnia magna* (Crustacea: Cladocera) being the most widely used freshwater species for chronic effects testing (OECD 1992). Daphnids are cyclical parthenogens, which can reproduce both by par-

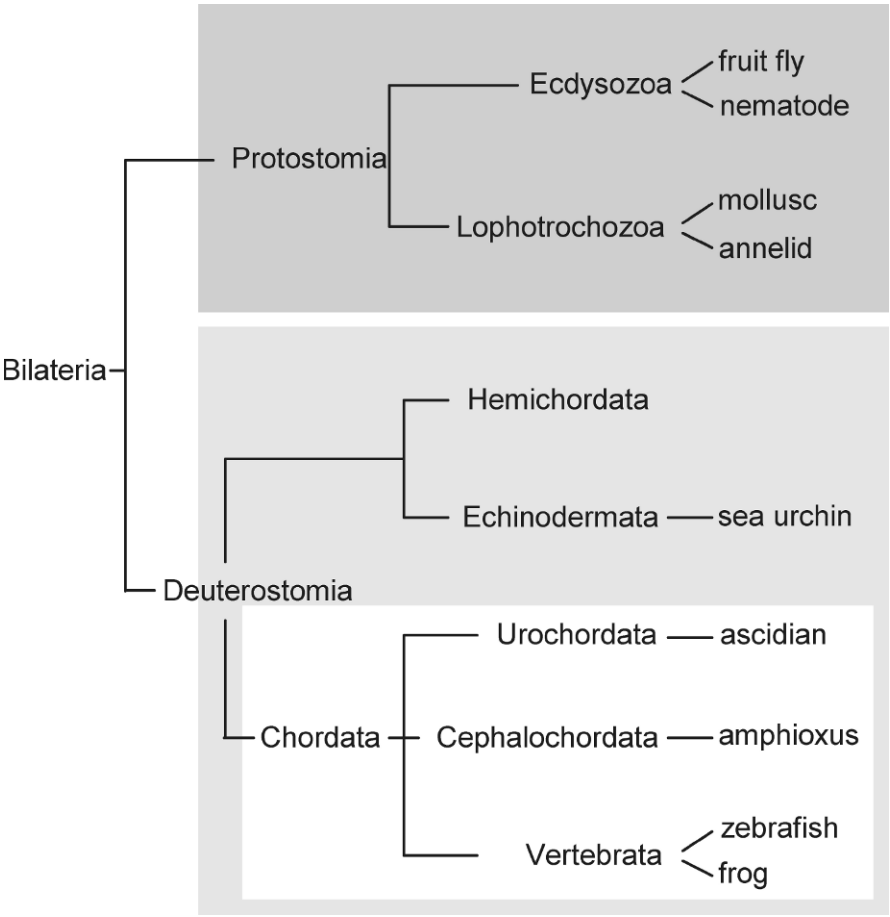

Fig.15.1. Phylogenic relationships in the animal kingdom, showing the vertebrates in the *lower white box* (adapted from Sea Urchin Genome Sequencing Consortium 2006). The current EMEA (2006) scheme usually requires chronic testing using ecdysozoans (*Daphnia magna*) and vertebrates (fish) plus algae (reviewed in Hutchinson 2007). See Tables 15.2 and 15.3 for a summary of freshwater and marine (saltwater) methods

thenogenesis and by sexual reproduction (which may be measured in terms of offspring sex ratio). With its ease of handling in the laboratory, and small volumes of test solution required for the 21d test protocol, in practical terms *Daphnia magna* has proven to be an ideal organism for routine testing of pharmaceuticals and other compounds, including any compounds which may have ecdysteroid or juvenile hormone disrupting activity (Debernard et al. 1994; Dinan et al. 2002; Tatarazako and Oda 2007). *Daphnia magna* is now being studied using genomic tools (Watanabe et al. 2005; Heckman et al. 2006, 2007) and biochemical approaches (Baldwin and LeBlanc 1994).

For pharmaceuticals that may partition to sediments (e.g., $\log K_{ow} > 4$) and hence require a $PNEC_{sediment}$ value, freshwater chironomids are widely used in Europe and

North America for sediment toxicity testing, as well as aqueous phase testing. Chironomid lifecycle tests have been applied to a wide range of organic compounds, especially agrochemicals and some pharmaceuticals (see review by Taenzler et al. 2007).

For both marine and freshwater hazard assessment purposes, copepods are a diverse taxon well-suited to the development of small-scale (static-renewal) lifecycle toxicity tests. In many ways, they have the practical utility of *Daphnia magna*, aided by the availability of methods for both aqueous phase and also sediment testing (see review by Kusk and Wollenberger 2007). A 21d lifecycle test is currently under OECD validation (Gourmelon and Ahtiainen 2007). To the author's knowledge, nematodes are not widely used in regulatory ecotoxicology; however, methods have been published using the model organism *Caenorhabditis elegans* (Anderson et al. 2001; Dengg and van Meel 2004) (Table 15.2).

15.5 Annelids and Mollusks (Lophotrochozoans) as Test Species

As reviewed by Ingersoll et al. (1995), freshwater annelids are periodically used for chronic ecotoxicity testing and can be adapted for use in both sediment and aqueous phase testing. Protocols of typically 28–40 d are typically used (for example, Liebig et al. (2005) showed bioaccumulation of 17α-ethinylestradiol in the aquatic oligochaete *Lumbriculus variegatus* over a 35 d period. Marine annelid test methods are also available; however, to date there is a paucity of published data on pharmaceutical testing with these organisms, either in 28d lifecycle tests or short-term tests (48h embryo development tests; see Hutchinson 2007).

With more than 130 000 species identified, mollusks are one of the most diverse and species-rich phyla of the animal kingdom being only second to the arthropods. Freshwater mollusks are being increasingly used to test pharmaceuticals (e.g., analgesics,

Table 15.2. Examples of freshwater invertebrate species used for assessing developmental or reproductive endpoints in current or potential future standard protocols (after Hutchinson 2007)

Species and chronic test endpoint
▪ Annelida: Oligochaete (*Lumbriculus variegatus*) – reproduction up to 28 d
▪ Arthropoda – Crustacea: Water flea (*Daphnia magna*) – neonate production and growth up to 21 d
▪ Arthropoda – Insecta: Non-biting midge (*Chironomus riparius*) – reproduction in two-generation test (approx. 45 d)
▪ Cnidaria: Hydroid (*Hydra vulgaris*) – development up to 17 d
▪ Mollusca – Bivalvia: Zebra mussel (*Dreissena polymorpha*) – spawning and embryo development after 2 d
▪ Mollusca – Prosobranchia: Freshwater mudsnail (*Potamopyrgus antipodarum*) – embryo production up to 56 d
▪ Mollusca – Pulmonata: Pondsnail (*Lymnaea stagnalis*) – reproduction up to 56 d
▪ Rotifera: Rotifer (*Brachyonus calyciflorus*) – fertilisation and reproduction up to 2 d

synthetic androgens and estrogens, Selective Serotonin Reuptake Inhibitors), and this diverse group of organisms is being considered for future OECD test guideline development (Gourmelon and Ahtiainen 2007; Hutchinson 2007). Table 15.3 summarizes how the embryos of marine bivalve mollusks may also be used as a rapid and sensitive test method for a variety of organic and inorganic compounds.

15.6 Echinoderms as Test Species

Echinoderms (starfish and sea urchins) are exclusively marine animals and are among the most familiar seashore animals. Echinoderms are also of interest as a possible alternative to fish in light of their key phylogenetic position, since in contrast to other widely employed invertebrates (such as arthropods and mollusks), they are deuterostomians and therefore closely related to vertebrates (Fig. 15.1). For this reason, they show some similarities with vertebrates in terms of physiological processes and hormonal pathways and have been used in cancer research (Pagano et al. 2001). Pharmaceuticals have been included in several recent toxicity studies using embryonic or larval stages (using small scale tests of usually <4 d), which are generally considered more sensitive than adults (reviewed by Sugni et al. 2007). The recent characterization of the sea urchin genome is a significant step toward developing a long-term 'knowledge bridge' between pharmaceutical target proteins (Sea Urchin Genome Consortium 2006).

15.7 Ascidians as Test Species

Ascidians, or sea squirts, are invertebrate chordates that belong to the earliest branch in the chordate phylum (the subphylum Urochordata or Tunicata; see Fig. 15.1). Ascidian larvae possess a prototypical chordate body plan that includes a dorsal neural tube,

Table 15.3. Examples of saltwater invertebrate species used for assessing developmental or reproductive endpoints in current or potential future standard protocols (after Hutchinson 2007)

Species and chronic test endpoint
▪ Annelida: Polychaete (*Ophryotrocha diadema*) – reproduction up to 28 d
▪ Arthropoda – Crustacea: Copepods (eg *Amphiascus tenuiremis*) – reproduction up to 21 d
▪ Ascidia: Tunicata Sea vase (*Ciona intestinalis*) – embryo development after 1–2 h
▪ Mysids (*Americamysis bahia*) – reproduction up to 28 d
▪ Echinodermata: Sea urchins (e.g., *Paracentrotus lividus*) – embryo development up to 4 d
▪ Mollusca – Bivalvia: Pacific oyster (e.g., *Crassostrea gigas*) – embryo development after 2 d and longer term reproduction over several months
▪ Mollusca – Prosobranchia: Periwinkle (e.g., *Littorina littorea*) – imposex development up to 3 months
▪ Rotifera: Rotifer (*Brachionus plicatilis*) – reproduction up to 2 d

an axial notochord flanked by muscles, and a ventral endodermal strand. Gastrulation and neurulation involve cellular rearrangements that are comparable to those seen in vertebrates, except that ascidian embryos are composed of just a few hundred cells whereas comparable vertebrate embryos contain many thousands of cells. The resultant motile larva (almost 1 mm long) is analogous to the amphibian tadpole. This larva ultimately undergoes metamorphosis into a sessile, filter-feeding adult. The cellular simplicity of the ascidian larva is mirrored by its small, compact genome (for the model species *Ciona intestinalis*), again representing a significant aspect of developing a long-term 'knowledge bridge' between pharmaceutical target proteins (Corbo et al. 2001). To date, there are no available studies for pharmaceuticals on ascidians, although Dolcemascolo et al. (2005) have used *Ciona intestinalis* to assess the effects of tributyltin. It is important that research be undertaken to fill this data gap using pharmaceuticals to assess whether or not such invertebrate data could replace tests with vertebrate (e.g., amphibian and fish embryo-larvae).

15.8 A Rough Guide to Protein Targets and ADME Complexity

As discussed earlier in this chapter, modern molecular pharmacology focuses on APIs that target specific proteins in human patients (namely: receptors, ion channels, enzymes and transporters (Rang et al. 2003). As is also well established from mammalian pharmacology, however, in vivo biological responses to a drug are dramatically influenced by species-specific ADME (adsorption, distribution, metabolism and excretion). Moreover, for prodrugs (e.g., aciclovir, candoxatril, cyclophosphamide, flutamide and levodopa), metabolism into the pharmacologically active form may also be species and tissue specific (Oliyai and Stella 1993; Rang et al. 2003). Precise quantitative predictions of toxic effects between species based on MOA is likely to represent a major challenge due to the concurrent ADME complexities in different species. Hence, it is probably prudent to use the 'protein target MOA' approach as a rough guide for selecting small aquatic animal models (and plants) in an intelligent testing strategy for pharmaceuticals (ECETOC 2007). In mammals, for example, it is known for muraglitazar, there is a ten-fold difference in systemic clearance values across mice, rats, dogs, and monkeys (Hosagrahara et al. 2006). Similarly, sildenalfil shows short-elimination half-lives in rodents but almost ten-fold higher half-lives in dogs and humans (Walker et al. 1999). Other examples include diclofenac (Sellers et al. 2004), lovastatin (Halpin et al. 1993) and rosiglitazone (Balfour and Plosker 1999). Drug-drug (chemical-chemical) interactions are also of importance in an ADME context (Prueksaritanont et al. 2002; Rang et al. 2003). Given the need to balance target protein MOA and ADME aspects, a rough guide to help select aquatic animal models is shown in Table 15.4.

15.9 Opportunities and Limitations

This chapter briefly reviews some of the key scientific developments in the evolutionary biology of the animal kingdom, juxtaposed to the ethical and economic desirability to optimize the use of small invertebrates for testing pharmaceuticals that may enter

Table 15.4. A rough guide to using mammalian MOA and ADME information as a guide to the selection of small aquatic animal models

Therapeutic (or toxic) MOA target protein	ADME processes	Examples
(1) Known or predicted that the a given aquatic species does not have the specific protein target;	(1) Regardless of metabolic and other ADME processes, assume this aquatic species will be relatively insensitive to compound via its therapeutic MOA;	(1) Oestrogen receptor absent from Ecdyzoans; in chronic studies, crustaceans and insects relatively insensitive to 17α-ethinylestradiol compared to molluscs and fish (Hutchinson 2007);
(2) Known or predicted that the invertebrate species does have the specific protein target;	(2) If ADME data indicates the compound (or a pharmacologically active metabolite) does not reach target tissues, then this aquatic species may be relatively insensitive to the compound;	(2) Compounds like flutamide are weakly active as an endocrine disrupter in fish and invertebrates, possible due to limited biotransformation into the active metabolite hydroxyl-flutamide (Panter et al. 2004);
(3) Known or predicted that the invertebrate species does have the specific protein target.	(3) If ADME data indicates the compound (or a pharmacologically active metabolite) will probably reach target tissues, then this aquatic species may be relatively more sensitive to the compound.	(3) Ibuprofen is more toxic to snails than to daphnids or fish in chronic studies, possible due to limited detoxification (Pounds et al. 2007).

aquatic ecosystems. There is a vast literature on the genomics and development of model invertebrates (e.g., sea urchins and ascidians) that are relatively closely related to fish and other vertebrates (Fig. 15.1). Importantly, the knowledge of molecular evolution (e.g., genomics, proteomics, phenotypic anchoring) in diverse animals continues to grow rapidly, potentially representing a resource that can be 'borrowed' to guide selection of aquatic toxicology test protocols for pharmaceuticals. This is rather analogous to the growing interest in using zebrafish as a drug discovery tool (see review by Zon and Peterson 2005).

There are, however, some important limitations to the practical application of the concepts raised in this chapter, lack of data being the key short-term problem. For example, this lack of data is especially pertinent opposite the need to develop an invertebrate comparative developmental and reproductive toxicity database for a range of reference pharmaceuticals. However, learning from the ongoing OECD mammalian, fish and invertebrate work on endocrine disrupter test validation, this would be feasible by selecting a limited range of pharmaceuticals and related compounds (e.g., 17α-ethinylestradiol, fadrozole and flutamide) (Gourmelon and Ahtiainen 2007). Establishment of defined laboratory cultures of model invertebrate species ought to be feasible by adopting methods used in biomedical laboratories.

Pending the evaluation of the comparative sensitivity of organisms represented in Fig. 15.1, the most promising test methods would then need to be validated in terms of their intra-laboratory repeatability and inter-laboratory reproducibility. As shown by a decade of ongoing validation work for endocrine disrupter test methods in fish and amphibians, this is a major task, although certainly this should be quicker and cheaper

if the focus stayed on small invertebrates species (e.g., embryo-larval development and growth tests). Another limitation is that fish toxicity testing is needed anyway in order to provide a basis for fish bioconcentration testing, although in the author's experience this is not a routine situation for most hydrophilic human pharmaceuticals and their metabolites. A further 'political' limitation could be reluctance by regulators to move away from aquatic toxicity data generated on traditional species (e.g., fathead minnows or zebrafish) to non-arthropod invertebrates (e.g., echinoderms or ascidians). One option to give the necessary scientific reassurance of comparability would be for the 'training set' of pharmaceuticals to have already been tested in fish embryo-larval toxicity tests, as per the recent EMEA (2006) guidance document. As noted in Table 15.4, whole MOA protein targets may be conserved across species; ADME aspects also need to be an important consideration.

It is hoped that the ideas briefly outlined in this chapter may stimulate wider debate over future opportunities to help replace, reduce and refine the use of fish in routine testing of pharmaceuticals. It is also important to recognize that fish chronic testing will be warranted in certain situations where there is substantial scientific uncertainty over the use of surrogate (invertebrate) test species, or possibly where pharmaceuticals are detected in ecosystems with commercially important fisheries. However, this case-by-case scenario should not prevent research into a suite of plant and invertebrate test methods for routine application to support generic $PNEC_{freshwater}$ and $PNEC_{marine}$ values. Generation of data on reference chemical data on both freshwater and marine species will also help clarify the basis for $PNEC_{freshwater}$ and $PNEC_{marine}$ comparisons (Leung et al. 2001; Wheeler et al. 2002).

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On the Ecotoxicology of Pharmaceutical Mixtures

T. Backhaus · J. Sumpter · H. Blanck

16.1 Introduction

The catastrophic decline of vulture populations on the Indian subcontinent due to Diclofenac poisoning, a nonsteroidal pain killer, is probably the most prominent case demonstrating that the occurrence of pharmaceuticals in the environment can lead to severe ecotoxicological effects. The birds were exposed by feeding on carcasses, originating from cattle previously treated with diclofenac (Oaks et al. 2004). Another example would be the contraceptive ethinylestradiol (EE2), which impairs the reproduction of exposed fish populations at environmentally realistic concentrations (Nash et al. 2004; Sumpter et al. 2006; Purdom et al. 1994). However, the situation is less obvious for the vast majority of other pharmaceuticals. In a number of recent ecotoxicological studies it was concluded that clear ecotoxic effects of the investigated pharmaceuticals are only to be expected at concentrations well above environmentally realistic levels. Hence, the current risk to the environment has often been assessed as being negligible, (e.g., Han et al. 2006; Miege et al. 2006; Wilson et al. 2004), or limited to certain cases (Brain et al. 2006; Lienert et al. 2007). However, several authors have also pointed out that our current knowledge on the ecotoxicity of pharmaceuticals is rather limited, especially with respect to possible chronic effects (Crane et al. 2006; Stuer-Lauridsen et al. 2000; Carlsson et al. 2006).

Furthermore, pharmaceuticals do not occur as isolated, pure substances in an environmental compartment. As a broad range of different substances is used simultaneously in human and veterinary medicine in any given area, pharmaceuticals are present as *multi-component mixtures* in the environment. Furthermore, most pharmaceuticals will either be transformed by physical and chemical processes in the environment and/or taken up by some organism and subsequently biotransformed. Thus from an environmental perspective, even individual pharmaceuticals ultimately have to be regarded as a multi-component chemical mixture (parent compound plus degradation products and metabolites).

16.1.1 Mixture Effects are of Special Concern

Not only is the occurrence as multi-component mixtures typical for the environmental exposure situation of pharmaceuticals. Two characteristics also make their joint toxic effects a major issue for hazard and risk assessment:

1. The ecotoxicity of a mixture is almost always higher than the effects of its individual components; and
2. A mixture can have a considerable ecotoxicity, even if all components are present only in low concentrations that do not provoke significant toxic effects if acting singly on the exposed organisms.

These characteristics are demonstrated for two multi-component pharmaceutical mixtures in Figs. 16.1 and 16.2. In both cases, the components are present in concentrations that individually provoke only low, non-significant effects. However, the resulting mixture effect is even higher than 50% in the case of the ten-component mixture of quinolone antibiotics (Fig. 16.1) and higher than 15% in the case of the fourteen-compound mixture (twelve pharmaceuticals plus two additional toxicants, Fig. 16.2). Further details of both studies can be found in (Backhaus et al. 1999, 2000). Significant mixture effects from low-effect individual concentrations (EC_{05}) were also observed in a study by Fent and coworkers for a mixture of cimetidine, fenofibrate, furosemide and phenazone (Fent et al. 2006). Several other studies demonstrated simi-

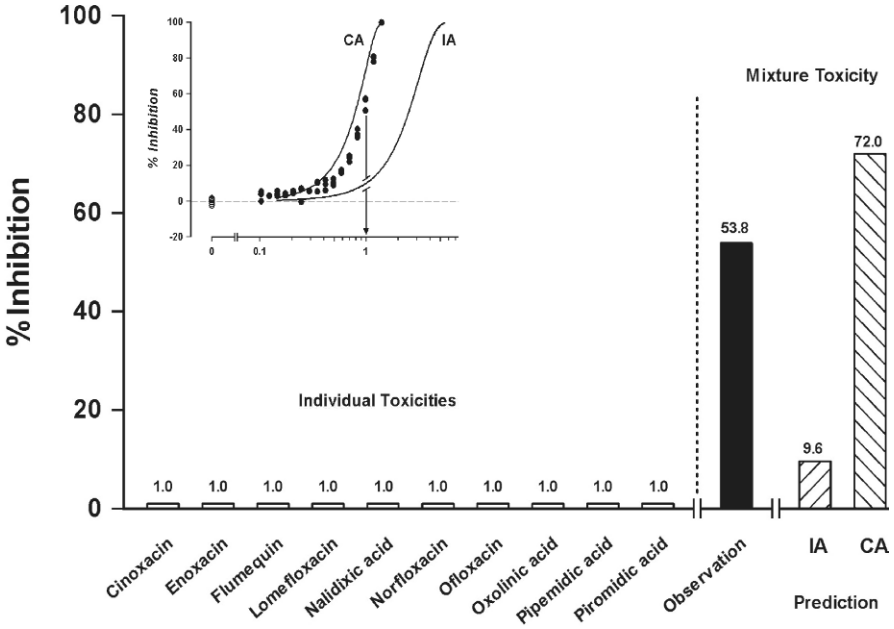

Fig. 16.1. Observed and predicted mixture toxicity of a ten-component mixture of quinolone antibiotics. Experiments were conducted in a chronic bioluminescence-inhibition assay with the gram-negative bacterium *Vibrio fischeri* (for details see Backhaus et al. 1999). Mixture ratio: EC_{01} of the components. *CA*: predicted mixture effect/mixture toxicity by concentration addition; *IA*: predicted mixture effect/mixture toxicity by independent action (see later for a discussion of these concepts). *Inset*: comparison of predicted concentration response curves with experimental observations. *Vertical arrow* indicates the mixture concentration at which every component was present at exactly its EC_{01} (0.95 μmol l^{-1}). *Main figure*: Comparison of the predicted mixture effects for this concentration with the experimentally observed mixture effect and the underlying single substance effects. It should be noted that each individual EC_{01} concentration is well below the corresponding NOEC

lar patterns for multi-component mixtures of herbicides (Faust et al. 2001, 2003), priority pollutants (Walter et al. 2002) and endocrine disrupters (Silva et al. 2002; Rajapakse et al. 2002; Brian et al. 2005).

Even mixtures of only comparatively few compounds often show a similar pattern. A mixture of fluoxetine and clofibric acid killed more than 50% of a water-flea (*Daphnia*) population after an exposure of six days, although the components were present at concentrations that did not provoke significant effects individually (Flaherty and Dodson 2005). In the same study, a significant shift in sex ratio was observed after an exposure to a three-component mixture of erythromycin, triclosan and trimethoprim – again at a mixture concentration at which all components were present at concentrations that did not provoke significant individual effects.

Binary combinations of clofibric acid and carbamazepine as well as diclofenac and ibuprofen show clear mixture effects in acute *Daphnia* tests, although each individual component was present in a concentration below its individual no observed effect concentration (NOEC) (Cleuvers 2003).

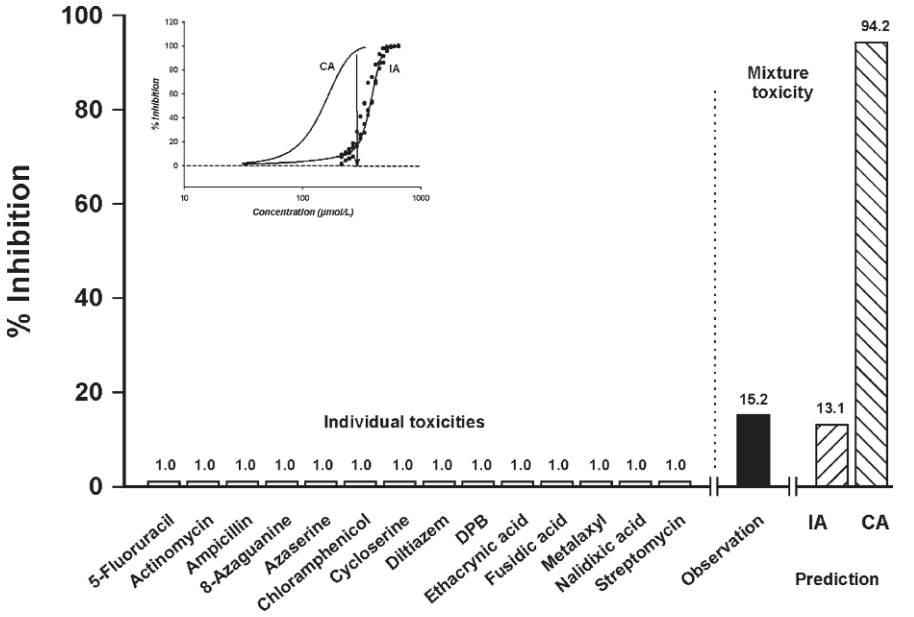

Fig. 16.2. Observed and predicted mixture toxicity of a fourteen-compound mixture (12 pharmaceuticals + dodecylpyridiniumbromide (DPB) and actinomycin). Experiments were conducted in a chronic bioluminescence-inhibition assay with the gram-negative bacterium *Vibrio fischeri* (for details see Backhaus et al. 2000). Mixture ratio: EC_{01} of the components. *CA*: predicted mixture effect/mixture toxicity by concentration addition; *IA*: predicted mixture effect/mixture toxicity by independent action (see later for a discussion of these concepts). *Inset*: Comparison of predicted concentration response curves with experimental observations. *Vertical arrow* indicates the mixture concentration at which every component was present at exactly its EC_{01} (0.95 µmol l^{-1}). *Main figure*: Comparison of the predicted mixture effects for this concentration with the experimentally observed mixture effect and the underlying single substance effects. It should be noted that each EC_{01} concentration is well below the corresponding NOEC

Finally, if trimethoprim was present at its NOEC concentration, the concentration-response curve of sulfamethoxazole and sulfadiazine was shifted by a factor of 4–5 towards higher toxicities in a recent study with unicellular algae (Eguchi et al. 2004).

In view of this evidence, it has to be concluded that recording the ecotoxicity of individual pharmaceuticals is a vital first step – but insufficient alone to assess the environmental risk of pharmaceuticals. Mixture effects have to be taken into consideration. This holds especially true as the compliance with environmental quality targets of individual pharmaceuticals does not safeguard against mixture effects because of the low-dose behavior of chemical mixtures.

16.2 Approaches for Studying Mixture Toxicities

Developing a conceptually sound and empirically well underpinned understanding of the ecotoxicity of pharmaceutical mixtures is a task of major importance in order to

1. facilitate the assessment whether and to what extent a given or anticipated exposure situation poses an environmental risk and determine those compounds that are the main causes for the observed toxicity (*retrospective assessment*), and to
2. establish environmental quality targets that adequately consider mixture effects (*prospective assessment*).

Depending on the actual aim, different methodologies can be applied, each with specific pros and cons. They can be divided into two major classes: whole-mixture tests and component-based approaches.

16.2.1 Whole-mixture Tests of Pharmaceutical Mixtures

A chemical mixture can be directly tested – simply as if it was a single chemical. Such a whole-mixture biotesting of pharmaceuticals can either be conducted using complex environmental samples (Schallenberg and Armstrong 2004) or laboratory-generated mixtures (Han et al. 2006; Wilson et al. 2004; Fent et al. 2006; Eguchi et al. 2004; Brain et al. 2004; Richards et al. 2004; Esacher et al. 2005; Cleuvers 2003, 2004, 2005; Christensen et al. 2006, 2007; Pomati et al. 2006; Borgmann et al. 2007). In the latter case, arbitrary exposure scenarios can be simulated and tested. Whole-mixture approaches are also often combined with component-based modeling approaches in order to verify the quality of the applied mixture concepts (see below).

A recent example of the whole-mixture approach for a mixture of pharmaceuticals is the study by Pomati and coworkers, in which the effects of a mixture of thirteen human pharmaceuticals to human embryonic cells were analyzed (Pomati et al. 2006). At assumed environmental exposure levels, cell growth was significantly inhibited. Results from more ecologically oriented studies can be found in a series of publications from the University of Guelph which describe the ecotoxicology of various pharmaceutical mixtures in aquatic microcosms (Wilson et al. 2004; Brain et al. 2004; Richards et al. 2004). For example, the impact of a mixture of four tetracyclines on plankton structure and function was documented by Wilson and coworkers (Wilson

et al. 2004). Effects on algal communities were observed only in concentrations greater then 200 nmol l^{-1}, which is actually well above environmentally realistic concentrations. Zooplankton was not affected significantly at the tested concentrations. However, it should be pointed out that the effects on the bacterial populations in the microcosms were not recorded, although these organisms are vastly more sensitive to tetracyclines than algae. For example, an EC_{50} of 4 nmol l^{-1} chlorotetracycline has been determined in a simple single species assay with *Nitrosomonas* (Halling-Sørensen 2001).

Borgmann and coworkers analyzed the effects of a seven compound pharmaceutical mixture on the amphipod *Hyalella* (Borgmann et al. 2007). At environmentally realistic concentrations, a significant change in sex ratio as well as small, non-significant reductions in survival and number of offspring was observed. In order to maximize the number of replicates and hence the statistical power, only one mixture concentration was tested. Hence, the study does not allow mixture NOECs to be estimated or any margin of safety to be determined.

The direct ecotoxicological testing of a given chemical mixture closely resembles the assessment of individual chemicals and therefore does not require mixture-specific methodologies. Nevertheless, when applying regression techniques for concentration-response analysis, the mixture ratio requires special attention. In a situation in which the ratio is at least approximately constant over the tested concentrations, standard procedures can be applied. However, if there are major changes in the mixture ratio between the tested mixture concentrations – either due to different degradation kinetics of the individual components in experiments with prolonged exposure or due to the specific design of the experiment (e.g., Brain et al. 2004) – regression techniques are of only limited use. Under these circumstances, the EC_{50} values that result from the interpolation between tested concentrations are extremely difficult to assess, as it is unclear which specific mixture composition lead to the assumed 50% effect.

The joint ecotoxicity of a complex environmental sample can in principle be assessed without knowing its chemical composition. For example, a study by M. Schallenberg and A. Armstrong investigated the effects of water from a drainage area that was supposedly contaminated by a mixture of veterinary antibiotics on the bacterial community of a supposedly uncontaminated lake (Schallenberg and Armstrong 2004). The authors did see sporadic ecotoxic effects of the drainage water but were not able to connect them with a specific exposure towards veterinary antibiotics, as the actual contamination of the different drainage water samples was not determined. The study clearly demonstrated the limits of ecotoxicological studies that investigate complex environmental samples without analytical determination of the actual exposure situation.

The results of a whole-mixture study only allow an assessment of the mixture actually tested within the tested concentration range and with a specific number of components and a certain mixture ratio. There is, however, a considerable dynamic in the number of pollutants and their concentrations in the environment, resulting in a virtually unlimited number of actual environmental mixtures. Hence, the application of whole mixture tests for prospective studies is limited. Comparing results from a whole-mixture study in terms of a Predicted No Effect Concentration (PNEC) with a Predicted Environmental Concentration (PEC) implicitly assumes that both PEC and

PNEC refer to the same mixture, i.e., with an identical composition and mixture ratio. Otherwise, the resulting mixture risk quotient (PEC/PNEC ratio) only allows very limited conclusions (Brain et al. 2004).

Furthermore, the contribution of the individual compounds to the observed mixture toxicity and their specific interactions cannot be inferred from a whole-mixture study alone. For example, in a study by Richards and coworkers, strong and unexpected fish mortalities were observed after exposure to a three-component mixture of fluoxetine, ibuprofen and ciprofloxacin (Richards et al. 2004). Although the authors hypothesize that it could be either an unexpected high single substance toxicity of fluoxetine or synergistic mixture effects, the actual reasons for the observed high mixture toxicity remain to be elucidated. Similarly, in the study by Pomati et al. it remained unclear how cyclophosphamide – a component in the test mixture which actually stimulated cell growth if applied singly – affected the overall growth-inhibiting effects of the mixture (Pomati et al. 2006).

16.2.2 Component-Based Approaches

In view of these limitations, several methods have been developed that are based on developing a quantitative relationship between the chemical composition of a mixture and the individual toxicities of its constituents on the one hand and the overall toxicity of the mixture on the other hand. These approaches are often summarized as "component-based" or "bottom-up" approaches and can be roughly grouped into three classes:

1. *Physiologically based pharmacokinetic/pharmakodynamic (PBPK/PD) models*

PBPK/PD models aim at precisely modeling the pharmacokinetic and pharmacodynamic behavior of each component in an organism, in order to finally develop expectations on their joint toxicity. This methodology mathematically describes the specific uptake, distribution and receptor binding of chemicals and their mixtures in an organism. Hence, PBPK/PD models are always specifically tailored to a particular organism and require detailed knowledge on its physiology, such as for example the exposed body surface or ventilation rate. Also specific data on the involved mixture components are needed, such as partitioning coefficients and metabolic rate constants. This approach hence has considerable demands in terms of input parameters. For example, Krishnan and coworker list some forty-five parameters that build up this type of model (Krishnan et al. 1994). PBPK/PD models have been applied in only a few animal test systems (such as rodents) and usually with the aim of human health oriented hazard and risk assessment (Yang et al. 1995a,b, 1998). They are unlikely to be useful for the assessment of the ecotoxicological effects of mixtures of pharmaceuticals in the foreseeable future.

2. *Empirical models*

Purely empirical models are at the other extreme in terms of a priori demands on biological knowledge. They empirically correlate the concentrations of the individual

components' effects with the effects of a mixture, yielding an $n + 1$ dimensional hyperplane, with n being the number of mixture components. For binary combinations, the hyperplane is a three-dimensional concentration-response surface, while the hyperplanes of multi-component mixtures are beyond simple visualisation.

Without any expectation of the shape of the hyperplane, the requirement in terms of experimental input data increases exponentially with the number of mixture components. For example, the concentration response surface of a binary mixture can be fairly accurately described with $5 \times 5 = 25$ test concentrations. However, such a five-level full factorial design would require $5^{10} = 9.8$ million test concentrations for a ten-component mixture, which is obviously completely unrealistic. Even then, conclusions would be limited to the specifically tested set of components only.

For such multi-component mixtures, screening designs are an option in which only an adequately chosen fraction of the possible combinations is actually tested, i.e., the experimental effort is focused on selected parts of the hyperplane. The major challenge is thus to identify the most important combinations to test and to skip those that are considered less important. The design that optimally balances the required experimental effort versus the achievable knowledge gain is specific for each study and study goal. Common designs include simplex-designs, D-optimal-designs and various types of screening or distance-based designs. However, whatever design is chosen, the larger the discrepancies between experimental power and number of mixture components, the rougher the final description of the mixture hyperplane.

Empirical mixture approaches are tailored towards analyzing mixture-ratio and concentration-dependent characteristics of a mixture. They are confined to the particular set of compounds that make up the test mixture, which limits their applicability in environmental research where varying mixture compositions have to be considered. The major domain of these approaches is the development of pharmaceutical products, during which the number of considered components is manageable and constant. Further details can be found, e.g., in the review by Gabrielsson et. al (2002).

3. *Concentration Addition and Independent Action: Simple, General Concepts for Predicting and Assessing Mixture Toxicities*

Concentration Addition (CA) and Independent Action (IA, also called Response Addition or Effect Multiplication) based approaches take an intermediate position between the previous two groups of component-based approaches. Of all methodologies, they have the biggest potential for ecotoxicological assessments of chemical mixtures and have been applied in both retrospective as well as prospective assessments. We will hence describe their fundamental assumptions and characteristics as well as current empirical evidence in the context of pharmaceutical mixtures in greater detail in the following section.

CA and IA are concepts that allow expected mixture toxicities to be described, depending on the toxicities of the individual compounds and their concentrations in a mixture, using simple assumptions about the similarity or dissimilarity of the mechanisms of action of the mixture components. The concepts can also be found under various other names (Faust et al. 2001) and are implemented in a diverse set of models for predicting or assessing mixture toxicities (see compilations in Boedeker et al. 1990, 1992; Berenbaum 1989; Kodell and Pounds 1991; Grimme et al. 1994).

16.2.3 Concentration Addition

CA was first formulated as a concept for a general pharmacology of pharmaceutical mixtures in two publications by the German pharmacologist Loewe in 1926/27 (Loewe 1927; Loewe and Muischnek 1926). For a mixture of n components, the concept can be mathematically expressed as the following:

$$\sum_{i=1}^{n} \frac{c_i}{\mathrm{EC}_{x,i}} = 1 \tag{16.1}$$

where c_i gives the concentration (or dose) of the i th component in a n-compound mixture, which elicits x% total effect, and $\mathrm{EC}_{x,i}$ denotes the concentration of that substance which provokes x% effect if applied singly. Every fraction $c_i/\mathrm{EC}_{x,i}$ – also termed a "toxic unit" – gives the concentration of a compound in the mixture scaled for its relative potency. If the sum of the toxic units of the mixture components equals 1 at a mixture concentration that provokes exactly x% effect, the mixture behaves according to CA. Under these circumstances and as long as the concerned toxic unit remains unchanged, any given mixture component can be exchanged by another chemical without changing the overall mixture toxicity. This behavior is assumed typical for compounds that share the same receptor, i.e., have a similar pharmacological mechanism of action. Hence, CA is thought to describe the toxicity of mixtures composed of similarly acting chemicals.

Equation 16.1 is equivalent to (Altenburger et al. 2000):

$$\mathrm{EC}_{x,\mathrm{mix}} = \sum_{i=1}^{n} \frac{p_i}{\mathrm{EC}_{x,i}} \tag{16.2}$$

Here p_i denotes the relative fraction of chemical i in the mixture, i.e., $\Sigma p_i = 1$.

Equation 16.2 allows the direct calculation (prediction) of an EC_x-value for the mixture based on the relative proportions of each of the mixture components as well as their individual toxicities. It should be noted here that CA does not directly allow the expected effect from a certain mixture concentration, $\mathrm{EC}_{\mathrm{mix}}$, to be calculated. Iterative approaches are needed for this purpose.

It should be pointed out that the general formulation of CA in Eqs 16.1 and 16.2 neither assumes any specific shape of the concentration-response curves of the components nor any "parallelism" between the curves. Even if all chemicals in a mixture share an identical receptor binding site, differences (e.g., in the toxicokinetic behavior of the substances) might lead to concentration-response curves that are not parallel and have effect-level dependent potency factors between the individual concentration-response curves. This might especially occur if the responses of the exposed organisms are observed on a higher, integrating level, such as reproduction. Also the biometrical description of the individual concentration-response data might exert an influence on the observed parallelism. If all of the component's curves are described by only one, inflexible model (e.g., the classic Probit model), the resulting curves might

appear to be more dose-parallel than they would appear after a biometrical analysis that uses more flexible models or even different models for different components (Scholze et al. 2001).

From a mathematical perspective (see Eq. 16.1), CA simply represents the weighted harmonic mean of the individual EC_x-values, with the weights just being the fractions p_i of the components in the mixture.

16.2.4 Independent Action

In contrast to CA, the alternative concept of IA assumes that the mixture components act dissimilarly (Bliss 1939). IA can be mathematically formulated as

$$E(c_{\text{mix}}) = 1 - \prod_{i=1} (1 - E(c_i)) \tag{16.3}$$

where $E(c_{\text{mix}})$ denotes the effect that the total mixture at a concentration $C_{\text{mix}} = \Sigma c_i$ provokes and $E(c_i)$ are the effects that the individual components would cause if applied singly at that concentration at which they are present in the mixture. Equation 16.3 shows that IA simply follows the statistical concept of independent random events (Bliss 1939).

Equation 16.3 allows the direct calculation of the mixture effect that is expected as a result from an exposure to a mixture of chemicals. However, the expected EC_x values (e.g., the EC_{50}) cannot directly be calculated. Iterative methods have to be applied for this purpose (Backhaus et al. 2000).

16.2.5 Input Requirements

The two concepts are only applicable to mixtures of known composition since they require knowledge on the toxicities of each mixture component. However, they operate in principally different ways. IA uses single substance effects, $E(c_i)$, for predicting a mixture effect, $E(c_{\text{mix}})$, while CA is based on effect concentrations (Ec_x values) and predicts an effect concentration of the mixture, $EC_x(\text{mix})$. EC_x values are the result of a concentration-response analysis. Hence, at least a considerable part of the concentration-response curves for all mixture components needs to be at hand for the application of CA. Concentration-response curves also allow the calculation of individual $E(c_i)$-values and therefore also provide the necessary input data for an application of IA. However, in contrast to CA, IA does not rely on concentration-response curves. It can also make use of single experimentally observed effect values as input data, although the robustness of such values has to be critically evaluated.

The predictive power of a concept obviously cannot be better then the quality of the underlying single substance concentration-response curves. If only one, inflexible model (e.g., the classic Probit model) had been used for their determination, the resulting curves might be biased, especially in the low- or high-effect region. This bias will be carried over and impact the mixture toxicity predictions (see examples and discussion in Cleuvers 2004, 2005; Scholze et al. 2001). It might therefore be necessary

to use models that are more flexible and perhaps also employ different models for the different mixture components (Scholze et al. 2001).

Both concepts make use of toxicity data on each individual substance. Therefore, the overall data requirements for calculating CA- or IA-expected mixture toxicities obviously increase with an increasing number of mixture components. However, a major advantage of the CA-concept is that the information needed per component is constant and does not depend on either the mixture ratio or on the number of chemicals in the mixture. If, for example, the EC_{50} of a mixture is to be estimated, the EC_{50} for each component has to be available (see Eq. 16.2). These values are the necessary and sufficient input values, independently of whether a binary or a fifty-component mixture is analyzed. This is in sharp contrast to IA, for which the needed input information changes with the number of mixture components as well as the mixture ratio. Imagine, for example, two components of which each provokes a 29% effect when applied singly at a certain concentration. When combined to a binary mixture, IA predicts a 50% combined effect. In a ten-component mixture, however, each component needs to be present only at a concentration that would give 6.7% individual effect in order to provoke the same mixture effect of 50%. That is, when more and more compounds are present in the mixture, lower and lower individual $E(c_i)$s need to be estimated in order to be able to calculate a 50% mixture effect. This is a serious drawback of IA, as it tremendously increases the input requirements when dealing with multicomponent mixtures.

CA always operates on a common effect level, the x in Eqs. 16.1 and 16.2. For example, for calculating the EC_{50} of a mixture, the EC_{50}s of all components have to be available. Hence, CA does not allow the EC_{50} of a mixture to be calculated that is composed of substances which individually only show low biological activity (e.g., for which individual EC_{50}s are beyond water solubility). To overcome this limitation, Fent and coworkers extrapolated from the observed small individual effects of the mixture components to high effects, which were experimentally not observed (Fent et al. 2006). One should be aware, however, that such an extrapolation introduces additional uncertainty in the prediction that is hard to quantify and thus might influence the final assessment.

16.2.6 Synergism and Antagonism

The terms "synergism" and "antagonism" refer to situations where the observed mixture toxicity is higher or lower, respectively, than an expected joint action. Hence, those terms can only be used with a clear reference to the particular concept used for formulating the expected mixture toxicity. It also follows that from the (non)-compliance of observed data with the predictions, no strict inferences on the principal accuracy of the applied concept is possible (the so-called "assessment dilemma", Boedeker et al. 1992). For example, in Fig. 16.2 it is shown that the mixture toxicity of the fourteen compounds is better estimated by IA than by CA. On this basis, it can be either concluded that the mixture components have a dissimilar mechanism of action – or that they have a similar mechanism of action and behave antagonistically in relation to CA. Only in connection with the fact that experimental data follow the IA-predictions over the complete curves and using the available mechanistic background informa-

tion on the mixture components was it concluded that indeed the assumption of a truly independent action seems to hold. Such decisions can obviously become rather problematic if the observed mixture toxicities are not in firm agreement with either concept and/or if the information on the modes and mechanisms of action in the exposed organisms is incomplete.

16.3 Empirical Evidence on the Predictive Power of CA and IA

Current empirical knowledge unanimously shows that the toxicity of mixtures that are composed of pharmaceuticals for which a similar mode or mechanism of action has been described in the target organisms can be predicted by CA. Figure 16.1 gives one example of the precise predictions that CA provided for the toxicity of ten-component quinolone mixtures (Backhaus et al. 1999). A similarly high predictive power of CA was also observed by M. Cleuvers for mixtures of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen and acetylsalicylic acid in a study with daphnids and algae (Cleuvers 2004), as well as for mixtures of the β-blockers propranolol, atenolol and metoproplol (Cleuvers 2005). Also, studies with binary mixtures of selective serotonin reuptake inhibitors citalopram, fluoxetine, fuvoxamine, paroxetine and sertraline did not find any significant deviations from CA-expected mixture toxicities in studies with algae and daphnids (Christensen et al. 2007). Estrogenic mixture effects of furosemide and 17β-estradiol as well as furosemide and phenazone followed CA expectations closely in a study by Fent and workers, employing the yeast estrogen screen (Fent et al. 2006), although small effect-level-dependent deviations were observed. In the same studies, deviations from CA expectations were observed for several mixtures containing pharmaceuticals of only low individual toxicity. These deviations were at least partly attributable to the fact that the CA-calculations largely relied on extrapolations (see discussion above and in Fent et al. 2006). Finally, even in a multi-species test with sewage sludge bacteria, the toxicity of a binary mixture of the two quinolone antibiotics oxolinic acid and flumequine followed the predictions made by CA (Christensen et al. 2006).

Comparatively few studies with mixtures of dissimilar pharmaceuticals have been documented in the scientific literature. The results from the only multi-component study that we are aware of with strictly dissimilarly acting pharmaceuticals are given in Fig. 16.2. IA predicted the mixture toxicity very well over the whole range of tested concentrations and mixture ratios (Backhaus et al. 2000). An algal toxicity study with the five dissimilar pharmaceuticals propranolol, sulfamethoxazole, ethinylestradiol (EE2), diclofenac, ibuprofen, and the herbicide diuron resulted in a mixture toxicity that followed IA expectations in the lower tested concentration range and CA in the region of higher concentrations (Escher et al. 2005). As four of the components (sulfamethoxazole, EE2, diclofenac, ibuprofen) were classified as acting primarily as baseline toxicants in algae and hence sharing an identical mode of action, a two-stage prediction combining CA and IA according to (Junghans 2004) improved mixture toxicity predictions.

Studies with binary mixtures of dissimilar pharmaceuticals provide a somewhat heterogeneous picture. While the toxicity of a binary mixture of clofibric acid and carbamazepine to algae was indeed predictable by IA, the effects of the same mixture

to daphnids could be better described by CA (Cleuvers 2003). A mixture of diclofenac and ibuprofen was slightly more toxic to daphnids than predicted by both of the concepts, while it had an intermediate toxicity to algae (Cleuvers 2003). The toxicity of binary mixtures of oxytetracycline + florfenicol, oxytetracycline + oxolinic acid and erythromycin + florfenicol could be described by IA in an assay with activated sludge microorganisms (christensen et al. 2006). However, in the same study, clear synergistic effects to algae were observed for mixtures of oxytetracycline + erythromycin and florfenicol + erythromycin. In these cases both CA as well as IA underestimated the observed mixture toxicity. Several other binary mixtures showed a joint action somewhere between CA and IA.

This heterogeneous pattern could point to misclassifications of the modes of action of the mixture components in some of the test organisms, as the assessment of the components' (dis)similarity is largely based on argumentation by analogy from knowledge in the target organisms or QSAR approaches that have not been validated for pharmaceuticals so far.

However, the results could also indicate interactions between the mixture components. Chemical as well as pharmacokinetic interactions between the components can lead to higher or lower mixture effects than expected from conceptual predictions. In a multi-component mixture, a plethora of interactions might occur, shifting the overall joint toxicity in both directions – and thus ultimately canceling each other out. This might be the reason why the predictive power of CA and IA seems to be higher for multi-component mixtures than it is for mixtures of comparatively few compounds. This pattern has also been observed for mixtures of narcotic chemicals and pesticides (Warne and Hawker 1995).

It should be noted that empirical evidence on the capability of CA and IA to accurately predict the toxicities of multi-component pharmaceutical mixtures is currently scarce, and in the documented multi-component studies the mixture ratios were adjusted to the toxicities of the individual components. Hence, no single component dominated the mixture, which might very well be the case for environmentally realistic mixtures, as has already been demonstrated for herbicide mixtures (Junghans et al. 2006). Binary interactions might then lead to deviations from the conceptual expectations, if they occur between the most important components.

A quantification of the documented deviations between CA-predicted and observed mixture toxicities is hampered by the multitude of different data analyses, aggregations, visualizations and documentation gaps in the different publications. Nevertheless, it can be preliminary concluded that mixture toxicities much higher than predicted – which would be most dangerous from an environmental risk assessment perspective – have not been recorded yet. The ratio between predicted and observed effect concentrations (e.g., EC_{50}-values) always seem to be lower than a factor of 5, with the vast majority of studies showing a clearly lower ratio.

Neither CA nor IA make any assumption on the targeted biological system nor do they consider any specific properties of mixture components beyond their pharmacological (dis)similarity. This is both a strength and a weakness of the concepts. On the one hand, this simplicity allows general rules for mixture toxicity assessment to be established, which is essential for considering the joint action of chemicals in regulatory guidelines. On the other hand, it cannot be assumed that these concepts actually describe biological reality, except perhaps in biologically very simple systems. Even

if all components of a mixture are similarly or dissimilarly acting, factors such as additional (unspecific) binding sites, differences in toxicokinetics and/or biotransformation pathways will interfere with the predictions and the toxicity of the mixture. Hence, assuming an appropriate experimental power in terms of accuracy and precision, differences between CA- or IA-expectations and the mixture toxicity that is actually observed will always become apparent. From a chemical risk assessment perspective, the crucial question therefore might not be whether deviations between simple concepts and complex biological realities can be observed, but whether CA and/or IA are oversimplistic. That is, can their predictive power be classified as sufficient or are size and direction of deviations unacceptable?

16.3.1 Should Mixture Effects be Generally Expected from Low-Effect Concentrations of Individual Pharmaceuticals?

As already mentioned in the introduction, a range of published mixture studies concluded that even low-effect concentrations of individual pharmaceuticals may lead to severe mixture toxicities (Backhaus et al. 1999, 2000; Flaherty and Dodson 2005; Cleuvers 2003). This does not necessarily imply synergistic effects, since this pattern actually is in concordance with the expectations from CA and IA.

According to CA, *every* toxicant that is present in the mixture contributes to the overall toxicity, in direct proportion to its toxic unit (Eqs. 16.1 and 16.2). In contrast, IA implies that only those components contribute to an overall toxicity that are present in the mixture at a concentration whose effect – if that concentration would have been applied singly – is greater than zero (Eq. 16.3). This implies that in the case of an IA-compliant mixture, i.e., a mixture of dissimilar pharmaceuticals, it would be sufficient to keep all individual compounds below their individual No Effect Concentrations (NECs) in order to safeguard against mixture toxicities.

This characteristic of IA has often been mistaken to mean that no mixture toxicities are to be expected if all components are present at concentrations below their individual No Observed Effect Concentrations (NOECs) (Cleuvers 2005). However, these values are defined as the highest test concentration where the response of the exposed organisms cannot be significantly distinguished from the response of untreated control organisms. Hence, a NOEC is based on the statistical failure to detect an effect – which does not imply that there is no effect in reality. That is, "No *Observed* Effect Concentrations" are no "No Effect Concentrations." In fact, NOECs can correspond to effect levels as high as 10%-30% (Moore and Caux 1997). Hence, from a mixture perspective, also for an IA-compliant mixture, combination effects are to be expected even if all components are present at or below their individual NOEC values – as long as enough components are present in the mixture. Figure 16.2 demonstrates this phenomenon for a mixture of fourteen dissimilarly acting compounds. The same pattern was also demonstrated by Faust et al. (2003) as well as Walter et al. (2002).

Especially from a mixture perspective, NOECs thus never describe an environmentally "safe" concentration. Whether certain fractions of individual NOECs – such as PNECs, which are based on NOECs divided by an assessment factor – are environmentally acceptable from a mixture perspective depends on the specific exposure situation and particularly on the number of involved components.

16.4 CA and IA in the Context of Risk Assessment of Pharmaceutical Mixtures

CA and IA can only be applied to mixtures of known chemical composition. Their main use might therefore be the definition of environmental quality targets, as instruments to indicate what pharmaceutical mixtures we are willing to tolerate e.g., in an effluent or in a river system. The concepts might also be useful tools in retrospective assessments, as they can be employed to determine the contribution of analytically determined pharmaceuticals to the total toxicity of an environmental sample, e.g., in the context of whole effluent toxicity testing.

In this context, a major question concerns how to choose between the two competing concepts for a particular mixture assessment. Existing experimental evidence indicates that the similarity or dissimilarity of the molecular mechanisms of action is a valid criterion. However, selecting the most appropriate concept for each particular mixture of interest leads to a multitude of practical problems. First of all, our mechanistic knowledge on pharmaceuticals is still very scarce from an environmental perspective. Although information on their human pharmacology and toxicology is usually substantial, it is to be expected that the compounds will show new "ecotoxicological" modes of action as soon as they enter the environment and come into contact with the huge variety of organisms inhabiting any given environmental compartment.

Pharmaceuticals might also have multiple mechanisms of action in the environment. Furthermore, their mechanisms of action will often be different for different exposed organisms and will depend on the analyzed biological endpoints. EE2, for example, is obviously a highly specific estrogen in fish but was classified as a baseline toxicant in algae (Escher et al. 2005). Taken together, these issues make the case-by-case decision between CA and IA extremely difficult.

If – due to the lack of available scientific knowledge, a specific mixture composition or resources for a particular study – it is not possible to rigorously select "the correct" concept, how can we at least roughly approximate mixture effects? Under the assumption that there are no strong interactions between the mixture components (see discussion below), the most promising possibility would be to use CA, disregarding the question of (dis)similarity of the components that make up the mixture in question.

Using CA as a default approach is preferable over the use of IA for two reasons: (*i*) the demands of CA in terms of input data are lower than for IA, especially for multicomponent mixtures (see above) and (*ii*) CA usually predicts the higher mixture toxicity. That is, the application of IA would risk an underestimation of the actual mixture toxicity, if the concept is applied to a mixture of pharmaceuticals that in fact have a largely identical mode or mechanism of action. On the other hand, using CA for estimating the toxicity of a mixture of actually dissimilarly acting pharmaceuticals runs the risk of an overestimation of the real mixture toxicity, which is in concordance with the precautionary principle ("better safe than sorry")?

However, using CA as a default approach is only justifiable if on average only minor errors are to be expected when the concept is used for mixtures that are not solely composed of similarly acting compounds. It has been proven that relevant differences between both IA and CA predictions may occur only when the mixture contains a

considerably large number of mixture components that all have rather steep or flat concentration-response relationships (Junghans et al. 2006; Drescher and Boedeker 1995; Faust 1999). Consequently, in all available studies that comparatively assessed both predictions, only minor differences in terms of EC_{50}-values between the CA- and IA-predicted concentration-response curves have been observed, with CA typically predicting slightly higher toxicities.

In view of this empirical evidence, we therefore suggest starting a hazard or risk assessment of pharmaceutical mixtures with the expectation of a CA-concordant behavior as the "null hypothesis." If supporting experiments can be conducted, they could then be tailored towards refuting this hypothesis, analyzing critical confounders and establishing the direction and magnitude of expectable deviations.

16.5 Regulation and Management

In view of the widespread occurrence of pharmaceuticals in all major environmental compartments and their inherent high biological activity, it is not surprising that stakeholders from governments, industries and academia rank those compounds among the top five surface and groundwater contaminants that need additional management in the U.S. and Europe (Doerr-MacEwen and Haight 2006). Mixture effects have been named by the interviewees as one of the major sources of uncertainty, hampering appropriate management strategies. It has even been suggested by O'Brian and Dietrich (2004) that the issue is so complex that it might be more economic to simply modernize existing sewage treatment plants in order to prevent the entry of pharmaceutical mixtures into the environment in the first place. Although we agree with the commendable goal to modernize STPs to prevent environmental pollution with pharmaceuticals as much as reasonably possible, we feel that this line of reasoning falls too short, as STPs will never be able to completely eliminate pharmaceuticals from effluents. Systematic knowledge on the joint action of pharmaceuticals will hence remain critical to define STP clean-up goals.

Currently the joint action of pharmaceuticals is not considered within European regulatory documents on pharmaceuticals. Even more importantly, regulatory documents that specifically tackle the assessment of mixture toxicities are still missing in Europe, in contrast to the US system where specific guidelines for mixture toxicity assessment are available at least for human-health oriented risk assessments (US EPA 2000, 2002).

It is far from clear how the consideration of mixture effects could be implemented within current environmental risk assessment frameworks for the market authorization of pharmaceuticals. Right now, applicants are only obliged to estimate the environmental risk of "their own" pharmaceutical. Structures that facilitate or even obligatorily demand cooperation between applicants whose pharmaceuticals are expected to co-occur in the same environmental compartment are missing.

The situation is further complicated by the fact that as soon as mixture effects are considered, the estimated risk would always be dependent on the current market (exposure) situation. This is in sharp contrast to current procedures in which the environmental risk of a pharmaceutical is determined in an artificial setting in which it is assumed that the assessed substance is the only present toxicant.

According to EU directives 2001/83/EC and 81/852/EEC and their amendments, all new pharmaceuticals have to undergo an environmental risk assessment before they are introduced to the market. According to the current guidance documents by the European Agency for the Evaluation of Medicinal Products (EMEA), market authorization is granted without an ecotoxicological assessment if the expected environmental concentrations are below set thresholds (Predicted environmental concentrations (PEC) in the aquatic environment below 0.01 $\mu g\ l^{-1}$ for human pharmaceuticals, PEC below 10 $\mu g\ kg^{-1}$ in soil or 0.1 $\mu g\ l^{-1}$ in groundwater for veterinary medicines) (EMEA 1998, 2006).

Especially for mixtures of similarly acting substances, such a priori cut-off values might not be sufficiently protective against possible mixture effects. All available evidence shows that the total toxicant load (total sum of toxic units) matters in such a case, not the individual concentrations. For mixtures of dissimilarly acting substances the picture is less clear, but even for this mixture type, pronounced mixture effects from an exposure to low concentrations of individual pharmaceuticals can be expected.

16.6 Open Questions, Challenges, Recommendations

The awareness of pharmaceuticals as environmental pollutants has emerged only during the last decade. Therefore, information on actual occurrences of pharmaceutical mixtures in the various environmental compartments in terms of mixture composition and concentrations of the individual components is still limited. Also, concentration-response relationships and critical concentrations at which pharmaceutical exert ecotoxic effects (EC_{50}-values, NOECs) still need to be recorded for the majority of pharmaceuticals and organisms.

Mixtures of pharmaceuticals pose even further challenges. As pharmaceuticals are constantly produced and consumed for treating a broad range of illnesses, diseases and infections in humans and animals, it can be assumed that environmentally realistic pharmaceutical mixtures usually contain substances with a wide range of different modes and mechanisms of actions. The investigation of the joint toxicity of such mixtures requires appropriate, highly integrative endpoints that might be rather different from the chemical- and mode-of-action specific endpoints often used for the investigation of ecotoxicological effects of pharmaceuticals so far. Vitellogenin induction has, for example, proven to be a robust and reliable endpoint for the investigation of EE2 effects. However, this endpoint will be of little use for analyzing the effects of a mixture that also contains, for example, beta-blockers and SSRIs. Only endpoints such as growth or reproduction are ultimately able to capture the joint action of such mixtures.

CA and IA build a link between single substance toxicities and the toxicities of their mixtures and hence provide a framework for systematically investigating and ultimately predicting the joint action of pharmaceuticals. Both of the concepts assume that all components of the mixture provoke the same type (direction) of effect. However, this is not always the case for pharmaceuticals. In some cases, the components of the mixture might actually provoke opposite, counteracting effects. An example of such a situation can be found in the recent study by Pomati et al. (2006), where growth stimulating effects of cyclophosphamide were observed, while a mixture containing this compound severely inhibited the growth of exposed cell lines. Another example

would be mixtures of estrogens such as EE2 and anti-estrogens such as tamoxifen. So far it is unclear whether and to what extent the effects of those compounds would cancel out, how often this type of mixture actually occurs in the environment and how this special situation could be conceptualized, modeled and connected to our current thinking on the ecotoxicity of chemical mixtures.

The two concepts are inherently simple, which makes them applicable to a whole range of different mixtures and organisms. In particular, both of the concepts assume that the compounds do not interact, neither in the pharmacokinetic nor in the pharmacodynamic phase. However, a range of sometimes severe drug interactions are described in human toxicology and pharmacology, all of which would hamper the predictive power of CA and IA. For example, numerous pharmaceuticals are known to interact with cytochrome P450 enzymes, belonging to the CYP superfamily, either as inhibitors (e.g., fluoxetine, ketoconazole), substrates (e.g., verapamil, atorvastatin) or inducers (e.g., rifampin, barbiturates). As the cytochrome P450s are responsible for a large part of the Phase I biotransformation in almost all animals, plants, fungi and bacteria, it is to be expected that any interference of a compound with this enzyme system also affects the toxicity of other simultaneously present compounds by inhibiting or enhancing their metabolism.

However, these and other interactions have been primarily investigated for situations of therapeutic relevance only, i.e., dealing mostly with two- or three-compound mixtures at high concentrations of the individual substances. As of now, it is largely unclear how these observations relate to environmental situations in which exposed organisms are exposed over long periods to multi-component mixtures at low-effect concentrations of the individual components. There is hence an urgent need to systematically explore whether, how often and to what extent deviations from conceptual predictions occur in environmentally realistic settings and to unravel their mechanistic foundation. Such knowledge would facilitate developing scientifically sound as well as pragmatically useful mixture toxicity guidelines based on the classical concepts of Concentration Addition and Independent Action.

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Chronic Mixture Toxicity of Pharmaceuticals to *Daphnia* – The Example of Nonsteroidal Anti-Inflammatory Drugs

M. Cleuvers

17.1 Introduction

17.1.1 Background

The release of pharmaceuticals and their metabolites to the environment is an issue of increasing importance. This emission to the aquatic environment took place in large amounts for many years, in fact mostly as complex mixtures via the effluents of sewage treatment plants and sludge. Because the importance of this diffuse pollution of the aquatic environment has been recognized, the European Medicines Agency has recently published a guideline on the environmental risk assessment of medicinal products for human use (EMEA 2006). Nevertheless, the number of studies dealing with the effects of pharmaceutical residues on aquatic organisms and risk assessment is still limited (Henschel et al. 1997; Backhaus and Grimme 1999; Webb 2001; Cleuvers 2002, 2003; Jones et al. 2002; Ferrari et al. 2003; Schwaiger et al. 2004; Triebskorn et al. 2004), particularly regarding long-term studies. Only very few studies have observed the effects of mixtures (Silva et al. 2002; Cleuvers 2004, 2005).

Among the detected substances, nonsteroidal anti-inflammatory drugs (NSAIDs), including compounds used as analgesics, belong to one of the most important groups of pharmaceuticals worldwide, with an estimated annual production of several kilotons. In Germany, for example, 93.5 million prescriptions for these substances were written in 2002, with a transaction volume of about 1562 million Euro (Schwabe and Paffrath 2003). Additionally, some of these drugs are sold as "over the counter" (OTC-drugs) without prescription, so that actual consumption is certainly even higher. As a result of this high application rate as well as the drugs' pharmacokinetics (half-life, urinary and fecal excretion, metabolism, etc.), analgesics and anti-inflammatory drugs can reach considerable (up to $>1\ \mu g\ l^{-1}$) concentrations in the environment (Stumpf et al.1996; Ternes 1998, 2001; Ternes et al. 1998; Heberer et al. 1998, 2002). Some substances could be found in very low doses even in drinking water (Heberer et al. 2001a). Findings of diclofenac and ibuprofen have also been reported from other countries, for example, from Swiss lakes and rivers (Buser et al. 1998, 1999; Tixier et al. 2003; Tauxe-Wuersch et al. 2005), as well as from the United Kingdom (Ashton et al. 2004), Brazil (Stumpf et al. 1999), Spain (Farre et al. 2001), Greece (Heberer et al. 2001a; Koutsouba et al. 2003), and the United States (Heberer et al. 2001b), to name just a few.

Previous studies (Cleuvers 2003, 2004) revealed that acute toxicities were relatively low, with half-maximal effective concentration (EC_{50}) values obtained using *Daphnia* in the range from 68 to 166 $mg\ l^{-1}$ and from 72 to 626 $mg\ l^{-1}$ in the algal test. Thus,

acute effects of these single substances seem to be quite improbable. With EC_{50} values of 23.6 (ibuprofen), 23.8 (diclofenac) and 38.2 mg l^{-1} (naproxen), chronic ecotoxicity was somewhat higher (Cleuvers and Heinrichs 2008), but still the values are far above the concentrations detected in surface water.

Otherwise, scientists were alarmed when evidence was found that diclofenac residues in dead cattle that have been treated with diclofenac were responsible for the drastic cases of death of vultures in Pakistan, which fed on the carcasses (Oaks et al. 2004). A population decline of >95% was noted. Consequently, these vultures have been listed as critically endangered. Meanwhile diclofenac has been banned for the treatment of cattle. Moreover, it could be shown that diclofenac can affect kidney and gill integrity in trout populations in environmentally relevant concentrations (Hoeger et al. 2005). These examples make clear that we have to face the occurrence of unexpected effects.

17.1.2 Mixture Toxicity

Obviously, drug residues – like other contaminants – always occur as mixtures. Thus, it is necessary to understand the mechanisms of their joint action to predict and assess mixture toxicity, particularly if the pollution is considered to be chronic. For this purpose, ecotoxicologists use concepts originally developed by pharmacologists in the first half of the 20th century (Loewe and Muischnek 1926; Bliss 1939) to predict the toxicity of mixtures. In the present study, the concept of concentration addition (Altenburger et al. 2000; Faust et al. 2001) was applied.

Concentration addition (or LOEWE additivity; Altenburger et al. 1996) is based on the idea of a similar action of single compounds, which means in a strict sense that single substances should show the same specific interaction with a molecular target site in the observed test organism (Pöch 1993). However, it has been shown that the concept of concentration addition is also applicable to non-reactive, nonionized organic chemicals, which show no specific mode of action but whose toxicity toward aquatic species is governed by hydrophobicity (Deneer et al. 1988; Van Loon et al. 1997). The non-specific mode of action of such compounds is called narcosis or baseline toxicity (Van Leeuwen et al. 1992; Verhaar et al. 1992). The potency of a chemical to induce narcosis is entirely dependent on its hydrophobicity, generally expressed by its $\log K_{ow}$.

Mathematically, the concept of concentration addition for a mixture of n substances is described by (Berenbaum 1985, following Eq. 17.1):

$$\sum_{i=1}^{n} \frac{c_i}{\mathrm{EC}x_i} = 1 \tag{17.1}$$

where c_i represents the individual concentrations of the single substances present in a mixture with a total effect of x%, and $\mathrm{EC}x_i$ are those concentrations of the single substances that would alone cause the same effect x as observed for the mixture. According to Eq. 17.1, the effect of the mixture remains constant when one component is replaced by an equal fraction of an equally effective concentration of another compound

with the same mode of action. It is important to know that concentration addition means that substances applied at less than their individual "no observable effect concentrations" (NOECs) can nevertheless contribute to the total mixture effect.

In previous studies with daphnids and algae (Cleuvers 2004), a quantitative structure–activity relationship (QSAR) approach showed that acute effects of diclofenac, ibuprofen and naproxen are most probably due to nonpolar narcosis rather than to any other specific effect. Mixture toxicity of the compounds could be accurately predicted using the concept of concentration addition. In general, if predicted effects differ less than 20% from the measured values, the mixture toxicity is considered to follow the chosen concept.

Considering that the release of pharmaceuticals to the aquatic environment occurs ubiquitously and permanently, subsequently it was observed whether not only the acute effects, but also chronic mixture effects on *Daphnia magna* are predictable via the concept of concentration addition.

17.2 Materials and Methods

17.2.1 Test Compounds

The sodium salts of diclofenac (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid; CAS No. 15307-79-6), ibuprofen (α-methyl-4-[isobutyl]phenylacetic acid; CAS No. 31121-93-4), and naproxen ((S)-6-methoxy-α-methyl-2-naphtaleneacetic acid; CAS No. 26159-34-2) were supplied in analytical grade by Sigma-Aldrich (Taufkirchen, Germany).

For assessing mixture toxicity, a third of the calculated EC_x (with $x = 5, 10, 20, 50, 80$ and 100) of each substance, as obtained in the *Daphnia* reproduction test, was used. Provided that the substances follow the concept of concentration addition, according to Eq. 17.1 the combination effect of the mixtures should add up to a total effect of around 5, 10, 20, 50, 80, or 100%, respectively (Table 17.1).

17.2.2 *Daphnia* Reproduction Test

Daphnia tests using the water flea *Daphnia magna* Strauss were conducted according to Annex V of Directive 67/548/EEC, Part C.20, "*Daphnia magna* reproduction test," which is equivalent to OECD 211, "*Daphnia magna* reproduction test" (1998). Daphnids were bred in ADaM, a culture medium imitating natural freshwater (Klüttgen et al. 1994). Total hardness, Ca to Mg ratio and alkalinity were adjusted according to ISO 6341 (ISO 1996) resulting in a pH of 7.8 and a total hardness of 2.5 mmol l^{-1}. Experiments were run at temperatures of 20 ±1 °C and photoperiods of LD 16:8 (about 20 $\mu E\ s^{-1}\ m^{-2}$).

For the *Daphnia* reproduction test, ten daphnids individually held at each test concentration and in the control series were used. Culture volume was 100 ml. The daphnids were fed three times a week (corresponding to media changes) with a concentrated algal suspension of *Desmodesmus subspicatus* (0.1–0.2 mg C/animal/day). The test duration was twenty-one days. Offspring produced by each parent animal was

Table 17.1. $EC_x/3$-values ($mg\ l^{-1}$) of the tested drugs as obtained in *Daphnia magna* reproduction tests as a basis for the test on mixture toxicity

	Diclofenac-Na	Ibuprofen-Na	Naproxen-Na
$EC_5/3$	5.05	2.41	2.35
$EC_{10}/3$	7.11	3.43	3.59
$EC_{20}/3$	8.56	4.74	5.75
$EC_{50}/3$	11.04	7.87	12.73
$EC_{80}/3$	13.88	12.38	26.20
$EC_{100}/3$	22.64	22.97	64.18

removed and counted daily. The measured endpoint for the calculation of EC values and the NOEC was the number of living offspring produced by each parent animal.

17.3 Results

Table 17.2 shows the mortality of the adult daphnids as well as the accumulated number of neonates per surviving adult *Daphnia*. In the control and in the second treatment ($EC_{10}/3$), one *Daphnia* died, resulting in a mortality value of 10%. In the third treatment ($EC_{20}/3$), mortality was 20%, whereas no mortality occurs in the higher treatments. Thus, no dose-dependent mortality could be observed. However, a clear influence on the body length of the daphnids could be observed. Moreover, the total number of offspring decreased in a dose-dependent manner, and in the highest treatment level, reduction of offspring per adult *Daphnia* was almost 100% compared to the control.

Figure 17.1 shows the empirically measured mixture toxicity compared to the calculated values and the effects of the drugs when applied singly. The prediction was quite good, particularly at higher concentration levels. The measured effects of the mixture were much higher than the ones expected from the measured effects of the single substances. For example, at the $EC_{80}/3$ level, a simple addition of the single effects would result in an inhibition of merely 21.8%, while the measured effect was 3.6 times higher (78.3%). In the same way, at the highest concentration an addition would lead to an inhibition of about 50%, while in fact the reproduction was totally disabled.

17.4 Discussion

Pharmaceuticals are emitted permanently as complex mixtures to the aquatic environment, and their nearly ubiquitous occurrence in mostly low concentrations is well documented (Heberer 2002). Thus, we have to consider that generally chronic effects are more likely than acute effects. Moreover, tests with single substances have only a limited significance for a risk assessment, because obviously considerable combination effects can also occur if some or even all substances were applied in concentrations near or below their individual NOEC. One consequence is that the use of individual NOECs is not suitable when assessing the environmental risk of substances occurring in mixtures.

Table 17.2. Influence of the mixture of tested drugs on mortality, body length and reproduction as obtained in the *Daphnia magna* reproduction test

Concentration (EC_x/3)	Mortality (%)	Body length[a] (mm)	Accumulated number of neonates per adult *Daphnia*	Inhibition relative to control (%)
Control	10	3.15	91.9	0
EC_5/3	0	3.05[b]	76.4	16.9[b]
EC_{10}/3	10	3.01[b]	74.0	19.5[b]
EC_{20}/3	20	3.11	77.4	15.8
EC_{50}/3	0	2.91[b]	57.9	36.99[b]
EC_{80}/3	0	3.01[b]	19.9	78.34[b]
EC_{100}/3	0	2.62[b]	0.3	99.67[b]

[a] At day 21.
[b] Statistically significant effect; *t*-test, $\alpha = 0.05$, one-sided.

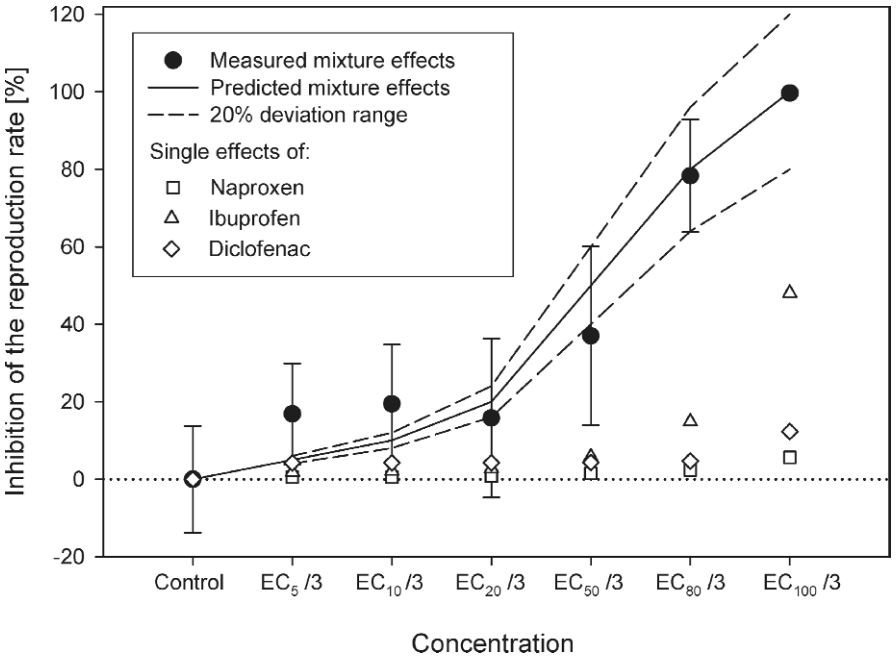

Fig. 17.1. Measured and predicted mixture effect of the observed NSAIDs compared to the effects of the single substances

In the current study, the reproduction decreased in a dose-dependent manner. In the highest treatment level, the reduction of offspring per adult *Daphnia* was almost 100% compared to the control. Remarkably, this nearly complete inhibition of repro-

duction occurred without any mortality of the adult daphnids. This means that this destructive effect on the *Daphnia* population would be totally overlooked by an acute test with the same concentrations. This implies that more data about chronic effects, e.g., on fish and benthic macroinvertebrates, like gastropods or oligochaetes, are indispensable to improve the environmental risk assessment of pharmaceuticals. Although the concentrations used in this study were at least a thousand times higher than naturally occurring concentrations, we have to consider that only three substances have been tested in this study, while mixtures of some hundred or even more substances occur in the environment. Moreover, newer studies (Hoeger et al. 2005; Triebskorn et al. 2007) revealed that effects on kidney and gill integrity in fish are already observable in environmentally relevant concentrations of 0.5 $\mu g\, l^{-1}$. Thus, we should be careful when stating that the observed environmental concentrations are safe and without measurable effects. The observed decrease in reproduction seems to be associated with a reduction in body length (see Table 17.2), but while the mean body length of the animals increased from the treatment $EC_{50}/3$ to the treatment $EC_{80}/3$, at the same time the number of neonates per adult *Daphnia* clearly decreased. Thus, there must be another mechanism responsible for the strong reduction of neonates.

All tested drugs are nonsteroidal anti-inflammatory drugs and are considered to have the same mode of action in humans. They inhibit the cyclooxygenases, the key enzymes catalyzing prostaglandin biosynthesis, which is inter alia responsible for the genesis of pain and inflammation (Vane and Botting 1998; Vane 1971). This inhibition is responsible for the analgesic and anti-inflammatory effect of these drugs. Furthermore, prostaglandins show other functions; they can cause contractions or atony of muscles in different organs with effects on blood pressure and circulation. In addition, some prostaglandins act to protect cells in the gastrointestinal tract, and their inhibition by NSAIDs with the possibility of internal bleeding is one of the most important side effects of these pharmaceuticals. There are a few publications about the occurrence of prostaglandins in non-mammalian animals (Bundy 1985; Nomura 1988) such as fish, amphibians, birds, and in invertebrates like corals, sponges, coelenterates, mollusks, crustaceans, insects as well as in marine algae and higher plants, where they are considered to play different roles, e.g., in defense mechanisms of plants. Several studies revealed that they also play an important role in reproduction, immune response and temperature regulation of insects (Stanley-Samuelson 1994; Rowley et al. 2005). Unfortunately, no literature is available about the occurrence in daphnids, so it is unclear whether the test species used in this study could exemplify a specific effect of NSAIDs. Thus, additional biotests with vertebrates like fish could be a useful tool to assess the environmental risk of nonsteroidal anti-inflammatory drugs.

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The Ecotoxicological Effects of Pharmaceuticals (Antibiotics and Antiparasiticides) in the Terrestrial Environment – a Review

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18.1 Introduction

Generally speaking, terrestrial ecotoxicology plays a minor role in the evaluation of pharmaceutical "side effects" as compared to aquatic toxicology. This is likely to be caused by the typical exposure pathways of drugs: human pharmaceuticals usually do not reach the terrestrial compartment except via the application of sewage sludge on agricultural soils. However, the situation is quite different for veterinary pharmaceuticals, which are mainly deposited in the terrestrial environment. Drugs like antibiotics or anthelmintics (also called antiparasitics or parasiticides) are regularly applied to intensively reared productive livestock (in particular cattle, swine, sheep, and horses). Residues can thus reach pasture soils directly via livestock feces, and they reach agricultural soils indirectly via the manure collected in stables (Halling-Sørensen et al. 1998).

Veterinary pharmaceuticals often act in a biocidal way, meaning that they actively impact target organisms like bacteria or invertebrates. In this respect they resemble pesticides, which are the chemicals most intensively studied both in the scientific and regulatory context. In fact, there are several examples where the same chemical is used for both purposes. Therefore, the same problems may occur after application, i.e., the respective chemical does not only affect their targets but also non-target organisms. In particular, the main classes of veterinary pharmaceuticals show such side effects (Boxall et al. 2004). Targets of parasiticides (e.g., blow flies or ascaricid roundworms) and non-targets (e.g., dung flies or saprophagous nematods) belong to the same taxonomic groups (Diptera and Nematoda, respectively). The same is true for effects of antibiotics on pathogenic germs and soil bacteria being important for nutrient cycling.

Some veterinary pharmaceuticals have such a high biocidal activity that their effects on non-targets in the soil compartment became obvious quite early. For example, the impact of parasiticides on the survival of dung beetles has been studied already in the mid-1970s (e.g., Blume et al. 1976).

The number of publications concerned with terrestrial effects of pharmaceuticals has increased recently, greatly improving our understanding of the risk of veterinary drugs. In this review, we will give an overview for parasiticides and antibiotics. Further, we provide conclusions for the risk assessment process of pharmaceuticals triggered by recent findings.

18.2 Methods

For literature searches, the SCOPUS database was used (covering about 15 000 journals, mainly in the areas of science, technology and medicine), together with the following search strategy for words in title, abstract or keywords:

- All pharmaceuticals: (ecotox* OR effects) AND (pharmaceutical* OR drug) AND (terrestri* OR soil OR feces OR manure);
- Antiparasiticides: (ecotox* OR effects) AND (antipara* OR antihelmin* OR parasiticid*) AND (terrestri* OR soil OR feces OR manure);
- Antibiotics: (ecotox* OR effects) AND (antibioti* OR antimicrobia*) AND (terrestri* OR soil OR feces OR manure).

In addition, literature not identified by these strings but known to the authors has been taken into account (about 30). However, many investigations have been pursued in a regulatory context, meaning that they are not publicly accessible or not included in literature databases.

18.3 Results and Discussion

18.3.1 Parasiticides

These compounds are used therapeutically in cattle, horses, pigs, and sheep against a wide range of parasitic worms, mites and insects to name just the most important groups. Because of the huge impact these parasites can have on the well-being of domestic animals and on the economic situation of farmers, such treatments are considered to be absolutely necessary in modern agriculture.

The information on the effects of these pharmaceuticals on non-target organisms in the terrestrial environment, in particular soil ecosystems, has been summarized and reviewed several times quite recently (Edwards et al. 2001; Boxall et al. 2004; Floate et al. 2005; Wardhaugh 2005; Kolar and Kozuh Erzen 2006). Their main conclusions can be summarized as follows:

- As chemically very different active ingredients belong to this class of drugs, no general statement on their environmental risk is possible. Instead, the respective exposure situation (e.g., way and frequency of application) in combination with the biocidal properties and persistence has to be assessed;
- Basic information about their fate (e.g., persistence or adsorption) in the environment, including manure and dung, is often missing. Data on the influence of environmental factors like temperature or soil properties on their behavior is particularly scarce;
- Toxicity varies more than six orders of magnitude: while compounds like ivermectin affect arthropods in the µg/kg (dung) to mg/kg (soil) range, others like praziquantel

act very specifically, thus showing no toxicity at environmentally relevant concentrations (up to 1 000 mg kg^{-1}). However, for many active ingredients no (or only acute) terrestrial ecotoxicity data have been published;
- The environmental subcompartment most at risk is dung originating from treated livestock because of direct exposure to non-target organisms closely related to the target species. The active ingredient or metabolites can occur in dung in high concentrations (up to several mg/kg dw). At the same time, dung and the related dung community play a central role in the nutrient cycling of pastures;
- The delay in dung decomposition is also the best (only?) example showing that effects of veterinary pharmaceuticals can cause economically important damages: Undecomposed dung pats covering pastures do impact their use for cattle grazing (i.e., pasture "fouling");
- Usually, no risk is expected from the use of these drugs during manufacturing, disposal or the treatment of pets.

However, despite the impressive amount of information compiled during the last twenty years, there are still serious gaps in the knowledge about the environmental risk of parasiticides. For example, despite early efforts to study the environmental risk of these drugs on different investigation levels (laboratory as well as directly in the field), the relevance of test results under realistic conditions is not yet clear. Secondly, most work focused on a small number of compounds, in particular ivermectin and related avermectins. In addition, results reported in the literature seem to be contradictive (e.g., there are nearly as many studies reporting no effect of ivermectin on dung degradation as those finding just the opposite). Finally, despite recently published guidelines on assessing the potential risk of new antiparasitics (VICH 2005; see last chapter), test result evaluation and higher tier testing remain an open question.

In the following, new findings since 2004 will be presented, and the open questions listed above will be addressed. The developments on the regulatory level will be addressed in a separate chapter. Due to limited space, selected topics will be highlighted.

18.3.1.1 *Exposure by Parasiticides*

Despite the wide usage of parasiticides, sparse monitoring data are available. However, in a recent study performed on farms in the United Kingdom (Boxall et al. 2005), concentrations of 0.112 (doramectin) to 1.85 (ivermectin) mg kg^{-1} dung were found. At the same sites, concentrations of these compounds in soil were significantly lower (up to 0.046 mg kg^{-1}). For dung and soil, the concentrations measured were also lower than those estimated using worst-case exposure models (VICH 2005). While there are at least some data about the fate of parasiticides used on cattle, data from sheep are much less common. In a study performed in Slovenia, it could be shown that high concentrations of abamectin and doramectin could be found in feces (0.2–0.8 mg kg^{-1} and 0.4–1.2 mg kg^{-1}, respectively) during the first twenty days after treatment, reaching concentrations of about 0.2 mg kg^{-1} after seventy and fifty days, respectively. Concentrations in soil were much lower and compounds disappeared quickly: after twenty days, no drug residues were determined (Kozuh Erzen et al. 2005).

18.3.1.2 *Effects on Dung Organisms*

A newly developed test method with the beetle *Aphodius constans* was applied to ivermectin. Cattle dung of different ages, containing different concentrations of ivermectin, as well as mixtures of highly-contaminated spiked dung with untreated control dung were studied (Lumaret et al. 2006). The LC_{50} using dung directly obtained from treated cattle ranged from 470 to 692 µg active substance (a.s.)/kg dung (dry weight; d.w.) and 67 to 97 µg a.s./kg dung (fresh weight; f.w.) (LC_{50}: lethal concentration). Using mixtures, the outcome was almost identical: 770 to 781 µg a.s./kg dung (d.w.); 109 to 132 µg/kg dung (f.w.). In comparison to the LC_{50} values obtained when ivermectin was spiked in control dung at several concentrations, the LC_{50} values were again very similar (LC_{50} 880–985 µg a.s./kg dung (d.w.)). The effects of ivermectin on ecologically relevant dung beetles obtained in a standardized test method reflect the results from field studies and are in the range of environmentally relevant concentrations.

During a long-term (ten-month) field study performed on two farms in South Africa, no effect of ivermectin or the acaricide fluazuron on local dung beetle communities (up to seventy-two species!) was found (Kryger et al. 2005). By using multivariate analysis methods it could be shown that abundance and diversity of these beetles mainly depend on non-chemical factors like climatic conditions. Unfortunately, the exposure of these beetles was not measured simultaneously, thus limiting the comparability of these results. Despite this constraint, the performance of field studies as part of the environmental risk assessment of parasiticides is favored due to their ecological realism. However, when performing such studies one must be able to assess the – often very complex – results. In addition, the test setup and statistical evaluation has to be adapted carefully in order to ascertain that ecologically relevant effects can indeed be detected (Maund et al. 1999). For example, in studies measuring the effects of ivermectin on dung degradation it was found that for several days after application, until degradation of the compound, dung organisms could not invade the dung pats (Floate 1998). Usually, such a short delay might not be considered to be relevant, but recently Lee and Wall (2006a,b) could not only identify the normal succession pattern of dung organisms in cattle pats in South England but could also prove that a delay of as little as two days following pat deposition causes a significant reduction in both the insect population size and the subsequent rate of pat degradation.

Little work has been done with dung organisms other than insects. For example, Grønvold et al. (2004) found that ivermectin and fenbendazole affect the survival of the nematode *Pristionchus maupasi* at concentrations higher than 3 mg dung/kg (w.w.) and 10–20 mg dung/kg, respectively, thus clearly higher than the concentrations occurring in the feces after routine application of these drugs.

New studies with oligochaetes have confirmed the low toxicity of avermectine parasiticides: for example, measuring several parameters describing the life history of the deep-burrowing (= anecique) *Lumbricus terrestris*, it could be shown in laboratory tests that ivermectin has no negative impacts on survival, growth or population dynamics of this very important species, which is considered to be an ecosystem engineer (Svendsen et al. 2002, 2005). The same lack of effects was also found when testing the

effects of moxidectin on another anecique species, *Aporrectodea longa*, in sheep and cattle dung (Svendsen and Baker 2002). However, while the inclusion of endpoints like the intrinsic growth rate is an important step towards ecological realism, the lack of information on the drug concentrations in the feces used considerably limits the usefulness of this study for environmental risk assessment. A similar approach focusing on population parameters has been used in tests with the collembolan *Folsomia candida*, showing that the effect of ivermectin depends inter alia on the density of the test organisms which may have consequences for environmental risk assessment (Noël et al. 2006).

Eprinomectin did not affect survival or biomass of the earthworm species *Lumbricus terrestris* in laboratory tests at concentrations up to 0.43 mg kg^{-1} dung (w.w.) or 3.3 mg kg^{-1} dung (Halley et al. 2005). This study is especially noteworthy because it is one of the very few reporting effect concentrations per wet and dry weight, thus vastly improving the comparability of these data. A relatively low LC_{50} value (17.1 mg kg^{-1} soil) – but still much higher than actual field concentrations – of avermectin B_{1A} was found in laboratory tests with the compost worm *Eisenia fetida* in artificial soil (Sun et al. 2005). This study is remarkable because it could be shown that this drug does not have the potential to be accumulated in the earthworm, thus confirming that there is no risk of secondary poisoning of predators.

18.3.1.3 *Relative Risk of Different Veterinary Drugs*

Using a newly developed test with the temperate dung beetle species *Aphodius constans*, the survival of beetle larvae was determined after being exposed to four parasiticides (ivermectin, moxidectin, dicyclanil, and praziquantel), representing different treatment regimes, modes of action and effect levels (Hempel et al. 2006). This study is noteworthy because several compounds were included that had not been studied before. The test was performed in the laboratory (duration three weeks), using fresh dung as well as formulated (dried, grounded and re-wetted) dung as test substrate. Ivermectin was the most toxic substance (LC_{50} 880–980 µg a.s./kg dung (d.w.)), followed by dicyclanil (LC_{50} 1.5–6 mg a.s./kg dung (d.w.)) and moxidectin (LC_{50} 4–5.4 mg a.s./kg dung (d.w.)), while praziquantel showed very low toxicity (LC_{50} > 1 000 mg a.s./kg dung (d.w.)). The toxicity in fresh and formulated dung differed by a factor between 1.1 and 4, which was not always significant. Using data from ivermectin and moxidectin, the test results are of the same order of magnitude as those known from other studies.

In a comparative study of fourteen parasiticides, Yoshimura et al. (2005) found huge differences in the effects of these compounds on hatching inhibition of the cricket *Gryllus bimaculatus*. For example, the results indicate that the avermectine moxidectin is by a factor of 100–200 less toxic than ivermectin and doramectin, belonging to the same chemical class. However, the exposure situation used in the test (filter paper) is very artificial, thus not allowing the extrapolation of these results to field situations.

In a series of comparative laboratory and field studies, Svendsen et al. (2002, 2003, 2005) could show that ivermectin and the benzimidazole compound fenbendazole do not affect earthworms. However, the disappearance of dung was affected by the avermectin but not by the benzimidazole compound.

18.3.1.4
Development of New Test Methods and Comparability of Test Results

Due to the fact that parasiticides can cause negative effects on dung organisms and their functions, the responsible agencies in the European Union (as well as in North America and Japan) require data on the effects of new veterinary pharmaceuticals on dung organisms as part of the environmental risk assessment process (see Phase II, Tier A of the VICH Guidance Paper 2005). Since no standard test guideline exists so far (De Knecht and Montforts 2001), the SETAC advisory group DOTTS (Dung Organism Toxicity Test Standardization) developed and validated a standard test method with two dung fly species (*Musca autumnalis, Scathophaga stercoraria*) according to the requirements of the Test Guideline Program of OECD (OECD 2006). In addition, a guidance document describing the testing of the temperate dung beetle species *Aphodius (Agrilinus) constans* as well as the Mediterranean/tropical species *Onthophagus taurus* is under development (Chapman et al. 2003). Without standard test methods it is not possible to provide comparable and scientifically sound data to the authorities responsible for the environmental risk assessment of veterinary pharmaceuticals. However, no test guidelines or guidance papers are available so far on higher tiers (i.e., semi-field or field level; see below).

18.3.1.5
Contradictive Findings

The potential influence of veterinary drugs, in particular of parasiticides like avermectins on the degradation of dung, has been studied many times, but the results are variable (e.g., Floate et al. 2005). Effects can vary in time (e.g., between years or seasons) and space (e.g., negative impacts in field studies in Australia, Canada and France; no impact in Brazil or Germany). Several possible reasons for these differences were discussed in the literature (Edwards et al. 2001), among them the influence of the respective formulations or application regimes (e.g., dung from cattle treated with sustained-release boluses is usually more toxic than other treatments) or the climatic conditions (e.g., it is known that ivermectin is more persistent in winter (DT value: 91–217 days) than in summer (DT value: 14–56 days). Lumaret et al. (2002) pointed out that the respective composition and diversity of the dung-feeding fauna may be another important factor to be considered when studying the influence of such antiparasitics on dung degradation. For example, the concentration of drugs in the dung is high enough to affect dung insects for a period of about a few weeks. The impact of such drugs certainly depends on the point in time when they are excreted, i.e., whether this excretion happens during a crucial period in the life cycle of important dung fly or beetle species. Since most of the common antiparasitics are much more toxic to arthropods, it is also important whether the dung degradation at a specific site is more influenced by sensitive beetles and flies (as at warm and dry sites like in southern Europe) or by other, less sensitive organisms like earthworms (e.g., at cool and moist sites like in Northern Europe). Under suitable site (soil type, weather) conditions, the negative impact of ivermectin on dung arthropods may even be overridden by the feeding activity of earthworms (Svendsen et al. 2003). Finally, it is not clear how much differences in the test methodology might have influenced the outcome of

degradation studies. Therefore, it is recommended to modify and standardize existing test methods for degradation of the dung itself. Further, testing the degradation of the parasiticidal compound in the dung matrix has to be standardized (Kreuzig et al. 2006). In this respect, the structure (i.e., the species composition) as well as the function (i.e., dung degradation) should be covered.

18.3.2 Antibiotics

Antibiotics are a heterogeneous group of compounds that are used against bacterial infections in farm animals, both therapeutically and prophylactically. Usage figures are scarce, but rough estimations show that about 54 mg antibiotics are consumed per kg of farm animal body mass in Europe (Ungemach 2000), based on data from FEDESA (1998). Similar to parasiticides, antibiotics can reach the terrestrial compartment via manure or excreta of treated animals.

The physicochemical fate and environmental concentrations of antibiotics in soil have been the subject of a range of recent studies. Several classes of antibiotics have been detected in field soils, and the sorption behavior and degradation has been studied to a large extent (Boxall et al. 2002; Halling-Sørensen et al. 2003; Hamscher et al. 2002, 2005). Very roughly speaking, soil concentrations of tetracycline antibiotics reach maxima of a few 100 $\mu g\ kg^{-1}$ and remain mostly stable after the manuring period, whereas concentrations of other groups of antibiotics such as sulfonamides and macrolides at the same sites were close to or below the detection limit (Hamscher et al. 2002, 2005).

In contrast, fewer publications have dealt with the effects of antibiotics on soil biota. In the following sections, recent findings on effects on different taxa will be summarized, structured by the organism and endpoint studied.

18.3.2.1 *Early Investigations: Soil Bacteria*

Effects of antibiotics on the microbial component of soil biota have already been studied relatively early on. Interestingly, in some experiments, antibiotics served as a positive control for the establishment of new methods for the effect testing of soil contaminants (Tu 1978). The selective inhibition of soil bacteria by antibiotics was also used for the study of separate groups of soil microorganisms (Ingham 1985). A first experiment on the impact of antibiotics on the soil bacterial flora was undertaken by Colinas et al. (1994). Here, forest soil was spiked with oxytetracycline and penicillin at concentrations of 10 $mg\ kg^{-1}$ d.w. It occurred that the antibiotics decreased the total cell counts as well as the counts of active bacteria by approximately 80%.

18.3.2.2 *Plant Toxicity*

There are only few studies on the effects of antibiotics on plants. Batchelder (1982) found some plant (*Phaseolus vulgaris,* pinto bean) growth retardations in response to tetracycline antibiotics. Migliore et al. (1995, 1996b) focused on growth retardation of

different plants in liquid or solidified media with antibiotics. The most pronounced effects found were growth-inhibiting effects of enrofloxacin in *Phaseolus vulgaris* at 100 µg l^{-1} (Migliore et al. 2000). In soil assays, growth-inhibiting effects of sulfadimethoxime were found on growth of barley (*Hordeum distichum* L.) and corn (*Zea mays*) at 109 mg kg^{-1} (recalculated from application of sulfadimethoxime solutions of 300 mg l^{-1}) (Migliore et al. 1996a, 1998).

In multispecies test systems, it occurred that the sulfonamide sulfachloropyridazine has the potential to exert plant toxicity at concentrations of 100 mg kg^{-1} (Boleas et al. 2005a), which might be related to their structural similarity to sulfonylurea herbicides. In some cases, effects also occurred at a very low concentration (0.01 mg kg^{-1} decreased the stalk elongation of *Triticum aestivum* after seven days, but with an inconsistent dose-response curve, which might reduce the validity of this finding). Oxytetracycline also led to a clear growth inhibition of both *Brassica napus* and *Triticum aestivum* at concentrations of 0.01 mg kg^{-1} and higher in similar MS.3 multi-species test systems co-spiked with manure (Boleas et al. 2005b). In contrast, no growth retarding effects were observed when oxytetracycline was applied without co-addition of manure, nor when doxycycline was used instead of oxytetracycline (Fernández et al. 2004).

18.3.2.3
Toxicity to Invertebrates

Most of the antibiotics studied up to date show little direct toxicity to soil invertebrates. For instance, a 10% inhibition in reproductive parameters of *Folsomia fimetaria* (Collembola, springtails) and *Enchytraeus crypticus* (Enchytraeidae) was observed at concentrations between 61 and 149 mg kg^{-1} for the antibiotics tylosine and oxytetraycline (Baguer et al. 2000), as well as tiamulin, olaquindox and metronidazole (Jensen et al. 2003).

Neither the sulfonamide sulfachloropyridazine nor oxytetracycline affected *Eisenia fetida* (Lumbricidae, earthworms) survival at concentrations up to 100 mg kg^{-1} after seven- and twenty-one-day exposure in MS.3 multispecies test systems (Boleas et al. 2005a,b). Also, spiking of manure with doxycycline (7.5 mg ml^{-1}) did not affect earthworm survival in MS.3 soil microcosms that received the spiked manure (Fernández et al. 2004). However, it cannot be ruled out that indirect effects due to changes in soil food webs might affect soil-dwelling organisms at lower concentrations.

18.3.2.4
Soil Bacteria: Substrate-Induced Respiration

Substrate-induced respiration (SIR) serves as a frequently studied functional endpoint of the soil microbial community, and a test setup based on respiration after glucose amendment has been harmonized in an international guideline, OECD 217 (OECD 2000a).

It has long been known that excessive concentrations (in the range of >1 g kg^{-1}) of antibiotics selectively inhibit the bacterial respiration in soils, which has been applied to study soil bacteria versus soil fungi (e.g., Lin and Brookes 1999).

A first investigation focusing on the effects of antibiotics on SIR was performed in 1998, where an inhibitory effect of the polyether monensin on SIR was found (Pfeiffer

et al. 1998). Vaclavik et al. (2004) studied both the degradation of antibiotics and the inhibition of soil respiration by several classes of antibiotics. They found that the total degree of inhibition of glucose-induced respiration depended on the class of antibiotics: broad spectrum antibiotics led to higher inhibition, whereas the inhibition of macrolides, which act more specifically on Gram-positive bacteria, reached only 35% at a concentration of 1 000 mg kg^{-1}. Further, the dose-response relationships deviated from the sigmoidal shape often obtained with single-species tests, pointing to a non-normal distribution of the sensitivities of soil bacteria. For oxytetracycline and sulfachloropyridazine, concentrations of 10 mg kg^{-1} led to a decrease in respiration around 40 and 20%, and slight effects were also seen at 1 mg kg^{-1}. The test setup differed from the OECD requirements, as CO_2 development was monitored over twenty-four hours instead of twelve hours.

In similar experiments, Thiele-Bruhn and Beck (2005) found a dose-dependent inhibition of substrate-induced respiration by oxytetracycline and sulfapyridine, which occurred after an incubation time of 24 h. In a loamy soil, clear inhibition occurred only after a second 24-h cycle of glucose application and respiration monitoring. Concentrations of 6.2 mg kg^{-1} (sulfapyridine) and 19.1 mg kg^{-1} (oxytetracycline) reduced the glucose-induced respiration by 50%, whereas a 10% inhibition was noted at concentrations <1 mg kg^{-1}.

Recently, it was found that sulfadiazine delays the onset of SIR by several days at a concentration of 1 mg kg^{-1} (the lowest dose studied), a finding which was supported by changes in the composition of the microbial community at that concentration. In contrast, chlorotetracycline did not affect substrate-induced respiration even at concentrations of 50 mg kg^{-1} (Zielezny et al. 2006).

An inhibitory effect of oxytetracycline which was maintained during twenty-eight days of exposure has been observed in multispecies test systems, with a maximum of 40% reduction seen at 1 000 mg kg^{-1} (Boleas et al. 2005b).

In contrast, experiments by Hund-Rinke et al. (2004) showed an inhibition of substrate-induced respiration only at a soil concentration of 500 mg kg^{-1}. The main reason for this divergence might be that in the latter publication, soils were incubated for eight weeks before performing the substrate-induced respiration assessment. During these eight weeks, the bioavailable fraction of the antibiotics may be reduced through sorption and degradation (Halling-Sørensen et al. 2002), and the soil microbial community can adapt to the antibiotic (see Sec. 18.3.2.6).

In summary, effects of antibiotics on substrate-induced respiration have been found in a number of publications, and SIR turned out to be a sensitive parameter of the soil microbial community. This is in line with the growth-inhibiting mode of action of antibiotics, which can cause clear changes in the respiration profile. Testing of microbial respiration should, however, be long enough to monitor bacterial growth.

18.3.2.5
Soil Bacteria: Soil Enzyme Activities and Nutrient Cycling

Soil enzyme activities have long been used for determining the physiological state of the soil microbiota, and more generally, soil functioning. Enzymes occur both as part of living cells and as extracellular enzymes and cannot directly be correlated with the state of soil microorganisms in the latter case.

A first indication of an inhibitory effect was found by Pfeiffer et al. (1998), who determined a dose-dependent inhibitory effect of monensin on dimethylsulfoxide reduction at 17.6 mg kg^{-1}. Thiele-Bruhn and Beck (2005) and Thiele-Bruhn (2005) have studied the effects of tetracyclines and sulfonamides on dehydrogenase activity and Fe(III) reduction. Especially the Fe(III) reduction reacted sensitively to the antibiotic amendment, with a 10% reduction seen below 100 µg kg^{-1} for sulfadiazine, sulfapyridine and sulfadimidine (Thiele-Bruhn 2005). For tetracyclines and sulfonamides, EC_{50} values were between 1.2 and 319 mg kg^{-1}, depending on the soil characteristics and the substance tested (EC_{50}: concentration leading to an effect of 50% reduction). The Fe(III) reduction test is characterized by a relatively long exposure of seven days and anaerobic conditions, which might favor the detection of inhibitory effects of antibiotics. While anaerobic conditions can occur in moist grassland soil, these conditions are less representative for mostly aerobic arable soil.

Other investigators found time and setup dependent effects. In a microcosm experiment with oxytetracycline, effects depended upon co-addition of manure and switched between a dehydrogenase activity increase at day seven and inhibition at day twenty-one, whereas dose-dependent inhibitory effects were observed with the soil used for setting up the microcosms, with an EC_{50} around 100 mg kg^{-1}. Phosphatase activities were also increased at the lower concentrations of oxytetracycline of 0.01 and 1 mg kg^{-1}, but decreased at 100 mg kg^{-1} (Boleas et al. 2005b). Similar trends were seen in MS.3 multispecies test systems spiked with sulfachloropyridazine (Boleas et al. 2005a), with an increase in dehydrogenase activity after a seven-day exposure at concentrations of 1 and 100 mg kg^{-1} as the clearest effects observed.

Up to now, few investigations have touched upon the effects of antibiotics on the nitrogen cycle. Patten et al. (1980) have found that soil nitrogen mineralization did not differ between soil microcosms spiked with manure of beef cattle receiving oxytetracycline or chlortetracycline as feed additives and microcosms with antibiotic-free manure. However, the potential of oxytetracycline to inhibit nitrification was shown in nitrifying batch reactors, where an inhibition of 50% was found in oxytetracycline concentrations between 100 and 250 mg l^{-1} (Campos et al. 2001). Interestingly, the lowest concentration tested (10 mg l^{-1}) severely changed the reactor biofilm structure, however, without affecting the nitrogen turnover.

In conclusion, soil enzymatic assays sometimes responded sensitively to antibiotic exposure, with effect concentrations that were comparable to effects seen in respiration assays. In other cases, the assays seemed to also depend on soil parameters other than the antibiotic, as seen from non-dose dependent results.

18.3.2.6
Soil Bacteria: Antibiotic-Induced Increase in Community Tolerance

Exposure of communities to toxic substances can lead to an increased overall tolerance, e.g., through disappearance of sensitive species, or genetic and physiological adaptations. An increase in community tolerance can thus be taken as a potentially specific and sensitive indicator of a toxic impact. This concept has been introduced as pollution-induced community tolerance (PICT) (Blanck 2002).

Recent investigations into community adaptations due to antibiotic exposure show that sulfonamides and tetracyclines can indeed induce PICT increases in soil micro-

cosms. In these investigations, PITC was taken as an indicator of antibiotic effects on community structure and measured through an analysis of the sensitivity of the whole bacterial community. An increase in antibiotic tolerance could be observed at concentrations of 7.3 mg kg^{-1} (10% increase in sulfachloropyridazine tolerance, Schmitt et al. (2004)), 1.3 mg kg^{-1} (doubling of oxytetracycline tolerance Schmitt et al. (2006a)) and >100 mg kg^{-1} (doubling of tetracycline tolerance Schmitt et al. (2006a)).

Changes in the structure of the microbial community have also been observed with phospholipids fatty acid (PLFA) analyses: high doses of tetracycline (500 mg kg^{-1}) reduced the microbial diversity (especially of Gram-positive bacteria), and led to a decrease in the diversity of bacterial versus fungal PLFAs after a soil exposure of eight weeks (Hund-Rinke et al. 2004).

It has been argued that structural changes in the bacterial community do not necessarily affect soil functioning, as some functions can be performed by a wide range of soil microorganisms ("functional redundancy") (Degens 1998; Griffiths et al. 2000). On the other hand, specialized soil functions might profit from a higher diversity, such as soil disease suppressiveness (Garbeva et al. 2004).

18.3.2.7 *Resistance*

As early as in 1979, it has been noted that antibiotics administered as food additives might increase the level of resistance in Gram-negative intestinal bacteria, and that this in turn might pose a public health risk (Lebek 1979). Through the application of manure, both antibiotics and resistant intestinal bacteria can reach agricultural soils. However, there is also a significant background of resistance in soil bacteria (D'Costa et al. 2006), which complicates an assessment of the influence of antibiotic use. This background of resistance is thought to originate from streptomycetes and other soil microorganisms, which produce antibiotics as a means of defense against surrounding organisms. Together with the biosynthesis pathways for antibiotics, resistance mechanisms have evolved and have since then been transferred to other soil bacteria, such that a huge diversity of resistance genes can be found in soil isolates.

However, there might be differences in the quantity of resistance rather than in the diversity of genes. Few publications have explicitly dealt with the hypothesis that manure quantitatively enhances the occurrence of antibiotic-resistant bacteria in soil. In one of those publications, the influence of antibiotics themselves as selective agents has been tested against the input of resistant bacteria with manure. It has been found in microcosm studies that manure amendment raises the quantities of tetracycline resistance genes in soil to a detectable level, but that even unrealistically high concentrations of tetracyclines do not have the same effect (Schmitt et al. 2006b). Similarly, Hund-Rinke and coworkers (2004) found in microcosm experiments that the presence of tetracycline resistance genes was rather linked with manure amendments than with the exposure to tetracycline. While there was a decline in the number of resistance genes detected after manure addition over twenty-six weeks in this experiment, Agersø et al. (2006) were able to detect the gene *tet*(M) for twenty-two weeks in microcosms receiving manure or a *tet*(M) bearing strain. In contrast, the frequency of phenotypical resistance reached the background level after ninety days, suggesting that resistance genes might be more "persistent" than their original bacterial carriers.

In field studies, a slight effect of manuring on resistance in soil-borne bacteria has first been detected by Jensen et al. (2001). Other researchers found that the application of manure leads to an increase in the frequency of especially tetracycline-resistant bacteria in soils. However, the tetracycline resistance declined to levels comparable to a non-manured soil over five months, and there was no significant increase in macrolide and streptomycin resistance (Sengeløv et al. 2003). In the same soils, the tetracycline resistance gene *tet*(M) showed elevated concentrations directly after manuring, and frequencies were higher in agricultural soils than in garden soils (Agersø et al. 2004). Macrolide resistance in soil bacteria has been found to be related to the use of tylosin as feed additive by Onan and LaPara (2003), who found that both the percentage of resistant bacteria increased and the types of resistant bacteria shifted from streptomyces-like bacteria to α- and β-proteobacteria. The local antibiotic usage pattern and soil properties might thus also contribute to the resistances observed at a given site. Recently, the mobility of resistance genes when connected to mobile genetic elements such as integrons has gained attention. Tetracycline resistance genes found in soil bacteria were often linked to class 1 integrons, harbored several resistance genes, and could be transferred to zoonotic pathogens such as *E. coli* (Agersø and Sandvang 2005). In a recent microcosm study, it has been shown that manure treatment introduced class 1 integrons carrying sulfonamide resistance to soil and that integrons persisted for at least two months. Indications of the presence of resistance genes on mobile elements were also found in plasmid transfer studies. Soil amendment with manure and sulfadiazine (in relatively high concentrations of 10 and 100 mg kg^{-1}) led to higher frequencies of resistance, both in culturable bacteria and in the total community (Heuer and Smalla 2007).

Up to now, no distinction between resistance occurring due to application of antibiotics for the treatment of animals and resistance occurring due to 'natural' processes in animal intestines or soils has been made.

To summarize, there is increasing evidence that the application of manure of antibiotic-treated farm animals can quantitatively increase antibiotic resistance in soils. However, in order to evaluate the role of "environmental" resistance as a public health risk, more data is still needed on the human exposure to environmental resistance genes. This exposure then has to be compared to acquisition of resistance through the food chain, through human antibiotic use, and transfer of bacteria between humans, especially in hospitals.

18.3.3 Other Drugs

For compounds other than antibiotics and parasiticides, the literature search did not yield any results. It is possible that some publications have been missed due to a different indexing system in the article, but still the conclusion can be made that data on effects of human drugs on the terrestrial system are very scarce. This can be related to their exposure route: as human pharmaceuticals reach the terrestrial compartment mainly after application of sewage sludge, the terrestrial exposure is thought to be small.

Actually, those few isolated observations found refer to compounds which are either well known and are used in high amounts (e.g., acetylsalicylacid (ASS)) or which

belong to different usage classes (e.g., coffein). Most often β-sitosterol has been studied in this respect, but since this chemical is naturally occurring as a phytoestrogen and it is mainly classified as a dietary supplement, it will not be considered here.

Very high concentrations of acetylsalicylacid (10–100 ppm) sprayed on the mycelium of parasitic fungi (e.g., *Phytphthora nicotianae* var. *parasitica* or *P. citrophthora*) can inhibit their growth (Walker 1988). When spraying an ASS solution (180.2 mg l^{-1}) on the leaves of beans (*Phaseolus vulgaris*), the plants reacted by increasing their number of flowers and pods (Larque-Saavedra and Lang 1988). It is uncertain whether effects gained under such artificial conditions have any relevance for real field situations. The same can be said about the effects on compost worms (*Eisenia fetida*), where these animals were exposed to ASS, coffein, phenmetrazin (psychoanalepticum), theophyllin (an antiasthmaticum), or eserinsalicylate (no current use known) dissolved in filter paper. Since this test situation does not resemble exposure in soil, the only conclusion gained was a relative classification of the toxicity of these compounds compared to other chemicals (Roberts and Dorough 1984).

18.3.4 Terrestrial Effects in the Current Guidance Documents

According to the new regulations on the registration of veterinary and human pharmaceuticals in the European Union, the USA and Japan, terrestrial test results can be required for the environmental risk assessment (CHMP 2005; VICH 2005). For both veterinary and human drugs, it must be secured in Phase 1 of the ERA whether exposure to the environment is possible. Environmental exposure is assessed from the use pattern and the predicted environmental concentration of the compound. A trigger value of 100 µg kg^{-1} for soil has been set; below this value environmental exposure is not assumed to bear ecotoxicological risks. These trigger values have been questioned because the original datasets used for their derivation were not comprehensive with respect to the number of substances and endpoints. Actually, a threshold value of 1 µg kg^{-1} soil was proposed (Montforts 2005). Veterinary drugs that breach the trigger value and those belonging to pharmaceutical classes known to be toxic (in particular, parasiticides) have to proceed to effect testing in Phase 2. Effects of human drugs in soil are only assessed if they show a potential for adsorption ($K_{oc} > 10\,000$), meaning that they can enter the terrestrial compartment via sewage sludge (CHMP 2005).

For human pharmaceuticals, degradation in soil (No. 307; OECD 2002) and effects on the transformation of nitrogen by soil microorganisms (No. 216; OECD 2002b), the growth of terrestrial plants (No. 208; OECD 2004a), the mortality of earthworms (No. 207; OECD 1984), and the reproduction of collembolans (No. 11267; ISO 1999) have to be tested. So far, very few human drugs have been assessed this way.

In Phase 2A, veterinary pharmaceuticals have to be assessed more intensively (VICH 2005). Firstly, the same tests as for human drugs have to be performed. However, instead of the earthworm acute test, either the earthworm or the enchytraeid reproduction test is required (No. 222/220; OECD 2004b,c). This is one of the very rare cases where ecotoxicological testing starts with a chronic and not an acute test because – considering the concentrations occurring in the environment – acute effects like mortality are unlikely. For parasiticides, additional tests with dung flies and dung beetles must be performed (OECD 2006). In case effects are found higher than the trigger

values defined for each individual test system, further tests are required in Phase 2B. Besides an elongation of the duration of the microbial test and an increase in the number of plant species to be tested, more earthworm and dung organism tests are possible, but so far no decision has been made as to the type of testing required.

18.4 Conclusion

The effects of antibiotics have been most intensively studied for soil bacteria, in line with their mode of action. However, effects on plants at relatively low concentrations have been detected for sulfonamides and tetracyclines, at least in soil-free systems. The emerging picture for antimicrobial effects is that effects have been found at concentrations of a few mg/kg and in some instances below the action limit of 100 µg kg^{-1} soil, a concentration at which some groups of antibiotics have also been detected in soil. Further, even within a structural homogeneous group of antibiotics, the observed effects vary significantly as has been seen from other investigations with much higher effect concentrations. Figure 18.1 highlights these two observations by comparing a range of observed soil concentrations with effect concentrations (based on publications reporting EC_{50} values). Overall, the wide range of effect concentrations might to some extent be caused by differences in soil properties, which both shape the microbial community and determine the bioavailable concentrations of the sometimes highly sorptive substances.

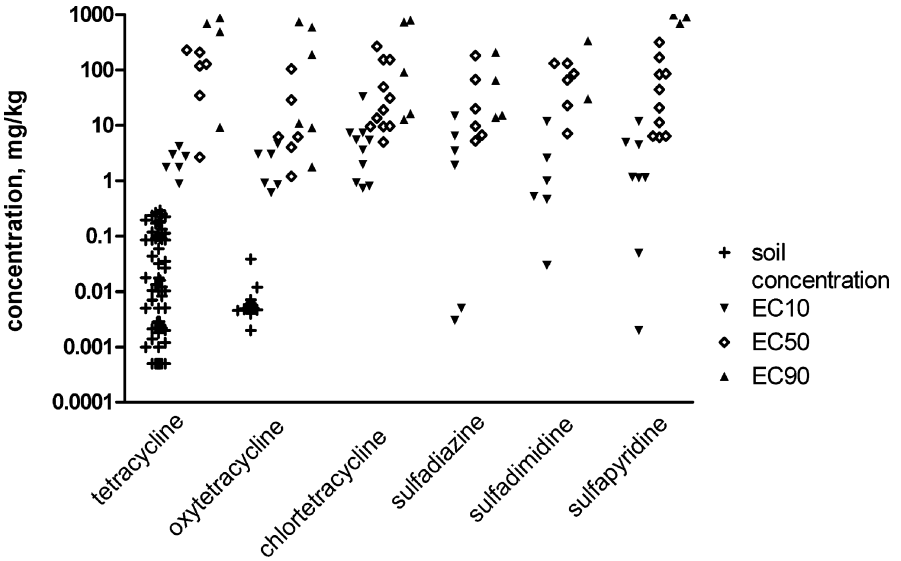

Fig. 18.1. Compilation of soil concentrations and effect concentrations of antibiotics, including effects on substrate-induced respiration and Fe(III) reduction. Data taken from Thiele-Bruhn (2005), Thiele-Bruhn and Beck (2005), Vaclavik et al. (2004), and Hamscher et al. (2000, 2002, 2005)

The possible increase in the occurrence of antibiotic resistance in the terrestrial compartment is a phenomenon that is linked to public rather than to ecosystem health. The role (and origin) of animal farming-related resistance versus the natural environmental background of resistance is however not yet fully known. Human exposure to antibiotic resistance via the terrestrial environment has also not yet been investigated in detail. In order to assess the relevance of resistance in the terrestrial environment, further research seems thus appropriate.

Among veterinary drugs, parasiticides are known to have the potential to negatively affect soil invertebrates and soil functions at environmentally relevant concentrations. The best-known examples are avermectins, causing effects on dung organisms and dung degradation. Despite a huge number of studies during the last decades, this concern has only recently been confirmed in standardized tests. In parallel, tests on the degradation of chemicals in dung have to be standardized.

However, there are gaps remaining concerning information on other active ingredients than ivermectin (e.g., synthetic pyrethroids; Wardhaugh 2005). In addition, the results of many existing tests and particularly field studies are difficult to compare due to differences in study design and conduction of the test. Furthermore, both abiotic (e.g., soil conditions) as well as biotic (composition of the dung-degrading invertebrate community) factors can influence the outcome of such studies.

With respect to drug classes outside of antibiotics and parasiticides, it is obvious that information on their effects in the terrestrial environment is nearly completely lacking.

For a relatively short time, the environmental risk of veterinary drugs has to be assessed according to EU guidelines. Regarding the trigger value below which no assessment of the environmental effects of drugs has to be performed, it can be stated that the mere existence of such a trigger deviates from the risk assessment paradigm of comparing exposure and effects as laid down in several other risk assessment frameworks for biologically active compounds such as pesticides or biocides.

With respect to the evaluation of veterinary antibiotics, it could be suggested to include the carbon mineralization (OECD No. 217) test along with the nitrogen mineralization test, as effects on substrate-induced respiration have regularly been observed. The standardized OECD test setup would however need adaptations in order to capture possible antibiotic effects. Firstly, the OECD guidelines 216 and 217 focus on effects persisting for twenty-eight days, while many investigations found effects during the first week after antibiotic exposure. As soil exposure to antibiotics coincides with "exposure" to manure and as manure degradation is crucial for soil health and soil fertility, we postulate that effects occurring during the peak manure degradation (in the first days after manure application) should be regarded as adverse. Secondly, substrate-induced respiration testing as described in OECD 217 potentially leaves growth-inhibiting antibiotic effects undetected: Only the first hours of respiration are assessed, while antibiotic-related growth retardation occurs at later stages. Here, an adaptation of the testing protocols seems necessary.

Regarding parasiticides, the testing procedure is well described for lower-tier testing. However, more guidance is surely needed on test data evaluation and higher-tier testing (e.g., the inclusion of structural parameters such as taxonomic diversity of the dung organism community). Only reliable data from all levels of investigation (labo-

ratory to field) will answer the question whether antiparasitics can impact dung organisms and dung degradation not only locally (as has been shown) but also, based on modeling, regionally (Wardhaugh 2005).

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Odorants – Potent Substances at Minor Concentrations: The Ecological Role of Infochemicals

U. Klaschka

19.1 Introduction: Ecosystems are Controlled by Odors

19.1.1 Odor Worlds

Imagine you are in a different world: You got lost in a forest. The smell in your nose tells you that it is night (explanation see under (1) below). You sniff carefully and notice some faint smell of your wife, indicating that she is about one kilometer away in a certain direction (explanation see under (2) below). You direct your steps to meet her. On your way you are assaulted. You immediately call for help by changing your perfume (explanation see under (3) below). As you have perceived the odor of this aggressor several times already, your body continues building an armature to deter this enemy better next time (explanation see under (4) below). Finally, you happily arrive at home where your wife is. Some hours later a giant enters your house. He destroys your stock and eats up your children, but you cannot help but welcome him and invite him for dinner, only because he uses your home perfume (explanation see under (5) below).

Does this sound like science fiction? All these examples are part of everyday life in nature for individuals other than man:

1. Caterpillars (*Mythimna separata*) live on host plants that emit different blends of volatiles during day and night. The caterpillars perceive whether it is night or day by these different smells without the need of any other stimulus such as light (Shiojiri et al. 2006);
2. Female moths attract males over long distances with their sexual pheromones;
3. Plants infested by caterpillars can "cry for help" by emitting odors that attract the parasites of their invaders. These herbivore induced plant volatiles are frequent examples of multitrophic chemical communication interactions. As most parasites are active during the day, the plants emit the volatile chemical cues mainly during the day. This is one reason why some plants emit different "perfumes" during the day from those they emit during the night (see explanation (1) above) (Oldham and Boland 1996);
4. Some zooplanktic species can form neck spines, helmets and other defensive structures in the presence of the odor of their predators (Larsson and Dodson 1993; Tollrian and Harvell 1999);

5. Invaders in ant colonies are attacked unless they carry the body odor of the ant species and even the exact blend of the specific colony. There are many examples of this camouflage, e.g., beetles in ant states, parasitic ants in ant colonies, butterflies in ant nests or beetles in termite states. The invaders feed on the brood or are even fed by the workers like a cuckoo.

Many organisms live in odor landscapes. Myriads of interactions controlled by chemicals occur that are unnoticed by man. The role of chemical communication for the survival of ecosystems cannot be overestimated. Chemicals released by biotic or abiotic sources are ubiquitous, the interactions extremely complex, and the sensitivities very high. Chemoreception plays a fundamental role in the origin of life. During ontogenesis, cells move along chemical gradients. In the beginning of evolution, chemoreception was the primary way to communicate with the extracellular space. The evolution of chemical communication affected both the senders and the receivers. This led to strong interrelationships and interdependencies, which resulted in coevolution (e.g., flowers and pollinators) or an "arms race" (e.g., defense against aggressors). The complex chemical communication systems of today are the results of the selection pressure to find food, distinguish between predator and non-predator, conserve energy, or find a mate.

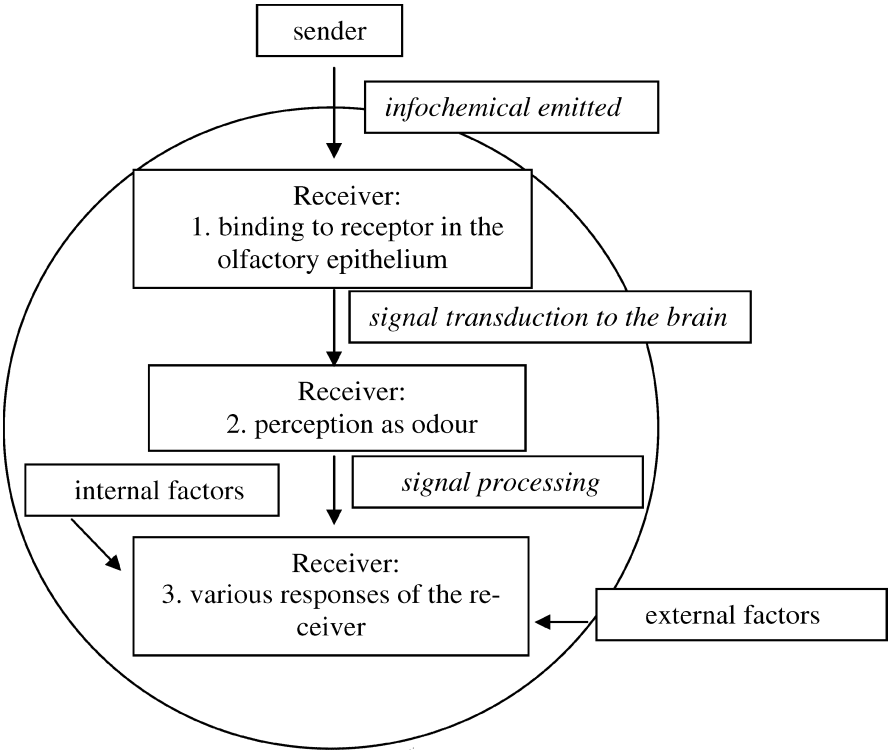

Fig. 19.1. Simplified model of the chemical communication which explains the difference between the chemical emitted and the odor perceived

There are several good reviews dealing with aspects of this topic (Bradbury and Vehrencamp 1998; Brönmak and Hansson 2000; Burks and Lodge 2002; Dodson et al. 1994; Hara 1992; Harborne 1993; Lass and Spaak 2003; Oldham and Boland 1996; Pohnert et al. 2007; Tomba et al. 2001; Wyatt 2003; Zimmer and Butman 2000). Although much progress has been made in this field in the last decades, the knowledge base has not caught up with its importance.

19.1.2 Emission of Chemicals versus Perception of Odors

It is essential to make a clear distinction between the chemical emitted and the odor perceived. In animals the simplified reaction sequence can be described as such: A sender releases substances. Some molecules reach the olfactory epithelium of a receiver and bind to receptors. The receiver can belong to the same species or to a different species as/than the sender. The receptor cell is stimulated and the signal is transferred to the brain leading to the perception of an odor. Subsequently, several physiological, behavioral or even morphological reactions of the receiver are possible. It is important to realize that the sensitivity of the receiver renders a chemical an odorant. If the receiver is not sensitive to the chemical stimulus, the respective chemical is no odorant for this organism (see Fig. 19.1).

19.1.3 Terminology

Chemicals that steer behavior can be classified according to their observed roles in the communication between individuals (see Table 19.1).

The definitions may give the impression that a substance plays only one role. But the same chemical may warn the prey, at the same time it may attract a mate or demonstrate dominance over a conspecific. The detection of a specific role in the ecosystem does not mean that this is the only role. As the terminology in Table 19.1 does not seem to be helpful for the purpose of this article, I will use the general term "infochemical" or "chemical cue" for chemicals involved in communication.

Table 19.1. Terminology of infochemicals (Dicke and Sabelis 1988; Koene and de Maat 2002; Wyatt 2003; Larcher 1995)

Semiochemical	Generic term for chemicals involved in animal communication
Allelochemical	Chemical involved in animal communication between species
Pheromone	Chemical used for the communication within a species
Kairomone	Allelochemical which benefits the receiver
Allomone	Allelochemical which deceit other animals or are used as propaganda
Allohormone	Chemical that induced a direct physiological response bypassing sensory organs
Synomone	Allelochemical which benefits the receiver and the sender
Phytoalexin	Chemicals produced as a method of defense in plants

19.1.4
Properties of Odor Signals

Odor signals are well suited for medium time frames: they last longer than sounds, vibrations or short-lived visual signals such as movements, but they do not last as long as long-lived visual signals such as morphological changes. The signals disappear with diffusion or degradation of the compounds. Therefore, degradability is of utmost importance. Odor signals are also well suited for the medium spatial range: They do not reach as far as penetrating sounds but reach farther than touch or taste. In contrast to light or sound, there is no linear scale such as the spectra of wavelengths or frequencies; instead, there are an unlimited number of odor qualities, depending on the number of chemicals and of the olfactory receptors in the receiver. One reason for the universality of chemical communication is the fact that chemicals can induce the biochemical signal transduction chain directly without the need to transform a physical signal in subsequent biochemical reactions. Chemical communication is independent of light. It is effective at night, in caves, at the bottom of lakes or the sea, in groundwater, in turbid water, in water habitats with hiding places such as plants or rocks. Odors cannot be avoided, as most animals cannot shut their noses. The perception can only be reduced by leaving the emission source. Organisms that are exposed to constant high concentrations of infochemicals become insensitive due to adaptation.

19.1.5
Potent Substances

The more we know about chemicals steering behavior, the more we know how sensitive this endpoint is. Concentrations controlling behavior may be 0.1–5.0% of the LC_{50} found in standard tests (survival of rainbow trout) (Little et al. 1993). Changes of behavior may also be much faster than other endpoints. For example, the swimming activity of rainbow trout was influenced after 96 h, whereas the growth was only affected after 30 d (Little et al. 1990, 1993). Reduced movements lead to a lower chance of escape from a predator. They can also lead to reduced feeding, which by itself may lead to increased vulnerability. Therefore subtle sublethal effects can affect the ecosystem as a whole.

I want to give a short summary showing the impact of chemicals on the behavior and survival of organisms. Most examples will be aquatic organisms. My special purpose is to connect ecology with ecotoxicology leading to the final question: Do anthropogenic compounds interfere with the framework of odor communication? This report might inspire the discussion on whether additional endpoints for behavioral ecotoxicology need to be considered in the future.

19.2
Sender – Infochemicals – Receiver

19.2.1
Sender: Who and What Emits Infochemicals and When?

Animals emit chemicals at various locations of their bodies and act therefore as multiple senders for infochemicals. The odor sources of mammals are, for example, sali-

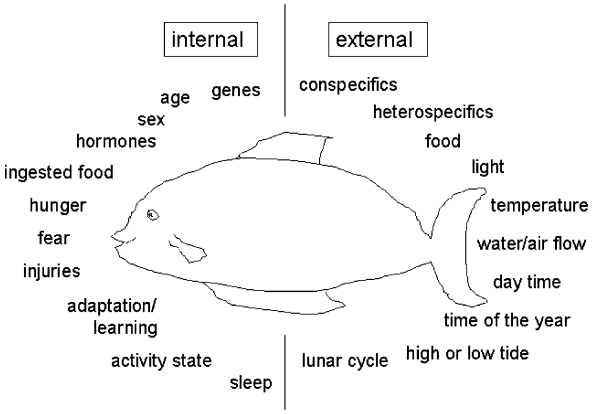

Fig. 19.2. Factors influencing the infochemical emission pattern of a sender

vary glands, lungs and trachea, the liver, gall bladder, kidney, ureter, bladder, urethra and urine, intestine, rectum and feces, and the anal region, as well as glands on the feet, legs and skin. Odorants can be produced by enzymes in specific glands, they can be metabolic by-products or they can originate from ingested food plants. For example, some insects use dietary toxic chemicals (produced by the plants to deter or kill insects feeding on them) for the production of their pheromones. One example is wood terpenes used by male bark beetles for the attraction of females. Infochemicals can also be produced by bacteria. For example, body cavities which are moist and warm such as armpits allow bacteria to grow and produce metabolic compounds, some of which are volatile. Plants are also important senders of infochemicals, especially flowers, host plants, food plants, injured plants or dead plant material (Larcher 1995; Pichersky 2004; Polya 2003). Many odorants are released during the degradation process of dead organic material. Not all substances that are released by organisms are infochemicals. Some infochemicals are known, while others may not play a role in chemical communication because there is no receiver. And many substances might be infochemicals, but so far the receivers have not been found or studied.

Senders release different blends of infochemicals depending on multiple internal and external factors as shown in Fig. 19.2. For example, the release pattern changes considerably during the lifetime of an organism: catfish (*Plotosus lineatus*) releases phosphatidylcholine only at an early developmental stage for school forming, whereas the older fish do not aggregate and do not secrete phosphatidylcholine any more (Matsumara 2004). Sexual pheromones are mainly emitted at times of increased sexual activity or fertility and can change after mating (Schiestl and Ayasse 2000). An example of light/day time influencing the odor emission pattern was given in Sect. 19.1.1 (3).

19.2.2 Infochemicals: What is Known About Their Chemical Identities?

Air or water can be transmission medium of infochemicals. Both media allow a wide distribution by diffusion or with the air or water current. Odorants released into air are usually volatile at ambient temperature, they are small, i.e., with a molecular weight under 300, and they are nonionic, mostly hydrophobic substances. Odorants released

into water are soluble in water, and the molecules may be larger than those emitted into air. Some organisms can perceive aquatic as well as aerial odorants. For example, the amphibian *Xenopus* has a bipartite olfactory epithelium: the dorsal section is open in the air; the ventral section is open in the water (Eisthen 1997).

The stability of the substance determines how long a signal can be perceived. Usually natural infochemicals have a persistence ranging up to several days or weeks, which means that the sender does not need to be present when the receiver perceives the message (Bolhuis and Giraldeau 2005). It is important to know the degradation products in air or water. For example, the odorant α–pinene, emitted by plants, oxidizes in the air to *cis*- and *trans*-verbenol and verbenon, which are also odorants (Payne et al. 1986). Concentration changes are essential for the role of infochemicals in communication. Higher concentrations can have different meanings than lower concentrations (see also Fig. 19.3).

In earlier studies, scientists used biological probes, such as skin extract, dead fish, tissue extract, or feces for their experiments. They were not able to characterize the chemicals involved. Much progress has been achieved lately due to the technical improvements of the detection methods for minor concentrations, which allowed the identification of some natural infochemicals (Oldham and Boland 1996; Tollrian and Harvell 1999; Toshiaki 1992; Wyatt 2003). The best analytical methods are needed to identify the substances involved and to determine the effective concentrations. The chemical characterization is difficult due to the following reasons:

- Any chemical might be an infochemical (examples in Fig. 19.4);
- The effective concentrations can be very small (Table 19.2), above noise and below physiological concentrations in tissues (Fig. 19.3) (Carr 1988);
- Additional substances might be present in the test that do not function as infochemicals and might be there at higher concentrations;
- Stereoisomers can have different effects, as the binding to the olfactory receptor can be very specific;

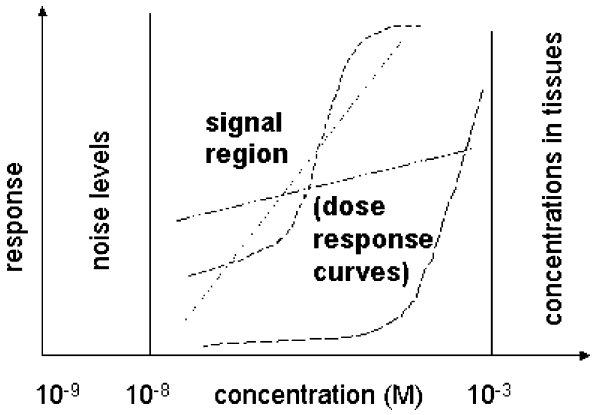

Fig. 19.3. Substances that are ubiquitous can be infochemicals in a concentration range above the noise levels and below the concentrations in tissues. Dose response curves can be found in this concentration window. This was shown e.g., for nucleotides or amino acids (Carr 1988)

- A substance identified may be only part of a cocktail which is relevant in nature. Many identified infochemicals are ubiquitous substances. It is possible that additional chemical factors which have not been identified so far are involved (Boriss et al. 1999);
- Suitable bioassays are needed to confirm that a substance is really the infochemical under scrutiny.

Some infochemicals are small chemicals such as ammonia or nitric oxide, whereas others are complex molecules such as steroids. Infochemicals can be waste products or by-products of the sender or they were originally designed for other purposes, such as defense, bactericidal action, and physiological regulation. In other cases, they are synthesized specifically for chemical communication, such as sexual pheromones. Elephants and some moth species use the same enzymes for their pheromone production. That is why unrelated species can have identical pheromones (Wyatt 2003).

Plant scents are usually secondary plant compounds, e.g., terpenoids, phenolics, alkaloids, amines or other compounds. Fish, e.g., teleosts, use various substance classes for communication: amino acids, bile salts, nucleotides, gonadal steroids and prostaglandins (Carr 1988; Rolen et al. 2003). Wounded or decaying prey in water emit low molecular metabolites such as amino acids, nucleotides and organic acids which are used as infochemicals for food finding and recognition by predators (Brönmark and Hansson 2000). Figure 19.4 shows that aquatic infochemicals can belong to various chemical groups.

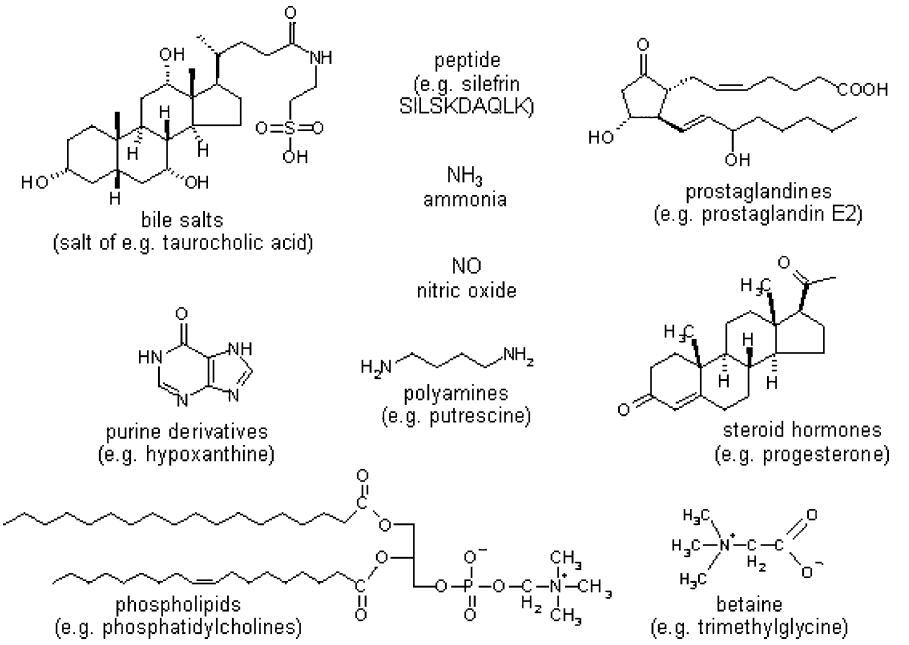

Fig. 19.4. General chemical groups of aquatic infochemicals shown with some representative examples

19.2.3 Receiver: When and How Does a Receiver React?

Every organism constantly meets a multitude of chemicals present in its environment. Many chemicals will remain unnoticed because the organism has no sensor to perceive it. The prerequisite for odor perception is an interaction of the infochemical with a receptor in the olfactory epithelium of the receiver, which initiates the signal transduction that leads to odor detection. The odorant receptors are encoded by a multigene family of up to several hundred genes and form the largest family of G-protein coupled receptors as was shown in various animal taxa (Dryer 2000; Olender et al. 2004; Young and Trask 2002).

The receiver will use this neuronal stimulus to answer the questions about its surroundings: is there something to eat, a predator, a mate, a competitor? And it will react in a suitable way. Therefore infochemical stimuli can result in a multitude of reactions (Fig. 19.5). I want to highlight only a few facts that are necessary to place the findings into the ecotoxicological perspective and to give some examples of the aquatic compartment (Table 19.2).

Reactions do not have to be stereotyped. Understanding a reaction is therefore not an easy task due to the following factors:

- Many chemical signals will be present at the same time, and the organism must decide the following: Which is the most important signal? Which is the best reaction? The experiment must clearly show that a reaction is dependent on a certain signal;
- The reaction patterns are controlled by internal as well as external factors, in the same way as described for senders in Fig. 19.2. For example, the activity state can have a strong influence on the response. Usually resting organisms are used in laboratory experiments. Zimmer-Faust et al. (1996) found out that walking crabs react to chemicals at concentrations around 500 times lower compared to quiescent crabs. External and internal factors are closely connected, e.g., the circadian rhythm steers the activity pattern of the organism and adjusts it to the light period;
- Reactions can be very prominent in some individuals whereas others do not respond at all. The individual differences can be due to genetic variance or due to experience (see Sec. 19.2.5 and example in Table 19.2; Brown and Smith 1998);
- Organisms can adapt to certain situations. Their behavior does not follow a simple mechanistic pattern. For example, a young fish that recognizes the odor of its schoolmates will return to its school if possible. But if its original school has disappeared, it can learn the odor of a new school and join them (Matsumara et al. 2004);
- It is to be assumed that complex multitrophic interactions are widespread in foodwebs, but they have been described only in a few examples (see Sec. 19.1.1 (3)). Experimental setups are simplified models of the interaction of one or two organisms with infochemicals and can rarely reflect the complex natural situation;
- The receivers respond to very low concentrations of infochemicals as shown in Table 19.2 and Fig. 19.3;
- The reactions are not always proportional to the concentrations of the infochemicals. The reaction may be different at high compared to low concentrations (see example in Table 19.2 Vardi et al. 2006);

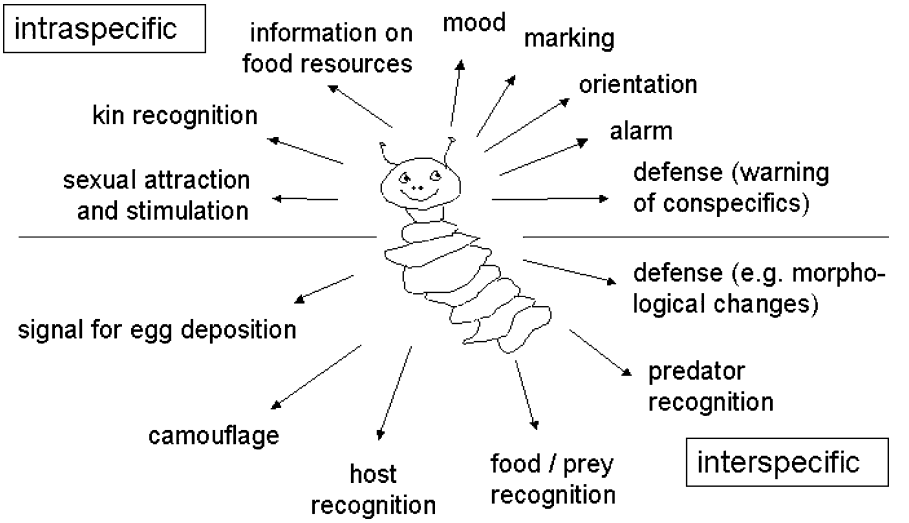

Fig. 19.5. The infochemicals supply the receiver with a variety of information about its surroundings. The multitude of subsequent behavioral reactions, as shown in the figure, can be observed in experiments (intraspecific: receiver is a conspecific to the sender, interspecific: receiver belongs to a different species)

- Some infochemicals can be perceived by one species only, whereas others are chemical cues for many. For example the alarm signals released by a prey can be an alert also for other species that are the prey of the same predator;
- Some reactions are honest, others can be used to cheat others, e.g., sexually deceptive orchids (Schiestl and Ayasse 2001) or camouflage described in Sec. 19.1.1 (5).

19.2.4 Applications

The knowledge about the significance of chemical communication has been leading to some economically important applications of infochemicals (Wyatt 2003). Pheromones are used to control the reproduction of farm animals. They can be used to temper the aggressive behavior of animals. The behavior of honey bees can be influenced to increase pollination and control swarming. Major applications in pest management utilize the chemical cues of many moth species, aphids, beetles or nematodes.

Applications in the aquatic compartment are few so far: Sex pheromones can be used to control reproduction of fish in aquacultures. Recently, pest control became possible for the aquatic compartment. Sea lampreys are eel-like parasites that have been serious pests in the American Great Lakes, because they damage the local fisheries. Gramling (2005) was able to rebuild the pheromone used by lampreys to locate their spawning waters. This discovery could be used to lure the fish away from their successful spawning sites. Barnacle settlements can be reduced by lectins that inhibit the effect of the adult settlement pheromone (Matsumara et al. 1998).

Table 19.2. Examples of aquatic senders, infochemicals and receivers described in the literature

a Sender b Infochemicals (conc. range) c Receiver	Reaction of the receiver Remarks	Reference
Fish		
a Larval sea lampreys (*Petromyzon marinus*) b Mixture of bile acids e.g. petromyzonol sulfate, allocholic acid, petromyzonol (10^{-5}–10^{-13} mol l^{-1}) c Adult conspecifics	Orientation of migratory fish; Adults of other lamprey species who have similar requirements for their spawning regions, are attracted as well	Fine et al. 2004, Li and Sorensen 1997
a Catfish (*Plotosus lineatus*) b Mixture of phosphatidylcholines c School mates	Forming schools, kin recognition	Matsumara et al. 2004
a Channel catfish (*Ictalurus punctatus*) b Purine-N-oxides, e.g. hypoxanthine-3-N-oxide c Conspecifics and some sympatric heterospecifics	Alarm pheromone, released from epidermal club cells upon mechanical skin damage; The nitrogen oxide functional group acts as the chief molecular trigger	Brown et al. 2000, Brown et al. 2003
a Goldfish (*Carassius auratus*) b Polyamines e.g. putrescine, cadaverine, spermine (10^{-8}–10^{-7} mol l^{-1}) c Conspecifics	Electrophysiological responses of olfactory receptor neurons, behaviour: swimming, feeding, nudging; Dose response curves were set up and the independence of receptor sites analyzed	Rolen et al. 2003
a Hungry and satiated perch (*Perca fluviatilis*) b Unknown substances c Dytiscid beetles (*Acilius sulcatus*)	Predator avoidance; The beetles can discriminate between chemical cues from hungry and satiated perch and even between perch satiated on fish versus those satiated on dytiscids	Abjörnsson et al. 1997
Amphibia		
a Red legged frog tadpoles (*Rana aurora*) b Ammonia and other substances c Conspecifics	Alarm; Disturbed tadpoles warned their conspecifics	Kiesecker et al. 1999 Carpenter 1999
a Male sword tailed newt (*Cynops ensicauda*) b Silefrin (peptide of 10 amino acids) c Conspecifics	Attracting females; A closely related species uses a different female attracting compound	Yamamoto et al. 2000
a Predator b Unknown c Early life stages of the common frog (*Rana temporaria*)	Defense (delay of hatching and change of morphology of the tail fin in tadpoles); Predator odours have influence on the early life history and morphology of the prey	Laurila et al. 2001

Table 19.2. *Continued*

a Sender b Infochemicals (conc. range) c Receiver	Reaction of the receiver Remarks	Reference
Amphibia (continued)		
a Predators of western toad tadpoles (backswimmers, waterbugs, snakes) b Unknown c Western toad tadpoles (*Bufo boreas*)	Distinguishing between predators and non-predators; Visual stimuli are not necessary to distinguish between predators and non-predators	Kiesecker et al. 1996
Crustaceae		
a Crayfish (*Astacus leptodactylus*) b Urine and signal substances in urine c Male conspecifics	Information about aggressiveness, dominance	Breithaupt et al. 2002
a Water from a mussle bed b Unknown c Blue crabs (*Callinectes sapidus*), spiny lobsters (*Panulirus interruptus*)	Prey attraction; Walking crabs react to chemicals at around 500 times lower concentrations compared to quiescent crabs	Zimmer-Faust et al. 1996
a Male rock shrimp (*Rhynchocinetes typus*) b Unknown c Female rock shrimps	Mate searching: Male shrimps use visual signals to find females, females use chemical signals to select males	Diaz and Thiel 2004
Various species from other taxa		
a Predator b Unknown c Zooplankton, barnacles, bryozoans, snails tadpoles and fish	Morphological defense structures, e.g. neck spines, helmets	Larsson and Dodson 1993
a Oyster (*Crassostrea virginica*), (juvenile and adult) b Peptides (10^{-8} mol l^{-1}) c Larval conspecifics	Larval settlement; Quantitative structure-activity relationships (QSARs) were set up	Browne et al. 1998
a Crushed flatworms (*Dugesia dorotochephala*) b Fish odour with conspecific cues c Conspecifics	Predator avoidance; Flatworms do not respond to fish odour without the odour of crushed conspecifics	Wisenden and Millard 2001
Plants		
a Water cress (*Nasturtium officinale*) b Glucosinolates c Herbivores	Feeding deterrence; Glucosinolates are hydrolyzed to isothiocyanates	Newman et al. 1996
a Diatoms (*Phaeodactylum tricornutum*, *Thalassiosira weissflogii*) b Aldehydes, e.g. decadienal (decadienal leads to the formation of nitric oxide) c Herbivores	Low concentrations are antifeedants and make the diatoms resistant. High concentrations are toxic to the diatoms and end the algal bloom. The aldehydes regulate the populations of both diatoms and their feeders	Vardi et al. 2006

19.2.5 What Test Designs Were Used in Basic Research?

The experimental designs used for tests of chemically induced behavior vary to a large extent: senders were whole healthy organisms (e.g., fish: sea lamprey, perch (hungry and satiated)), blindfolded organisms (e.g., crayfish), dead organisms or crushed conspecifics, and plants (injured plants, flowers, host plants, food plants). In some setups, no sender was used, but chemicals were added directly to the receiver, either as unknown complex mixtures such as skin mucus, agar blocks with skin mucus of a sender, washing water of intact organisms (e.g., clams) or in some cases single substances (e.g., specific bile acids, single peptides). The receivers were healthy organisms at various life stages (e.g., fish, amphibia), blindfolded organisms (e.g., crayfish) or artificially anosmic animals (e.g., by blocking the nasal cavities of fish with dental cement). In some receivers the olfactory stimulus was recorded by electroolfactograms (e.g., the fish is anaesthetized, immobilized and the skin of the nose is removed to expose the olfactory epithelium rosette), electroencephalograms or electroantennograms (for insects).

The behavioral responses tested were manifold: locomotion, attraction (e.g., tested in two-choice bioassay systems, Y-maze olfactometers), orientation, turn behavior, shelter seeking, flight, swimming, diel vertical migration, freezing (staying motionless), slowing down, school formation, food searching, appetite (saliva production), predation, food uptake, predator avoidance, competition, aggression, disgust, reproduction, spawning, sexual stimulation, mating, movement of a single organ (antenna, eye, leg, wing), morphological changes, and uttering a sound. Some responses may be interconnected (e.g., flight, shelter seeking and predator avoidance). In many experiments, the responses were video recorded followed by a computer-based analysis.

Organisms usually meet several stimuli at a time, which often comprise in addition to odorants visual or acoustical signals (e.g., Kiesecker et al. 1996). Most authors very carefully tried to exclude factors that could bias the result and took care of possible visual stimuli, temperature effects, day/night rhythms, influence of the food or the time needed for an organism to become familiar with the laboratory situation. However, as chemical communication is not fully understood, there could well be some influences that haven't been detected yet, which would affect the outcome of an experiment. Here are some additional caveats:

- Chemical cues might be undetected and still influence the outcome of the experiment. There might be senders that are more potent than the sender under scrutiny, e.g., the feces of the receiver. In some occasions, bacterial contamination (which release odorants or toxic substances) proved to be responsible for the reaction found. The unintended presence of anthropogenic substances can also affect the natural communication (Larsson and Dodson 1993);
- An animal can do only one thing at a time! Is the observed action the reaction to the chemical stimulus or to something else? It must be verified that the reaction to infochemicals is tested and that other factors (such as food quality, effects of toxic compounds) are excluded. The reaction tested might be the result of a different reaction chain (e.g., ingestion of the substance and not reaction with olfactory receptors);

- The interpretation of a behavior is not always evident: Is a swimming activity due to flight, stress, or the pursuit of food or social contact? It is also difficult to recognize a reaction as a causal response to the signal and not just as an arbitrary movement. Sometimes the different reactions might not be independent: e.g., hypoactivity, low food uptake, diminished response to external stimuli or lethargy can be correlated;
- The experimental setup is an artificial and often stressful situation for the animal, which might in turn lead to an artificial reaction. If a fish that is used to swimming in a school (e.g., fathead minnows) is tested alone in an aquarium, the stress of being alone might bias the outcome of experiments (Lawrence and Smith 1989);
- Wild-captured animals may have learned the significance of a certain infochemical in the wild and respond differently in the laboratory situation compared with animals that were raised in a different environment. Diverging results could therefore be due to the pre-laboratory lives of the test organisms (Kiesecker et al. 1996; Brown and Smith 1998).

The conclusion of these findings is that testing behavioral endpoints as a reaction to signal substances is not an easy task. Many possible factors need to be controlled very carefully to obtain a valuable test result.

19.2.6 The Infochemical Effect: Anthropogenic Substances Can Act as Infochemicals

Anthropogenic chemicals can act as infochemicals and interfere with the communication of environmental organisms. This is called "infochemical effect" (Klaschka, in press). Some man-made chemicals are identical to natural infochemicals (Table 19.3). The author analyzed the role of fragrances, which are obvious odorants for man. Many fragrances in perfumes have identical chemical structures to those produced by flowers as pollinator attractants. Some fragrance ingredients in anthropogenic perfumes are infochemicals for various groups of organisms, as shown in Table 19.3. For more examples see Klaschka and Kolossa-Gehring (2007).

It must be assumed that some anthropogenic substances -other than fragrances- will be able to act as infochemicals as well. This assumption is supported by the large variety of compounds that are shown to be infochemicals in nature (Fig. 19.4). The receiver cannot distinguish between a natural infochemical and an anthropogenic

Table 19.3. Compounds used in anthropogenic fragrance preparations and their roles as infochemicals in nature (Wyatt 2003; Sigma Aldrich Catalogue)

Compound	Function	Family and Genus
Benzaldehyde	Trail pheromone Defense Male sex pheromone	Bee, *Trigona*, Apidae Ant, *Veromessor*, Formicidae Moth, *Pseudaletia*, Amohipyrinae
2-Tridecanone	Alarm pheromone Defence	Ant, *Acanthomyops*, Formicidae Termite, *Schedorhinotermes*, Rhinotermitidae

chemical, which bind to the same olfactory receptor. The concentrations of infochemicals that induce behavioral changes can be extremely low. Standardized tests should help to answer the question: at what concentrations do anthropogenic chemicals influence behavior?

Anthropogenic substances can also interfere in some other detrimental way on chemical communication: e.g., male salmons were rendered anosmic for the female pheromone in the presence of sublethal levels of the pesticide carbofuran, and the male reproductive system was therefore not stimulated (Waring and Moore 1997).

Analysis of chemical communication and behavioral changes has been neglected in ecotoxicology so far. As described in Sec. 19.2.5, testing of behavior is not simple. But taking into account the huge impact infochemicals have on ecosystems, it is necessary to find out whether suitable standard test designs can be proposed and what they might look like. New standardized test designs are needed to elucidate the infochemical effect of anthropogenic substances.

19.3 Summary and Outlook: Will the Infochemical Effect Change Ecotoxicology?

Odorants are potent substances at minor concentrations!

Changes of behavior due to disturbed chemical communication may be even more detrimental to the survival of a species than toxic effects. Reduced food uptake, ignoring the predator (see example in Table 19.2, Brown and Smith 1998), impaired mating behavior, or other inappropriate behavior can lead to population declines, even if the individuals are perfectly healthy. The more we learn about chemical communication, the clearer it becomes that many organisms are very sensitive to subtle chemical cues in the environment.

Much work has been done, but we are far from understanding the communication nets in ecosystems. Research is challenging as interactions are very complex, and an interdisciplinary approach is needed that includes chemistry, ecology, ecotoxicology, molecular biology and physiology. Even nature conservation needs to be considered, as information on infochemicals might help to understand recent changes in ecosystems. For example, ammonia in water is released as a chemical distress signal by alerted tadpoles that warn their conspecifics (Carpenter 1999; Kiesecker 1999). How do the tadpoles react to increased ammonium concentrations in surface waters? Could the understanding of chemical communication be a clue to explaining population changes due to anthropogenic discharges?

So far it has not been analyzed how anthropogenic infochemicals interfere with the chemical communication in natural ecosystems. This is an important task for the future. Increasing knowledge about the function of anthropogenic chemicals as infochemicals might very well lead to a change in ecotoxicology: a new very sensitive end point – called the *infochemical effect* – will give more realistic information about the impact chemicals have on environmental organisms.

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Part IV
Risk Assessment

European Developments in the Environmental Risk Assessment of Pharmaceuticals

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20.1 Legal Background

The authorization procedure for new medicinal products addresses the potential environmental risk of the use of pharmaceuticals. Prior to marketing, the applicant is required to demonstrate that the use of the medicinal product is safe for the environment. Recently, new regulatory guidance documents have been released on the assessment of potential environmental risks (ERA) of both human and veterinary medicinal products. These guidelines are not legally binding but in general are followed by the parties involved, i.e., industry and regulatory bodies. The existing European guideline for veterinary medicines (EMEA 1997) was replaced by an internationally harmonized concept (VICH 2000, 2004). A supporting document giving guidance on details of, e.g., exposure assessment for Europe was adopted in 2007 (EMEA 2007). Fourteen years of work concluded in a long-awaited European guidance document of human medicines (EMEA 2006).

From a legal point of view, the environmental risk assessment of (non immunological, non-genetically modified organism containing) veterinary and human pharmaceuticals has been established with the EC Directives 81/851/EEC and 93/39/EEC, respectively (EEC 1981 and EEC 1993). Recently, these directives have been replaced by the new Community Codes 2001/83/EC (EC 2001a) for human medicines and 2001/82/EC (EC 2001b) for veterinary medicines.

Amendments were introduced by EC Directives 2004/27/EC and 2004/28/EC. These directives extended the legal background of an environmental risk assessment from new medicines to all types of new applications, including generics, bibliographical applications, extensions and Type II variations.

Furthermore, Directives 2004/27/EC and 2004/28/EC introduced a definition of the potential risks arising from the use of human and veterinary medicinal products and the overall risk benefit analysis. While the descriptions of a potential risk in both directives are quite similar, there is a striking difference regarding the risk benefit balance (Table 20.1): A potentially serious risk on the environment can interfere with the authorization of a veterinary medicinal product if risk mitigation is not possible and another product for the specific veterinary indication is available. However, this is not the case for human medicines, where legislation focuses on risk reducing mitigation measures only.

The environmental safety of veterinary medicinal products is also addressed in the frame of pharmacovigilance, the post authorization surveillance system that shall take into account any available information related to the lack of expected efficacy, off-label

Table 20.1. Definition of risk and risk benefit analysis in the legal framework for the authorization of human and veterinary medicinal products

2004/27/EC, human medicines	2004/28/EC, veterinary medicines
Art 1 28. Risks related to use of the medicinal product:	Art 1 19. Risks relating to use of the product:
– Any risk relating to the quality, safety or efficacy of the medicinal product as regards patients' health or public health;	– Any risk relating to the quality, safety and efficacy of the veterinary medicinal products as regards animal or human health
– Any risk of undesirable effects on the environment;	- Any risk of undesirable effects on the environment
Art 1 28a. Risk-benefit balance: An evaluation of the positive therapeutic effects of the medicinal product in relation to the risks as defined in point 28, first indent.	Art 1 20. Risk/benefit balance: An evaluation of the positive therapeutic effects of the veterinary medicinal product in relation to the risks as defined above.

use, and investigations into the validity of the withdrawal period and potential environmental problems arising from the use of the product (Article 73). At present, what kind of information (e.g., environmental concentrations, effects monitoring) should be gathered and in what way this can be done are under discussion. The legal framework does not foresee reporting environmental problems to the human pharmacovigilance system.

20.2 The EU Guidance Document on the Environmental Risk Assessment of Medicinal Products for Human Use

First drafts in the early 1990s envisaged a consolidated guidance concept for environmental risk assessment of both veterinary and human medicinal products. The guideline covered both the prediction of the environmental concentration and the assessment of fate and effects in the aquatic and/or terrestrial compartment. A base set of data on the ecotoxicity of veterinary and/or human pharmaceuticals was required irrespective of the extent of environmental exposure.

Then in the mid-1990s, the risk assessment procedure was divided into two phases. In addition, an exposure threshold value or "action limit" was introduced between the estimation of the predicted environmental concentration (PEC) in the first phase and the test requirements in the consequent second phase. This assessment was outlined in three draft documents: a guidance paper on the preliminary Phase I risk assessment procedure distinctly for veterinary and human pharmaceuticals, and in addition a guidance paper covering the in-depth Phase II risk assessment of both veterinary and human pharmaceuticals together. The action limits separating the two phases from another were 1 ng l^{-1} for surface water and 10 µg kg^{-1} for soil. The joint Phase II paper included extensive guidance on the assessment of human drugs in domestic sewage treatment systems, the assessment of veterinary drugs on grassland inverte-

brates and terrestrial vertebrate wildlife as well as the assessment of both human and veterinary drugs on aquatic organisms, soil, fauna, and crops.

A new draft dating from 1995 raised the action limit for the Phase II assessment of human medicinal products from 1 to 10 ng drug substance/l surface water. In the same year, the U.S. Food and Drug Association (FDA) initiated a retrospective review (FDA 1997) on ecotoxicity data submitted in environmental assessments over the preceding decade. These data showed approximately 90% of the results (dose effect concentrations) being 1 μg l^{-1} or greater, but only 10% being between 1 ng l^{-1} and 1 μg l^{-1}. In this respect, in 1997 the FDA implemented a Note for Guidance paper in which all drugs entering the aquatic compartment at levels below 1 μg l^{-1} ($PEC_{EFFLUENT}$) were exempted from a detailed risk assessment (FDA 1998). The EU maintained the action limit of 10 ng l^{-1} by sticking to a safety factor of 10 and taking into account the variations between USA and EU in estimating the PEC formula.

Extensive discussions on the environmental risk assessment of veterinary and human drugs in the EU resulted in further proposals: As a next step, the ambitious concept of a joint Phase II guidance document was abandoned, and individual concepts were elaborated separately for veterinary and human pharmaceuticals. Then in 1996, substantial progress was achieved when the EMEA/CVMP guidance paper on the environmental risk assessment of veterinary drugs (EMEA 1997) was finalized and implemented in the EU.

In 2001, a discussion paper on the ERA of human drugs (EMEA 2001) was released for public consultation. The EU Committee Scientifique de Toxicologie, Ecologie et Ecotoxicologie (CSTEE) (EC 2001c) and several Member States strongly opposed this document, mainly because it was not in line with the accepted approaches for the environmental risk assessment of other chemicals, e.g., the lack of a logically tiered structure and the sole focus on short-term toxicity in the aquatic environment.

In 2002, the Safety Working Party (SWP) at the European Medicines Agency (EMEA) initiated extensive redrafting in an expert group on environmental risk assessment of human medicinal products. Discussions were fruitful: Two much improved documents CPMP/SWP/4447/00 draft (EMEA 2003, EMEA 2005) were released by the EMEA Committee for Human Medicinal Products (CHMP) before the finalized guidance paper was adopted in 2006.

20.2.1 General Principles

The environmental risk assessment considers environmental exposure from the use of the medicinal product only. An evaluation of the environmental risk arising from drug production and/or disposal of used or unused pharmaceuticals is outside of the scope of the guidance document.

The protection goals of the risk assessment cover aquatic and terrestrial ecosystems, microorganisms in sewage treatment plants and top predators. Any benefit/risk analysis is outside of the scope of the guideline. The focus of the environmental risk assessment is on the active ingredient, not the formulation. If indicated, the potential impact of excipients and of the active metabolite in case of a pro-drug should be considered.

In describing a two-phased and logically tiered approach, the note for guidance is similar to the established guidelines for veterinary medicines and for feed additives. However, the exposure calculation, the action limits in Phase I and II and the data requirements are suited to the specific environmental exposure to human pharmaceuticals.

20.2.2 Phase I Risk Assessment

In step one, the pharmaceutical substance concentration expected to occur in the aquatic environment is calculated. If this value ($PEC_{SURFACEWATER}$) is below a defined action limit of 0.01 μg l^{-1}, it is assumed that this specific medicinal product is unlikely to represent a risk to the environment and the assessment stops. In case the calculated concentration in surface water exceeds the action limit of 0.01 μg l^{-1}, a Phase II environmental fate and effect analysis is required. However, when known effects from related substances or results of biological studies indicate an unusually high potential for ecotoxic effects, then a substance-tailored Phase II ERA should be carried out irrespective of the predicted environmental concentration. Criteria for the so-called "however clause" are currently elaborated (UBA FKZ-Nr.: 370765400, Test strategies for human pharmaceuticals with potential effects at low concentrations), although hormonal substances have been earmarked already.

To calculate the Phase I surface water concentration ($PEC_{INITIAL}$) in a standard risk assessment, the guidance document recommends using a simple algorithm including a default market penetration factor (F_{PEN}). This F_{PEN} resembles the proportion of a population using a human medicinal product on a daily basis. It is derived from the 95th percentile of the market success of 800 individual drug substances in Germany and considered representative of the EU. The guideline gives an option to deviate from the F_{PEN} value if data is available for a more realistic estimation. These data should be published, e.g., epidemiological studies, designated orphan drug status or medicine statistics. In-house data are not acceptable. It is important to note that F_{PEN} refinement in Phase I focuses on the indication, not on the estimated sales forecast for the specific product.

The PEC calculation is based on a worst-case scenario assuming that

- The pharmaceutical is evenly used over the year and throughout the geographic area;
- The medicinal product enters the environment via the sewage water system. The calculation of the $PEC_{INITIAL}$ calculation does not take into account other paths of environmental exposure (i.e., atmosphere, soil);
- There is no degradation or retention of the medicinal product in sewage treatment plants;
- The drug substance is not metabolized in the patient. The so-called *total residue approach* – which assumes equal toxicity of a drug substance and metabolites so that the PEC estimation may rely on 100% excretion of the parent compound – avoids extensive and resource-intensive metabolite testing in the early stages of the environmental risk assessment;
- Complete mixing of the effluent in the surface water is assumed with a dilution factor of 10.

Vitamins, electrolytes, amino acids, peptides, proteins, carbohydrates, and lipids are exempt from a Phase II assessment, because they are unlikely to result in a significant risk to the environment. Similarly, vaccines and herbal medicinal products are also exempt due to the nature of their constituents. The only experimental datum required in Phase I is the *n*-octanol/water-coefficient (K_{OW}). In most cases, this value is already generated when the drug substance is characterized and may be cross read from other parts of the dossier.

20.2.3 Phase II Risk Assessment

In the second phase, information on the physical, chemical, and toxicological properties are obtained and assessed in relation to the extent of the environmental exposure. Phase II is split into two tiers, Tier A and Tier B. The screening information base data set in Tier A allows for a rapid prediction of the environmental risk associated with the use of the medicinal product. Risk quotients, i.e., the comparison of the PEC with the predicted no-effect concentration (PNEC), characterize the risk to the aquatic compartment and to microorganisms in the sewage treatment plant. Additional assessment criteria are given with fate data, i.e., the adsorption/desorption coefficient, the n-octanol/water partitioning coefficient, or the potential for biodegradation. If a risk or a hazard is identified in Tier A, then the assessment proceeds to Tier B.

Tier A Risk Assessment

At the beginning of Phase II, a Tier A base data set on the fate and the effects of the pharmaceutical in the environment is required to allow for rapid identification of risks and/or hazards associated with the use of the product (Table 20.2). This data set comprises investigations in the aquatic compartment only. In general, all studies should be performed in compliance with OECD test guideline methods. However, information on the physicochemical properties, e.g., water solubility, dissociation constants, and vapor pressure may be cross-referenced from other sections in the dossier.

Table 20.2. Recommended tests/guidelines in Phase II Assessment Tier A

Test	Proposed guideline
Adsorption - Desorption Using a Batch Equilibrium Method Ready Biodegradability Test	OECD 106/ OECD 121/OPPTS 835.1110 OECD 301
Aerobic and Anaerobic Transformation in Aquatic Sediment Systems	OECD 308 (if justified from result of OECD 301)
Activated sludge respiration inhibition test	OECD 209
Algae, Growth Inhibition Test	OECD 201
Daphnia sp. Reproduction Test	OECD 211
Fish, Early Life Stage Test	OECD 210

The potential for degradation is investigated in an OECD 301 "ready" biodegradability study. If a substance is not readily biodegradable, then its fate should be investigated in an OECD 308 water/sediment study.

The sorption behavior of substances in sewage sludge is given with the adsorption coefficient (K_{OC}) for sewage sludge. If the average K_{OC} value exceeds 10 000 l kg^{-1}, then a Phase II assessment of Tier B for fate and effects in the terrestrial compartment is recommended, unless the substance is readily biodegradable.

If the K_{OW} of a drug substance exceeds the value of 1000, then its direct uptake by fish from the surrounding water (bioconcentration factor) should be experimentally reported in Tier B. Having determined the ratio of steady-state concentration of the drug substance in fish, an assessment of bioaccumulation from water, sediment and diet should be conducted in accordance with the EU TGD (EU 2003). It should be noted that the requirements of the OECD 305 Test Guideline only consider the test suitable for stable organic substances. If a substance is readily biodegradable, then the test may be waived. This is also the case for substances with a hydrolysis half-life <12 hours if none of the hydrolysis products has a $\log K_{ow} > 3$.

Effect studies on the aquatic base data set comprising of algae (OECD 201), *Daphnia* (OECD 211) and fish (OECD 210) allow for risk characterization in surface water. The effects assessment for *Daphnia* and for fish deviates from established risk assessment procedures by requiring the results for chronic endpoints from the start. This approach was chosen since (*i*) applying established assessment factors to results from short-term studies may not be conservative in light of high acute chronic ratios for a number of medicinal products, (*ii*) long-term study endpoints are more appropriate to investigating potential effects of pharmaceutical substances than lethality and (*iii*) the aquatic compartment is continuously exposed to sewage treatment plant (STP) effluents. For the effects assessment of antimicrobials, blue-green algae are recommended, since they are considered more sensitive than green algae. The $PNEC_{AQUATIC}$ is determined from the effect concentration of the most sensitive species tested and applies an assessment factor of 10. In addition, a $PNEC_{MICROORGANISMS}$ is likewise determined from the activated sludge respiration inhibition test (OECD 209). Following the risk-quotient approach, the $PEC_{SURFACEWATER}$ is compared to the $PNEC_{AQUATIC}$ and the $PEC_{EFFLUENT}$ to the $PNEC_{MICROORGANISMS}$ representing the aquatic ecosystem and the microbial communities of sewage treatment plants, respectively.
If at Tier A level, no risk and no hazard is identified, then the assessment is complete.

Tier B Risk Assessment

If at Tier A level a risk or a hazard is identified, then the risk assessment advances to the next tier. Tier B is the ultimate step in the risk assessment and resembles a substance-specific iterative process of PEC and/or PNEC refinement. To ensure consistent decision making, Tier B uses standard risk assessment approaches from the EU Technical Guidance Documents (EU 2003) on the risk assessment of new substances, existing chemicals and biocidal products. At Tier B, level three general options for risk refinement are given:

One option is effects testing of metabolites. If these data are available, then the total residue approach may be resolved and risk quotients are generated for the indi-

vidual excreted fractions of the active and its metabolites. The Tier A test recommendations should be followed for effects testing of metabolites.

The second Tier B refinement option uses a model for the recalculation of the $PEC_{SURFACEWATER}$. This model has been adapted from the EU TGD local emission scenario. In addition to the parameters given in the formula for the calculation of the $PEC_{INITIAL}$, the model takes into account the adsorption of the drug substance to sewage sludge and its biodegradability in the sewage treatment plants. The output value may be used for the Tier B risk characterization for the drug substance where it is compared with the PNEC of the compartments (aquatic/terrestrial) under concern.

The third option is further effects studies with the drug substance (total residue) from the TGD or alternative approaches. PNEC refinement of the total residue by using higher Tier effects studies, e.g., micro- or mesocosms, is not recommended but testing a wider range of taxa may be an option.

20.2.4 Outcome

Risk mitigation measures proposing safety and precautionary measures to limit environmental exposure should be included in the Summary of Products Characteristics (SPC) and the Product Leaflet (PL). The guideline recommends that – even for medicinal products that do not require special disposal measures – package leaflets (patient information leaflets) should include the following general disposal advice: "*Medicines should not be disposed of via wastewater or household waste. Ask your pharmacist how to dispose of medicines no longer required. These measures will help to protect the environment.*"

20.3 One Year after Adoption of the Guideline – Experiences

The German Medicines Act provides that the Federal Environment Agency (UBA) is responsible for the ERA. UBA started assessing the environmental impact of veterinary and human pharmaceuticals in an authorization routine in 1998 and 2003, respectively. Since then, UBA assessed around 180 veterinary and around 240 human pharmaceutical formulations. Filtering concepts established between UBA and the authorization agency responsible for veterinary medicines focused the ERA on antibiotics, parasiticidal substances and analgesics. Cytostatic medicines, hormones and contrast agents dominated the human medicine dossiers assessed by UBA.

With the adoption of the EMEA guidelines in 2006, EU Member States' authorities started systematically assessing the environmental risk of human pharmaceuticals irrespective of the type of application (e.g., new pharmaceutical substance, variation, or generic) and procedure (central, national, mutual recognition, or decentralized). There is a regular exchange of opinions among regulators of the EU Member States on the implementation of various issues of the guideline in authorization practice. Agencies which have not assessed the environmental risk of pharmaceuticals before are gradually building up capacities. The authors consider a CHMP environmental risk assessment working party at the EMEA useful for both an official contact point for industry and a discussion forum for regulators. As the revision of the guidelines is

scheduled five years after implementation, this would also be a useful forum for discussions on how to use the knowledge gathered by research and agencies for improving the guideline.

With a finalized document at hand, industry becomes adapted to the requirements of the guideline, and since 2006 dossier quality is increasing steadily. A number of events on animal and human health regulatory issues informed pharmaceutical companies on technical, structural and legal aspects of the ERA. Contacts were knit between regulators and industry on specific issues, e.g., test design and PEC refinement.

Phase I

The *Phase I PEC calculation* dominated discussions between applicants and assessors after guideline CHMP/SWP/4447/00 came into force. This does not come as a surprise, since this Phase I criterion decides on whether experimental studies, consuming resources and time are necessary to demonstrate safe use of the product for the environment. Pharmaceutical companies tend to present in-house data taking into account product related information, e.g., market share, number of patients treated, etc. to refine the Phase I default market share (F_{PEN}). Risk assessors have no instruments to assess the plausibility of these assumptions and frequently reject these considerations. A good example of plausible Phase I F_{PEN} refinement are orphan medicinal substances where CHMP and the pharmaceutical company agreed on the designated orphan drug status with a defined number of patients per year in the EU region.

For *extensions and variations*, the guideline requires a full environmental risk assessment if the application results in a significant increase of exposure. Unfortunately, the guideline lacks cut-off criteria for "significant increase" and a discussion on the potential increase of patients substantiated by medicinal statistical data (e.g., IMS health) is required with any new indication, application form or strength of a medicinal product.

For *generic applications*, the guideline also lacks clear guidance on data requirements stating that for these application types "the expert should provide a rationale for the absence of an ERA, taking into consideration a possible significant increase of environmental exposure to the drug substance." Without additional guidance, the authorization of generic products which fail this criterion, e.g., non-prescription medicines, may result in duplication of data on the environmental safety with the 2nd, 3rd etc. generic. However, if the applicant demonstrates that the use of the generic compound is not likely to lead to a significant increase of exposure, e.g., prescription medicines, then the ERA stops. In this case, data gaps on fate and effects of successfully marketed substances prevail. A sustainable approach would require systematic testing of existing substances in a monograph system. In light of the high number of medicinal substances marketed, such a monograph system should prioritize use volumes, pharmaceutical groups and/or mode-of-action.

Phase II

Study requirements regarding specific tests and individual test designs have repeatedly been discussed between regulators and pharmaceutical companies. In fate assess-

ment, the application of the transformation Test Guidelines for soil and water/sediment systems can be adapted to human pharmaceuticals. Extensive discussions are still needed to include experience from the toxicological assessment and focus the attention on mode-of-action-related approaches.

Regarding the fate of the pharmaceutical excreted into the surface water, the guidance document recommends OECD Test Guideline 308. The investigation of the fate and behavior of the substance in the STP is mostly neglected. Only a brief look is given on the ready biodegradability of the test substance in activated sludge. Comparing the ready biodegradability of human pharmaceuticals against degradation in the water/sediment system, it becomes apparent that only few substances are readily biodegradable in activated sludge systems. Hence, the guideline emphasizes generating data on the fate of the pharmaceutical in a test system resembling surface waters. In this context, it is important to note that a number of EU cities have poor STP performances. Good standards in STP technology are frequently undermined by limited storm flow capacities after rain events and leaking sewer systems. Once the fate profile of major pharmaceutical groups in surface water bodies is clarified, then the guideline may place more emphasis on the removal efficacies of STPs, e.g., recommending OECD Test Guideline 303.

According to the guideline, *exposure of agricultural lands* with sewage sludge triggers a terrestrial risk assessment requiring data on fate and effects. The trigger criterion is the adsorption/desorption of the active ingredient according to OECD Test Guideline 106 adapted to sludge. Regarding effects testing in soil, the requirement for an acute earthworm study (OECD 207) is disputable since the test system is insensitive to medicinal substances. Limited experience gained in authorization procedures so far reveals low predicted environmental soil concentrations and moderate to low toxicity to soil organisms. This results in low risk ratios for the soil compartment exposed with human pharmaceuticals indicating an acceptable risk for the environment. While the result of the soil degradation study may therefore be irrelevant for PEC refinement, the DT_{50} is still needed to evaluate whether the substance is persistent in soil or not. With the implementation of the REACh regulation, the PBT criteria now cover persistence in the terrestrial environment. Choosing a pragmatic approach when applying the OECD Test Guideline 307, it is considered sufficient to focus on transformation of the test substance in two different well-characterized soils (instead of four soil types) unless the degradation rate is deemed for PEC_{SOIL} refinement.

An exposure assessment for *groundwater* is required. The guideline envisages risk characterization based on the PEC for groundwater and the PNEC for aquatic invertebrates. The PEC groundwater calculation is based on a simple algorithm and should be replaced in the future by a more sophisticated model. An approach for the calculation of exposure via bank filtration is currently developed by UBA (UBA FKZ 370764400). The need for more precise modeling of groundwater exposure is given with the protection goals of the groundwater Directive 80/86/EEC (EEC 1980). Recently the commission confirmed that relevant environmental community legislation should be equally considered during the authorization procedure. A substance classified as a dangerous substance according to Directive 80/86/EEC should be prevented from entering groundwater. Being organohalogenic compounds, a range of antibiotics are Annex I candidates of Directive 80/86/EEC.

According to the authors' opinion, the demanding task of the upcoming years is the improvement of the *effects assessment* for pharmaceuticals. The guideline encourages applicants to provide results from "acceptable test guidelines and approaches and methods … which are capable of providing an equivalent environmental risk assessment." Considerations on alterations of the guideline approach for fate assessment have been given above. Three major questions arising for the effects assessment are the following:

- How do we find criteria to identify intended pharmacological mechanisms (or side effects) in patients, which may result in potential detrimental effects for populations at the observed and/or predicted environmental concentrations? It goes without saying that one key for solving this question lies in the rich toxicology part of the dossier;
- How do we find a common understanding on test systems capable of reflecting those criteria? Discussions are needed on the potential for extrapolating results of toxicological origin, histopathologic studies, traditional biomarkers, omics techniques, etc. to potential effects on the population level in the environment;
- How do we integrate these molecular, biochemical, cellular or physiological indicators in the assessment scheme for pharmaceuticals? Can novel, non-OECD approaches in the effects assessment serve as a screening tool or even replace the effects test battery given in Phase II Tier A of the guideline?

Some regulatory agencies tend to foster improvement of the assessment techniques by waiving OECD effects tests when mode-of-action targeted ecotoxicological studies are presented. Other EU member states stick to the traditional trophic level approach, since ecological relevance and sensitivity of biomarkers are still under discussion. Reflections from the pesticide and industrial chemicals' world on the use of biomarkers as "signposts" or "traffic lights" in authorization should help regulators in finding harmonized concepts for human pharmaceuticals.

A serious risk for the environment arising from the use of human pharmaceuticals does not interfere with its authorization. This is contrary to the regulation of other substances and products, e.g., pesticides, biocides, veterinary pharmaceuticals and industrial chemicals listed in Annex IX of the REACh legislation.

So far, the majority of applications reported no risk at the end of the Phase II Tier A assessment. Subsequent fate and effect testing and modeling in the 2nd tier demonstrated a safe use of those substances that entered Tier B for refinement of the ERA. However, some medicinal substances with PBT properties were authorized, contradicting the goals of the European Union to cease exposure of the environment with PBT substances. At present, the only action regulatory bodies can take is reporting those incidents to the OSPAR Commission.

20.4 Outlook

The revision of the guideline is scheduled five years post implementation. Guideline CHMP/SWP/4447/00 aims at investigating traditional OECD Test Guideline endpoints known from the ERA of biocides, pesticides and industrial chemicals. Discussions

already started on how to adapt the assessment scheme and test requirements to pharmaceuticals, e.g., by taking into account the mode-of-action of pharmaceuticals and making better use of the information available in the toxicology part of the dossier. A literature study commissioned by UBA is currently evaluating whether the standard base data set is appropriate for cytostatics or if a mechanism-based approach should be applied for these compounds, for example. The threshold value 10 ng l^{-1} triggering experimental testing, which is based on a limited data set, will be revaluated in the upcoming years using the effect data collected in research projects and authorization procedures.

An active substance-specific, prioritized monograph system can avoid duplication of data in authorization procedures. Such a program would also fill data gaps on the environmental safety of successfully marketed compounds that enter the environment at high loads.

Quantitative structure-activity relationships (QSARs) are increasingly used in environmental risk assessment. Models developed and tested for pharmaceutical compounds might become a useful tool for determining specific parameters or assist in designing experimental test strategies.

Currently the environmental risks arising from the use of human medicines is outside the benefit/risk analysis. To address the environmental safety, the Community Code requires risk mitigation measures if a risk is identified. For human medicines with numerous point sources and chronic exposure on a regional scale, risk mitigation measures known from biocides, veterinary medicines and pesticides are not feasible. Discussions with health care professionals should explore practically and economically feasible risk mitigation measures for human medicines beyond authorization. Risk communication is considered to be a key component in novel approaches for risk management of human medicines.

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The State and the Future Development/Perspective of Environmental Risk Assessment of Medicinal Products for Human Use: Aspects of Its Regulations in Japan

Y. Yoshioka

21.1 Historical Background for the Regulation of Chemicals

Japan has learned much from the experiences of public nuisance. Among them, Itai-itai disease, Minamata disease and Yusho are old and most famous in Japan. These incidents showed that chemical pollution could lead to serious health and environmental damage if it was not controlled.

Itai-Itai disease was caused by cadmium with softening of the bones and kidney failures, and patients of this disease suffered with severe pain in the joints and the spine. Cadmium was released into the Zintu River from mining companies. Cadmium accumulated in people through contaminated food, and 184 victims have been legally recognized as sufferers of this illness since 1967. In 1992, the average annual health expense compensation was U.S.$6.8 million, and agricultural damage was compensated with U.S.$16 million per year. Another U.S.$5.6 million was invested annually to reduce further pollution of the river.

The first patient with Minamata Disease was reported as an individual suffering from neurological symptoms of unknown cause in 1956. The disease was caused by methyl mercury compound, which poisoned the central nervous system. Methyl mercury compound accumulated in fish and shell fish in the Minamata Bay through the food chain. Consequently, the disease occurred when the inhabitants ate high amounts of these foods. About 3,000 persons were certified by the end of 2006 and more than 3 000 persons have requested certification. The direct total payment to the certified patients amounted to approximately U.S.$13 billion by March 2001.

A mass poisoning, the so-called Yusho incident, occurred in western Japan in 1968 because of cooking oil accidentally contaminated by heat-degraded polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). About 14 000 persons were estimated to be affected, but only 1 860 persons were certificated as affected. PCBs are persistent organic pollutants and the environmental transport of PCBs is complex and global.

These historical incidents affected the Japanese policy of chemical regulations. Japan enacted the Law Concerning the Examination and Regulation of Manufacture, etc. of Chemical Substances, or the so-called Chemical Substances Control Law (CSCL) in 1973. CSCL regulated manufacture and import of persistent, bioaccumulative and toxic chemical substances, such as PCBs. The government could conduct hazard assessment of chemical substances for human health prior to their manufacturing or import under CSCL. Japan was a pioneer of the pre-manufacturing evaluation and regulation system for new chemical substances, because there were no similar statutes in the world at that time. The law was amended in 1986 to regulate the persistent,

not bioaccumulative but toxic chemical substances. CSCL was again amended in 2003 to deal with environmental safety. Japan has turned from hazard assessment to risk assessment with this revision.

21.2 Regulation of Chemicals in Japan

The regulations on chemicals are categorized by waste, discharge and manufacture/import regulation. Chemicals are also classified by the purpose of regulation with special focus on use. Many laws and acts cooperate to keep the environment safe. From the point of environment precautionary protection, the major part of chemical regulation is covered by the CSCL.

There are some chemicals not regulated by CSCL; (1) chemicals under stricter regulation due to other laws such as specified toxic substances, stimulants, and narcotics; (2) chemicals controlled by other laws such as agricultural chemicals, pesticides, pharmaceutical products, cosmetics, food additives, fertilizers and so on; (3) disposals; and (4) household products containing harmful substances. These chemicals are used for a definite purpose, and CSCL targets the chemicals for general use.

21.3 Regulation on General Chemicals

21.3.1 The Number of New Chemicals Submitted

The numbers of general chemicals submitted for manufacture or import in Japan at levels of more than 1 t yr^{-1} and up to 1 t yr^{-1} were approximately 300 per year and 14 000 per year in 2004, respectively. The latter increased greatly over fifty years, but the former remained constant. This shows a trend of the limited production of diversified products.

21.3.2 Outlines of Chemical Substance Control Law

The CSCL is composed of two parts; (1) a pre-evaluation system for new chemical substances and (2) regulations against chemical substances depending on chemical properties and possibilities of exposure. The CSCL also deal with the existent chemicals registered at the time of the CSCL enforcement. The number of existent chemicals is about 20 000, and they are regulated in the same way as new chemicals except the nation and the enterprise cooperate to certify chemical safety. The cooperation launched in 2007 as the Japan Challenge Program.

The scheme of the evaluation procedure is illustrated in Fig. 21.1. New chemicals for the intermediates, use in closed systems and export only are exempted from pre-evaluation because there is little or no possibility of exposure in Japan. Otherwise, anyone who wants to produce/import new chemicals has to report to the authorities first.

If the amount of chemical production/import does not exceed one ton nationwide, then the chemical is permitted with no further claim for one year, although yearly

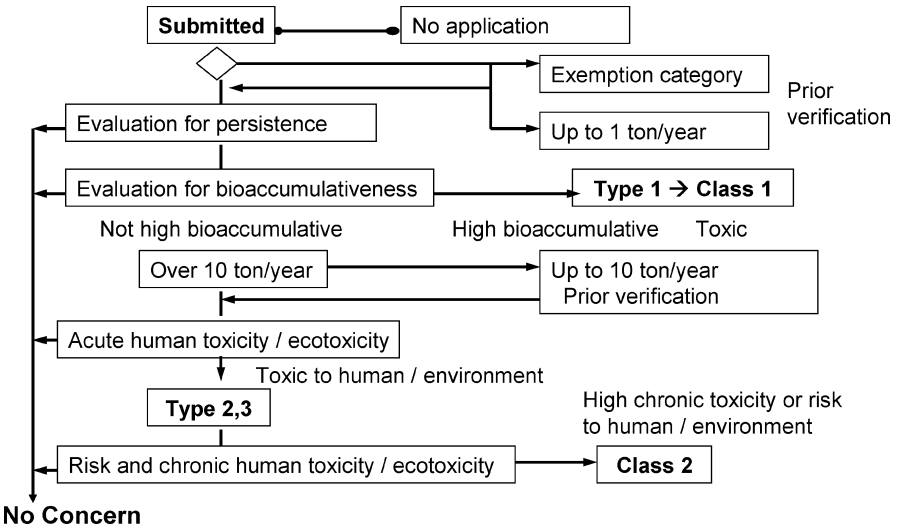

Fig. 21.1. Procedures for chemical regulation by Chemical Substances Control Law (CSCL)

application is necessary (exemption category). This is because chemicals in low volume are considered not to be risky even if the chemicals are toxic. This is called preverification. The accredited company must abide by the claims postulated. To ensure the abidance, items such as chemical name, actual amount manufactured/exported, or any changes in the verified content should be reported every year. Risk assessment for human health and environment based on the existing knowledge is still applicable, but it is seldom applied. If it is not verified, then notification of manufacture/import is required.

21.3.3
Test Guidelines and Procedures

Tests in the process should follow test procedures provided by the notice of the Director-General (of the Ministry of Health, Labor and Welfare; the Ministry of Economy, Trade and Industry; and the Ministry of the Environment). Test procedures are equivalent to the corresponding Organization for Economic Cooperation and Development (OECD) Test Guidelines with some notes (see Table 21.1). All tests should be carried out under proper laboratory practice. The standards concerning test facility and good laboratory practice operations are provided by the Director General.

21.3.4
Regulatory Classification of Chemicals

When a chemical substance is readily biodegradable (OECD 301C), no further investment is requested and its production/import is accepted. OECD 302C may be carried out if 301C shows a decline. When a chemical is highly bioaccumulative (bio-concen-

Table 21.1. Ecotoxicological tests registered and frequently used in Japan

Test		Endpoint/standard for Type III (mg l^{-1})
301C	Ready biodegradability; Modified MITI Test (I)	BOD ≥ 60%; Ready biodegradable
302C	Inherent Biodegradability: Modified MITI Test (II)	In specific case
305	Bio-concentration: Flow-Through Fish Test	BCF ≥ 5 000
201	Algae growth inhibition	72 h EC50 ≤ 2 and NOEC (rate) ≤ 0.2
202	*Daphnia* sp. acute immobilization	48 h EC50 ≤ 1–10
211	*Daphnia magna* reproduction	21 d NOEC ≤ 0.1
203	Fish, acute toxicity	96 h LC50 (*Oryzias latipes*) ≤ 10
210	Fish, early-life stage toxicity	30 d NOEC (*Oryzias latipes*) ≤ 0.1
218	Sediment-water chironomid toxicity (spiked sediment)	20–28 d NOEC (*Chironomus yoshimatsui*)
206	Avian reproduction	8 + (2 – 4) + (8 – 10) + 2*w* NOEC for Class 1

tration factor is equal to or more than 5 000 by OECD 305), the chemical is regarded undesirable for production. It will be classified as a Type I Monitored Chemical, and strict regulation will be applied (production and import are prohibited in practice). If the Type I Monitored Chemical is determined to be highly toxic to human health or the environment (by OECD206 chronic test), then it is classified as a Class I Specified Chemical Substance, and production/import is not allowed. Table 21.2 shows the classification conditions for regulation.

If the amount of chemicals which is not highly bioaccumulative is up to 10 ton, then the specific rule for low production chemicals will be applied, and production/import will be permitted. Ex post monitoring is carried out for the exempted chemicals to ascertain whether the admitted conditions are followed.

Chemicals manufactured or imported at more than 10 tons per year will be examined for human toxicity and ecotoxicity. Ecotoxicity will be determined mainly from the test results of acute toxicity tests (OECD 201, 202, 203). Chronic toxicities are estimated from acute data with practical acute/chronic ratio. Once chemicals are determined hazardous to human health or to the environment, they are classified as Types II and III Monitored Chemicals, respectively. Production volume must be reported annually for both types. The chemicals in both types are investigated for risk associated with the degree of exposure and potential long adverse effects. If a potential risk is accepted, then the applicant is ordered to perform a long-term toxicity investigation of the chemical substance. The test methods to be used for measuring the chronic ecotoxicity are OECD 201, 211, 210, 218. The necessity of the sediment-water chironomid toxicity test (OECD 218) is determined on the status of residue in the environment. When a risk is considered to be serious through the investigation, it will be regulated as the Class II Specified Chemical Substance.

At the end of 2006, the number of Class I, II and Type I, II, III chemicals are 15, 23 and 28, 836, 51, respectively.

21.4 Regulation of New Agricultural Chemicals

Agricultural chemicals must be registered prior to production or use because they are used in the open environment and can be toxic to environmental organisms as well as human health. Specific care is necessary for their use in the paddy fields because an agricultural chemical may easily flow out to the stream and cause serious damage. A risk-based assessment system for environmental safety was introduced to the agricultural chemical regulation in 2005.

The concentration of a pesticide in a paddy field is easy to estimate because the quantity of pesticide sparged per area is prescribed. The pesticide will be sparged on the paddy field (1×5 km) in a definite area (10×10 km) with a definite borderline of a stream and the number of days in operation. The pesticide flows into the tributary and joins the main river. The evaluation point is downstream from the confluence. This is the scenario employed for the agricultural chemicals. A predicted environment concentration (PEC) is calculated step by step, refining the value of the parameters by the tests (see Table 21.3).

Table 21.2. Classification conditions for regulation of the chemicals that are not biodegradable

Category	Accumulation	Chronic for Human	Environmantal toxicity	Exposure
Type I	High	Unknown	Unknown	–
Class I	High	Yes for human or high predatory environment organisms		
Type II	Low	Suspicious	–	–
Type III	Low	–	Yes	–
Class II	Low	Yes for human or environment organisms (chronic)		Possible

Table 21.3. Registration procedure for agricultural chemicals

Predicted Environment Concentration (PEC) Tier1 PEC : Estimated by calculator Tier2 PEC : Paddy; water pollution test Field; outflow test /drift test Tier3 PEC : Paddy; concentration test in paddy field /drift test etc.	
Acute effect concentration 201 Algae growth inhibition test: 202 Daphnia magna immobilization test: 203 Fish Acute Test:	(AEC = Min.[$AEC_{f,d,a}$]) UF AEC_a = 72 h – EC50 AEC_d = 48 h – EC50 × (0.1–1.0) AEC_f = 96 h – LC50 × (0.1–1.0)
If necessary, tests like microcosm test/field simulation test etc. will be carried out.	

The Acute effect concentration (AEC) is defined as the minimum of $AEC_{a,d,f}$ (OECD 201, 202 and 203) instead of the normal predicted no-effect concentration (PNEC). An uncertainty factor (UF; 0.1–1.0) corresponds to sensitivity of the test organism and the number of the test species reported. Chronic toxicity is not considered. If the PEC/AEC ratio is equal to or more than 1.0, then preventive measures are essential. Otherwise, the agricultural chemical will not be registered.

21.5 Regulation on Veterinary Medical Products

The Regulation on veterinary medical products (VMPs) in Japan links to International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). VMPs will be regulated for environment safety as described in Environmental Impact Assessment for VMPs; Phase I in 2000 and Phase II in 2004. Japan raised public opinion over VICH GL 38 (eco-toxicity Phase II) in 2004. Currently, the regulation procedure for VMPs in Japan requests no data on ecotoxicity. The municipal law will be revised after the agreement is reached in the meeting for VICH.

21.6 Regulation on Human Health Products

21.6.1 The Status of Drug Pollution and Regulation in Japan

Approval or permission is necessary under the Drugs, Cosmetics, and Medical Instruments law before medical supplies are produced, imported or sold by a business. An applicant should make a report on their quality, effectiveness, and safety. For example, documents about the origin or process of discovery, physical chemical property, stability, acute toxicity for humans, pharmacological action, absorption / distribution, and examination results of clinical tests are essential, but no ecotoxicological test is requested now.

Japan has not paid much attention to the ecological effects of drugs. Recent developments in chemical pre-evaluation consensus urged Japan to conduct political and technological research of human health products.

Kunikane et al. (2006) reported a detection of pharmaceuticals in the sewage works and a river in Japan. Eleven out of twelve pharmaceuticals searched were detected from raw and treated sewage water from eight sewage works. The concentration levels in the sewage effluents were similar to those in the EU and USA. They also detected sixteen agents out of twenty-eight antipyretic anodynes searched in the Tamagawa River below the sewage works, and maximum concentration was 0.312 $\mu g\ l^{-1}$.

21.6.2 Framework for Pharmaceutical Regulation

From the viewpoint of environmental safety, the differences between pharmaceuticals and general chemicals are negligible. The differences are limited to the scenario

for disposal and the quality and quantities of information on the product. Limited routes in disposals of pharmaceuticals result in the easy estimation of a PEC. A pharmaceutical has a lot of information about physiochemical properties and human effects and fate in the body. High physiological potentials for human health may lead to high toxicity to environmental organisms. However, many countries, including Japan, will devise a separate evaluation system for pharmaceuticals.

21.6.3 Regulation of Pharmaceuticals in Japan

The Ministry of Health, Labor and Welfare organized a research group to make a concept on the regulation of pharmaceuticals for environmental safety until 2007. The discussion is ongoing and no one can show a clear conclusion. The points anticipated are as follows:

The regulation system will be similar to that of general chemicals in Japan and the Guideline by EMEA (2006). The scenario for disposal may be limited to surface water, because Japanese citizens will throw the unused pharmaceuticals into the wastebins at home, and most of the garbage (70%) will be burned at the refuse incineration plant. The landfill of the sewage sludge which may adsorb the pharmaceuticals will be ignored because incineration is the main process of sludge treatment in Japan. The cut-off by production volume will be introduced.

The handling of the metabolites is the greatest problem. Once the group decides to regulate pharmaceuticals with their metabolites, a test and evaluation system will be rather complicated and many ecotoxicological data should be prepared for submission depending on the number of the metabolites. The group may propose to establish a risk-benefit analysis committee for the pharmaceuticals which have a high risk for environmental organisms and are an important contribution to human health.

Ecotoxicological tests should be followed by the OECD test guidelines under proper laboratory practice. The registration will be judged by the PEC/PNEC ratio or ΣPECi/PNECi. The management method of the existent pharmaceuticals is vague, but re-evaluation with ecotoxicological data may be brought in after a grace period.

References

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Shoichi Kunikane et al. (2006) Research on the existence of physiologically active substances in drinking water sources and control by drinking water treatments. Research Committee, Ministry of Health, Labour and Welfare, FY2004-2006 (in Japanese)

Deterministic and Probabilistic Environmental Risk Assessment for Diazepam

J. O. Straub

22.1 Introduction

Concern about trace pharmaceutical residues in the environment started with the first reports in the 1970s (Hignite and Azarnoff 1977). Especially in the 1990s and early 2000s, many widespread further detections, thanks to a steadily increasing analytical sensitivity down to picogram-per-liter dimensions, have consistently fueled this concern, particularly as pharmaceuticals are developed for specific and high biological activity.

As one consequence, coordinated international and national research programs on the environmental presence, fate, effects and assessment of risks of micropollutants that include or focus on pharmaceuticals were initiated from the 1990s onward (e.g., COMPREHEND 2002; ERAVMIS 2004; REMPHARMAWATER 2003; POSEIDON 2005/2006; ERAPharm 2007; NoMiracle 2005; START 2007; USGS 2002). Furthermore, an environmental risk assessment (ERA) for active pharmaceutical ingredients (APIs) was added to the requirements for new drug registration both in the European Union (EU), the USA and Australia. Formal guidelines for an ERA for human APIs were published in the USA in 1998 (FDA 1998) and in the EU in 2006 (EMEA 2006), after a thirteen-year-long development (see Straub 2005). Comparable guidelines are currently in development in Canada and Japan. In addition, certain individual countries, e.g., Sweden and the United Kingdom, have started looking into potential environmental risks from 'old' APIs that are already on the market.

Doing an ERA for existing APIs is particularly interesting because in many instances, there are both fate and effects data, as well as use and sales information and measured environmental concentrations (MECs) available. These allow an ERA not only in a prospective manner, i.e., based on extrapolations as for new APIs, but also based on actual measured data, adding realism and allowing an evaluation of methodologies for predicted environmental concentrations (PECs) and ERAs in general (Straub 2006; Straub and Stewart 2007). This contribution is the first extensive ERA for diazepam, respectively for the whole family of benzodiazepines.

22.2 Diazepam Basic Data and Methods

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one; $C_{16}H_{13}Cl\,N_2O$; CAS 439–14–5; Fig. 22.1; American Chemical Society 2003) is one of the first benzodiazepines used in medicine: an API with anxiolytic, sedative, muscle-re-

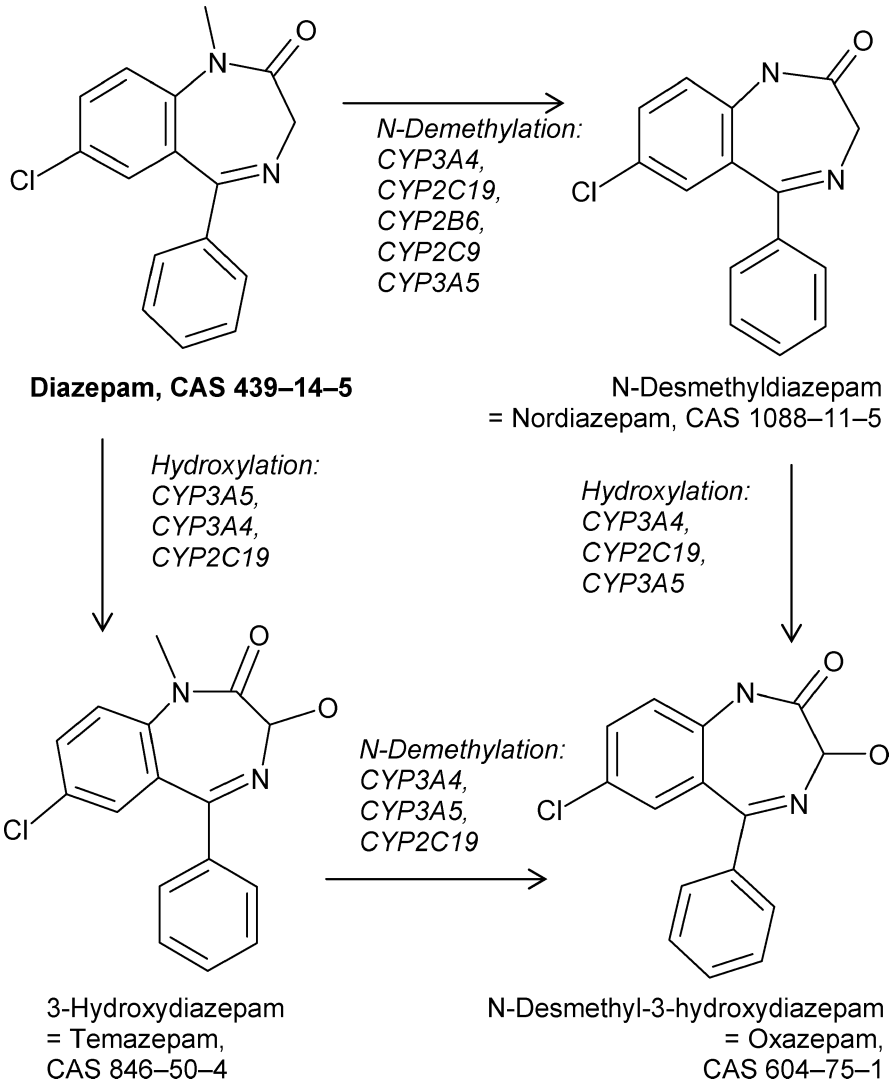

Fig. 22.1. Diazepam, its Phase-I metabolic pathways, the cytochrome P450 (CYP) enzymes involved and the corresponding metabolites (based on Ono et al. 1996)

laxant, anti-convulsive and anti-epileptic properties (F. Hoffmann-La Roche 2005). Diazepam was originally developed by F. Hoffmann-La Roche Ltd. in the 1960s.

The Defined Daily Dose (DDD) for diazepam is 10 mg per patient (WHO 2006); the maximum admissible daily dose is given as 60 mg (Martindale 2005). Subsequent to oral application, diazepam shows rapid uptake, high bioavailability and plasma protein binding (Martindale 2005). Hepatic metabolism is the main pathway for elimi-

nation of diazepam in man; the important cytochrome-P450 Phase-I enzymes (Ono et al. 1996) are shown in Fig. 22.1. The Phase-I metabolites (nordiazepam, temazepam and oxazepam) are also pharmacologically active; moreover, they have a longer half-life than the parent diazepam, which results in an overall elimination half-life of up to 48 h (F. Hoffmann-La Roche 2005; Martindale 2005). Phase-II metabolism consists of glucuronidation or sulfatation of diazepam or the Phase-I metabolites, in particular oxazepam as the main excreted metabolite (Ono et al. 1996). Human excretion of diazepam is mainly via the urinary pathway in the form of conjugates. Quantitative estimates of the relative amounts of diazepam and its metabolites as a fraction of total excretion vary widely. An older reference (van der Heide and Hueck-van der Plas 1984) reports up to 50% of orally ingested diazepam to be excreted as the parent via the urinary pathway. In contrast, based on several literature data, Lienert et al. (2007) report a mean estimate of approximately 11% of ingested diazepam being excreted as the parent or its glucuronide conjugate. At the low end, according to former Roche benzodiazepines researcher Dr Roland Amrein (pers. comm.), most, i.e., approximately 95% of ingested diazepam is metabolized and partly conjugated, meaning that only 5% is excreted as the parent or its conjugate.

Physicochemical, environmental-fate and ecotoxicological basic data for diazepam were investigated in company reports, public databases and open literature. Data gaps were identified and the following environmental fate and effects tests were commissioned at contract laboratories working under GLP or ISO 17025 quality assurance systems. All tests were performed according to the respective guidelines, with independent controls and analytical HPLC confirmations or scintillation counting. They include an inherent respirometric biodegradation test, prolonged to eighty-four days, following OECD guideline 302C except for using only one activated sludge from a municipal sewage works, with determination of the parent by HPLC (Häner 2005a); an ultimate anaerobic biodegradation test according to ISO guideline 11734 with three different initial concentrations of diazepam (Häner 2005b); growth inhibition tests with green algae *Desmodesmus (Scenedesmus) subspicatus* (Häner 2005c) and with blue-green algae *Synechococcus leopoliensis,* both according to OECD 201 (Häner 2005d); a 21-d waterflea reproduction test with *Daphnia magna* according to OECD 211 (Peither 2006a); a developmental toxicity test with aquatic larvae of the midge *Chironomus riparius* according to OECD 219 (Memmert 2005), which included a screening bioaccumulation assessment with radio-labeled diazepam in an additional test vessel over eleven days (Memmert 2005); and last, an early-life-stage test with the zebrafish *Danio rerio* according to OECD 210 (Peither 2006b).

Annual sales data for diazepam for the years 1995–2003 were collated from the IMS Health Database (IMS Health/IMS MIDAS 2004) for Western Europe (the EU-fifteen member states Austria, Belgium, Denmark, Finland, France, Germany, Greece, Italy, Ireland, Luxemburg, Portugal, Spain, Sweden, The Netherlands and the United Kingdom, plus Switzerland and Norway). The average total for these years was divided by the total population for Western Europe, taken from Eurostat (Eurostat 2001), and by 365 days per year, to arrive at an average daily use in micrograms per inhabitant and day. The same procedure was followed for Germany, where most of the measured environmental concentrations (MECs) have been determined, for later comparison of predicted environmental concentrations (PECs) with the MECs.

Local PECs were extrapolated according to the EU EMEA 2006 guideline for ERA of human pharmaceuticals (EMEA 2006), using the default penetration factor F_{pen} of 0.01 and the maximum daily diazepam dose of 60 mg per patient (Martindale 2005) for the crude EMEA Phase I local PEC, or by using the average daily use per inhabitant in Western Europe instead of F_{pen} and maximum daily use, entering either 50% or 95% metabolism in patients to model high and low PECs, respectively, and an empirical sewage works elimination rate (similar to EMEA Phase IIB local PEC, including the empirical average instead of maximum daily dose). Refined regional PECs for the whole of Western Europe and for Germany were computed using the European Union System for Evaluation of Substances, EUSES 2.0 (ECB 2005), which includes the formulae of the EU Technical Guidance Document (TGD) for ERA (European Commission 2003). Diazepam basic data were entered as well as the average annual use. The EUSES 'Region' was reconfigured using geographical, hydrological and population data for Germany (Eurostat 2001, CIA 2005), entering the German average annual use as well as metabolism and fate data as above.

For probabilistic assessment (Solomon et al. 1996), published European diazepam surface water MECs were back-distributed, combined and percent-ranked to form a MEC distribution as described earlier (Straub 2006; Straub and Stewart 2007). Briefly, MEC data given as a range or as percentiles with maxima were back-distributed per publication, based on total number of samples, number below limit of quantitation (LOQ), median, 90th or 95th percentile and maximum value, to a theoretical fraction of detections per 1-ng l^{-1}-gradation in a concentration range from ≤ 1 ng l^{-1} up to 500 ng l^{-1}. Then, both the precisely known numbers (given single values, maxima, the median from those studies with an odd number of analyses) and the expected fractions of detections per ng/l-gradation were summed up, multiplied by 100 and divided by the total number of samples plus 1, in a percent-ranking procedure. From the 500 values computed, only those were kept for plotting (SigmaPlot 8.0, SPSS, Point Richmond, CA, USA) where at least one actual analytical determination was certain. The associated regression line allowed the graphic estimation of the overall median and 95th percentile MEC values (MEC_{95}), based on the total of European, mainly German, data. The same procedure was used for combining published European sewage treatment plant (STP) effluent MECs. These combined MEC distributions can then be compared with the PECs. All existing and new acute 50%-effects (EC_{50}, LC_{50}) data and the chronic no-observed-effect concentrations (NOECs) from ecotoxicity test results were also collated and percent-ranked to form acute and chronic species sensitivity distributions (SSDs), respectively (Solomon et al. 1996). Where more than one EC_{50} was available for a given species, these were geometrically averaged, in order not to overrepresent single species in the SSDs.

22.3 Results

All located published and company-internal environmental fate and effects data including the new test results are listed in the Diazepam Data Tables 22.A1–22.A4 (see Appendix). Table 22.A1 shows physicochemical, mobility and bioaccumulation data; Table 22.A2 collates degradation and environmental fate information; Table 22.A3

gives PECs and MECs for diazepam for Western Europe and Germany; and lastly, Table 22.A4 presents the ecotoxicity test results.

The PECs for Western Europe and Germany are based on the annual use of diazepam in these areas, as listed by IMS Health (IMS Health/IMS MIDAS 2004). The mean annual European use for the years 1995–2003 was approximately 6 300 kg (range 6 460–6 170 kg a^{-1}, with a decreasing trend in time), which corresponds to an average use of 44.83 µg inhabitant^{-1} d^{-1} (385 million inhabitants in Western Europe in 2000; Eurostat 2001), while the yearly use in Germany during 1995–2003 was about 1 140 kg (range 1 180–1 110 kg a^{-1}, also with a decreasing trend), corresponding to 37.95 µg inh^{-1} d^{-1} (82.3 million inhabitants in Germany; Eurostat 2001). Thus, the average *per capita* use of diazepam in Germany was around 15% below that of Western Europe.

22.3.1 Environmental Exposure and Fate Assessment

Environmental Exposure

Manufacture and Formulation. Wastes from chemical synthesis are disposed of through high-temperature incineration with flue gas scrubbing. Traces of APIs originating from cleaning operations of galenical manufacturing and formulating equipment may be released to sewage systems and reach the aquatic compartment after passage through an STP. Such losses were estimated at 0.2% of the total, based on a balance of materials of galenical production of solid medicines at Roche Basle (Hörger et al. 2005). As a single API is only formulated intermittently, during one to a few days per year and manufacturing plant, galenical production is estimated to lead to local and temporary exposures only.

Proper Use. Diazepam has a high bioavailability on oral administration. Metabolism is mainly through hepatic pathways, with extensive formation of pharmacologically active Phase-I metabolites, which are further Phase-II-conjugated. Excretion is mainly renal (F. Hoffmann-La Roche 2005), hence, all excreted substance will collect in sewage. Between 5% (R. Amrein, pers. comm.) and maximally 50% (van der Heide and Hueck-van der Plas 1984) of ingested diazepam is excreted as the parent, partly conjugated. A higher rate of metabolism is supported by measurements in Italian sewage works, where around three times as much N-desmethyldiazepam as diazepam was found both in the influent and effluent (Castigliono et al. 2006).

Disposal. Overaged galenical dosage forms of diazepam, returned by distributors or from Roche warehouses, are incinerated in qualified installations. The product disposed of directly by customers consists of dosage forms, mainly tablets containing diazepam. These will end up in special waste or domestic refuse, which is either incinerated or dumped into approved landfills. In the case of irresponsible disposal, nonused drugs may be drained into domestic wastewater and enter a communal STP (START-News 2006).

Environmental Fate

Sewage Treatment. Most domestic wastewater in Europe is treated in STPs (European Commission 2003). Based on data from other APIs (Möhle and Metzger 2001),

deconjugation of glucuronides may be expected. Diazepam was not biodegradable in two standard aerobic biodegradation tests (Häner 2005a; Gröner 1981); hence, no rapid elimination in STPs may be assumed. This is supported by two laboratory STP models (POSEIDON 2005/2006; Clara et al. 2002), where no significant removal was found. On the other hand, an inherent biodegradation test (Häner 2005a) prolonged to eighty-four days showed 70% primary degradation of diazepam, evidenced by (non-identified) metabolite HPLC peaks, but little sorption (max. 11% by DOC). In working STPs in Austria (Clara et al. 2002) and Belgium (van der Ven et al. 2004), some (from unquantified up to theoretically 83%) removal was noted based on measured influent and effluent concentrations. However, the analytical determination of diazepam was hindered by signal-to-noise problems in these difficult matrices (Clara et al. 2002; van der Ven et al. 2004). In contrast, in six STPs in Italy, the loads of diazepam in the effluent were slightly greater than in the influent (influent mean 0.4 (range ND–1.4) mg/d/1 000 inhabitants vs. effluent 0.5 (ND–1) mg/d/1 000 inhabitants; Castiglioni 2006). The same picture emerged for the metabolite N-desmethyldiazepam, which supports deconjugation or higher deconjugation than removal. Therefore, while some elimination seems possible in individual cases, low removal rates overall are expected for diazepam in sewage treatment (Joss et al. 2006). Based on these reports, STP elimination for the PEC models was set to zero.

Published $\log K_{OW}$ values range between 2.58 and 2.99 (F. Hoffmann-La Roche 2005; Ran et al. 2002; Di Guardo et al. 2001; Hilal et al. 1996; Stuer-Lauridsen et al. 2000) and one measured liposome/water distribution coefficient agreed with a $\log D_{lipw}$ of 2.79 (Escher et al. 2002), all suggesting insignificant adsorption to sewage sludge. In contrast, another lipid-microsome/water distribution coefficient translates to a $\log D_{lipw}$ of 4.76 (Omran et al. 2001), predicting some adsorption to sludge. However, based on measured sludge/water partition coefficients (K_d) of 7.9–15.8 l kg^{-1} (Clara et al. 2002) respectively 44 ±26 l kg^{-1} (POSEIDON 2005/2006; Joss et al. 2006) for primary sludge and 39.1–100 l kg^{-1} (Clara et al. 2002) respectively 21 ±8 l kg^{-1} (POSEIDON 2005/2006; Joss et al. 2006) for secondary activated sludge, no significant removal through adsorption to sewage sludge may be expected for diazepam. Based on a graph by Joss and colleagues (2006), less than 2% sorption to sludge is predicted. As shown in a sediment/water fate study (Mamouni et al. 2005, see below), sorption and bound residue formation is an important environmental process for diazepam in the long term. However, in the case of diazepam, sorption is not rapid and the average hydraulic retention time in an STP of only a few hours is far too short for diazepam to partition to activated sludge to a significant degree.

Anaerobic Sludge Digestion. An ultimate anaerobic degradation test resulted in zero degradation but showed toxicity at a nominal concentration of 150 mg diazepam l^{-1} (Häner 2005b). In contrast, at lower initial concentrations (30 and 20 mg l^{-1}) there was consistently an initial biodegradation, as evidenced by the day-13 inorganic carbon (IC) production, which was the higher the lower the diazepam starting concentration was; but this turned into negative degradation, in comparison with blank sludge anaerobic IC production, by day 64 (Häner 2005b). This is tentatively interpreted as a partial initial primary degradation, showing basic nontoxicity to anaerobic bacteria and anaerobic degradability of diazepam itself, which results in one or more metabolites that are inhibitory to anaerobic bacteria, slowing further degradation so much

that the net IC production compared to the blank sludge becomes negative. In support of these results, a paper on anaerobic digestion concludes that 'data does not allow to discriminate between no or a partial removal (0 to 60%)' during anaerobic digestion (POSEIDON 2005/2006). In another STP the same authors found 10–50% elimination (POSEIDON 2005/2006), which was recently refined to 20–50% diazepam removal in mesophilic and 30–60% in thermophilic anaerobic digestion, after sludge adaptation (Joss et al. 2006). This is taken to confirm significant (>10%) anaerobic degradation of the minor part of diazepam ending up in surplus sludge.

Soil. Due to the small fraction of diazepam adsorbing to activated sludge and to its partial degradation in anaerobic sludge digesters, no significant concentrations in treated sludge are expected. In an experiment where composted sludge containing radio-labeled diazepam was spread on three soil types, low mobility was confirmed by ~90% of applied ^{14}C-diazepam remaining in the top 10-cm layer and only about 1% moving deeper than 15 cm in 48 h (Oppel et al. 2004). Low mobility is further supported by a soil lysimeter study, where diazepam was not detected at 0.4, 0.8 and 1.2 m depth nor in the deeper groundwater after irrigation of a field with treated wastewater containing the compound at an unstated concentration; the authors noted that 'most (>80%) [was] sorbed or degraded' (POSEIDON 2005/2006). In spite of low soil adsorption coefficients in OECD 106 laboratory tests ($K_d = 13–20\ l\ kg^{-1}$ in silty soil respectively clayey silt with solution application, $K_d = 4–7\ l\ kg^{-1}$ in the same soil types with application in aged sludge; Kreuzig et al. 2003), which are suspected to be so low due to the comparatively short equilibration time, diazepam in soil is not highly mobile, or else it is degradable, or both.

Landfills. A minor amount of diazepam may be expected to end up in landfills from the disposal of municipal or industrial wastes or of sewage sludge. Indeed, diazepam was detected in concentrations of 453 and 192 ng l^{-1} in two landfill leachates in Germany (Schneider et al. 2004) and of ~10–40 µg l^{-1} in groundwater near a Superfund landfill in New Jersey, USA, 'in which pharmaceutical manufacturers disposed of chemicals' (Genicola 1999). These two reports show that under certain circumstances, diazepam may be mobilized from unknown concentrations in landfills into the groundwater. On the other hand, if the initial partial biodegradation evidenced in anaerobic degradation tests (Häner 2005b) and sludge digestion (POSEIDON 2005/2006; Joss et al. 2006) can be translated to landfills, some (unquantifiable) anaerobic biodegradation may also be expected there.

Groundwater. Diazepam may theoretically enter the groundwater compartment through the application of treated or untreated wastewater and of sewage sludge, through leaching from landfills or through river bank or sediment infiltration from surface waters. However, in spite of the landfill leachate analyses up to 40 µg l^{-1} (Schneider et al. 2004; Genicola 1999) listed above, diazepam was not detected in 105 German groundwater monitoring well samples with an LOD of 6.9 ng l^{-1} (Sacher et al. 2001) nor in seven additional German groundwater samples with an LOD of 20 ng l^{-1} (Wolf et al. 2004). Hence, as diazepam can only be detected close to landfills, this confirms limited mobility or removal during groundwater passage. Sorption and/or degradation was also the conclusion of the POSEIDON EU project report (POSEIDON 2005/2006), which found (*a*) low mobility in surface soil in a lysimeter study (see above); (*b*) no change in groundwater diazepam concentration after the first twenty-five days of subsurface flow, with the initial concentration already below the

LOQ; however, after an additional fifty days all data were below LOD; and (*c*) a total of 10–50% elimination of diazepam in groundwater in the saturated zone over the duration of more than ten weeks (POSEIDON 2005/2006). Based on data from a sediment/water fate study described below (Mamouni et al. 2005), slow adsorption to organic matter with subsequent formation of immobilized, covalently bound residues or possibly biological degradation is predicted to be the fate of diazepam in soils and groundwater. In conclusion, with the exception of sampling sites close to landfills, no significant groundwater concentrations of diazepam are expected.

Surface Waters. Based on laboratory model STPs (POSEIDON 2005/2006; Clara et al. 2002), measurements in actual plants (Clara et al. 2002; van der Ven et al. 2004) and two mathematical STP models (Episuite 3.12, U.S. EPA and Syracuse Research Center 2004; SimpleTreat v.3.1, RIVM 2003), the bulk of the diazepam will remain in solution and pass right through an STP. On release of the effluent into receiving waters, dilution will take place, resulting in low but measurable concentrations of diazepam and its metabolites in the aquatic compartment. Diazepam is reasonably soluble in water (36–50 mg l^{-1}; Häner 2005c,d; Ran et al. 2002; Di Guardo et al. 2001; Yalkowski and Pinal 1993; Chen et al. 2002). With a base dissociation constant pK_b of 3.3–3.4 (Hilal et al. 1996; Stuer-Lauridsen 2000; Halling-Sørensen et al. 1998), diazepam will be present in neutral, unionized form over the environmentally relevant *p*H range of 5–9. Diazepam has no hydrolysable bonds and was shown to be hydrolytically stable in the short term in aqueous solution in the dark at 22 °C over 120 h by HPLC (Häner 2005a). Based on the calculated Henry's Law constant ($K_H = 2.9 \times 10^{-4}$ Pa m^3 mol^{-1}; Ran et al. 2002; EPISuite 2004; SPARC Online Calculator 2005), diazepam will not volatilize from water, nor will its metabolites (data not shown); hence, partitioning to the air is not expected.

In the uppermost layer of surface waters, diazepam may be expected to degrade pretty rapidly by photolysis, based on reports showing approximately 20% aquatic photodegradation in 2 h at 300 nm wavelength (intensity not stated) to 2-methylamino-5-chlorobenzophenone, with opening of the benzodiazepine ring (Cornelissen 1980) and a photolytic half-life of about 36 h in distilled water in a photoreactor (West 2005), with degradation following pseudo-first-order kinetics and resulting in the formation of various photoproducts with longer half-lives (West 2005). A simplified photostability test in lower light intensities in an algal test light cabinet at 22 °C concurred in 25% loss by HPLC after 120 h, compared to a stable dark control (Häner 2005a). Hence, aquatic photodegradation is estimated to be an important environmental fate process for diazepam in the top layer of surface waters.

Water/Sediment. In two sediment/water-system fate studies according to OECD guideline 308 under aerobic conditions (Mamouni et al. 2005; Löffler et al. 2005), the partitioning dynamics and persistence of diazepam were investigated. In the first study (Mamouni et al. 2005), partitioning into the sediment compartment proved to be comparatively slow, even though eventually after thirty days approximately 80% of [^{14}C] activity was to be found in the sediment compartment, uniformly in both the river and pond systems. While 75% of [^{14}C] activity could be extracted from the sediment, between 5 and 8% proved to be non-extractable. While this is a relatively slow rate of bound residue formation, it is a significant, final removal of the substance. Roughly 21% of [^{14}C] activity was still in the water phase on day 30 and nearly 20% on day 60, reflecting slow and limited partitioning to the sediment compartment, while 0.1% $^{14}CO_2$ on day 30 and still below 1% on day 60 confirmed diazepam to be recalcitrant

to biomineralization. By day 60, however, less than 80% was confirmed by HPLC to be diazepam, while the sum of the water-phase and sediment-extractable [^{14}C] activity still correspondeds to around 90% of the total, which shows that a significant fraction of more than 10% must have undergone primary degradation. The water-phase half-life in the river sediment/water system in this first test was about ten days; in view of the flat slope of the formation of bound-residues, no useful extrapolation to total system half-life was possible in the first study (Mamouni et al. 2005). In the second study (Löffler et al. 2005), the authors calculated a total system half-life of 311 ±26 days and a DT_{90} of over one year. Also in the second water/sediment test over 100 days (Löffler et al. 2005), less than 2% $^{14}CO_2$ was reported, which confirms that biomineralization is not an important environmental fate for diazepam. While the DT_{50}s and DT_{90}s in this paper constitute extrapolations, they are certainly correct as to the magnitude of the duration.

Conclusion. Diazepam is an API that is excreted to a minor fraction (5%, possibly up to half of administered) as the parent or its conjugate. In STPs, conjugates are expected to be cleft again to the parent. Only very limited elimination by adsorption may be assumed during sewage treatment, and that small fraction is expected to be anaerobically degraded at least in part, while most diazepam will enter receiving waters with the STPs effluent. In the surface waters, the substance will be diluted and advected, transported onwards with the flow. In the superficial layers, diazepam is expected to be photodegraded rapidly. However, as only a small fraction of the surface water bodies receive sufficient sunlight for efficient photodegradation, most diazepam is expected to slowly adsorb to undissolved solids in the water column (and in part sink to the bottom with these) or it may adsorb directly to sediment. In the sediment, slow formation of bound residues will render diazepam bio-unavailable and effectively take it out of environmental circulation. In anoxic sediment layers, additionally, some anaerobic biodegradation is likely. A part of the diazepam will be transported with rivers to the sea, where the same fate mechanisms are expected. Diazepam disposed of by land-filling or land-spreading of sewage sludge may undergo partial biodegradation under anaerobic conditions; in soils or groundwater it will be relatively immobile and slowly form bound residues.

22.3.2 Environmental Effects Assessment

Microorganisms. Diazepam was not toxic to aerobic sewage sludge in the biodegradation toxicity controls with NOECs of 30 mg l^{-1} (Häner 2005a) and 100 mg l^{-1} nominal concentration (NC) (Gröner 1981). While it was inhibitory in the first anaerobic degradation test at 150 mg l^{-1} starting concentration (Häner 2005b), it was not, or at least not initially, at 30 and 20 mg l^{-1} (Häner 2005b); later inhibition in these tests is interpreted to argue for toxicity by a metabolite. Further, it was not toxic to bacteria with a short-term NOEC of 10 000 mg l^{-1} NC (Calleja et al. 1993, 1994; Calleja and Persoone 1993) nor to fungi and yeasts with EC_{50}s ≥ 290 mg l^{-1} NC (Botsford 2002; Koch et al. 1993) or in the prokaryote mutagenicity assay (F. Hoffmann-La Roche 2005). Therefore, at expected low concentrations no disruption of the biological step in STPs is expected nor is there any indication of toxicity towards microorganisms in the environment.

Algal Toxicity. Diazepam proved to be relatively toxic to algae, with a lowest 50% biomass effect concentration E_bC_{50} of 0.61 mg l^{-1} average measured concentration (AMC) over 72 h in an OECD 201 test with the green algae *Desmodesmus subspicatus* (Häner 2005c). The corresponding growth rate E_rC_{50} was 3.11 mg l^{-1} AMC, while the respective nominal values were 7.23 and 22.8 mg l^{-1} NC and the NOEC was <2.56 mg l^{-1} NC (Häner 2005c). On prolongation of this test beyond the regulatory 72 h to 14 d, however, almost full recovery of the algae in the 6.4 mg l^{-1} and 16 mg l^{-1} NC test vessels was seen when compared with the controls, with the long-term LOEC being 16 mg l^{-1} NC and the NOEC 2.56 mg l^{-1} NC (Häner 2005c).

In an OECD 201 test with the cyanobacteria *Synechococcus leopoliensis,* the 72-h E_bC_{50} was 3.5 mg l^{-1} AMC, the E_rC_{50} was >11.9 mg l^{-1} AMC and the NOEC 0.67 mg l^{-1} AMC (Häner 2005d). In nominal concentrations, the E_bC_{50} was 18.2 mg l^{-1} NC, the E_rC_{50} > 40 mg l^{-1} NC and the NOEC 6.4 mg l^{-1} NC. On prolongation of this test to 7 d (the cells lysed later on, but also in the controls), there was complete recovery at 16 mg l^{-1} NC (= 7-day NOEC) and partial recovery at 40 and 100 mg l^{-1} NC, all compared with controls, with no EC_{50} being reached (Häner 2005d). This recovery in both *Desmodesmus* and *Synechococcus* (Häner 2005c,d) shows either adaptation of the algae to diazepam or degradation of the test substance. The latter is also suggested by photodegradation data, which were arrived at by exposing 48 mg diazepam l^{-1} in distilled water in algal test light cabinets, resulting in 25% loss by HPLC over 120 h at 22 °C, in contrast to zero loss in the dark control (Häner 2005a).

The above examples show the importance of nominal concentrations, because in these algal tests ((Häner 2005c,d), the measured diazepam concentrations dropped rapidly to below HPLC LOQ, hinting at degradation or adsorption to the algae (with concomitant higher local exposure). Further, in the literature the test concentrations for the most part are not analytically confirmed; hence for a fair comparison with literature data, nominal concentrations should be used. For instance, for the brackish water green algae *Tetraselmis chuii,* a 96-h EC_{50} of 16.5 mg l^{-1} NC and a NOEC of 7.9 mg l^{-1} NC were determined in an OECD 201 test (Nunes et al. 2005). This compares very well with the effect levels found in terms of nominal concentrations in the two aforementioned algal tests over the regular test duration.

Acute Toxicity to Invertebrates. The available acute toxicities of diazepam to invertebrates range from >1 to 10 000 mg l^{-1} NC. The LC_{50} of >1 mg l^{-1} for the freshwater polyp *Hydra vulgaris* (Pascoe et al. 2003) actually refers to an acute NOEC; at 10 mg l^{-1}, all the polyps were dead, but also in the corresponding solvent control, hence no dependable LC_{50} can be derived from this test. The lowest EC_{50} located was 4.3 mg l^{-1} for the waterflea *Daphnia magna* (Lilius et al. 1995); in the same publication, *D. pulex* had an EC_{50} of 12.7 mg l^{-1} (Lilius et al. 1995), while in two other papers the *D. magna* EC_{50} is given as 14.1 mg l^{-1} (Calleja et al. 1993; Calleja and Persoone 1993). For another freshwater crustacean, the fairy shrimp *Streptocephalus proboscideus,* the same authors give a 24-h EC_{50} of 100 mg l^{-1} and for the freshwater rotifer *Brachionus calyciflorus* a very high 24-h NOEC of 10 000 mg l^{-1} (probably reached by using a solvent) (Calleja et al. 1993; Calleja and Persoone 1993). For marine invertebrates, a 24-h EC_{50} of 67 mg l^{-1} was published for the crustacean *Artemia salina* (Calleja et al. 1993; Calleja and Persoone 1993) and a 48-h EC_{50} of 12.16 mg l^{-1} for the related *A. parthenogenetica* (Nunes et al. 2004, 2005). In addition, for the saltwater rotifer *Brachionus plicatilis,* a very high 24-h NOEC of 10 000 mg l^{-1} was given (Calleja et al. 1993; Calleja and Persoone 1993).

Acute Toxicity to Vertebrates. Acute vertebrate toxicity is mainly represented by an older fish test with the rainbow trout *Oncorhynchus mykiss,* which showed a 96-h NOEC of 50 mg l^{-1} NC and an LC_{50} of 84 mg l^{-1} NC (Gröner 1981). Recently, a 96-h LC_{50} of 12.7 mg l^{-1} was published for the euryhaline fish *Gambusia holbrooki* in brackish water (6 g NaCl l^{-1}) (Nunes et al. 2005). In a pharmacological test exposing common eels, *Anguilla anguilla,* to 0.5 mg l^{-1} NC during sixty minutes, nonsignificant effects on heart rate (depression), electrocardiographic parameters (QRS-waves: elevation; T-waves: depression) and respiratory rate (depression) were described, occurring mainly during the first thirty minutes of exposure (Hassan Mourad 1992); these effects are considered pharmacological, i.e., low- to medium-level effects expected from a benzodiazepine based on the mode of action of this particular class of APIs, rather than toxic. Neurophysiological research with an induced-epilepsy model in tortoises, *Testudo graeca,* also showed expected pharmacological effects in the low-dose range (comparable to human therapeutic doses on a mg/kg-bodyweight basis) and excessive effects at higher doses (Servít and Strejcková 1974).

Cytotoxicity and Biomarkers. Several in vitro cytotoxicity tests with fish cells confirm low cellular toxicity from diazepam, with a 3-h EC_{50} of 659 mg l^{-1} in fresh rainbow trout hepatocytes in a Rubidium Leakage test (Lilius et al. 1994), a 24-h EC_{50} of 103 mg l^{-1} in the trout liver cell line R1 in a Neutral Red Uptake (NRU) test (Castaño et al. 2003), a 24-h EC_{50} of 103 mg l^{-1} in the *Poeciliopsis lucida* hepatoma cell line 1 (PLHC–1) in a methyl thiazolyl tetrazolium (MTT) cleavage test (Caminada et al. 2006), a 24-h EC_{50} of 125 mg l^{-1} in PLHC–1 in an NRU test (Caminada et al. 2006) and a 24-h EC_{50} of 175 mg l^{-1} in the rainbow trout gonadal cell line 2 in an MTT test (Caminada et al. 2006). In crustaceans (Nunes et al. 2006), low oxidative stress in *A. parthenogenetica* due to 48-h exposure to diazepam was described, with an overall NOEC of 5.86 mg l^{-1} for the following biomarker enzymes: total and selenium-dependent glutathione peroxidase, glutathione reductase, superoxide dismutase, glutathione-S-transferase and thiobarbituric-acid-reactive substances. The same NOEC for cholinesterase activity was taken as evidence for low neurotoxic stress due to diazepam (Nunes et al. 2006).

Baseline Toxicity. Baseline or membrane or narcotic toxicity describes the expected effect concentration based on physical partitioning of a substance into and interference with the function of phospholipid cell membranes (Escher et al. 2002). Normally, the *n*-octanol/water partition coefficient $\log K_{OW}$ is used as a substitute for lipid partitioning, but in the case of diazepam there is a measured lipid-membrane/water partition coefficient $\log D_{lipw}$ of 2.79 (Escher et al. 2002) as well as one measured in a different way and recalculated in this ERA as $\log D_{lipw}$ of 4.76 (Omran et al. 2001). This allows the calculation of diazepam baseline toxicity using the formulae given in the EU TGD (European Commission 2003). These QSAR values can then be divided by the lowest experimental toxicities for the same groups of organisms. As long as the toxic ratio (TR; baseline/experimental, both in millimolars) is less than 10, a substance is assumed to exert its toxic actions purely through baseline toxicity. In the case of diazepam, based on a $\log D_{lipw}$ of 2.79 and on the lowest ecotoxicity test results in NC per group, the TRs are 8.8 for algae, 7.3 for daphnids and 3.9 for fish (calculations not shown; note that by using the recalculated $\log D_{lipw}$ of 4.76 the TRs would be even lower). The calculated TRs argue for baseline toxicity as the mode of toxicological action of diazepam. Baseline toxicity is also supported by a recent publication by Lienert and colleagues (2007).

In conclusion, on the whole diazepam showed moderate to low toxicity by NCs in several acute tests with organisms from different systematic groups. By mean measured exposure, green algae show a slightly higher susceptibility, but this may be an artifact caused by the decrease in concentration; this hypothesis is supported by both algae showing recovery on prolongation of the test. Based on biomarker and cytotoxicity data as well as on TRs, diazepam is judged to exert its toxic effects through baseline toxicity only.

Chronic Ecotoxicity. Chronic ecotoxicity data are available for cyanobacteria, green algae, hydrozoans, daphnids, aquatic insect larvae and fish. Due to the fact that algae divide several times during the regular test duration of seventy-two hours, the algal NOEC is accepted as a chronic endpoint. The 72-h NOEC for *Desmodesmus* was <2.56 mg l^{-1} NC, with the 14-d NOEC at 2.56 mg l^{-1} NC, and for the blue-green *Synechococcus* the 72-h NOEC was 6.4 mg l^{-1} NC, with the 7-d NOEC at 16 mg l^{-1} NC (Häner 2005c,d).

For invertebrates, a semi-static (with regular media exchange to ensure consistent test substance concentrations) daphnid reproduction test according to OECD 211 with *D. magna* resulted in an overall 21-d NOEC of 0.9 mg l^{-1} NC respectively 0.8 mg l^{-1} AMC (Peither 2006a). Similarly, a twenty-one-day sediment toxicity test according to OECD 219 with aquatic larvae of the midge *Chironomus tentans* (Insecta) resulted in an overall NOEC of 1 mg l^{-1} NC added to the water compartment (Memmert 2005). Chronic endpoints in the hydrozoan *H. vulgaris* show widely divergent NOECs (Pascoe et al. 2003): While the feeding and behavioral NOEC was 1 mg l^{-1} over seventeen days, the same authors reported a regeneration LOEC of 10 µg l^{-1}, the only tested concentration for this particular endpoint (Pascoe et al. 2003), meaning that the NOEC would be lower still. As there is some doubt as to the validity of this test system, both of these values will be used in the risk assessment and also discussed later.

Lastly, an Early Life Stage test according to OECD 210 in the zebrafish, *Danio rerio,* resulted in an overall 35-d NOEC of 273 µg l^{-1} AMC and an overall LOEC of 2 570 µg l^{-1} AMC (Peither 2006b). For the risk assessment, the NOEC will be used.

22.3.3 Risk Assessment

PECs, MECs and PNEC

PECs. Since many MECs are available for diazepam, mainly from Germany, PECs will be calculated for the whole of Western Europe and for Germany alone. According to the formulae in the guidelines referenced, the following initial surface-water PEC values are extrapolated (Table 22.1; see also Fig. 22.2).

The high EMEA PEC of 300 ng l^{-1} corresponds to a local PEC according to the TGD (European Commission 2003), i.e., maximum daily *per capita* use in 1% of the population, no human metabolism, no elimination in sewage works and a default receiving waters dilution factor of 10, but no further partitioning or elimination within the surface water bodies. The two EUSES PECs are regional PECs (European Commission 2003) based on actual diazepam sales and geographical, hydrological and population data for Germany; they assume either 50% (high PEC) or 5% (low PEC) excretion as the parent, no elimination in sewage works, standard dilution, but additionally envi-

Table 22.1. Diazepam initial PECs

PEC type	Value (ng l^{-1})	PEC calculated using
Local $PEC_{surface\ water}$ EU EMEA Phase I	300	EMEA guideline (EMEA 2006)
Regional $PEC_{surface\ water\ high}$ D	3.29	EUSES 2.0 (ECB 2005)
Regional $PEC_{surface\ water\ low}$ D	0.327	EUSES 2.0 (ECB 2005)

Local PECs do not include surface water fate while regional PECs do. High PEC uses 50%, low PEC uses 5% excretion as diazepam. *D:* Germany.

ronmental fate data, viz., an overall surface water half-life of thirty-four days based on an OECD 308 water/sediment study (Löffler et al. 2005).

MECs. There are many MECs for diazepam, mainly from Germany (POSEIDON 2005/2006; Ternes 1998, 2000; Ternes et al. 2001; BLAC 2003), both for sewage works effluents and surface waters. For the surface waters, out of a total of 236 single data located, the highest trustworthy (i.e., not erroneously cited, as in Debska et al. 2004) single MEC value was 33 ng l^{-1}, while all median MECs were below the respective LOD or LOQ, ranging from 10 to 30 ng l^{-1}. The highest surface water MEC worldwide is from the United States, where a range of 3–62 ng l^{-1} was published for Lake Mead, Nevada (Snyder et al. 2001). For a total of 188 effluent samples from 91 STPs in Germany, the highest single MEC was 100 ng l^{-1} (BLAC 2003), while in the same publication both the 90th percentile and the median were below the LOQ of 20 ng l^{-1}. In corroboration, the POSEIDON report noted that 'diazepam was hardly detected in STP influents and effluents from six European countries' (POSEIDON 2005/2006). The highest effluent MEC worldwide is from a Belgian STP, where an exceptional 660 ng l^{-1} was determined (beside a similarly exceptional influent of 1180 ng l^{-1}; van der Ven et al. 2004). These many MECs allow a comparison with the PECs and an estimation of the realism of the PEC derivations. The EMEA guideline local surface water PEC is in the range of the highest STP effluents, but certainly not representative of measured surface water concentrations. In contrast, both of the two EUSES regional PECs for Germany, that lie below 10 ng l^{-1} which is the lowest LOD in most German MEC papers (POSEIDON 2005/2006; Ternes 1998, 2000; Ternes et al. 2001; BLAC 2003), are much closer to the median MECs.

In order to extract more information from the published MECs for diazepam, which are normally given as summary data plus LOD or LOQ and number of samples or determinations, the MEC data per publication were evenly back-distributed and added in a weighted way across different publications, following a procedure developed earlier (Straub 2006). The composite data were plotted on a logarithmic vs. probability graph and the associated regression line then allowed the graphic estimation of the overall 50th and 95th percentile MEC values (median or MEC_{50}, respectively MEC_{95}), based on 236 single, mostly European data (Fig. 22.2). The composite MECs can then be compared with the PECs and with both PNECs and probabilistically derived HC_5 (hazardous concentration for 5% of species) values.

Based on 236 single measurements, the MEC_{50} is approximately 6.4 ng l^{-1} and a realistic worst-case MEC_{95} is about 27 ng l^{-1} (Fig. 22.2). Comparing the EMEA local PEC

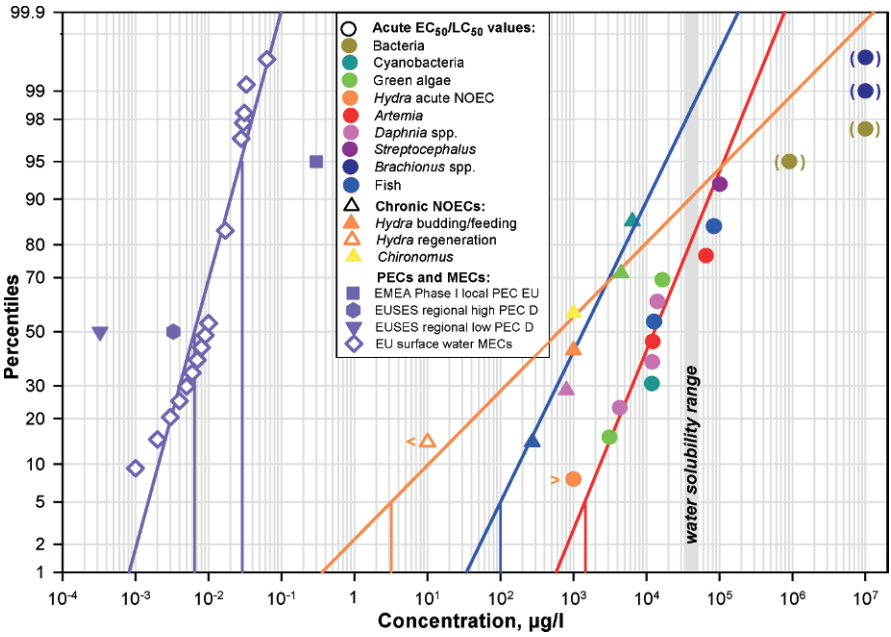

Fig. 22.2. Composite surface waters MECs with regression line and PECs (EMEA Phase I local EU PEC, EUSES D high PEC, EUSES D low PEC) as well as acute and chronic ecotoxicity data for diazepam. Two *Hydra* chronic values, a behavioral NOEC and a doubted regeneration LOEC are differentiated, with their respective chronic-based regressions (re-ordering of the other chronic values is not shown). Acute ecotoxicity values above 100 mg l^{-1} (higher than twice the water solubility) are marked in *brackets* and were not used for the acute SSD. *Droplines* show MEC_{50} of ~6.4 ng l^{-1} and MEC_{95} of ~27 ng l^{-1} as well as the acute HC_5 of ~1.3 mg l^{-1} and the chronic HC_5s of ~100 or <~3 µg l^{-1}, depending on the *Hydra* results

with this MEC_{95} results in a factor of 11 while comparing the median with the EUSES German high PEC results in a factor of about 2. Based on these comparisons, the crude EMEA local PEC is too high by about one magnitude; this is basically not so far off the mark for a very simplistic estimate. On the other hand, the EUSES high PEC accounting for 50% of the parent excreted as such is very close to the median, while the EUSES low PEC based on only 5% excretion as parent is lower by about a magnitude, making it as distant from the median MEC of 5 ng l^{-1} as the EMEA local PEC is from the corresponding MEC_{95}. In contrast to a local PEC, the regional German PEC encompasses environmental fate and partitioning (European Commission 2003). For the purposes of deterministic and probabilistic environmental risk assessment for diazepam, the derived MEC_{50} and MEC_{95} values will be used.

PNEC and HC_5. The deterministic PNEC is extrapolated by dividing the lowest EC_{50} or LC_{50} from acute ecotoxicity tests or the lowest NOEC from at least three chronic tests by an assessment factor. The latter expresses the uncertainty in extrapolating from acute to chronic toxicity, if applicable, and for taking into account inter- and intraspecies variability. With better, i.e., chronic, data both the uncertainty and the assessment factor decrease (from 1000 to 10, respectively, with three different species each). Ac-

Table 22.2. Diazepam PNECs

PNEC type	Value (μg l^{-1})	PNEC based on
$PNEC_{water\ acute}$	4.3	*Daphnia* EC_{50} 4.3 mg l^{-1}
$PNEC_{water\ chronic\ high} = PNEC_{EMEA}$	27.3	*Danio* NOEC 273 μg l^{-1}
$PNEC_{water\ chronic\ low}$	<1	*Hydra* LOEC 10 μg l^{-1}

Note: The $PNEC_{water\ chronic\ high}$, derived from the fish (*Danio*) NOEC of 273 μg l^{-1} (Peither 2006), corresponds to the formal PNEC in the EMEA guideline (EMEA 2006), which is based on the lowest of three chronic NOECs for the standard test organisms algae, *Daphnia* and fish and an assessment factor of 10; it assumes a *Hydra* NOEC of 1 mg l^{-1}.

Table 22.3. Diazepam straightforward HC_5s

HC_5 type	Value (μg l^{-1})	HC_5 based on
$HC_{5\ acute\text{-}based}$	~1 300	(All acute data)
$HC_{5\ chronic\text{-}based\ high}$	~100	*Hydra* NOEC 1 mg l^{-1}
$HC_{5\ chronic\text{-}based\ low}$	$\lesssim$3	*Hydra* LOEC 10 μg l^{-1}

cording to the EU TGD (European Commission 2003), the following PNECs are derived for diazepam (see Table 22.2).

Based on the ecotoxicological effects regressions in Fig. 22.2, the probabilistic straightforward HC_5s are the following (see Table 22.3).

Deterministic Risk Ratios and Probabilistic Margins of Safety

In deterministic ERA, a risk ratio (RR) of PEC/PNEC or MEC/PNEC smaller than one suggests no evident risk for a given substance. In probabilistic ERA, the outcome is usually given as a margin of safety (MOS) between HC_5 and MEC_{95}. This MOS can be transformed to the corresponding RR through inversion ($RR = MOS^{-1}$). All deterministic and probabilistic RRs are smaller than one, most of them significantly so (Table 22.4). Only the EMEA Phase I PEC divided by the chronicle low PNEC results in an RR of >0.3, due to the *Hydra* regeneration value of 10 μg l^{-1} being a LOEC and not a genuine NOEC; but even this worst-case RR is not unambiguously larger than one. Based on all available data, no long-term negative effects on the aquatic environment need to be expected from the current use of diazepam.

22.3.4 Sediment Risk

Diazepam will partition to the sediment and, in time, form bound residues. Partitioning dynamics in surface waters are expected to be slower than in the OECD 308 tests, due to a different water-volume to sediment-surface ratio. However, the formation of

Table 22.4. Diazepam aquatic RRs and MOSs

Risk derivation	Calculation					Result
Acute-based deterministic RRs						
$PEC_{surface\ water}$ EMEA ÷ $PNEC_{water\ acute}$	300	ng l^{-1} ÷	4.3	µg l^{-1} =		0.07
$PEC_{surface\ water\ high}$ D ÷ $PNEC_{water\ acute}$	3.29	ng l^{-1} ÷	4.3	µg l^{-1} =		0.0008
$PEC_{surface\ water\ low}$ D ÷ $PNEC_{water\ acute}$	0.327	ng l^{-1} ÷	4.3	µg l^{-1} =		0.00008
MEC_{50} ÷ $PNEC_{water\ acute}$	~6.4	ng l^{-1} ÷	4.3	µg l^{-1} =		~0.0015
MEC_{95} ÷ $PNEC_{water\ acute}$	~27	ng l^{-1} ÷	4.3	µg l^{-1} =		~0.006
Chronic-based deterministic RRs						
$PEC_{surface\ water}$ EU ÷ $PNEC_{water\ chronic\ high}$	300	ng l^{-1} ÷	27.3	µg l^{-1} =		0.01
$PEC_{surface\ water\ high}$ D÷$PNEC_{water\ chronic\ high}$	3.29	ng l^{-1} ÷	27.3	µg l^{-1} =		0.0001
$PEC_{surface\ water\ low}$ D÷$PNEC_{water\ chronic\ high}$	0.327	ng l^{-1} ÷	27.3	µg l^{-1} =		0.00001
MEC_{50} ÷ $PNEC_{water\ chronic\ high}$	~6.4	ng l^{-1} ÷	27.3	µg l^{-1} =		~0.0002
MEC_{95} ÷ $PNEC_{water\ chronic\ high}$	~27	ng l^{-1} ÷	27.3	µg l^{-1} =		~0.001
$PEC_{surface\ water}$ EU ÷ $PNEC_{water\ chronic\ low}$	300	ng l^{-1} ÷	<1	µg l^{-1} =		>0.3
$PEC_{surface\ water\ high}$ D ÷ $PNEC_{water\ chronic\ low}$	3.29	ng l^{-1} ÷	<1	µg l^{-1} =		>0.003
$PEC_{surface\ water\ low}$ D ÷ $PNEC_{water\ chronic\ low}$	0.327	ng l^{-1} ÷	<1	µg l^{-1} =		>0.0003
MEC_{50} ÷ $PNEC_{water\ chronic\ low}$	~6.4	ng l^{-1} ÷	<1	µg l^{-1} =		>0.006
MEC_{95} ÷ $PNEC_{water\ chronic\ low}$	~27	ng l^{-1} ÷	<1	µg l^{-1} =		>0.03
Straightforward probabilistic RRs						
MEC_{50} ÷ $HC_{5\ acute}$	~6.4	ng l^{-1} ÷	~1 300	µg l^{-1} =		~0.000005
MEC_{50} ÷ $HC_{5\ chronic\ high}$	~6.4	ng l^{-1} ÷	~100	µg l^{-1} =		~0.00006
MEC_{50} ÷ $HC_{5\ chronic\ low}$	~6.4	ng l^{-1} ÷	≲3	µg l^{-1} =		>0.002
MEC_{95} ÷ $HC_{5\ acute}$	~27	ng l^{-1} ÷	~1 300	µg l^{-1} =		~0.00002
MEC_{95} ÷ $HC_{5\ chronic\ high}$	~27	ng l^{-1} ÷	~100	µg l^{-1} =		~0.0003
MEC_{95} ÷ $HC_{5\ chronic\ low}$	~27	ng l^{-1} ÷	≲3	µg l^{-1} =		>0.009

PECs, MECs PNECs and HC_5s from Tables 22.1–22.3 and Fig. 22.2.

bound residues is not dependent on this ratio, resulting in less bioavailable diazepam in the sediment, as predicted by environmental partitioning models (Di Guardo et al. 2001; Level III Model v2.70 2002). Multiplying the empirical sediment K_d of 3 l kg^{-1} (Löffler et al. 2005) with the MEC_{95} of ~27 ng l^{-1} results in a worst-case sediment PEC of ~81 ng diazepam kg^{-1}. In support, Italian measurements reported less than the LOD of 9 ng kg^{-1} (Zuccato et al. 2000). Comparing both PEC and MECs with the single chronic NOEC of 1 mg l^{-1} NC for *Chironomus* (Memmert 2005), applying the sediment

K_d as above and the TGD assessment factor of 100 (European Commission 2003), there is no indication of risk to the sediment compartment.

22.4 Discussion and Refinement of the ERA

22.4.1 PECs and MECs

Based on the above PEC and MEC comparisons, the EMEA local PEC is too high by about one magnitude. While this is all right for a very simplistic estimate, most likely it is relatively close for the wrong reasons. The EMEA Phase I PEC assumes the maximum daily dose (MDD) for a fixed penetration factor F_{pen} of 1% of the population. This F_{pen} is based on the cumulative population fraction taking 95% of all medicines consumed at the defined daily dose (DDD) in Germany in 2001 (Rönnefahrt 2005), meaning that most single APIs were actually used by a lower fraction. Additionally, the MDD is not the one generally prescribed. Dividing the average annual diazepam sales of 6 300 kg for Western Europe (IMS Health/IMS MIDAS 2004) by the MDD of 60 mg day^{-1} and 365 day a^{-1} results in 287 671 statistical consumers out of a total population of 385 million (Eurostat 2001), which corresponds to an F_{pen} of 0.075%. For the DDD of 10 mg/day, the F_{pen} increases to 0.45%. Based on 1 140 kg a^{-1} for Germany (IMS Health/IMS MIDAS 2004) with 82.3 million inhabitants (Eurostat 2001), MDD corresponds to an F_{pen} of 0.063% and DDD to 0.38%. Hence, the EMEA default F_{pen} of 1% is too high; for diazepam, a refined F_{pen} of 0.38–0.45% is more realistic.

The EUSES high PEC, on the other hand, is very close to the median MEC, even if probably again for the wrong reasons. First, for an API that is largely metabolized, like diazepam where an excretion as the parent of 11% (Lienert et al. 2007) or only 5% (R. Amrein, pers. comm.) may be more realistic than the 50% (van der Heide and Hueck-van der Plas 1984) on which the high PEC is based (as also supported by the 3:1 ratio of N-desmethyldiazepam to diazepam in Italian STPs; Castiglioni et al. 2006), even this high PEC is below the actual median MEC. Moreover, the EUSES low PEC (5% excretion) is lower again by a magnitude, making it as distant from the median MEC of ~5 ng l^{-1} as the EMEA local PEC is from the corresponding MEC_{95}. In contrast to a local PEC, regional PECs encompass environmental fate and partitioning (European Commission 2003). Based on a water/sediment fate study with diazepam (Löffler et al. 2005), a surface water half-life of thirty-four days was entered. While this study was certainly performed correctly, as shown by comparable dimensions for the aquatic half-life in a second OECD 308 study with diazepam (Mamouni et al. 2005), the volumetric and interface characteristics of these lab tests were originally developed for a water ditch simulation in pesticide risk assessment, but not for larger surface waters overall. This means that in particular those half-lives that are a function of partitioning to the sediment, in contrast to degradation within the water phase, are not directly transferable to a surface water ERA for APIs.

Another inherent estimation error of the PECs may derive from the surface water dilution factor, the TGD default for which is 10 (European Commission 2003). The actual dilution factor for the existing MEC dataset of diazepam can be approximated by compiling and integrating the published STP effluent MECs in the same way as the

surface water MECs before. The comparison of these two MEC regressions (Fig. 22.3) results in a dilution factor for the available data, again mostly from German measurements, of only 1.8. This low dilution factor is possibly biased by lower-than-average effluent or higher-than-average surface water sampling sites or a combination of both. Moreover, the TGD was developed for chemical ERA, with few point sources only, in contrast to pharmaceuticals which enter surface waters in a very diffuse fashion and often show a background concentration that is absent in the case of chemicals. Hence, pending careful further case-by-case corroboration, the low factor of 1.8 should not be used in general for pharmaceutical ERAs. Specifically for the present diazepam ERA, however, refining the PECs with this dilution factor of 1.8 results in the following (see Table 22.5).

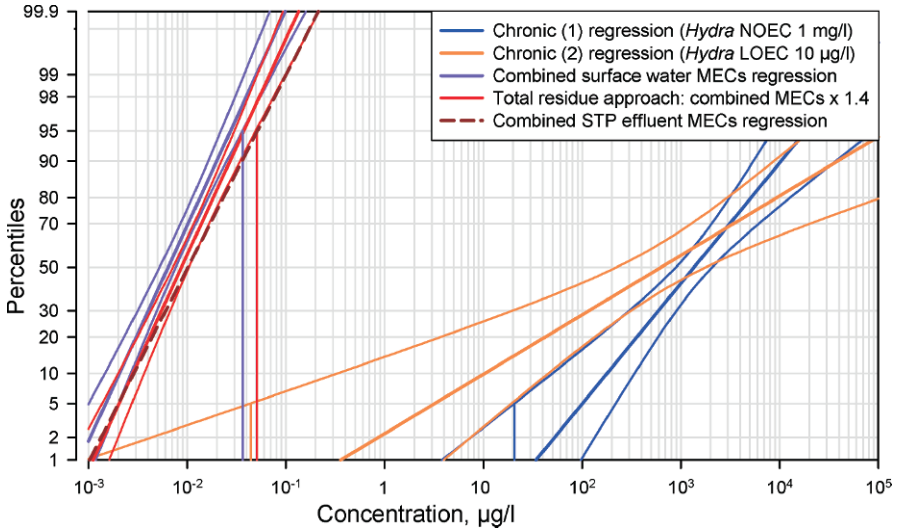

Fig. 22.3. Refined probabilistic ERA for diazepam. Two chronic regressions, (1) rejecting and (2) accepting the *Hydra* LOEC of 10 µg l^{-1} as a valid NOEC, with their 95% CIs and the intersection of the lower CI with the 5th percentile as the *blue* (1) and *orange* (2) probabilistic PNECs. The surface water MEC distribution (*lilac* with 95% CI) was multiplied by 1.4 (*red regression* with 95% CI) to reflect the combined toxic potential of diazepam and its metabolites as calculated by Lienert et al. (2007). The combined STP effluent MEC regression is shown as a *dashed brown line*; please note the latter does not include the exceptionally high 660 ng l^{-1} value, which would exert inordinate leverage on the regression line

Table 22.5. Diazepam refined PECs

PEC type	Initial PEC (ng l^{-1})	Refined PEC (ng l^{-1})
Local $PEC_{surface\ water}$ EU EMEA Phase I	300	1 667
Regional $PEC_{surface\ water\ high}$ D	3.29	18.3
Regional $PEC_{surface\ water\ low}$ D	0.327	1.8

Initial PECs from Table 22.1.

Now the EMEA local PEC becomes inordinately high and likely to be extremely unrealistic in comparison with the highest recorded surface water MEC of 62 ng l^{-1} (Snyder et al. 2001) and the highest STP effluent MEC of 660 ng l^{-1} (van der Ven et al. 2004). The high and low EUSES PECs are reasonably close to the median MEC, although possibly the refined low EUSES PEC may still be too low.

The local and regional PECs based on actual sales may assume wrongly that all diazepam sold will also be used. Some of the unused or overaged medicines will be disposed of through sinks or toilets (START 2007; START-News 2006; Kuspis and Krenzlok 1996; Bound and Voulvoulis 2005). In addition, there is evidence of a significant degree of patient noncompliance in taking their prescribed medicines (Donovan and Blake 1992). This leads to part of these APIs being disposed of by direct draining into wastewater, without human use and metabolization, resulting in higher (in case of diazepam, assuming only 5% human excretion of the parent, much higher) influent concentrations into sewage works. Hence, possibly, the comparatively high MEC_{50} in comparison with the low EUSES PEC based on actual sales and a dilution factor of 1.8 for diazepam may be indirect evidence of partial patient noncompliance. If one assumes 95% loss through human metabolism and 0% loss on direct draining of diazepam into wastewater, and that the fate parameters entered in the EUSES model are reasonably correct, calculation suggests that a direct-draining, noncompliant patient fraction of 13.5% is sufficient to increase the low PEC from 1.8 to the median MEC of 6.4 ng l^{-1}. In partial corroboration, a recent enquiry in Germany found that 15.7% of polled households would habitually to rarely dispose of (overaged) solid medicinal products by draining them into municipal wastewater (START-News 2006).

22.4.2 The *Hydra* Chronic Data Crucial for PNEC and HC_5

The chronic PNEC and HC_5 depend directly on the validity, or not, of the *Hydra* regeneration LOEC of 10 µg diazepam l^{-1} (Pascoe et al. 2003). In the first acute test phase, five *Hydra* each were exposed to 10, 100, 1 000 or 10 000 µg l^{-1} for seven days, with medium and solvent controls. In the second chronic feeding and asexual budding test phase, the same animals were exposed for ten more days. In the third regeneration test phase, again the same animals, pre-exposed to 10 µg l^{-1} or solvent control for seventeen days, had their digestive region tubes (DRTs) dissected; these were transferred to new vessels with 10 µg diazepam l^{-1} or solvent and observed for three more days for a predefined regeneration score. Five additional excised DRTs from *Hydra* not previously exposed were put in 10-µg l^{-1} or control vessels. In the acute test, the 10-µg l^{-1} group was significantly different from, i.e., not as fit as, the solvent controls; there was no difference in the 100- and 1000-µg l^{-1} groups, while in both the 10 000-µg l^{-1} test group and its solvent control all were dead, suggesting solvent toxicity. In the feeding and budding test there was no significant difference between the three remaining diazepam concentrations and their solvent controls. In the regeneration test, there was no effect on non-pre-exposed DRTs, but a significant inhibition of the pre-exposed ones, with a regeneration score from zero (= median) to the score of the solvent controls (Pascoe et al. 2003).

This report is difficult to evaluate, due to precisely that group which had a significantly lowered fitness score in the first acute phase being used for the third regenera-

tion phase, without any such fitness score given for the feeding and budding test phase. Only five pre-exposed DRTs were used in the regeneration, which increases the statistical uncertainty, but at least one did reach the extent of regeneration of the solvent controls. Due to only one treatment concentration in the regeneration test, there is no possibility of deriving a dose-response relationship or a NOEC. Finally, in view of the solubility of diazepam, it is not clear why any solvent was used at all.

Widespread toxic effects of *Hydra attenuata* in thirty Ukrainian bottled drinking water and nine well water samples were recently reported (Arkhipchuk et al. 2006). In all cases, twenty-one-day chronic endpoints (budding rate followed by sublethal and lethal effects) were the most susceptible parameters. But then, only thirteen out of thirty-nine water samples had no sublethal, only fifteen had no significant lethal effect and only four had no adverse effect on budding (Arkhipchuk et al. 2006). In view of these results, it may be legitimate to ask whether the *Hydra* test system is really dependable, specifically regarding false-positives.

In conclusion, there is reasonable uncertainty as to the *Hydra* regeneration LOEC. Lacking better data, respectively pending confirmation of this LOEC or establishment of a NOEC, however, the value of 10 $\mu g\ l^{-1}$ is provisionally utilized as a NOEC for the derivation of a chronic PNEC and HC_5.

22.4.3 Persistence, Bioaccumulation and Toxicity Considerations

With an experimental sediment half-life >60 days (Mamouni et al. 2005) or an extrapolated 311 ±26 days (Löffler et al. 2005), diazepam is a very persistent substance according to the TGD criteria ($t_{½} \geq 60$ days in water, which was not reached in both tests, but $t_{½} \geq 180$ days in sediment, which was; European Commission 2003). Regarding the toxicity criterion (chronic ecotoxicity NOEC < 10 $\mu g\ l^{-1}$; European Commission 2003), diazepam would qualify as toxic if the doubted *Hydra* regeneration LOEC of 10 $\mu g\ l^{-1}$ (Pascoe et al. 2003) is accepted as a valid result; pending experimental clarification, this question cannot be definitively answered. Therefore, the bioaccumulation properties are decisive for classifying diazepam as possibly PBT (persistent, bioaccumulative and toxic), vPvB (very persistent, very bioaccumulative), or not at all. Based on the experimental octanol/water partition coefficients, with all log values below three (F. Hoffmann-La Roche 2005; Ran et al. 2002; Di Guardo et al. 2001; Stuer-Lauridsen et al. 2000), no significant accumulation is to be expected, but no experimental bioaccumulation data were available for confirmation. The developmental test with aquatic larvae of *Chironomus riparius* (Memmert 2005) was therefore expanded with one additional vessel containing radio-labeled diazepam in the water phase. After eleven days' exposure, the following bioconcentration factors (BCFs) were determined: BCF larvae/water = 62.5 $l\ kg^{-1}$, BCF larvae/total sediment = 9.7 $l\ kg^{-1}$ and BCF larvae/sediment pore water = 280.8 $l\ kg^{-1}$ (Memmert 2005). None of these BCFs would qualify for a classification according to the TGD. Moreover, the highest BCF is judged to reflect the partitioning of diazepam from the pore water to the organic sediment fraction (resulting in depletion in the pore water) rather than actual uptake by the larvae, as the *Chironomus* larvae live mostly in the detritus layer on the sediment surface and are not genuine in-benthic sediment dwellers. Low diazepam uptake was recently confirmed in *Gammarus pulex* in a sediment-free system, with a 48-h BCF of

35 l kg^{-1} (Netherton et al. 2007). Hence, while diazepam is very persistent and potentially toxic, it is not bioaccumulative based on logK_{OW} values as well as two nonstandard tests; therefore, it is neither PBT nor vPvB.

22.4.4 Going Probabilistic With Fewer Chronic Tests

In 2005, Ragas and colleagues (2005) showed in a presentation that in a deterministic setting, additional chronic tests above the three required by the EMEA Guideline (EMEA 2006) would at best leave the PNEC where it was and at worst lower it still more but could never raise it. Hence, there is no incentive for doing any additional chronic tests within a deterministic ERA framework. For an investment in additional chronic testing to be potentially worthwhile, a higher-tier ERA should follow probabilistic procedures. According to the TGD, at least ten chronic NOECs should be available for an SSD, which is explained with the 'confidence to be associated with a PNEC derived by statistical extrapolation' (European Commission 2003). But this creates a wide discrepancy between the minimum of chronic tests for deterministic (3) and probabilistic (10) ERA, which again is not an incentive for additional tests. Ragas and coworkers (2005) argued that the uncertainty of SSDs is well characterized by the 95% confidence intervals (CIs) of the regression line, which depend both on the distance of the single data points from the regression and on the absolute number of data points. The enhanced uncertainty due to comparatively few data would be reflected by a wider CI, in particular in the high and low percentile ranges, where the HC_5 is determined. Figure 22.3 shows the 95% CIs for both the chronic regressions (accepting the *Hydra* regeneration LOEC of 10 μg l^{-1} as valid or not) and also for the MEC regression. Designating the MEC_{95} upper CI as the refined MEC_{95} (lilac dropline in Fig. 22.3) and the chronic HC_5 lower CI as the refined chronic HC_5 or probabilistic PNEC (blue respectively orange droplines in Fig. 22.3), both now including a measure of uncertainty, the MOS becomes approximately 530 for the blue $PNEC_{prob}$, respectively 1.2 for the orange $PNEC_{prob}$, while the corresponding RRs are 0.002 respectively 0.8.

22.4.5 Including Metabolites, Going Semi-Deterministic Again

Assuming that most diazepam will be excreted in the form of metabolites, with 11% (Lienert et al. 2007) or possibly only 5% excreted as the parent (R. Amrein, pers. comm.), this means that in the worst case, the MECs reflect only 5% of administered diazepam while 95% of the original activity remains unaccounted for. A useful ERA, however, needs to consider major metabolites as well. It is not practicable to test all diazepam metabolites to the same extent as the parent; hence, some assumptions and approximations must be used to address the question.

Phase-I metabolites in general are more water-soluble than the parent, as this facilitates excretion. Higher water solubility correlates with lower lipophilicity and through the latter, also with lower baseline toxicity (European Commission 2003; Escher et al. 2002). Escher and colleagues (2006) showed experimentally for several beta-blockers that the parents act by baseline toxicity. They then calculated the metabolite ecotoxicity by QSAR, based on the assumption of baseline toxicity and on lower

lipophilicity, and presented a model for adding all these quantifiable toxicities in a weighted manner, as an overall ecotoxic potential. No ecotoxicity data are available for the diazepam metabolites. Assuming baseline toxicity for diazepam as well as for its metabolites and noting that all of the latter have lower QSAR logK_{OW} values than diazepam (data not shown), it seems reasonable to expect lower or equal, but not higher ecotoxicity for the metabolites compared to diazepam.

Enlarging their own model (Escher et al. 2006) to many APIs, Lienert et al. (2007) QSAR-calculated and combined the toxicities for diazepam and its metabolites. Assuming an average 11% excretion as the parent as well as given rates for the metabolites, as such or conjugated, they computed a combined toxic potential of 15% (range 5–23%) relative to 100% of ingested diazepam, or 1.4 times (15%/11%) the amount excreted (Lienert et al. 2007). As diazepam is not significantly removed in STPs, 1.4 times the uncorrected surface water MEC regression is used as the refined MEC distribution incorporating parent and metabolite total residue toxicity as per Lienert et al. (2007) (red line with 95% CIs and red dropline in Fig. 22.3), resulting in a refined risk graph.

For the blue NOEC regression, the probabilistic graph is simple to read. There is no vertical overlapping of the refined exposure distribution (red), and the blue NOEC distribution as well as their upper respectively lowers 95% CIs, which simply means that there is no risk. In contrast, the interpretation becomes more complicated if the *Hydra* 10 μg l^{-1} is accepted as a valid NOEC. Due to the orange regression being leveraged by the low *Hydra* value, which results in a flatter line, the lower orange 95% CI crosses the 1st percentile by close to 0.001 μg l^{-1}. The lower NOEC 95% CI does overlap the red upper refined MECs 95% CI over the range of 0.001–0.2 μg l^{-1}, which means that in this scenario there is some risk. With the probabilistic graph, however, this risk can now be characterized. For instance, for 95% of refined exposures, the red dropline in Fig. 22.3 crosses the lower orange 95% CI just above the 5th percentile.

Graphing these intersections between the refined exposures and lower SSD CIs results in Fig. 22.4. The values were kindly computed by W. Roelofs using the Webfram tool (DEFRA/Hart, no year); please note that results differ slightly from Fig. 22.3 due to a refined Bayesian calculation. This probabilistic risk graph allows the characterization of risk over the whole exposure range. It shows that in up to 90% of exposure situations the NOEC 95% CI is breached for not more than 5.4% of species and in 95% of situations for less than 6.2% of species; at 99.9% of exposures (higher than the highest surface water MEC ever determined) the potential risk increases to 10.5% of species. The shaded area shows potential risk, the white area indicates no risk. As there is an additional unquantified safe range between the NOECs and the genuine no-effect concentrations, Fig. 22.4 still depicts a worst-case scenario.

22.5 Conclusion

Various PECs for diazepam were calculated based on defaults and on actual use and available fate data, and compared with collated MECs. This comparison showed the very simple EMEA initial PEC derivation to result in a conservative overestimate, while refined PEC computations are in realistic dimensions. Being clearly below the average MEC, the refined PECs may evidence an amount of incorrect medicine disposal

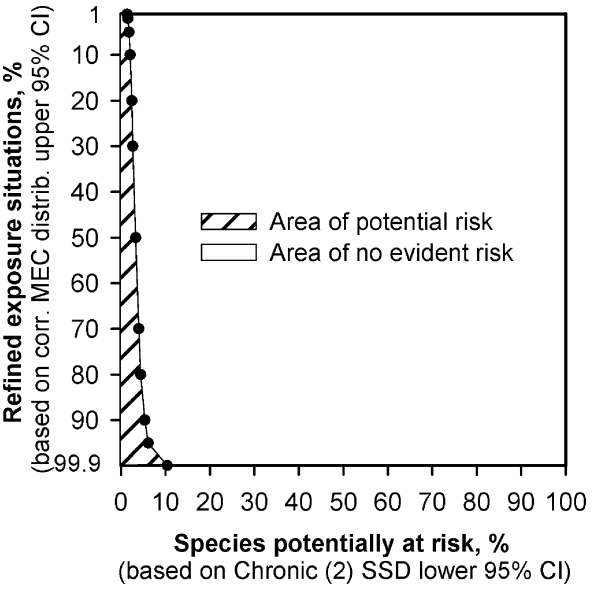

Fig. 22.4. Probabilistic risk graph for the Chronic (2) NOEC distribution CI, accepting the low *Hydra* value of 10 μg l^{-1} as a NOEC, and the refined, combined-toxic-potential-corrected MEC distribution; data points computed by W. Roelofs

by way of municipal wastewater and may suggest a certain incidence of noncompliance in taking prescribed drugs. PNECs for diazepam were derived by deterministic and probabilistic procedures, based on acute and chronic ecotoxicity data. Initial PEC/PNEC and MEC/PNEC ratios and probabilistic RRs do not indicate an environmental risk due to diazepam. Additionally, acknowledging the uncertainty of the MECs and the SSDs through use of the respective 95% CIs and incorporating metabolite toxicity based on a recent calculation model, any risk still fails to be shown in one probabilistic scenario, while a second scenario (provisionally accepting a doubted, very low chronic endpoint as valid) shows a very limited but quantifiable risk. Moreover, while diazepam qualifies as very persistent in the sediment compartment, there is reasonable doubt as to very high toxicity and, mainly, it does not bioaccumulate; hence, it is not a PBT substance.

Based on the data and procedures presented, the current use of diazepam in Western Europe does not cause significant concern for the environment.

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data; however, analysis of IMS Health data was independently arrived at by the author of the present paper on the basis of the data, and other information and IMS Health is not responsible for any reliance by recipients on the data or any analysis thereof.

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Appendix: Diazepam Data Tables

Table 22.A1. Physicochemical and distribution properties of diazepam

Property	Method/remark	Reference
Molecular mass		
284.74		American Chemical Society (2003)
Melting temperature (°C)		
125–126		Ran et al. (2002); Verschueren (1996); Yalkowski and Pinal (1993)
131–135		F. Hoffmann-La Roche (2005)
Vapour pressure (Pa)		
5×10^{-5}		Ran et al. (2002)
Solubility (mg l^{-1})		
50.6	Water	Ran et al. (2002)
50	Water	Chen et al. (2002)
49.5	Water	Yalkowski and Pinal (1993)
41	Water	Di Guardo et al. (2001)
36.6–48	Ecotoxicity test media, HPLC	Häner (2005c,d)
Henry's law constant (Pa mol^{-1} m^3)		
$K_H \sim 2.9 \times 10^{-4}$	Calculated: experimental VP/(WS/MW)	Based on Ran et al. (2002)
$K_H = 3.7 \times 10^{-4}$	QSAR	EPISuite (2004); SPARC (2005)
Dissociation constant		
$pK_b = 3.4$		Stuer-Lauridsen et al. (2000)
$pK_b = 3.3$		Halling-Sørensen et al. (1998)
***n*-Octanol/water partition coefficient**		
$\log K_{OW} = 2.99$		Ran et al. (2002); Di Guardo et al. (2001)
$\log K_{OW} = 2.82$		Stuer-Lauridsen et al. (2000)
$\log D_{OW} = 2.58$	pH 7.2	F. Hoffmann-La Roche (2005)
Lipid-microsomes/water partition coefficient		
$\log D_{lipw} = 2.79$	Egg yolk microsomes/water	Escher et al. (2002)
$K_p = 0.00308$	Phosphatidylcholine microsomes/water, which, normalizing molar concentra-tions, corresponds a $\log D_{lipw}$ of 4.76	Omran et al. (2001)
$\log D_{lipw} = 4.76$		
Solids/water distribution coefficient		
$\log K_d = 0.9–1.2$	$K_d = 7.9–15.8$ l kg^{-1}; primary sludge	Clara et al. (2002)
$\log K_d = 1.6–2.0$	$K_d = 39.1–100$ l kg^{-1}; activated sludge	Clara et al. (2002)
No significant sorption (<10%) to sludge		POSEIDON (2005/2006)
$K_d = 43.9 \pm 26.1$ l kg^{-1}	Primary sludge	POSEIDON (2005/2006)
$K_d = 21.1 \pm 7.6$ l kg^{-1}	Secondary sludge	POSEIDON (2005/2006)

Table 22.A1. *Continued*

Property	Method/remark		Reference
No significant sorption (<10%) to sludge (*continued*)			POSEIDON (2005/2006)
K_d = 3 l kg^{-1}	Sediment		Löffler et al. (2005)
K_{OC} = 192 l kg^{-1}	Sediment organic carbon		Löffler et al. (2005)
Partitioning to sediment phase in water-sediment system fate test (OECD308 aerobic, 0.042 mg ^{14}C-diazepam l^{-1} initial concentration)			Mamouni et al. (2005)
Time (d)	[^{14}C] in water	[^{14}C] in sediment	
0	100.4%	<0.1%	Mamouni et al. (2005)
1	83.4%	17.2%	Mamouni et al. (2005)
5	57.7%	45.2%	Mamouni et al. (2005)
14	33.4%	70.4%	Mamouni et al. (2005)
30	20.9%	81.5%	Mamouni et al. (2005)
60	~18%	~80%	Mamouni et al. (2005)
60	Bound residues in sediment ~12%		Mamouni et al. (2005)
60	Negligible (0.1%) $^{14}CO_2$ in air		Mamouni et al. (2005)
Low/slow mobility in soil	No leakage through 30 cm soil columns (3 soil types) in 48 h; ^{14}C-Diazepam applied on surface, 60–>70% recovered from top 5 cm, 25–30% from 6–10 cm, <10% from 11–15 cm, ~1% from 15–20 cm		Oppel et al. (2004)
Low adsorption to soils by application in solution or with aged sludge			Kreuzig et al. (2003)
K_d = 13 ±1 l kg^{-1}	Silty soil; solution application, OECD106		Kreuzig et al. (2003)
K_d = 20 ±4 l kg^{-1}	Clayey silt; solution application, OECD106		Kreuzig et al. (2003)
K_d = 4 ±0 l kg^{-1}	Silty soil; aged sludge application, OECD106		Kreuzig et al. (2003)
K_d = 7 ±1 l kg^{-1}	Clayey silt; aged sludge appl., OECD106		Kreuzig et al. (2003)
Bioaccumulation			
Experimental BCFs determined in parallel with *Chironomus riparius* emergence test, OECD 219, after 11 d exposure to 1 mg ^{14}C-diazepam l^{-1} in the water phase in additional test vessels; GLP			
BCF = 62.5 l kg^{-1}	*Chironomus* larvae/water		Memmert (2005)
BCF = 9.7 l kg^{-1}	*Chironomus* larvae/sediment		Memmert (2005)
BCF = 280.8 l kg^{-1}	*Chironomus* larvae/sediment pore water		Memmert (2005)
BCF = 35.4 l kg^{-1}	*Gammarus pulex*/water, 48 h, 0.1 mg ^{14}C-diazepam l^{-1}		Netherton et al. (2007)
BCF ~ 407 l kg^{-1}	QSAR, pH 4		American Chemical Society (2003)
BCF ~ 509 l kg^{-1}	QSAR, pH 7–10		American Chemical Society (2003)
BCF ~ 48.8 l kg^{-1}	QSAR, fish; based on logK_{OW} = 2.82 and predominantly hydrophobic structure		ECB (2005)

BCF: Bioconcentration factor; *MW:* molecular weight; *QSAR:* Quantitative structure-activity relationship; *WS:* water solubility; *VP:* vapour pressure.

Table 22.A2. Degradation, partitioning and environmental fate of diazepam

Characteristic			Reference
Stability (based on drug substance stability testing)			
Stable under normal conditions, some light sensitivity			F. Hoffmann-La Roche (2005)
Aerobic biodegradation in laboratory tests			
0%	21 d (100 mg l^{-1} diazepam, 200 mg l^{-1} activated sludge (dry weight), inherent manometric respirometry test)		Gröner (1981)
<5%	28 d and also <5% 84 d (BOD/ThOD, 30 mg diazepam l^{-1}, 100 mg activated sludge (dry weight) l^{-1}, prolonged inherent manometric respirometry test), ISO/IEC 17025;		Häner (2005a)
~70%	Primary degradation by HPLC, 84 d		Häner (2005a)
Anaerobic degradation in standard tests			
–39%	56 d (IC/ThIC, 150 mg l^{-1} SC, Ultimate Anaerobic Degradation, ISO11734), consistently negative degradation relative to blank IC hinting at toxicity to anaerobic sludge microorganisms at concentrations applied; ISO/IEC 17025		Häner (2005b)
14%	13 d (IC/ThIC, 30 mg l^{-1} SC, ISO11734)		Häner (2005b)
–28%	64 d (IC/ThIC, 30 mg l^{-1} SC, ISO11734)		Häner (2005b)
30%	13 d (IC/ThIC, 20 mg l^{-1} SC, ISO11734)		Häner (2005b)
–7%	64 d (IC/ThIC, 20 mg l^{-1} SC, ISO11734)		Häner (2005b)
Measured degradation/removal in STPs or STP models			
No significant removal in laboratory STP models, pseudo-first-order rate constant $k_{biol} < 0.1\ l \times g\ SS^{-1} \times d^{-1}$ (~$0.02\ l \times g\ SS^{-1} \times d^{-1}$, result only given as graph)			POSEIDON (2005/2006)
No elimination in laboratory STP model with sludge retention times of 1, 16 or 35 d			Clara et al. (2002)
Some elimination observed in Austrian STP, but not quantifiable due to LOD l^{-1} OQ respectively signal-to-noise ratio			Clara et al. (2002)
Measurements in 3 Belgian STPs 2001			Van der Ven et al. (2004)
Elimination rate	Influent, ng l^{-1}	Effluent, ng l^{-1}	
<83%	590	>100	Van der Ven et al. (2004)
44%	1180	660	Van der Ven et al. (2004)
Insufficient data	>100	>100	Van der Ven et al. (2004)
	(>100: detected but not quantifiable due to signal-to-noise ratio)		

Table 22.A2. *Continued*

Characteristic			Reference
6 Italian STPs 2004, loads given as mg/d/1000 inh, average (range), no elimination on the average			Castiglioni et al. (2006)
Elimination rate	Influent, ng l^{-1}	Effluent, ng l^{-1}	
	Diazepam		
Negative, deconjugation?	0.4 (ND–1.4)	0.5 (ND–1)	Castiglioni et al. (2006)
	N-desmethyldiazepam		
Negative, deconjugation?	1.1 (ND–19)	1.6 (0.5–13)	Castiglioni et al. (2006)
	Ratio N-desmethyldiazepam/diazepam		
	~2.8	~3.2	Castiglioni et al. (2006)
Anaerobic degradation in STPs			
10–50%	Measured elimination in anaerobic sludge treatment		POSEIDON (2005/2006)
0–60%	Anaerobic digestion in STPs:'data does not allow to discriminate between no or a partial removal (0 to 60%)'		POSEIDON (2005/2006)
Abiotic degradation			
None	Hydrolytically stable; 48 mg l^{-1} distilled water, 22 °C, dark, 120 h, HPLC		Häner (2005a)
~20%	Photodegradation (2 h, 300-nm radiation); photoproducts responsible for phototoxicity		Cornelissen et al. (1980)
25%	Aquatic photodegradation; 48 mg l^{-1} distilled water, 22 °C, algal light cabinet, 120 h, HPLC		Häner (2005a,c,d)
$t_{½}$ ~ 36 h	Aquatic photodegrad., 10 mg l^{-1} distil. water, Heraeus Suntest CPS; pseudo-first-order kinetics, formation of various photoproducts with clearly longer half-lives		West (2005)
$t_{½}$ ~ 12.96 h (=1.08 d, 12-h day)	QSAR atmospheric half-life, estimated for •OH-radical reactions, based on 1.56×10^6 •OH radicals cm^{-3}		EPISuite (2004)
Environmental fate			
Slow removal in water-sediment system (OECD308, aerobic, dark)			Löffler et al. (2005)
DT_{50} ~ 311 ±25 d	Whole water-sediment system		Löffler et al. (2005)
DT_{50} ~ 34 ±5 d	Water compartment only		Löffler et al. (2005)
DT_{90} > 365 d	Whole water-sediment system		Löffler et al. (2005)
DT_{90} ~ 113 ±17 d	Water compartment only		Löffler et al. (2005)
<2%	Mineralisation, 100 d		Löffler et al. (2005)

Table 22.A2. *Continued*

Characteristic		Reference
Very slow but significant removal through formation of bound residues in aerobic river/pond water-sediment system (OECD308, aerobic, dark, 60 d, 0.042 mg ^{14}C-diazepam l^{-1} initial concentration)		Mamouni et al. (2005)
$DT_{50} \gg 60$ d	Whole water-sediment system	Mamouni et al. (2005)
$DT_{50} \sim 10$ d	Water compartment only	Mamouni et al. (2005)
20.8/21%	In water in r/p w-s system, 30 d	Mamouni et al. (2005)
82.4/80.6%	In sediment (total) in r/p w-s system, 30 d	Mamouni et al. (2005)
74.5/75.5%	In sediment (extractables), 30 d	Mamouni et al. (2005)
8.0/5.2%	In sediment (non-extractables), 30 d	Mamouni et al. (2005)
0.1/0.1%	$^{14}CO_2$ in gas phase of r/p w-s system, 30 d	Mamouni et al. (2005)
Removal 10–50%	In groundwater, saturated zone, >10 weeks	POSEIDON (2005/2006)
Environmental partitioning		
Water 97.5% Sediment 2.53% Soil 8.05 × 10^{-5}% Air 5.7 × 10^{-5}% Persistence 761 h	Emission only into water; Mackay EQC Model v. 1.0, Level III: MW = 284 g mol^{-1}, MP = 125 °C, VP = 5 × 10^{-5} Pa, WS = 41 mg l^{-1}, $logK_{OW}$ = 2.99, $t_{½}$ (air) = 200 h, $t_{½}$ (water, soil) = 2 000 h, $t_{½}$ (sedim) = 20 000 h	Di Guardo et al. (2001)
Water 97.8% Sediment 2.14% Soil 0.02% Air 3.81 × 10^{-6}% Persistence 552 h	Emission only into water (0.719 kg h^{-1} = 6300 kg a^{-1}, model configured for western Europe); Mackay Level III v. 2.70 Model: MW = 284.75 g mol^{-1}, MP = 125 °C, VP = 5 × 10^{-5} Pa, WS = 50 mg l^{-1}, $logK_{OW}$ = 2.99, $t_{½}$ (air) = 25.9 h, $t_{½}$ (water) = 816 h = 34 d, $t_{½}$ (sediment, soil) = 7 464 h = 311 d	Level III Model (2002)

IC: inorganic carbon; *LOD:* limit of detection; *LOQ:* limit of quantitation; *MP:* melting point; *MW:* molecular weight; *ND:* not detected; *SC:* starting concentration; *SS:* suspended solids; *ThIC:* theoretically produced inorganic carbon; *VP:* vapour pressure; *WS:* water solubility.

Table 22.A3. PECs and MECs for diazepam

PEC or MEC types		Reference
Sewage Treatment Plant PEC		
PEC = 1.2 µg l^{-1}	Effluent; first STP PEC for NL, based on use data, no elimination, published 1984	Van der Heide and Hueck-van der Plas (1984)
Surface water PECs		
PEC = 0.44 µg l^{-1} (UK 1985)	First surface water PEC for UK river, based on use, no elimination and dilution, published in 1985	Richardson and Bowron (1985)
PEC = 0.3 µg l^{-1} (EU)	EU; crude EMEA 2006 ERA initial PEC, based on maximum daily dose of 60 mg, default penetration factor of 0.01, default sewage flow, of 200 l in h^{-1} d^{-1}, no human metabolism, no removal in STPs and default surface water dilution factor of 10	This work; EMEA (2006)
PEC = 0.00329 µg l^{-1} (D high)	D; EUSES 2.0 refined regional PEC high, based on 1140 kg annual consumption for Germany, 50% excretion as diazepam, physico-chemical basic data, TGD defaults, total surface water $t_{½}$ = 34 d, sediment $t_{½}$ = 311 d	This work; ECB (2005); European Commission (2003)
PEC = 0.000327 µg l^{-1} (D low)	D; EUSES 2.0 refined regional PEC low, based on 1140 kg annual consumption for Germany, 5% excretion as diazepam, physico-chemical basic data, TGD defaults, total surface water $t_{½}$ = 34 d, sediment $t_{½}$ = 311 d	This work; ECB (2005)
STP/effluent MECs		
MEC <1 µg l^{-1}	STP effluent concentration, GC; UK	Richardson and Bowron (1985)
? <1 µg l^{-1}	STP effluent? incorrect citation of ref. Waggott (1981), should refer to Richard and Bowron (1985)	Halling-Sørensen et al. (1998)
Max = 40 ng l^{-1}	1/20 STP effluents; D	Ternes (1998, 2000)
90%ile = 30 ng l^{-1}	20 STP effluents; D	Ternes (1998, 2000)
Med <30 ng l^{-1} (LOQ)	12/20 STP effluents; D	Ternes (1998, 2000)
ND <200 ng l^{-1} (LOQ)	STP influent, Hessia; D	Ternes et al. (2001)
ND <50 ng l^{-1} (LOQ)	STP effluent, Hessia; D	Ternes et al. (2001)
Max = 53 ng l^{-1}	1/14 STP effluent samples; D	Ternes et al. (2001)
Med <50 ng l^{-1} (LOQ)	13/14 STP effluent samples; D	Ternes et al. (2001)

Table 22.A3. *Continued*

PEC or MEC types			Reference
Measurements in 3 Belgian STPs 2001			Van der Ven et al. (2004)
Influent, ng l^{-1}	Effluent, ng l^{-1}	Elimination rate, %	
590	>100	<83	Van der Ven et al. (2004)
1 180	660	44	Van der Ven et al. (2004)
>100	>100	insufficient data	Van der Ven et al. (2004)
	(>100: detected but not quantifiable due to signal-to-noise ratio)		Van der Ven et al. (2004)
Max = 100 ng l^{-1}	153 STP effluents; D		BLAC (2003)
90%ile <?20? ng l^{-1} (LOQ)	153 STP effluents; D		BLAC (2003)
Med <?20? ng l^{-1} (LOQ)	153 STP effluents; D		BLAC (2003)
'Diazepam was hardly detected in STP influents and effluents from 7 European countries'			POSEIDON (2005/2006)
NQ ~10 ng l^{-1} (LOD) –<40 ng l^{-1} (LOQ)	Influent and effluent: STP D		POSEIDON (2005/2006)
ND	Influent and effluent: STPs A, CH, F, SF, SP		POSEIDON (2005/2006)
	(Not measured: PL)		POSEIDON (2005/2006)
ND <35 ng l^{-1} (LOD)	3 water reclamation plant effluents, California; USA		Soliman et al. (2004)
Surface water MECs			
'Trace', <1 ng l^{-1}	Resin extraction, River Lee tributary, UK; *Note:* substance only described as '1,4-Benzodiazepin-2-one derivative', probably but not certainly diazepam		Waggottt (1981)
MEC ~10 ng l^{-1}	River Lee; UK		Richardson and Bowron (1985)
Max <30 ng l^{-1} (LOQ)	30 samples/20 surface waters; D		Ternes (1998, 2000)
90%ile <30 ng l^{-1} (LOQ)	30 samples/20 surface waters; D		Ternes (1998, 2000)
Med <30 ng l^{-1} (LOQ)	30 samples/20 surface waters; D		Ternes (1998, 2000)
? ~10 ng l^{-1}	River water? Incorrect cit. of Waggott (1981), should refer to Richard and Bowron (1985)		Halling-Sørensen et al. (1998)
MEC 0.7, 1.2 ng l^{-1}	2 sampling sites, River Lambro; I		
MEC 0.5, 0.7 ng l^{-1}	2 sampling sites, River Po; I		Zuccato et al. (2000)
ND (<0.02 ng l^{-1} LOD)	1 sampling site, River Adda; I		Zuccato et al. (2000)
MECs 0.13, 0.21, 0.23, 0.29, 0.83, 1.16, 2.13 ng l^{-1}	Rivers Po (7 sites) and Lambro (1 site); I		Calamari et al. (2003); Zuccato et al. (2004)

Table 22.A3. *Continued*

PEC or MEC types		Reference
Surface water MECs (*continued*)		
ND (? <0.1 ng l^{-1} LOD?)	1 sampling site, River Po; I	Calamari et al. (2003); Zuccato et al. (2004)
MECs <1(LOD), 1.3 ng l^{-1}	3 sampling sites near Las Vegas; USA	Vanderford et al. (2003)
Max = 33 ng l^{-1}	11 surface water samples; D	Ternes et al. (2001)
90%ile = 17 ng l^{-1}	11 surface water samples; D	Ternes et al. (2001)
Med <10 ng l^{-1} (LOQ)	9/11 surface water samples; D	Ternes et al. (2001)
ND	A, D, CH, F, SF (number of samples not given)	POSEIDON (2005/2006)
	(Not measured: PL, SP)	POSEIDON (2005/2006)
Max = 33 ng l^{-1}	1/174 surface water samples; D	BLAC (2003)
90%ile <?20? ng l^{-1} (<LOQ)	174 surface water samples; D	BLAC (2003)
Med <?20? ng l^{-1} (<LOQ)	174 surface water samples; D	BLAC (2003)
Max 0.88 µg l^{-1}	Surface waters? Incorrect citation of Ternes et al. (2001), correct: max = 33 ng l^{-1}	Debska et al. (2004)
MEC 34, 31, 28, <30 ng l^{-1}	4 surface water samples, 1 < LOQ (30 ng l^{-1}); Romania	Moldovan (2006)
MEC 3–62 ng l^{-1}	Number of samples not given, Lake Mead, Nevada; USA	Snyder et al. (2001)
Soil Water/Groundwater MECs		
Not detected in lysimeters at 0.4, 0.8 and 1.2 m depth nor in deeper groundwater after irrigation of a field with treated wastewater containing diazepam at unstated concentration; 'most (>80%) sorbed or degraded'		POSEIDON (2005/2006)
No change in groundwater after first 25 d of subsurface flow, with initial concentration already below LOQ; all data were below LOD after an additional 50 d		POSEIDON (2005/2006)
ND (<6.9 ng l^{-1} LOD)	105 groundwater monitoring well samples; D	Sacher et al. (2001)
ND (<20 ng l^{-1} LOD)	7 groundwater samples; D	Wolf et al. (2004)
MEC 453 and 192 ng l^{-1}	2 landfill leachates; D	Schneider et al. (2004)
MEC ~10–40 µg l^{-1}	Groundwater near a Superfund landfill, 'in which pharmaceutical manufacturers disposed of chemicals', New Jersey; USA	Genicola (1999)
Drinking water MECs		
MEC ~10 ng l^{-1}	GC, drinking water; UK	Richardson and Bowron (1985)
(? ~10 ng l^{-1})	Potable water? Incorrect cit. of Waggott (1981), should refer to Richard and Bowron (1985)	Halling-Sørensen et al. (1998)

Table 22.A3. *Continued*

PEC or MEC types		Reference
Drinking water MECs (*continued*)		
Max <10	12 drinking water samples; D	Ternes (1998, 2000)
90%ile <10 ng l^{-1} (LOQ)	12 drinking water samples; D	Ternes (1998, 2000)
Med <10 ng l^{-1} (LOQ)	12 drinking water samples; D	Ternes (1998, 2000)
MEC 19.2–23.5 ng l^{-1}	2 drinking water samples, city of Lodi; I	Zuccato et al. (2000)
MEC 0.2 ng l^{-1}	1 drinking water sample, city of Varese; I	Zuccato et al. (2000)
ND <0.02 ng l^{-1} (LOD)	1 drinking water sample, city of Milano; I	Zuccato et al. (2000)
River sediment MECs		
ND (<9 ng kg^{-1} LOD)	4/4 sampling sites, rivers Lambro, Po (2 sites) and Adda; I	Zuccato et al. (2000)
Marine MECs		
ND (<5 ng l^{-1} LOD)	7/7 sites, coastal STP effluent plume plus 3 samples each on both sides, Marseille; F	Togola and Budzinski (2005)
ND	In particulate phase of effluent plume plus 3 samples each on both sides, Marseille; F	Togola and Budzinski (2005)
ND	The metabolite N-desmethyldiazepam was not detected, either; coastal STP effluent, Marseille, F	Togola and Budzinski (2005)

LOD: Limit of detection; *LOQ:* limit of quantitation; *Max:* maximum value; med: median; *ND:* not detected; *NQ:* not quantifiable.

Table 22.A4. Ecotoxicity of diazepam

Acute toxicity to fish		Reference
LC_{50} = 84 mg l^{-1} NC LC_{100} = 100 mg l^{-1} NC NOEC = 50 mg l^{-1} NC	*Oncorhynchus mykiss*, 96 h; OECD 203; tested using emulsifier	Gröner (1981)
LC_{50} = 12.7 mg l^{-1} NC	*Gambusia holbrooki*, OECD 203, brackish water (6 g NaCl l^{-1}), 96 h	Nunes et al. (2005)
EC_{low} = 0.5 mg l^{-1} NC	*Anguilla anguilla*, 60 min; slight, nonsignificant pharmacological effects on heart rate (↓), ECG (QRS-waves ↑, T-waves ↓) and respiratory rate (↓), mainly during first 30 min of exposure	Hassan Mourad (1992)
Chronic toxicity to fish		
NOEC = 273 µg l^{-1} AMC	Overall NOEC, NOEC for survival of larvae and juveniles, fish length and weight; OECD 210, *Danio rerio*, Early Life Stage Test; GLP	Peither (2006b)
LOEC = 2.57 mg l^{-1} AMC	Highest tested concentration; NOEC for embryo development, hatching rate, time to hatch and development rate of embryos; OECD 210, *D. rerio*, Early Life Stage Test	Peither (2006b)
Acute effects in other vertebrates		
EC_{low} = 0.27 mg kg^{-1} i.p. (range 0.12–0.68)	*Testudo graeca;* in the low-dose range (comparable to human doses) pharmacological effects, suppression of paroxysmal electroencephalographic activities of a turtle epileptic model	Servít and Strejcková (1974)
Acute toxicity to invertebrates		
EC_{50} = 14.1 mg l^{-1} NC	*Daphnia magna*, 24 h	Calleja et al. (1993); Calleja and Persoone (1993)
EC_{50} = 4.3 mg l^{-1} NC	*Daphnia magna*, 24 h	Lilius et al. (1995)
EC_{50} = 12.0 mg l^{-1} NC	*Daphnia pulex*, 24 h	Lilius et al. (1995)
EC_{50} = 100 mg l^{-1} NC	*Streptocephalus proboscideus*, 24 h	Calleja et al. (1993); Calleja and Persoone (1993)
NOEC = 10 000 mg l^{-1} NC	*Brachionus calyciflorus*, 24 h	Calleja et al. (1993); Calleja and Persoone (1993)
NOEC = 1 mg l^{-1} NC	*Hydra vulgaris*, semi-static, 7 d; *Note:* significant mortality at 0.01 mg l^{-1}, but none at 0.1 and 1 mg l^{-1}, possibly chance event/ statistical outlier	Pascoe et al. (2003)
LC_{100} = 10 mg l^{-1} NC	Possibly solvent toxicity as identical in solvent controls; *H. vulgaris*, semi-static, 7 d	Pascoe et al. (2003)
NOEC = 10 000 mg l^{-1} NC	*Brachionus plicatilis*, saltwater, 24 h	Calleja et al. (1993); Calleja and Persoone (1993)

Table 22.A4. *Continued*

Acute toxicity to fish		Reference
Acute toxicity to invertebrates (*continued*)		
EC_{50} = 67 mg l^{-1} NC	*Artemia salina*, saltwater, 24 h	Calleja et al. (1993); Calleja and Persoone (1993)
EC_{50} = 12.16 mg l^{-1} NC	*Artemia parthenogenetica*, saltwater, 48 h	Nunes et al. (2004, 2005)
Chronic toxicity to invertebrates		
NOEC = 0.91 mg l^{-1} NC	OECD 211, *Daphnia magna*, Reproduction Test, 21 d; GLP	Peither (2006a)
NOEC = 0.8 mg l^{-1} AMC		Peither (2006a)
NOEC = 1 mg l^{-1} NC	OECD 219, *Chironomus tentans*, Larval Emergence Test, 21 d; GLP	Memmert (2005)
NOEC = 1 mg l^{-1} NC	Endpoint feeding rate, *Hydra vulgaris*, semi-static, 17 d	Pascoe et al. (2003)
NOEC <10 µg l^{-1} NC (only tested concentration for regeneration)	Endpoint regeneration, *H. vulgaris*, semi-static, = 17 d	Pascoe et al. (2003)
Acute/chronic toxicity to algae		
E_bC_{50} = 7.23 mg l^{-1} NC	OECD 201, *Scenedesmus subspicatus*, green algae, 72 h; ISO/IEC 17025	Häner (2005c)
E_rC_{50} = 22.8 mg l^{-1} NC		Häner (2005c)
NOEC <2.56 mg l^{-1} NC		Häner (2005c)
E_bC_{50} = 0.61 mg l^{-1} AMC		Häner (2005c)
E_rC_{50} = 3.11 mg l^{-1} AMC		Häner (2005c)
NOEC = 0.035 mg l^{-1} AMC		Häner (2005c)
LOEC = 16 mg l^{-1} NC	On prolongation of test to 2 weeks almost complete recovery at 6.4 and 16 mg $l^{-1,}$ compared with controls; OECD 201, *S. subspicatus*, 14 d; ISO/IEC 17025	Häner (2005c)
NOEC = 2.56 mg l^{-1} NC		Häner (2005c)
EC_{50} = 16.5 mg l^{-1} NC	OECD 201, brackish water, *Tetraselmis chuii*, green algae, 96 h	Nunes et al. (2005)
NOEC = 7.9 mg l^{-1} NC		Nunes et al. (2005)
E_bC_{50} = 18.2 mg l^{-1} NC	OECD 201, *Synechococcus leopoliensis*, bluegreen algae, 72 h; ISO/IEC 17025	Häner (2005d)
E_rC_{50} >40 mg l^{-1} NC		Häner (2005d)
NOEC = 6.4 mg l^{-1} NC		Häner (2005d)
E_bC_{50} = 3.5 mg l^{-1} AMC		Häner (2005d)
E_rC_{50} >11.9 mg l^{-1} AMC		Häner (2005d)
NOEC = 0.67 mg l^{-1} AMC		Häner (2005d)

Table 22.A4. *Continued*

Acute toxicity to fish		Reference
Acute/chronic toxicity to algae (*continued*)		
NOEC = 16 mg l^{-1} NC	On prolongation of test to 7 d full recovery at 16 mg l^{-1} compared with controls, partial recovery at 40 and 100 mg l^{-1}, lysis of cells after day 7; OECD 201, *S. leopoliensis*	Häner (2005d)
Toxicity to micro-organisms		
NOEC = 100 mg l^{-1} SC	No significant inhibition of activated sludge; toxicity control in Roche inherent biodegradability test, 14 d	Gröner (1981)
NOEC = 30 mg l^{-1} SC	No significant inhibition of activated sludge, toxicity control in inherent respirometry test, 14 d; ISO/IEC 17025	Häner (2005a)
NOEC = 20 mg l^{-1} SC	Borderline (–7%) inhibition of anaerobic sludge compared to blank, probably due to primary metabolite(s) as there was no inhibition observed earlier up to 13 d; Ultimate anaerobic degradability, ISO11734, 64 d; ISO/IEC 17025	Häner (2005b)
NOEC = 10000 mg l^{-1} NC	*Photobacterium phosphoreum*, 5 min	Calleja et al. (1993); Calleja and Persoone (1993)
NOEC = 10000 mg l^{-1} NC	*Pseudomonas putida*, 5 min	Calleja et al. (1994)
EC_{50} = 908 mg l^{-1} NC	*Sinorhizobium meliloti*, 20 min	Botsford (2002)
EC_{50} = 290 mg l^{-1} NC	*Saccharomyces cerevisiae*, yeast growth inhibition test; 210 min, 30 °C	Koch et al. (1993)
Cellular biomarkers and cytotoxicity		
Low physiological stress due to short-term diazepam exposure in fish cell lines		
EC_{50} = 659 mg l^{-1} NC	Rubidium leakage test, fresh rainbow trout hepatocytes, 3 h	Lilius et al. 1994)
EC_{50} = 108 mg l^{-1} NC	Neutral Red uptake test, R1 trout liver cell line, 24 h	Castano et al. (2003)
EC_{50} = 103.4 mg l^{-1} NC	MTT assay, PLHC–1 fish cell line, 24 h	Caminada et al. (2006)
EC_{50} = 125.3 mg l^{-1} NC	Neutral Red uptake, PLHC–1 cells, 24 h	Caminada et al. (2006)
EC_{50} = 175.4 mg l^{-1} NC	MTT assay, RTG–2 fish cell line, 24 h	Caminada et al. (2006)
Low oxidative stress due to 48-h diazepam exposure in *Artemia parthenogenetica*		
LOEC = 6.13 mg l^{-1} NC	Increase in total glutathione peroxidase	Nunes et al. (2006)
NOEC = 8.83 mg l^{-1} NC	Selenium-dependent glutathione peroxidase	Nunes et al. (2006)
NOEC = 8.44 mg l^{-1} NC	For glutathione reductase	Nunes et al. (2006)

Table 22.A4. *Continued*

Acute toxicity to fish		Reference
Low oxidative stress due to 48-h diazepam exposure in *Artemia parthenogenetica* (*continued*)		
NOEC = 8.44 mg l^{-1} NC	For superoxide dismutase	Nunes et al. (2006)
NOEC = 8.44 mg l^{-1} NC	For glutathione-S-transferase	Nunes et al. (2006)
Low neurotoxic stress due to 48-h diazepam exposure in *A. parthenogenetica*		
NOEC = 5.86 mg l^{-1} NC	For thiobarbituric-acid-reactive substances (TBARS); the 7.04-mg l^{-1} LOEC is possibly a chance event/statistical outlier	Nunes et al. (2006)
LOEC? = 7.04 mg l^{-1} NC		Nunes et al. (2006)
NOEC = 8.44 mg l^{-1} NC		Nunes et al. (2006)
Published risk estimates and risk ratios		
'Few effects are to be expected' (based only on PEC; NL 1984)		Van der Heide and Hueck-van der Plas (1984)
RR = 0.013	PEC(DK)/PNECacute	Stuer-Lauridsen et al. (2000); Halling-Sörensen et al. (1998)
RR = 0.01	Max MEC(D)/PNECacute	Stuer-Lauridsen et al. (2000)
RR = 0.002	MEC(UK)/PNECacute	Römbke et al. (1996)
RR = 0.1	Max MEC(UK)/PNECacute	Römbke et al. (1996)

AMC: average measured concentration; *NC:* nominal concentration; *RR:* Risk ratio; *SC:* starting concentration.

Comparison of Prospective and Retrospective Environmental Risk Assessments of Human Pharmaceuticals

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23.1 Introduction

Prospective risk assessment is performed in the context of market authorization of a compound, whereas the retrospective risk assessment is generally aimed to identify the causes of adverse effects that have already occurred (Calow and Forbes 2003). For a large number of existing substances, an environmental risk assessment (ERA) was not required at the time of their introduction; rather it was based on data sets, which nowadays are not considered to be state-of-the-art. For such substances, a retrospective ERA may serve to define quality standards which mark levels of environmental safety. Ideally, the prospective and retrospective risk assessment should have the same outcome if the conceptual approach and the data used for both the pro- and retrospective risk assessment are likewise suitable. However, in several cases it appeared that the prospective exposure assessment underestimated the actual occurrence of a substance in the environment after a few years of use, or adverse effects were identified, which previously had not been anticipated (e.g., endocrine disrupting effects).

In this paper we will compare the prospective and retrospective environmental risk assessment approaches for human pharmaceuticals (pro- and re-ERA) which have been established within the European Union (EU) in recent years. We have chosen three existing pharmaceuticals for which sufficient data have been generated to compare pro-ERA and re-ERA for the aquatic compartment. Based on these examples, we will highlight and explain the differences between the pro- and re-ERA for human pharmaceuticals.

23.2 Conceptual Approaches for the Pro- and Re-ERA of Human Pharmaceuticals

The widely accepted principle of ERAs (EC 2003) consists of the comparison of predicted (PEC) or measured environmental concentrations (MEC) with predicted no-effect concentrations (PNEC). The PNEC is derived by applying assessment factors to the endpoint of an ecotoxicological test, which compensate for the uncertainties when extrapolating measured effects data from the laboratory to the real environment and from individual organisms to populations. The PEC/PNEC ratio indicates an unacceptable risk, if the ratio is equal to or exceeds 1. An ERA is a tiered approach, which, in the first instance, makes worst-case assumptions and then, if unacceptable risk is indicated, considers more realistic conditions by refining exposure and effects assessment. Risk characterization ratios above 1 require risk management measures.

When applying the guideline developed by the European Medicines Agency (EMEA 2006) for the pro-ERA of human pharmaceuticals, the following aspects have to be taken into consideration, in addition to the general risk characterization approach described above.

- At the lower tier (Phase I) an assessment of the persistence (P), bioaccumulation (B) and toxicity (T) of the pharmaceutical (PBT-criteria) is required, if the value assessing the potential for bioaccumulation ($\log K_{OW}$) is 4.5 or higher. Also in Phase I, the comparison of the PEC value for surface water with a defined trigger value determines whether the pro-ERA stops at Phase I or moves on to Phase II. Regardless of the PEC in Phase I, compounds which may affect the reproduction of organisms at low concentrations will enter the next tier (Phase II);
- At Part A of the higher tier pro-ERA (Phase II-A), and according to the main exposure route of human pharmaceuticals via effluent and sewage treatment plants into surface water, a risk assessment for the pelagic phase of the water compartment is performed. In case the aquatic PEC/PNEC ratio shows unacceptable risks, further environmentally more relevant studies are required at Part B of Phase II (Phase II-B) to refine the risk characterization ratio PEC/PNEC. In addition to the quantitative risk assessment for the pelagic compartment, physicochemical properties and fate (K_{oc}, transfer of the compound to the sediment, and $\log K_{OW}$) are determined in Phase II-A. If one or all measured values are higher than defined trigger values, further studies are required in Phase II-B, which allow assessing the risk for one or several additional compartments (for soil if $\log K_{oc} \geq 3$, for sediment if transfer of the substance to sediment is ≥10% within fourteen days, and for biota, i.e., indirect toxicity along food chains, if $\log K_{OW} \geq 3$);
- In Phase II-B of the tiered pro-ERA all compartment specific risk quotients should be smaller than 1. However, if one or more risk quotients indicate unacceptable risks, precautionary or safety measures should be proposed to reduce the exposure of the compound in the environment.

Although the pro-ERA is part of the marketing authorization procedure and presently only required for new pharmaceuticals, its procedures could also be applied to existing substances, which already occur in the environment. However, the re-ERA according to the Water Framework Directive (WFD; EC 2000) differs from the pro-ERA in a way that Annual Average Environmental Quality Standards (AA-EQSs) are determined, which indicate the need for protective measures for the aquatic environment if exposure concentrations measured in environmental monitoring programs (MECs) exceed the AA-EQS (Lepper 2005). If the MEC of a specific compound is higher than the EQS, unacceptable risks are stated and measures to reduce the exposure concentrations have to be applied. For the derivation of AA-EQS, chronic aquatic effects data are preferred to which safety factors are applied according to the EU Technical Guidance Document (TGD; EC 2003). Effects data generated according to the Directive 91/414/EEC concerning the placing of plant protection products on the market can also be used for the determination of EQS. Acute aquatic toxicity data may be used to derive AA-EQS if sufficient chronic toxicity data are not available.

A second kind of quality standard referring to possible acute effects of transient exposure peaks, the maximum acceptable concentration EQS (MAC-EQS), has been defined by Lepper (2005). The MAC-EQS of a specific compound should not be exceeded by the highest measurement (MEC_{max}) in environmental samples for that compound. To protect the benthic community $EQS_{sediment}$ are derived for all substances with a $\log Kp_{SPM\text{-}Water} \geq 3$ (partitioning between suspended particulate matter and water). This partitioning coefficient refers to the dimensionless form as it is described in the TGD (Eq. 24; EC 2003). However, as immissions to the aquatic environment normally occur in water first and because subsequent partition between water and settled sediment is normally rather slow, it is deemed appropriate to derive this MAC-EQS for water only.

23.3 Examples for Pro- and Retrospective Environmental Risk Assessments

Three human pharmaceuticals with different medicinal and physicochemical properties were selected as examples for the pro- and re-ERA (Table 23.1).

For the pro-ERA, PECs are estimated for surface water (Table 23.2). For the initial exposure assessment of surface water concentrations (PEC_{sw}), a rather crude model is proposed by EMEA (2006). In fact, the only variable in this model is the "maximum daily dose" of the pharmaceutical. At higher tiers of the ERA, more sophisticated models consider, for example, the human excretion profile of the pharmaceutical and removal of the compound during sewage treatment (EMEA 2006; Knacker et al. 2006).

MECs shown in Table 23.2 were derived from recently published data on measurements in surface waters (MEC_{sw}) in Germany (Liebig et al. 2006). The authors evaluated the appropriateness of these data according to criteria for monitoring data recommended by the TGD (EC 2003). Only those data were used to derive the MEC_{sw},

Table 23.1. Properties of the selected compounds as cited by Knacker et al. (2006)

	Carbamazepine (CBZ)	Sulfamethoxazole (SMX)	17α-Ethinylestradiol (EE2)
Pharmaceutical group	Antiepileptic	Antibiotic	Oral contraceptive
Chemical group	Tricyclic dibenzazepin derivative	Sulfonamide	Synthetic steroid
log K_{OW}	2.45	0.89	4.2
log K_{oc}	1.92–3.42	n.a.	2.28–4.0
log $Kp_{SPM\text{-}water}$ [a]	0.47–1.82	n.a.	0.75–2.4
Significant partition of the substance into the sediment	ca. 28% in 14 d	n.a.	n.a.

n.a.: Not available.
[a] Calculated according to TGD (EC 2003; equation 24).

Table 23.2. Predicted environmental concentrations for German surface water (PEC_{sw}) determined according to models for different ERA-phases (EMEA 2006) and measured environmental concentrations in German surface waters (MEC_{sw}) according to Liebig et al. (2006) in μg l^{-1}

Compound	PEC_{sw}			MEC_{sw}	
	Phase I	Phase II-A	Phase II-B		
CBZ	5.0	1.46	0.234	0.454	($n = 4$)
SMX	10.0	0.895	0.088	0.126	($n = 2$)
EE2	0.00013	0.00079	0.000076	0.00058	($n = 3$)

which were found to be adequate for use in exposure assessment. According to the TGD (EC 2003), the MEC of a specific compound corresponds to the mean of the 90th percentiles of individual sites.

In Table 23.3 results of the higher-tier pro-ERA are shown, which were determined according to the rules described by the EMEA Guideline (EMEA 2006). The most sensitive long-term effect endpoints available for the selected compounds were used, and the appropriate assessment factor was applied in order to determine the PNEC. In the case of sulfamethoxazole, a higher assessment factor was used since data were not available for all three trophic levels. For EE2, the risk characterization quotient (PEC/PNEC) is ≥ 1, indicating an unacceptable risk for surface water.

According to EMEA (2006), an assessment of the compartment sediment is required for CBZ since more than 10% of the substance is partitioned to the sediment (Löffler et al. 2005; cf. Table 23.1). For the risk characterization quotient, a $PEC_{sediment}$ calculated according to the TGD (EC 2003) was used, which results in a risk quotient of far above 1 for CBZ, indicating an unacceptable risk to the sediment. Using the measured concentration of 42 ng/g (90th-percentile of measured data; T. Ternes, pers. com. 2005) for CBZ in sediment, the value of the risk characterization ratio is >15. For SMX and EE2, no data are available on the transfer into sediment.

The $\log K_{OW}$ for EE2 is above the trigger value of 3 (cf. Table 23.1). This requires the assessment of bioconcentration in fish, which is not addressed in this paper. For a detailed description of the procedure and the outcome of the pro-ERA, see Knacker et al. (2006).

For the performance of the retrospective ERA (re-ERA), annual average environmental quality standards (AA-EQS) were derived according to Lepper (2005) in accordance with the requirements of the WFD (EC 2000). In analogy to the PEC/PNEC ratio of the pro-ERA, the AA-EQS are compared to MECs for the compartment's surface water and sediment within the re-ERA (Table 23.4). For CBZ, the risk characterization remained below 1, although the MEC was twice as high as the PEC. For SMX, a slight increase of the MEC (0.126 μg l^{-1}) compared to the PEC (0.088 μg l^{-1}) led to a MEC/AA-EQS ratio of >1 indicating unacceptable risk to the aquatic environment. For EE2, the MEC is approximately eight times higher than the PEC, leading to an unacceptable risk to surface water. The trigger value for the assessment of the compart-

Table 23.3. Prospective ERA (pro-ERA): Most sensitive long-term toxicity data (NOEC) available for the selected pharmaceuticals, derivation of predicted no effect concentrations (PNEC) by applying the appropriate assessment factor (AF) to the NOEC and risk characterisation (PEC/PNEC) according to EMEA (2006)

Compound	Species	NOEC (µg l^{-1})	AF	PNEC (µg l^{-1})	PEC[i] (µg l^{-1})	PEC/PNEC
CBZ	Water: *Ceriodaphnia dubia*	25[e]	10	2.5	0.234	0.09
	Sediment[a]: *Chironomus riparius*	<140 ng g^{-1} [f]	50[c]	<2.8 ng g^{-1}	1 170 ng g^{-1} [j]	>418
SMX[b]	*Lemna gibba*	10[g]	100[d]	0.1	0.088	0.88
EE2[b]	*Danio rerio*	0.0003[h]	10	0.00003	0.000076	2.53

[a] Assessment of sediment toxicity required since trigger value for "transfer into sediment" is met (cf. Table 23.1).
[b] No data on "transfer into sediment" available (cf. Table 23.1).
[c] AF = 50 since data for 2 sediment dwelling organisms available.
[d] AF = 100 since data are not available for 3 trophic levels.
[e] Ferrari et al. 2003.
[f] Oetken et al. 2005.
[g] Brain et al. 2004.
[h] Wenzel et al. 2001.
[i] PEC_{sw} according to the second refinement step (EMEA 2006).
[j] $PEC_{sediment}$: calc. according to TGD (EC 2003) based on the log K_{oc} = 1.92..

Table 23.4. Retrospective ERA (re-ERA): Most sensitive long-term toxicity data (NOECs) available for the selected pharmaceuticals, annual average environmental quality standards (AA-EQS) derived by applying appropriate assessment factors (AF) to the NOEC according to Lepper (2005); finally comparison of AA-EQS with measured environmental concentrations (MEC)

Compound	Species	NOEC (µg l^{-1})	AF	AA-EQS (µg l^{-1})	MEC (µg l^{-1})	MEC/AA-EQS
CBZ	*Ceriodaphnia dubia*[a]	25[b]	10	2.5	0.454	0.18
SMX	*Lemna gibba*	10[c]	100	0.1	0.126	1.26
EE2	*Danio rerio*	0.0003[d]	10	0.00003	0.00058	19.3

[a] Assessment of sediment is not required since trigger value is not met.
[b] Ferrari et al. 2003.
[c] Brain et al. 2004.
[d] Wenzel et al. 2001.

ment sediment ($\log Kp_{SPM\text{-}water} \geq 3$) is not met by any of the selected compounds. Therefore, an assessment of sediment is not required within the re-ERA.

In Table 23.5, the MAC-EQSs are compared with the MEC_{max} values for the selected pharmaceuticals. This re-ERA resulted in acceptable risks for all substances.

Table 23.5. Retrospective ERA (re-ERA): Most sensitive short-term toxicity data (EC_{50}) available for the selected pharmaceuticals; derivation of the maximum acceptable concentrations (MAC-EQS) by applying appropriated assessment factors (AF) to the EC_{50} values according to Lepper (2005); finally comparison of MAC-EQS with maximum measured environmental concentrations (MEC_{max})

Compound	Species	EC_{50} (µg l^{-1})	AF	MAC-EQS (µg l^{-1})	MEC_{max} (µg l^{-1})	MEC_{max}/ MAC-EQS
CBZ	*Daphnia magna*[a]	13 800	1 000	13.8	7.1	0.51
SMX	*Scenedesmus subspicatus*[b]	2 500	1 000	2.5	1.9	0.76
EE2	*Scenedesmus subspicatus*[c]	840	1 000	0.84	0.0067	0.008

[a] Ferrari et al. 2003.
[b] Liebig 2005.
[c] Kopf 1997.

23.4 Summary and Outlook

This paper highlights that the prospective environmental risk assessment for pharmaceuticals according to EMEA (2006) may lead to different results compared to the retrospective environmental risk assessment according to the WFD (Lepper 2005). For the pelagic sub-compartment, the main reason therefore may be found in differences between calculated exposure values (PECs) and measured environmental concentrations (MECs). Another reason for differences between the two risk assessment approaches is related to the fact that in principle, EQS values can be derived from short-term effect data, whereas – in the context of pharmaceutical assessment – PNEC values are derived exclusively from long-term effect data. It has been shown in the literature that for several biologically highly active substances, the assessment factor of 1 000 does not cover the acute to chronic ratio. We therefore propose in the case of pharmaceuticals that the EQS should be based exclusively on long-term effect studies.

When considering sediment, there is an important difference between the pro- and retrospective risk assessment with regard to the trigger value, which initiates the requirement of a risk assessment of the benthic sub-compartment. Furthermore, it has not been covered in this paper that the prospective risk assessment for sediment dwelling organisms is based on one species, whereas for the retrospective risk assessment data from up to three different species are required.

As proposed by the 'Strategy for a Future Chemicals Policy' (White Paper, CEC 2001) further efforts should be made to harmonize those approaches that guide the assessment of environmental risks of any substances released by human activities.

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Methodological Aspects Concerning the Environmental Risk Assessment for Medicinal Products; Research Challenges

M. H. M. M. Montforts

24.1 Introduction

Pharmaceuticals in the environment have been studied for several decades. The oldest references are on fate and behavior (Zondek and Sulman 1943; Soulides et al. 1962; Tabak and Bunch 1970). Studies into the effects of pharmaceutical residues in the environment appeared not long after that (Berland and Maestrini 1969; Manten 1971; Blume et al. 1976; Rurainski et al. 1977; Patten et al. 1980). Over time, several reviews on the use, emission, fate, occurrences, and effects of pharmaceuticals have been published, of which this book is the outstanding example (Roij and De Vries 1980; Römbke et al. 1996; Ternes 1999; Jorgensen and Halling-Sørensen 2000; Kümmerer 2001; Daughton and Jones-Lepp 2001; Dietrich 2002; Halling-Sørensen et al. 2002; Boxall et al. 2004).

Environmental risks of pharmaceuticals are on the agenda at the national and supra-national regulatory levels. The intrinsic pharmacological properties of medicines indeed warrant an environmental risk assessment at registration, as recent events have underlined. In particular, the alarming decline of vulture populations (up to 95%) that occurred in Pakistan in the late 1990s, which research has attributed to the use of the anti-inflammatory drug diclofenac in cattle (Oaks et al. 2004), brings the history of pesticide regulation back to mind. In the 1960s, populations of birds of prey alarmingly declined (up to 91%) in Europe, which was attributed to insecticides, substances for seed treatment, and rodent baits, which were all applied in agriculture for crop protection (Cramp 1963; Koeman et al. 1972; Mendenhall and Pank 1980; Hill and Fleming 1982). Regulatory responses to these particular findings involved, amongst others, the ban on certain pesticides[1]. In some early incidents with bird mortalities in Europe, however, the substances involved were not pesticides, but were in fact applied as veterinary medicines to cattle, either by feed or by pour-on applications (Ludke and Locke 1976; Hill and Mendenhall 1980; Henny et al. 1985).

These findings by themselves lead to the conclusion that regulatory measures taken for plant protection products with respect to the environment are also needed for medicines. These measures are not to be restricted to the issue of secondary poisoning. It has also been shown that the veterinary use of sheep dips in Scotland to com-

[1] Council Directive 79/117/EEC of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances.

bat scab and other parasites has caused surface water contamination and fish mortality (McVeigh et al. 1997), remedial treatment of fish resulted in high surface water concentrations of oxytetracycline (Staeb et al. 2004) and the use of ethinyl-estradiol as a contraceptive has contributed to estrogenic effects in fish (Purdom et al. 1994; Vethaak et al. 2002).

From a biological perspective, pesticides and medicines are rather similar types of substances. Two illustrative examples are warfarin and paracetamol. Therapeutic medicinal use of warfarin prevents thromboembolism, while the same compound used as a rodenticide very effectively kills rats and mice. Paracetamol is a well-known pain reliever that also effectively controls Brown Tree snakes when applied in baits (Johnston et al. 2002). The antibiotics oxytetracycline and streptomycin are applied as medicines and as foliage pesticides in crops (EC 2006).

Before the environmental risk management for medicines can be discussed in depth, we must define what we consider a veterinary or human medicine. Any substance or combination of substances presented for treating or preventing disease in animals is a veterinary medicine, also when administered with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions. Substances that are added to animal feed in order to increase animal production without preventing any specific illness are included with feed additives. Substances that are used for treating, diagnosing, or preventing disease, or restoring, correcting or modifying physiological functions in man are classified as human medicines. These are the definitions given in the EU directives 2001/79/EC, 2001/82/EC and 2001/83/EC on the marketing authorization for feed additives and medicines[2]. Although the term substances can point to either chemicals or vaccines, blood products or herbs, to name a few, only chemicals will be considered further in this review. When referring to medicines, terminology like pharmaceuticals, drug substances, drugs, and chemicals, compounds, and substances is used interchangeably. Please note that registration has concerns for a product: a veterinary or human pharmaceutical, containing active ingredients (substances) and excipients, and environmental quality policy deals with substances in compartments, and activities of legal persons concerning the emission of substances. A drug at registration is a product with a certain intended use, whereas a drug in the environmental quality policy is a substance (be it a parent compound, pro-drug, or metabolite) emitted to, or present in an environmental compartment.

This chapter focuses on research needs for environmental risk management and risk assessment of human pharmaceuticals. First of all, we will outline what the environmental protection goals could be and how risk assessment works in general terms. This provides us with some reference to compare the current risk assessment practices for human medicines. We will identify several research challenges, including the handling of metabolites in the environment and the alignment of product registration and environmental quality policy.

[2] The first EU Directive on medicines dates from 1965 and has been amended numerous times. Comprehensive reviews resulted in new directives on veterinary medicines in 1981 (81/851/EC and 81/852/EC), in 2001 (2001/82/EC and 2001/83/EC), and recently in 2004 (2004/27/EC and 2004/28/EC).

24.2 Risk Management

As a general observation, it is stated here that the primary goal of any environmental risk assessment should be risk mitigation and risk management. In order to mitigate or accept risks, a risk assessment has to be performed, both for products (e.g., drugs) and for activities (e.g., emission of drug residues). In Europe, product regulations are usually special laws, whereas regulations of activities (leading to discharges) related to environmental quality are considered general laws. Ideally, all pieces of legislation are geared to one another, clarifying what legislation prevails if there should be any overlap.

Medicines are regulated by special regulations in order to protect animal health, consumers, professional users, and the environment, as well as the internal market. The framework of the registration procedure and risk assessments consist of European Commission and Council directives and regulations on registration, European policy, case law, and global (trade) agreements. At registration it is possible to lay the burden of proof on the applicant, complying with the principle that the polluter pays. The decision-making process and the risk models should optimize (reduce) the costs to society in terms of environmental damage (due to false negatives, implying registration of harmful products) and economic damage (due to false positives implying refusal of harmless products). Also the assessment process itself should hamper neither product development nor timely action to remove hazards (Cranor 1997).

Management of polluting activities and environmental quality is the subject of a considerable number of directives and regulations on the quality of water, air and sludge, to name a few compartments. All this legislation operates on the assumption that all actions that may lead to pollution are forbidden unless a permit is granted by the competent authority. Thus, in order to emit or spread the residues of medicines, one needs a permit. The permit ought to regulate emission (e.g., prescribing application techniques) as well as the maximum permissible concentration of the substance in the environment. The competent authorities should thus derive these quality standards for all substances of interest. Also, they have to develop action plans for the local resource management[3] (Van Rijswick 2001).

Product authorization and environmental management constitute two policy realms to effectively manage the risk of pharmaceuticals. They also involve different competent authorities. In both realms, risk assessment can only be performed if the protection goals and the assessment methodology have been developed. Risk assessment is a key process in which both regulators and scientists determine the outcome (Joerges et al. 1997). Regulators, or risk managers, have to indicate what should be assessed (hazard identification). Legislation and policy documents concerning environmental quality are thus important sources of information on protection goals for product registration (Heyvaert 1999). Risk managers also have to specify what level of protection

[3] Medicines are acknowledged as a specific group of substances in the Netherlands' 4th Water Action Program (NW4, 1998).

should be employed for protection goals, since they have to make the risk-benefit decisions. This is the prerogative of the risk manager, and this activity should be under scrutiny of the public, whom the risk manager represents. Scientists are required to provide information on the relevance of certain hazards, on the difference that the chosen level of protection makes to the quality of the assessment and on the level of uncertainty associated with the assessment. Scientists should not be placed in a position where they have to decide on these elements, but they should inform the risk manager in a transparent way about the significance of his decisions. Finally, it is also the scientists' responsibility to assess the fate and effects of the substances in a way that addresses the chosen standards and effectively communicates the desired information for the risk-benefit analysis (Montforts 2007).

24.3 Risk Assessment: The Risk Model

The environment is at risk when a product reaches the environment. Emission and transport (mass transfer), transformation, concentration, and impact of substances are influenced by the environment, the substance and the receptor (e.g., the species or populations). Environmental variables such as soil, climate, and the biological receptors (communities, species) are subject to a considerable spatial and temporal variation. Because it is impossible to assess the risks of all combinations of substances and environmental factors, there is a well-established need to predict fate and exposure concentrations as well as associated risks. In order to do so, models of the environment and suitable values for the quantities (parameters) described by the models are needed. Two levels of modeling are discerned: the level of the complete risk model covering the environmental risk assessment process and the sublevel of the fate and effect models that function within that process.

The risk model includes all activities employed in the risk assessment process, including their harmonization and communication with the risk manager. In Fig. 24.1 below, the risk model is represented by the ellipses containing hazard identification, exposure and effects assessment, and risk characterization. Hazard identification is the stage at which possible effects (hazards) are characterized. Exposure assessment starts from the emission of the product to the different compartment and addresses all exposure routes, using emission and exposure models, as well as monitoring data. Effect assessment addresses all hazards identified, using dose-effect models and monitoring data, as well as the integration of the effect model results. Risk characterization combines the information gathered.

The risk model translates the protection goals in quantities: for example probabilities, concentrations, dosages, and risk quotients. The risk model is as good as the weakest link in the model, be it the protection goal, an exposure model or an effect model (including any overseen exposure or effect), the interpretation of effect data, or the integration of exposure and effect.

The rectangular boxes, from risk classification down to monitoring, belong to the stage of risk management. The characterized risk may be classified into categories that help in balancing the risk benefit analysis and in identifying what measures need to

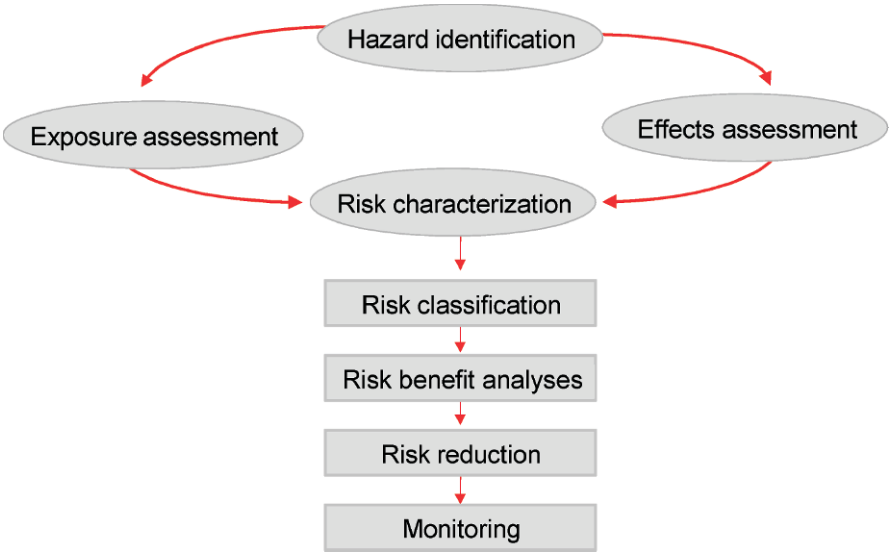

Fig. 24.1. The basic framework of risk management. Hazard identification, exposure and effects assessment and risk characterization are components of environmental risk assessment. Risk classification, risk-benefit analysis, risk reduction and monitoring are additional methods aimed towards risk management.

be taken. Post-approval, the monitoring data are indispensable as a check on the appropriateness of the risk management system.

Ultimately, the quality of the risk assessment that can be achieved will depend upon the adequacy of available data as well as the choice of model and modeling parameters (Dee 1994; WRc-NSF 2001). It is important to note that the model capabilities should have been reflected in the decision making process, for example in applying a worst-case scenario, in the use of safety factors, or in the selection of adequate confidence intervals (Brouwer et al. 1994; Resseler et al. 1997; Uffink and Van der Linden 1998; Van der Linden et al. 1999). Modeling at low concentration levels requires a rigorous understanding of all relevant transport, fate and effect processes, or it requires sufficiently high safety factors. Evidently, there should be good agreement between the protection goal and the methodology used to assess the risk, in the sense that it should be clear what situations the methodology represents, and what level of certainty the predictions have (cf. Forbes and Calow (2002) and Tarazona et al. (2002)). The risk model should thus produce predictions with reference to the quality levels that are pursued for the compartments. Exclusion of compartments (soil, surface water, groundwater, drinking water, and sediment) either because the risk is supposed to be covered through other submodels, or because models have yet to be developed, greatly impairs the risk characterization and thus the decision making.

In the next section, the risk model for human medicines is reviewed and regulatory needs and research challenges are indicated.

24.4 Risk Assessment at Registration of Human Medicines

The Directive 2001/83/EC, as amended, on the registration of human pharmaceuticals does not contain explicit environmental protection goals, only procedural directions. The Directive 2001/83/EC as amended by 2004/27/EC includes the risk to the environment in the definition of risk, but excludes the environmental risk from the risk/benefit assessment. Nevertheless according to article 8.3.(ca), an environmental risk assessment is required for every new request for authorization, including generics. The risk assessment approach is detailed in a guidance document, and emission to the environment is primarily foreseen through wastewater. The guidance addresses several of the challenges raised in the second edition of this book (Montforts 2004; EMEA 2006; Spindler et al. 2007).

In Phase I of the risk assessment, an assessment of Persistence, Bioaccumulation, and Toxicity (PBT assessment) according to the Technical Guidance Document for New and Existing Substances and Biocides (TGD) (EC 2003) is performed. If the logKow is >4.5, first persistency, then bioaccumulation, and finally toxicity should be assessed, with the aim of ruling out the possibility that the substance is persistent (P); then if it is persistent it is not bioaccumulating (B); and finally, if also bioaccumulating, not toxic (T). In this way, the possibility that PBT substances are marketed unnoticed is ruled out.

In Phase I a predicted environmental concentration (PEC) of 10 ng l^{-1} in surface water was proposed as a trigger value to proceed to a true risk-based assessment in Phase II. Particularly for sexual hormones, this trigger should, however, be ignored according to the guidance. It is generally accepted that genotoxicity is no special reason to skip the trigger like for estrogenic effects, since the effects of genotoxicity are revealed later than those of 'normal' toxicity in aquatic toxicity tests (Würgler and Kramers 1992; Roex et al. 2001). The scientific basis for the trigger value in itself is, however, poor for other therapeutic classes (Montforts 2005), but the guidance is clear that the trigger value may be revised if more long-term information is available.

The Phase II risk assessment is split into two tiers: tier A and a tier B. The predicted exposure concentration (PEC) in Phase II tier A is the same as in Phase I and is based on a simple dilution model, in which the total annual consumption is diluted over the total amount of wastewater produced. The concentration in wastewater is further diluted to surface water using a default dilution factor of 10. The presence of metabolites is basically ignored, so that the risk assessment is based on the total residue representing the most potent "substance." In most cases, this is the drug substance. Retention in wastewater treatment plants (WWTPs) can be not accounted for until Tier B. Only in tier B are human metabolites excreted in fractions below 10% excluded from the assessment. Fractions >10% can be further assessed along the lines set out for the drug substance. The predicted no-effect concentration (PNEC) for surface water is derived from a base set of chronic aquatic toxicity data in accordance with the TGD (EC 2003).

Besides water, also groundwater, soil and sediment are assessed. If >10% of a dosage in the water-sediment system is in the sediment after fourteen days, the risk to sediment-dwelling organisms needs to be investigated. If the K_{oc} for the drug substance is >10 000 l kg^{-1}, the risk to the soil compartment needs to be investigated, using laboratory tests on plants, earthworms, collembola reproduction, and nitrification. The

exposure concentration for groundwater is based on the German model Exposit and starts with the assumption that the groundwater concentration is one quarter of the surface water concentration after infiltration (Winkler 2001a,b). The PNEC for groundwater is based on the crustacean *D. magna*, since this taxon represents typical groundwater organisms rather well (Notenboom et al. 1999).

In view of this fairly straightforward approach, there are some research challenges that may surface during the assessment of particular compounds at hand. The discussion below contains several interesting issues. A comprehensive overview of new research challenges is also provided in the special issue of the *Drug Information Association Journal* 2:2007 (Montforts et al. 2007).

24.4.1 Metabolites versus Drug Substance

The Total Residue Approach, which is also adopted in the Veterinary International Conference on Harmonization (VICH) guideline on veterinary medicines (VICH 2000) assumes that with regard to ecotoxicity, the risk assessment based on the drug substance is in general protective for the risk posed by metabolites. One may even conclude that this risk model is overprotective. Thus this approach avoids multiple, hence expensive, metabolite studies in the early stages of risk assessment: if this presumably worst-case assessment demonstrates that environmental risk is absent, no further action is needed.

Should uncertainty remain on the absence of environmental risk after the assessment based on the most active ingredient, the EMEA guideline only guides the assessment of human metabolites excreted in fractions <10%, namely to discard these fractions. The strategy for metabolite fractions >10%, or for metabolite fractions generated within the environment, is not guided. Below follows a short overview of the existing approaches towards metabolite risk assessment in both safety testing of pharmaceuticals and risk assessment of pesticides. Based on the working experiences, a proposal for environmental risk assessment of metabolites from pharmaceuticals is made, which could be considered by national assessors and by the EMEA for review of the guideline.

The possibility that the adverse effects could be caused by a metabolite instead of the drug substance forces one to anticipate the impact of metabolites when assessing the risk of the drug substances. However, there may be several metabolites formed. To deal with this increasing complexity, a committee from the industry published their suggestions on handling metabolites in safety testing of drugs intended for human use (MIST) in 2002. Their suggestions included a definition of 'major' metabolites that should be forwarded to safety testing (Baillie et al. 2002): "*In the absence of other scientific considerations, a major metabolite is defined as one that accounts for 25% or more of the exposure to circulating drug-related material.*" This definition raised concerns, amongst others, based on the possibility of overseeing risky minor metabolites (Hastings et al. 2003), which gave the authors the chance to highlight what they considered to be the flexible nature of the definition (Baillie et al. 2003). Recently, a proposal was made to base the safety testing of metabolites not on relative abundance criteria, but to combine other information such as similarity to the parent drug and presence of chemically reactive substituents (Smith and Obach 2005).

The central, undisputed feature of metabolites in safety testing in these opinions is the fact that they are assessed against the drug substance circulating within the human body. Within the environment, however, depending on substance properties, metabolites may be present in a certain compartment, while the drug substance is not. The crucial question is thus whether the approaches presented within MIST are of help when dealing with the risk of (excreted) metabolites in the environment.

The assessment of metabolites in the environment differs between regulatory frameworks, amongst others, as a result of typical differences in the mode of action or differences in the use and destination of the product. In the publications on MIST, both industry and U.S. regulators acknowledge that using different criteria in other frameworks is justified and acceptable. The following examples of criteria in other frameworks were given:

- As a threshold for impurities present in human pharmaceuticals;
- For pesticide identification and 10% for pesticide environmental risk assessment both in the USA (OECD 2004) and in the EU (ECCO 2002);
- For metabolite residues in food-producing animals for human consumption.

While on the one hand there are major differences in route of application among different product types such as veterinary medicines, pesticides and medicines which justifies the choice of different criteria, on the other hand there is pharmacologically very little difference between the active substances. All three product categories involve pharmacologically active substances and all are categorized as substances with a specific mode of action within the broader realm of environmental chemicals (De Wolf et al. 2005). Since there is very little relevant information available on the impact of drug metabolites on the environment relative to the parent compounds, it is not warranted to define a unique strategy for pharmaceuticals. One needs to borrow information from other frameworks, of which the pesticide framework is one of the most documented.

The information available in the pesticide framework regarding the strategy designed for the assessment of metabolites is presented below. As a caution it should be noted that the studies presented below were based on acute aquatic data only and may fall short in the assessment of sublethal effects under prolonged exposure or in the assessment of the terrestrial environment.

Auteri et al. (2002) evaluated about 200 active ingredients and 130 metabolites of pesticides in a statistical approach. It was found that the frequency in which metabolites displayed an activity of more than 10% compared to parent substances was about one in ten (Auteri et al. 2002). In a complementary review by Sinclair and Boxall (2002), it was found that in 30% of selected cases, one metabolite was more toxic than the parent compound. However, this sometimes involved a pro-drug. In general, the presence of the toxophore, a higher accumulation potential, or a different mode of action were the reasons for the observed 'high toxicity' of the metabolite (Sinclair and Boxall 2002). Based on the abovementioned research, it was construed that the probability of any metabolite displaying an activity of more than 1/10 of that of the drug substances, is on the order of 0.1. Often this could be anticipated judging the metabolite structure and hydrophobicity. In some cases, however, a different mode of action could be expected.

A pragmatic approach towards metabolite safety assessment in the environment has been broadly supported in the past. In its opinion on metabolites, the EU Scientific Committee on Plants (SCP) stated the following (SCP 1999):

"*As to the 10% trigger, the SCP supports this as a pragmatic screening approach. However, it is recognized that metabolites occurring at lower levels may well be ecotoxicologically relevant. Hence, all available information and expert judgment should be used to assess if metabolites <10% give rise to particular concern. Such metabolites should then also be subjected to a risk assessment rather than a specific justification.*"

For practical purposes, in the EU aquatic risk assessment for pesticides, the 10% criterion is used to discern major metabolites from minor metabolites (ECCO 2002). Still, metabolites are to be addressed irrespective of the formation percentages, reflecting the SCP's opinion. In the guidance to specific risk assessment for pesticide authorization, the focus is on the relative exposure level in the environment. Should a metabolite be present in concentrations >10% compared to the parent compound, a further assessment is warranted. Metabolite identification is already required at 5%, providing the opportunity to make an expert judgment on the hazard of minor metabolites. In this approach to pesticides we recognize the principles of the MIST approach: a relative criterion for the circulating compounds (yet now within the environment), possibly extended with structure-activity research. The 10% criterion for metabolites in the environment is not only observed in the pesticide frameworks in the USA and the EU, but also for veterinary medicines in the global framework of VICH (CVMP 2004). The criterion for relative abundance of 10% is combined with other information such as similarity to the parent drug and presence of chemically reactive substituents. An assessment strategy for metabolites of pharmaceuticals could be developed along these lines.

24.4.2 Medicines in Environmental Quality Policy and Legislation

European legislation on water quality and product registration have created different liabilities for member states when it comes to evaluation and control of risks to water following the use of medicines, veterinary medicines and feed additives. Water quality legislation demands that at some point in time water quality complies with standards and offers a range of policy instruments. Firstly, quality objectives for the substances need to be set in a statutory regulation. Then these standards need to have their effects on policy, planning, and decision making. Enforcing the available legal instruments for water quality will not likely prove to be sufficient in controlling the discharge of these products, while at the same time the legislation on registration does not allow for a direct judicial review of the product use vis-à-vis quality standards. The following policy options to cover this deficit are discerned:

- Require setting quality standards for all substances in these products;
- Interlace these quality standards into other policy areas, like granting permits and general rules that replace individual permits. At registration quality standards can be used in an indirect way;
- Designate specific areas that need special protection;

- Make the environmental information gathered at registration publicly available in an active way;
- Regulate the use of (veterinary) medicinal products though product labeling and secondary legislation.

To achieve this, active cooperation between different regulatory bodies is necessary. Some opportunities lie in the harmonization of legislation and implementation. The methodology for risk assessment should be harmonized between quality standard setting and product risk assessment. Finally, there should be a direct judicial link between quality standards and the product risk assessment, in the sense that the risk assessment functions as a compliance check with environmental legislation (Montforts et al. 2006).

It is very well possible that existing European directives on the environmental quality of water already contain standards for medicines, even though the product group 'medicines' is not named in the environmental directives like Directive 2006/11/EC (replacing the 76/464/EEC) on surface water, Directive 80/68/EC on groundwater, and Directive 98/93/EC on drinking water. Clearly, the terms 'pesticide' and 'biocide' used in these directives do not refer to the product categories but rather to the nature of the substances reaching environmental compartments after production, use or disposal of products. After all, once the compound is in the environment, the authority responsible for the quality of that environment is concerned with the risk of the compound itself, and not whether it was a medicinal treatment or a pesticide application that caused the pollution. Medicines can be qualified as 'biocidal' because they are biologically active, and many are even used because of their biocidal properties (Montforts and De Knecht 2002).

24.5 Discussion

Several of the challenges identified in the first and second edition of this book have been addressed in the recent regulatory guidance documents (Montforts 2001; Montforts 2004). However, more research challenges lie within these three aspects of risk modeling. They are centered on the coherence of the risk model components, the connection between risk model and user, and the development of methodology for hazards yet to be addressed. Some have evolved from the recent experiences in practice, and include the following points:

- In Phase I the $\log K_{ow}$ determination is key to the PBT assessment. For highly lipophylic substances, a slow stirring method is preferred over a shake-flask (Tolls et al. 2003). However, medicines are often ionic and polar, and partitioning of ionic species is also a function of ionic strength and pH (Escher et al. 2000). To complete the assessment, environmentally relevant reference values need to be set for these characteristics;
- The Phase I PEC calculation depends on the estimate of the annual production, which should be well documented. When evaluating annual consumption estimates, data on disease prevalence, and on the choice of the medical practitioners between therapies, should seasonal and even regional differences also be taken into account (Cars

et al. 2000; Abbas and Kratz 2000; Baart and De Neeling 2001)? The Phase I calculation contains the term "penetration factor." What is meant here is the fraction of the general population that is using the medicine, and consequently it resembles the fraction of the wastewater that Phase I contaminated with medicine residues. Should the default penetration factor of 1% actually be overruled based on data of total consumption over the whole of the EU? On local scales, the penetration factor is higher. Since there are 200 hospital beds for every 10 000 inhabitants (Feldmann et al. 2002), the hospital contributes at least 2% of the wastewater to the local wastewater treatment plant. For the receiving water body, dedicated use of a certain medicine in hospitals could be considered to be represented, perhaps even underrepresented, by the default 1% penetration factor;

- How should information on mammalian toxicology for environmental risk assessment instead of long-term fish testing be handled (Huggett et al. 2005)? From an evolutionary perspective it is assumed that many receptors, enzymes, channels, and transporters have been preserved and are shared between different phyla. However, there are still limitations in extrapolating mammalian data based on internal concentrations to effects on aquatic species related to external concentrations. On the one hand, if receptors are rather universal, base set testing should be adequate for pharmaceuticals. On the other hand, if not only the presence of the intended receptors, but also the presence of other receptors, different sensitivities of these receptors to the drug substance, and different homeostatic processes of checks-and-balances are operative, base-set testing may not be sufficient. Base-set testing for pharmaceuticals should therefore always be followed by some extra (mode of action) driven assessment, in order to fulfill the requirement that actually *absence* of hazard should be demonstrated. For a case-based assessment of any substance based on mammalian data,
  - It should be unambiguously established that the sensitive receptors are homologous, indicating that there are no other relevant receptors;
  - There should be convincing data about the sensitivity of the receptors that allow comparison to the mammalian data;
  - The substance should be assessable with the blood-water partitioning QSAR. The existing relationships between $\log K_{ow}$ and blood-water partitioning are based on merely eleven non-polar compounds with $\log K_{ow}$ between 1.5 and 8 (Bertelsen et al. 1998; Fitzsimmons et al. 2001). Even for non-polar compounds within the proper $\log K_{ow}$ range, the results should be handled with great care. If a compound does not belong to this category, the blood-water partitioning should be determined experimentally;
  - More information on the relationship between internal and external concentrations should be available, amongst others what is the function of active transport?

This methodology proposed by Huggett et al. (2005) should be validated by the scientific community with pharmaceuticals, resulting in agreements on the model parameterization, the use of safety factors, and the applicability for waiving base-set requirements in the registration procedure.

- How should the predictions for the exposure be verified post-registration? To enable post-registration control and monitoring (for example of drinking water qual-

ity or discharge water quality) and emergency measures in the case of an accident, an analytical method for water is needed. The limit of quantification (LOQ) must be equal to 0.1 μg l^{-1}, or accordingly lower if the PNEC is lower. If this analytical method is already available from the ecotoxicity studies, this can be used, although the LOQ may not be sufficiently low. A requirement for an appropriate analytical method would solve this problem;

- A prediction of groundwater and drinking water exposure seems to have become very relevant both from an environmental and a public health point of view. Exposure via water, soil and landfill waste should be taken into account when performing a risk assessment at registration. A certain harmonization of environmental and public health concerns is needed from the risk manager to set an operative quality standard for risk assessors. Here it is clear that the interests of registration connect closely to the interests of environmental and public health safety;
- And finally, in case quality standards are not met, an indication of the actual impact or costs will be needed to come to a cost-benefit analysis. The registration process for medicines is the exponent of risk assessment (Di Fabio 1994). Policy makers, scientists and other interested parties (ESC 2001) should engage in a reconnaissance of expressing, scaling and weighing costs and benefits for society, including the environment.

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Part V
Risk Management

Strategies for Reducing the Input of Pharmaceuticals into the Environment

K. Kümmerer

25.1 Introduction

Opportunities for the reduction of the input of pharmaceuticals into the aquatic environment are possible through different approaches (Fig. 25.1; *www.start-project.de*; Schulte-Oehlmann et al. 2007; Götz and Keil 2007; Kümmerer 2007; Kümmerer and Schramm 2008). According to the principle of sustainability, the whole life cycle of a compound has to be taken into account to identify opportunities for risk management and risk reduction.

From a temporal perspective and type of measures, a combined strategy for input reduction/prevention seems to be appropriate within this setting. Three classes of measures can be identified (Fig. 25.1). All the three of them are needed for an effective reduction of the input of pharmaceuticals (and other chemicals) into the environment. The one that has been most extensively discussed within recent years is the technical one. As for the second one, we have to learn that environmental protection has to include the shareholders, the stakeholders and the people using the compounds, including patients, doctors and nurses, and pharmacists when seeking solutions that will work. The third strategy is emerging from the field of green chemistry (Anastas and Warner 1998). Until now it is the less developed one of the three approaches. However, in term of sustainability it seems to be the most promising one in the long run.

Development, disruption, usage and residue management is done by different stakeholders (Table 25.1).

25.1.1 Advanced Effluent Treatment

The objective of advanced effluent treatment is to further reduce the ecotoxicity, hormonal effects and pathogenic effects of the effluent. Within the last years, advanced effluent treatment has been studied extensively.

Much research and developmental effort is directed towards advances in municipal wastewater treatment aiming at reducing the effluent content of micropollutants and pathogens. The advanced treatment of effluents has been investigated using (photochemical) oxidation processes (e.g., Qiting and Xiheng 1988; Zwiener and Frimmel 2004; Ravina et al. 2002; Kiffmeyer 2003; Ternes et al. 2003; Watkinson et al. 2007; Strässle 2007; Isidori et al. 2007), filtration (Schröder 2002; Drewes et al. 2002; Heberer and Feldmann, this book), application of powdered charcoal (Metzger et al. 2005;

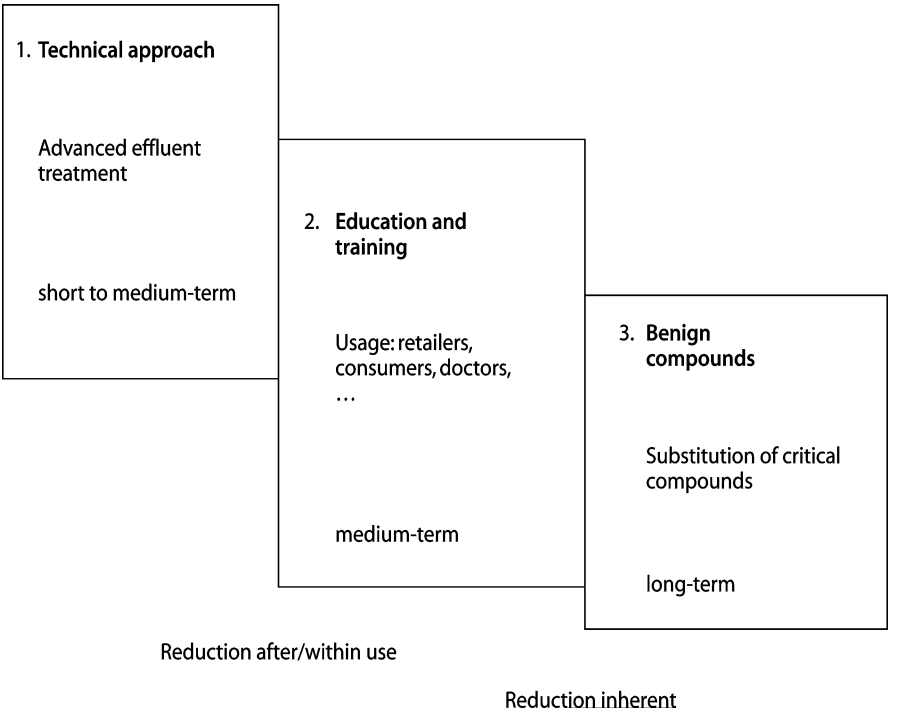

Fig. 25.1. Strategies to reduce the input of chemicals into the environment (Kümmerer 2007)

Nowotny et al. 2007) and constructed wetlands (Matamoros and Bayona 2006). Reviews on the advantages and disadvantages of the different technologies are available (Schulte-Oehlmann et al. 2007; Jones et al. 2007; Wenzel et al. 2008; Ternes and Joss 2006). All of these technologies have more or less specific shortcomings:

- Efficiency may depend strongly on the type of compound;
- None of the technologies can remove all of the compounds (Oiting et al. 1988; Ravina et al. 2002; Schröder 2002; Ternes et al. 2003; Wenzel et al. 2008);
- Will they work for new compounds in the future?
- Mutagenic and toxic properties have been found for the reaction products of (photo) oxidation processes (Isidori et al. 2005, 2007; Lee te al. 2007; Wei-Hsiang and Young 2008);
- Prolongation of the hydraulic retention time results in little improvement of the elimination rates only. It may cause high costs because of the necessity to enlarge the STPs;
- Resistance in bio-membrane reactors: Would the enrichment of antibiotics and resistant bacteria cause increasing resistance? No information is available on this topic;
- Resistance material will not fully be retained by membranes;
- Combined sewer overflow in the case of stormwater will not be treated;
- Sewage that infiltrated the ground is not treated due to leaking sewage pipes before it reaches the STP;

Table 25.1. Opportunities to reduce the input of pharmaceuticals into the environment

Who	Possible measures and activities
Pharmaceutical companies	Publication of data relevant for environmental assessment Publication of analytical methods and results
	Offering of suitable package size Integration environmental aspects in the development of new APIs and new therapies
	Dedication to green pharmacy
	Less over the counter products
	Establish take back systems where not already present
Patients	Improvement of compliance
	Intake of APIs only if necessary and only after prescription by a medical doctor
	Out dated medicaments not down the drain; instead return to pharmacy if take back system is established or into the household waste if appropriate (check with local authorities and pharmacies)
Pharmacists	Information of patients
	Participate in take back systems if appropriate (check with local authorities)
Hospitals	Integration of the delivering pharmacy/wholesaler into the handling of out dated medicaments
	Information of doctors and patients
Medical doctors	Prescription according to environmental criteria if alternatives are available
	Information of patients
Health Insurances	Keeping the necessary medical standards and demonstration of reduction potential and economical benefits
	Information of doctors and patients
Veterinary medicine	Restrictive prescription
	Improvement of compliance
	Improvement of hygiene
	Less exchange of animals between flocs and farms
	Information of farmers
Waste Water handling and treatment	Reduction of input by broken sewerage/piping
	Reduction of total water flow to be treated (separate piping of waste water and rain water) and thereby increasing concentration of APIs
	and costs
Drinking water treatment	Extended monitoring
	Advanced treatment if necessary
	Information of the general public

Table 25.1. *Continued*

Who	Possible measures and activities
Authorities	Initiation and back up of communication between all stakeholders
	Development of limits/thresholds for APIs in different environmental compartments and drinking water
Politics	Inclusion of APIs in environmental legislation
	More restrictive connection between environmental properties and authorization of human pharmaceuticals
	Improvement of legislation for the management of out dated medicaments

- They depend on a high energy input and a minimum water flow. Therefore, they may not be possible/affordable in less developed countries;
- Costs are not clear and whether they are affordable is not known. As for the costs, different authors present different data depending on the assumption made. It is questionable whether the additional costs are acceptable (Dohmann 1994; Jones et al. 2007);
- In principle, they are not compatible with sustainable development as they are end-of-the-pipe technologies and not affordable in all countries;
- Energy demand causes high CO_2 emissions (Jones et al. 2007).

The application of powdered charcoal seems to be a promising approach for advanced treatment. It removes not only pharmaceuticals but also some other classes of micropollutants, too. It avoids some of the critical points addressed above, but not all of them. However, it is like the other approaches in that it does not fulfill the criteria of sustainability. Additionally, slow sand filtration that is already established in some European STPs may be as effective.

Wenzel et al. (2008) investigated the advantages and disadvantages of advanced wastewater treatment of micropollutants using environmental life cycle assessment (LCA) and a literature review of advanced treatment performance. The LCA evaluation comprised sand filtration, ozonization and membrane bioreactors and assessed the effect of extending existing tertiary treatment with these technologies on a variety of micropollutants (heavy metals, endocrine disruptors, PAH, phthalates, and detergents). The authors assessed the 'environmental break-even' point where the removal of micropollutants and reduction in (eco-)toxicity will outweigh the increased resource and energy consumption. It was found in some of the studied scenarios that more environmental impact may be induced than removed by the advanced treatment. The study showed that for the three technologies, sand filtration has the best balance between prevented and induced impacts, and sand filtration proved to have a net environmental benefit under the assumptions used in the study. But the outcome of the study suggests that this is not always the case for ozonization and membrane bioreactors. Because of the limitations identified for the advanced effluent treatment, other (additional) approaches are necessary.

25.1.2 Training, Education and Information

Proper and effective risk management strategies need knowledge of sources. In this context, one has to know the size of substance flows associated with the different sources of pharmaceuticals such as households and hospitals. It has been found that hospitals are only of minor importance in terms of flows of pharmaceuticals into the environment. Therefore, an advanced effluent treatment would not be very effective. However, hospitals can reduce their contribution by different measures such as appropriate training and education of staff and patients.

Proper information for doctors, pharmacists and patients can contribute to the reduction of the input of APIs into the aquatic environment (*www.start-project.de*; Götz and Keil 2007; Gunnarson and Wenmalm, this book). Proper information on how to handle left over drugs will result in the reduction of the environmental burden of drugs. A major unknown with respect to drugs as pollutants is what fractions of drug residues occurring in the ambient environment result from discarding leftover drugs (Götz and Keil 2007; Ruhoy and Daughton 2007). Data are needed on the types, quantities, and frequencies with which drugs accumulate in households, hospitals, nursing homes, and rehabilitation hospitals. Absence of this data has prevented assessments of the significance of drug accumulation and disposal as a contributing source of drug residues in the environment.

In a mid-to long-term perspective prescription, therapy and consultation practices of physicians and pharmacists as well as the patients' use and disposal patterns of pharmaceuticals should be changed towards a higher environmental sensibility. The relationship between physicians and patients plays a key role within this strategy (*www.start-project.de*): Knowledge and information about the environmental relevance of pharmaceuticals raise the physicians' awareness of the problem in their consultation of patients. In order to assist the integration of the problem into the physicians' everyday practice, it has to be implemented in the medical education and advanced training by facilitators of education and health policy. Health funds can foster the demand for ecological alternatives by means of changes in the funding of pharmaceuticals and therapies. This increased demand can support the pharmaceutical industry in supplying a sustainable product range (e.g., varieties of packaging sizes and potencies).

Hospitals should use their opportunities to reduce the input through the proper application and disposal of pharmaceuticals. Hospitals and practical surgeries can contribute to the reduction of the input of micropollutants through several measures, e.g., using the proper cleaning or disinfecting agents in the amalgam separators of dental treatment units. The amount of contrast media necessary in MRI can drastically be reduced by modern technology (Ehritt-Braun et al. 1994). If an internal commission recommends a positive list of recommendable pharmaceuticals that is the basis for purchasing activities, the variety of products is reduced and savings will result. The furniture at the wards should not allow for too much storing space. This reduces the share of outdated medicaments and thereby the environmental burden if the internal system should allow the wards to give back not yet outdated, broken and not yet used packages to the pharmacy. A medical doctor who is a specialist in infectious

diseases should be present and can give advice on the proper use of antibiotics. Proper hygiene that is not too much, not too little, at the right place and the right time can also contribute to reducing infections and the need for pharmaceuticals and disinfectants.

25.1.3 Green and Sustainable Pharmacy

According to the principles of green chemistry (Anastas and Warner 1998), the functionality of a chemical should not only include the properties of a chemical necessary for its application, but also easy and fast degradability after its use. Taking into account the full life cycle of chemicals will lead to a different understanding of the functionality necessary for a chemical. In the present discussion, improvement of synthesis and renewable feedstock are very prominent, whereas the environmental properties of the molecules are somewhat underestimated. Applying these principles and the knowledge of green chemistry to pharmaceuticals is necessary in the future. One aspect of it is the third, long-term oriented strategy of Fig. 25.1 (Kümmerer 2007). It means that easy degradability after use or application is taken into account even before a pharmaceutical's synthesis ("benign by design"). Such an approach is not completely new. For example, it is quite common during the development of pharmaceuticals with respect to unwanted side effects. This can also result in economical advantages in the long run and will fit into green pharmacy (see Daughton, 2nd edition of this book; Daughton 2003; Kümmerer 2007). Examples of pesticides and detergents, complexing agents and some pharmaceuticals (Kümmerer et al. 2000; Kümmerer et al. 2007; Kümmerer and Al Ahmad 1997) demonstrate the feasibility of this approach.

25.2 Conclusion

It has been found that the most important sources of the emission of APIs into the aquatic environment are private households and not hospitals. Data presented here and other publications recommend that separate treatment of hospital effluent is not an effective approach to reduce the input of pharmaceuticals into the aquatic environment. Furthermore, it is not sufficient to aim for advanced municipal sewage treatment only. In medical treatment, there are possibilities of input reduction of APIs in hospitals that may be as efficient and less costly with less energy demand than advanced treatment. Substance flow management for APIs and risk management strategies as well as the allocation of economical resources have to take this into account. Other measures have to be taken into account too, such as information for patients, doctors and pharmacists on the proper handling of remainders and outdated medicaments, i.e., not to pour them down the drain. A perspective for the extended future is to design APIs for better removal or ideally for full mineralization after excretion in wastewater treatment. As a political approach for risk management it should be discussed whether some pharmaceuticals should be included into the POP convention (*http://www.pops.int/*) and/or the European Water Framework Directive (*http://ec.europa.eu/environment/water/water-framework/index_en.html*).

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COST ACTION 636 Xenobiotics in the Urban Water Cycle – A Network for Collaboration within Europe

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26.1 Introduction

Hazardous chemicals like many of the xenobiotic organic compounds are of rising concern in urban water management, since water supply, urban drainage and wastewater treatment systems were originally designed solely to solve other problems (supply of potable water, flooding prevention and sanitation). Thus, there is a need to understand, in an integrated manner, the sources, flow paths, fate and effects of hazardous chemicals on both humans and ecosystems. The main objective of COST Action 636 Xenobiotics in the Urban Water Cycle is to assess the role of xenobiotics in the urban water cycle and to set up strategies for minimizing their impact on humans and ecosystems as illustrated by the logo shown in Fig. 26.1 (COST Action 636 2007). The COST Action 636 provides the tools for the integration of knowledge and experience at the European level to create a critical mass required to assess the role of xenobiotics in the urban water cycle. The action includes a multidisciplinary approach with discussions and exchange of knowledge among experts and stakeholders on the specific topics listed below.

26.1.1 COST

COST – European Cooperation in the field of Scientific and Technical Research – is the oldest and most extensive system of research networking in Europe (COST 2007a). It is based on an intergovernmental framework for cooperation on research, agreed following a Ministerial Conference in 1971. The goal of COST is to ensure that Europe holds a strong position in the field of scientific and technical research through the support of European cooperation and interaction between European researchers. It aims to strengthen noncompetitive and pre-normative research in order to maximize European synergy and added values. Ease of access for institutions from non-member countries also makes COST a very interesting and successful tool for tackling topics of a truly global nature. To emphasize that the initiative came from the scientists and technical experts themselves and from those with a direct interest in furthering international collaboration, the founding fathers of COST opted for a flexible and pragmatic approach. COST activities have in the past paved the way for community activities, and its flexibility allows COST Actions to be used as a testing and exploratory field for emerging topics. The funds provided by COST support the coordination costs of the research networks (Actions), while the research funding for the networks is a responsibility for others (nationally and EU). The member countries participate on an "à la carte" principle and activities are launched on a "bottom-up" approach. One of

Fig. 26.1. Logo for COST Action 636 Xenobiotics in the Urban Water Cycle

its main features is its built-in flexibility. This concept clearly meets a growing demand and in addition, it complements the community programs.

COST had in the end of 2006 around 230 running actions and involved approximately 30 000 scientists from thirty-four European member countries and more than 160 participating institutions from twenty-three non-member countries and non-governmental organizations.

26.1.2 COST Action 636 Xenobiotics in the Urban Water Cycle

So far, thirty-one countries (COST 2007b) have signed up to participate in COST Action 636 Xenobiotics in the Urban Water Cycle. This includes more than 250 scientists and practitioners from more than 120 different departments, companies and municipalities in these countries, as well as Australia, Canada, Ukraine, and the United States. The action had its first meeting in the management committee in Brussels in 2005. This has been followed by several meetings at different venues all over Europe.

The aim of the following sections is to present the objectives and expected outcomes from this action and to illustrate that COST actions are excellent tools for improving the collaboration between scientists and practitioners on such a complicated and important topic as organic pollutants in the urban water cycle.

26.2 Objectives and Expected Benefit for Europe

The main objective of the action is, as mentioned above, to assess the role of xenobiotics in the urban water cycle and to set up strategies for minimizing their impact on humans and ecosystems.

There is an urgent need for collaboration between different disciplines in order to achieve this objective, but also in order to ensure that the research carried out in Europe on this issue is on the highest possible level and on the forefront in the world. Integration and consolidation of various nationally and internationally funded research projects within the COST Action 636 allows combining otherwise isolated results into a more conclusive picture and to identify gaps in our knowledge. Xenobiotics in the urban water cycle is a very complex issue and it is necessary that experts on urban water treatment (water supply, as well as waste- and stormwater handling) interact with e.g., analytical and environmental chemists, human- and ecotoxicologists, microbiologists and architects as well as industries. It is also important to involve different stakeholders (e.g., administrators in governmental and municipal organizations, different end user groups, decision makers and politicians) in the process.

This can be obtained by fulfilling the secondary objectives for COST Action 636:

- Identification of the most critical problems related to xenobiotics in the urban water cycle;
- Suggestion of different strategies for solving these problems, incl. identification of future research needs;
- Creation of a strong network between researchers and other experts all over Europe working on issues related to xenobiotics and the urban water cycle;
- Creation of a strong network among young researchers all over Europe working on issues related to xenobiotics and the urban water cycle;
- Stimulation of an active dialog between researchers/experts and stakeholders all over Europe dealing with issues related to the urban water cycle.

The following benefits from obtaining the objectives have been identified:

- Future priority areas for research regarding handling of urban water within Europe will be identified;
- Future strategies for handling of xenobiotics in the urban water cycle will be pointed out;
- Achievement of the critical mass of knowledge and experts needed to assess the role of xenobiotics in the urban water cycle;
- Assurance that stakeholders have access to the newest knowledge and the most competent expertise covering this interdisciplinary problem on xenobiotics in the urban water cycle;
- Assurance that the research in Europe is focused on issues that have the highest priorities among the stakeholders.

26.3 Organization and Activities

There are more than 100 000 xenobiotics on the market in the European Union. Approximately 30 000 of these are "everyday" chemicals, i.e., estimated to be used in volumes over one ton each year. It has been estimated that 70 000 xenobiotics may potentially be hazardous for humans and/or ecosystems. In order to assess the role of xenobiotics, information is needed with respect to the sources, flow paths, fate (transport, treatment, natural attenuation) and impact on humans, livestock and ecosystems. Furthermore, it is necessary to have suitable tools like chemical analytical methods or ecotoxicological test methods for collecting the information that is needed and assessing the potential risk. Four subtopics have been identified as focus areas for COST Action 636 in order to cover the objective. The identified subtopics that also correspond to the four working groups (WG) are:

1. Identification, sources and fluxes;
2. Methods for treatment
3. Impact assessment
4. Analytical issues

The most important activities in the action are the meetings, which are held twice a year and are intended to run for four years (start in March 2005 and end in March 2009). These meetings are organized as spring meetings for individual groups and autumn meetings for all groups at the same venue (for cross-activities and -objectives among the WGs) as well as a meeting for the management committee. The following meetings/workshops have been organized so far:

- COST 636 Workshop in Copenhagen, 6–8 September 2005 – WG 1, 2, 3 and 4. The workshop aimed at formulating action plans for all four working groups and established strong and fruitful collaboration between the groups;
- COST 636 Workshop in Ljubljana, 3–4 April 2006 – WG 4. The WG organized a workshop on analysis of xenobiotics in different environments. An intercalibration study was planned, and the possibility of preparing a book or review issues was discussed;
- COST 636 Workshop in Lisboa, 24–25 April 2006 – WG 1. The WG organized a workshop on sources for xenobiotics in the urban water cycle;
- COST 636 Workshop in Nancy, 27–28 April 2006 – WG 2. The WG organized a workshop regarding general aspects in assessing removal of xenobiotics;
- COST 636 Workshop in Duisburg, 20–21 June 2006 – WG 3. The WG organized a workshop on impact thresholds in the environment and humans;
- COST 636 Workshop in Vienna, 25–27 September 2006 – WG 1, 2, 3 and 4. WG 1 had a workshop with focus on sinks, processes and chemical properties. WG 2 had a workshop that was a continuation of the work initiated in Nancy regarding a fact sheet on processes and mechanisms in treatment technologies for drinking water, wastewater and stormwater. WG 3 had a workshop regarding the impact assessment of xenobiotics in the urban water cycle, started to prepare for a document on "the best biotest battery" and initiated the planning for a research school. WG 4 had a workshop on sampling and continued the discussions regarding organizing an

intercalibration study on estrogens in wastewater and writing a textbook about the analysis of xenobiotics in urban waters;

- COST 636 Workshop in Karlsruhe, 29–30 March 2007 – WG3 and 4. WG 3 had a workshop regarding the impact assessment of xenobiotics in different compartments and on gaps in impact assessment. WG 4 had a workshop on analysis of endocrine disrupting compounds and continued the planning of both an intercalibration study and a textbook. The two WGs had a common workshop on "Effect-related analytical methods – The game of gaps";
- COST 636 Workshop in Stockholm, 19–20 April 2007 – WG1. The WG organized a workshop on mitigation options, partly in collaboration with the EU-project ScorePP (ScorePP 2007);
- COST 636 Workshop in Rome, 23–25 May 2007 – WG2. The WG organized a workshop on biological processes for the biodegradation of xenobiotics as a basis for further work on removal pathways and technical realization. Aspects from wastewater treatment, drinking water treatment and stormwater treatment were tackled.

Short Time Scientific Missions (COST 2007a) will be offered to the action participants during 2007 with the purpose of strengthening the networks by allowing young scientists to go to an institution or laboratory in another COST member state for an exchange visit. The aim of the visit could be to learn a new technique, method or model, and to carry out experiments with instruments or set-ups not available at their own institutions.

Training schools are planned for 2008 within two working groups. The training schools are aimed at providing dissemination from the action activities or they will be used for intensive training in a new emerging subject (COST 2007a). Those that should be attending are basically but not exclusively young researchers according to COST´s rules. But training schools may also cover appropriate retraining as part of "life-long learning."

A website (COST Action 636 2007) has been established as an information exchange platform to facilitate contacts between researchers, experts and stakeholders. The website contains detailed information regarding the activities in the working groups, as well as invitations to meetings and minutes from the meetings. Specific information, such as PowerPoint presentations is presented on an Intranet, available for the participants in the working groups. A database summarizing and presenting relevant projects and demonstration projects dealing with xenobiotics in the urban water cycle is continuously filled and presented on the website.

The participants taking part in the activities within the action are encouraged to use this extraordinary network to identify collaboration partners for upcoming calls for research funding, such as the 7th Framework program from EU.

Other initiatives taken for dissemination of the outcome from the discussions in the action are extensive reviews on emerging topics to be included in peer reviewed journals or books.

The major outcome from this COST Action will be compiled in a report. The report will include summaries of the major findings from each working group. It will also contain chapters covering future strategies and priority areas for research regarding handling of xenobiotics in the urban water cycle, according to the identification of major benefits from the action. The report will also present the results from the

quantitative evaluation of this COST Action. For instance, the results from the questionnaires to stakeholders will be presented and discussed. Special attention will be given to present the outcome from the COST Action in a way appealing to both the scientific community and the stakeholders through for example a final international conference.

Furthermore, the research community has several established channels for publishing the results from scientific work among researchers. All these means are used, such as publications in journals, conferences research reports and web-publications, as well as presentations at international and national conferences and meetings.

26.3.1 Working Group 1: Identification, Sources and Fluxes

There are several lists available presenting priority pollutants jointly on national, European and worldwide levels; e.g., national drinking water directives, national sludge directives, national discharge regulations, OSPAR´s list over priority pollutants, WHO drinking water directives, etc. These lists include relatively few xenobiotics, compared to the number of compounds that potentially can be present in urban waters, and the regulators are often faced with questions like: "Are you sure that the most relevant xenobiotics are included?" This is why this COST Action will include presentations and discussions of work that are going on around Europe on identification of priority xenobiotics.

Pharmaceuticals, household chemicals, personal care products, clothing, foodstuffs and additives, building and road material, vehicles and industry are a few examples of the sources of chemical compounds to the urban water cycle. The present knowledge regarding the discharge of different xenobiotics to the urban waters is limited. For instance, release factors for organic xenobiotics from building materials to stormwater are rare in the literature. Even more surprising is that data regarding the consumption of individual xenobiotics included in personal care products is totally lacking. This COST Action will focus on fluxes to the environment, which can be used for prediction of environmental concentrations. New scenarios for source control/reduction are another focus area that is already identified.

An action plan was prepared at the first meeting in the working group, which states the following: "*The overall task for WG1 is to focus on fluxes to the environment, which can be used for prediction of environmental concentrations and identification of mitigation options. A mind map session resulted in some major areas to focus on: sources, sinks, processes, chemical properties, mitigation, storage, water cycle and fluxes.*"

26.3.2 Working Group 2: Methods for Treatment

The second focus area is methods for removal of xenobiotics in drinking water, waste- and stormwater. The activities should include presentations and discussions and will include state-of-the-art and new findings with respect to advanced chemical, physical and biological processes for treatment of aqueous streams containing xenobiotics with identification of pertinent efficiency criteria. Both "process innovation" (i.e., integration of chemical and biological processes and use of sequential reaction environment for

enhancing biotransformation) and "technological innovation" (i.e., high rate biofilm reactors and membrane reactors) will be included. Fate of xenobiotics during the treatment and different approaches for modeling of the treatment processes will also be considered. Technological challenges will be in focus and both low- and high-tech methods will be covered, as well as centralized and decentralized strategies. Attention will also be paid to sludge treatment and waste minimization.

An action plan was prepared at the first meeting in the working group, which states the following: "*The topics elaborated were the result of a systematic approach to break down the complex aspects of treatment technologies into units that can be dealt with by the expertise of the participants during specific meetings. Topics are selected in a way that they apply in the same way for all relevant treatment processes (stormwater, wastewater, drinking water). These units/topics form the base for the working plan of WG2: Assessing treatment efficiency, physical/chemical processes, biological processes, and modeling.*"

26.3.3 Working Group 3: Impact Assessment

Several questions can be raised regarding the impact of xenobiotics in the urban water cycle. The impact of xenobiotics on the water quality of surface and groundwater due to discharges of wastewater and stormwater has been given attention during the last decades. Most studies regarding the impact have been focusing on single compounds, although mixtures of compounds and dynamics in the loads would be of high relevance. There is also a need for evaluation of both short- and long-term exposure at environmental concentration (chronic versus acute toxicity). Xenobiotics and their impact on the applications for sludge, as well as the effects of xenobiotics on biological treatment processes, are examples of other areas where information is lacking.

There is an ongoing discussion in both the EU and in many European countries regarding new regulations and guidelines with respect to xenobiotics in the urban water cycle. The elevated number of substances of concern contributes to the complication of this issue. An active dialog between experts and regulators will improve the quality of the decisions taken and additionally ensure that the research will be focused on the most relevant issues.

An action plan was prepared at the first meeting in the working group, which states the following: "*The working group agreed on pursuing the long-term goal of developing a conceptual framework for impact assessment for xenobiotics in the urban water cycle. To address this objective, the following topics will be covered initially: Impact thresholds in the environment and humans, Complex composition – how is it dealt with in different countries?*"

26.3.4 Working Group 4: Analytical Issues

Methods suitable for assessment of the role of xenobiotics in the urban water cycle cover a wide range of methodologies. There is a need for identification and quantification of various different xenobiotics present in low concentration ranges and complex matrices based on relevant criteria. Calibration, validation, standards, etc. are

topics for discussion and intercalibration can be carried out within this COST Action's network.

An action plan was prepared at the first meeting in the working group, which states that *"there is a need for critical reviews of existing and recently developed methods as well as interlaboratory studies for improved quality assurance. The following activities have therefore been initiated: a critical review regarding analytical methods available for sampling and quantitative analysis of xenobiotics in different kinds of urban water matrices. The review will be published as a book or in a special issue of a peer-reviewed journal. Interlaboratory studies will be carried out also with the focus on xenobiotics in different kinds of urban water matrices.*"

26.4 Summary

Assessing the role of xenobiotics in the urban water cycle and setting up strategies for minimizing their impact on humans and ecosystems are challenging tasks. This work can only be accomplished by a strong network of researchers with different competences and experiences.

A COST action is an excellent tool for improving collaboration all over Europe on specific well-defined topics such as xenobiotics in the urban water cycle. It also provides good possibilities for collaboration outside Europe, however, without financial support from EU.

The combination of meetings/workshops, short-term scientific missions and training schools makes it possible for scientists to meet, discuss and make decisions for future collaboration on specific projects. Young researchers get the opportunity to establish a network and to try their ideas out before a relative large, heterogenic and critical audience.

In-between meeting activities such as interaction on an active website even further stimulate the interactions and hopefully this results in exchange and development of ideas with the purpose to improve our understanding of xenobiotics in the urban water cycle and how to decrease their impact on humans and ecosystems.

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Removal of Pharmaceutical Residues from Contaminated Raw Water Sources by Membrane Filtration

T. Heberer · D. Feldmann

27.1 Introduction

In recent years, pharmaceutically active compounds (PhACs) have been recognized as persistent residues mainly being discharged via municipal sewage effluents into the aquatic environment (Halling-Sørensen et al. 1998; Daughton and Ternes 1999; Kümmerer 2001; Heberer 2002; Boxall et al. 2004; Heberer and Adam 2005). In the meantime, more than 100 different PhACs have been detected at concentrations up to the µg/l level in sewage effluents, surface waters, bank filtrate, groundwater, and in a few cases even in drinking water (Heberer 2002; Heberer and Adam 2005). Thus, PhACs have also been recognized as potential contaminants of raw water sources to be used for the generation of drinking water. Besides other purification pretreatment or treatment techniques such as bank filtration (Heberer et al. 1997, 2001, 2002a, 2004; Brauch et al. 2000; Kühn and Müller 2000; Reddersen et al. 2002; Verstraeten et al. 2002; Zuehlke et al. 2004), artificial groundwater replenishment (Heberer and Adam 2004; Massmann et al. 2006), soil-aquifer treatment (SAT) (Drewes et al. 2002; Conroy et al. 2005), slow-sand filtration (Preuß et al. 2001; Ternes et al. 2002), ozonation (Andreozzi et al. 2002; Ternes et al. 2002, 2003; Huber et al. 2003, 2005; Hua et al. 2006) or filtration applying granular activated carbon (Ternes et al. 2002), membrane filtration using nanofiltration (NF) or reverse osmosis (RO) membranes is one of the most promising techniques for the removal of PhACs in drinking water production. In wastewater treatment, there is a growing demand for the use of membrane filtration techniques including microfiltration (MF), ultrafiltration (UF), membrane bioreactors (MBRs usually based on MF or UF technology), NF, and RO. Additionally, RO is also applied for desalination of brackish water and seawater. This chapter compiles the most recent data from studies investigating the potentials of different membrane filtration techniques for the removal of residues of PhACs, endocrine disrupting compounds (EDCs), and other trace organic compounds from municipal sewage and other raw water sources under the influence of municipal wastewater discharges.

27.2 Application of Membrane Bioreactors (MBR) using Micro- or Ultrafiltration Units

The large-scale application of membranes for municipal sewage treatment is still in its infancy. The first commercial plant using submerged membrane filtration was built in the early 1990s in Japan. Today, this technology is operational in more than 1 400 plants worldwide both for municipal sewage treatment applications and for in-

dustrial applications. In Europe, the first membrane bioreactor (MBR) plant was built by Wessex Water at Porlock and has been operational since 1998. Recently, some new sewage treatment plants applying MBR technology started operation in Germany, France, Spain, Italy and the UK. In 2006, there were more than 180 sewage and industrial plants in operation in Europe. (*http://www.kubota-mbr.com/low_references.html*)

MBR plants may generally be divided into two categories: those with membranes directly installed inside the nitrification basins and a second type where the membranes are located in an external downstream basin. Raw sewage is applied to microfiltration or ultrafiltration membranes of the MBR plants at a slightly negative pressure. Complexity of design and operation is higher when using separated membrane filtration basins caused by inevitably necessary recirculation streams. On the other hand, chemical cleaning of the surfaces is much easier and necessary to prevent the membranes from clogging with particles, residues or microbes (fouling). The use of MBR units in the treatment train of conventional sewage treatment plants (STPs) also has significant impacts on the operational parameters and thus on the sizing of the whole plant. In the first large-scale MBR plants, an upstream denitrification with an anoxic-to-aerobic zone ratio of 50:50 was used which is much different from conventional STPs (e.g., with a ratio of 20:80).

The first results from investigations of municipal STPs equipped with integrated MBRs using micro- or ultrafiltration membranes showed that removal of trace organic contaminants was comparable to those observed for conventional STPs (Clara et al. 2004a,b, 2005; Joss et al. 2005). Such results confirm the expectation that the micro- and ultrafiltration membranes are inappropriate to remove micropollutants directly by sieving (the molecular size is at least 100 times smaller than the pore size of the membranes). On the other hand, results from other studies indicate that extended sludge ages and contact times often reached in MBRs may in a few cases significantly improve the degradation and removal of certain trace organic contaminants. Kimura et al. (2005) investigated submerged MBRs and their potentials to remove selected pharmaceuticals including clofibric acid, diclofenac, ibuprofen, ketoprofen, mefenamic acid and naproxen. In these experiments, improved removal rates were observed for ketoprofen and naproxen when using MBRs instead of conventional STPs. Kimura et al. (2005) also stated that removal efficiencies of the pharmaceuticals by MBRs were dependent on their molecular structure such as number of aromatic rings or inclusion of chlorine. Snyder et al. (2007) also reported that some compounds (including dehydro-erythromycin, sulfamethoxazole and carbamazepine) are well-removed by MBR, while others are not. In some cases, concentrations of pharmaceutical residues appear to increase through the MBR, e.g., for meprobamate. This phenomenon has been documented by Snyder et al. (2007) on several occasions and might be explained by the cleavage of conjugated residues.

Zuehlke et al. (2006) studied the performance of two MBR pilot plants (PVDF modules with pore sizes between 0.1 and 0.2 µm and membrane surfaces between 8.5 and 8.9 m^2) operated in parallel to a full-scale STP fed with the same raw municipal sewage in terms of a project called "Immersed Membrane Filtration" (IMF project). They investigated the potentials to remove selected drug residues such as estrogenic steroids (estradiol, estrone, and ethinyl estradiol) and polar phenazone-type pharmaceuticals including their metabolites. More than 90% of the natural steroids estrone and estradiol and about 80% of the synthetic drug ethinyl estradiol were removed by

the conventional STP, respectively. Removal rates could further be improved by applying purification with MBR. Thus, estradiol and estrone were removed up to 99% and ethinyl estradiol up to 95%. For phenazone, propyphenazone, and formylaminoantipyrine, removal rates of only less than 15% were observed for the conventional STP. Significantly higher removal rates of 60 to 70% were monitored in the pilot plants after an initial adaptation period of approximately five months.

Zuehlke et al. (2006) also reported higher removal rates coinciding with higher ambient temperatures in the summer. Carbamazepine and AMDOPH (1-acetyl-1-methyl-2-dimethyl-oxamoyl-2-phenylhydrazide), a metabolite of the analgesic dimethylaminophenazone, were refractory in all sewage treatment processes and could not be removed by either conventional or MBR purification processes. The persistence of carbamazepine was also proven in the investigations reported by Clara et al. (2004b). As also shown in Fig. 27.1, carbamazepine was neither removed in the conventional STP nor in the MBR unit independent from the sludge age.

27.3 Application of Nanofiltration (NF) and Reverse Osmosis (RO)

Reverse osmosis (RO) is known for its application in desalination of seawater. High pressure-driven membranes such as nanofiltration (NF) or RO membranes can also be used for wastewater treatment and might also be applicable to remove pharmaceutical residues from contaminated raw waters to be used for the drinking water supply (Kimura et al. 2004). Despite higher operational costs, an increasing number of

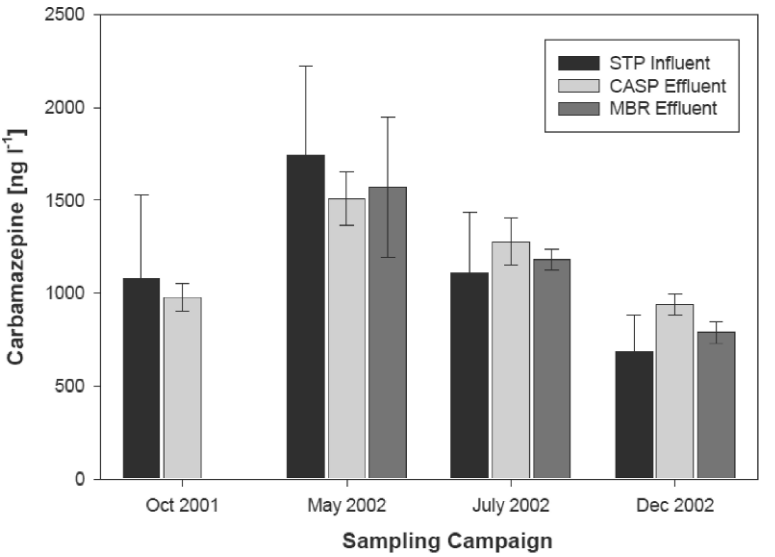

Fig. 27.1. Comparison of carbamazepine concentrations (median values, standard deviation) detected in sewage influents and in the purified effluents of a conventional STP (7000 person equivalents) and a MBR unit, respectively. Reprinted from Clara et al. (2004a) with kind permission from Elsevier Science Publishers Ltd

sewage or drinking water facilities are now using membrane filtration by NF or RO as the final purification method.

NF distinguishes itself from RO by only retaining multivalent ions, which makes it a very economic alternative when retention of monovalent salts is not required (Schäfer et al. 2003). The main objective for the use NF or RO filtration in water and wastewater treatment is the removal of small molecules including trace pollutants. However, the retention of such compounds is to date not completely understood (Schäfer et al. 2003). Nevertheless, several research studies have shown that residues of pesticides, pharmaceuticals, endocrine disrupters and other trace organics can be effectively removed from contaminated waters by RO and also by NF (Baier et al. 1987; Agbekodo et al. 1996; Ventresque et al. 1997; Van der Bruggen et al. 1998, 1999; Kiso et al. 2000, 2001a,b, 2002; Adams et al. 2002; Drewes et al. 2002; Heberer et al. 2002b, 2004; Ozaki and Lee 2002; Nghiem et al. 2002a–c, 2004a,b, 2005a,b, 2006; Wintgens et al. 2002; Schäfer et al. 2003; Kimura et al. 2003a,b, 2004; Xu et al. 2005; Escher et al. 2006; Nghiem and Schäfer 2006; Pronk et al. 2006; Schrader et al. 2006; Yoon et al. 2006, 2007; Snyder et al. 2007; Urase and Sato 2007).

Escher et al. (2006) investigated the removal efficiency of pharmaceuticals and hormones in different treatment processes of source-separated urine with bioassays. They concluded that filtration methods such as NF and electrodialysis were highly efficient with respect to toxicity reduction. The rejection of pesticide residues and related compounds by RO or NF membranes is influenced by the physical-chemical properties of the individual molecules including their molecular size, polarity, dipole moment, and charge (Van der Bruggen et al. 1998, 1999; Kiso et al. 2000, 2001a,b; Ozaki and Lee 2002; Schäfer et al. 2003). Additionally, rejection is influenced by the operating conditions of the membrane filtration process and by the properties of the individual membranes such as pore size, hydrophobicity, permeability, and charge (Kimura et al. 2003b).

In the past few years, several new studies have investigated the rejection capacities of membrane filtration for the removal of pharmaceutical residues and also provided an insight into mechanisms influencing the retention behavior of drug residues using different filtration techniques and materials.

In several research studies (Nghiem et al. 2002a–c, 2004a,b; Wintgens et al. 2002; Schäfer et al. 2003), the removal of estrogenic steroids by NF or RO was investigated. Schaefer et al. (2003) studied the retention behavior of estrone at RO and NF membranes. They concluded that size exclusion dominates retention with the tighter membranes. For NF membranes, exhibiting low retention of ions, both size exclusion and adsorptive effects appeared to be instrumental in maintaining high retention. Schäfer et al. (2003) assumed that these effects may be driven by hydrogen bonding between estrone and the membrane. Thus, deprotonation of estrone or high concentrations of sodium chloride led to a significant decrease in the retention of estrone at "open" NF membranes, but it did not affect retention by RO. In general, tighter NF and RO membranes retained estrogenic hormones more effectively, and the presence of organic matter in solution can enhance retention due to the interaction of such substances with estrogenic steroids (Nghiem et al. 2004a). In another study, Nghiem et al. (2004b) observed that adsorption or partitioning of steroid hormones to the membrane polymer is the dominant removal mechanism in the early stages of filtration. Due to the limited adsorptive capacity of the membranes, the final retention stabilizes when the

adsorption of steroids into the membrane polymer has reached equilibrium. Then, overall retention is lower than that expected solely based on the size exclusion mechanism. Nghiem et al. (2004a) pointed out that there is no cross-flow velocity effect on retention. However, an increase in trans-membrane pressure may lead to a decrease in the retention of steroid estrogens for some membranes.

Adams et al. (2002) evaluated conventional drinking water treatment processes including RO under typical water treatment plant conditions to determine their efficacy in the removal of seven common antibiotics including carbadox, sulfachlorpyridazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfathiazole, and trimethoprim. The experiments were conducted with synthetic solutions of both distilled/deionized water and Missouri River water fortified with the studied compounds. In these experiments, RO was shown to be effective in removing all studied antibiotics.

Yoon et al. (2006, 2007) investigated the potentials of NF and ultrafiltration membranes for the removal of various contaminants including endocrine disrupting compounds, pharmaceuticals and personal care products. They tested commercially available NF and ultrafiltration membranes by applying a commercial bench-scale stainless steel dead-end stirred-cell filtration unit to evaluate flat-sheet membrane specimens for the retention of the compounds. Yoon et al. (2006, 2007) observed a general separation trend due to hydrophobic adsorption as a function of the octanol-water partition coefficient between the hydrophobic compounds and porous hydrophobic membrane during membrane filtration in unequilibrium conditions. They concluded that an ultrafiltration membrane typically retains hydrophobic molecules due to hydrophobic adsorption, whereas NF membranes retain many PhACs due to both hydrophobic adsorption and size exclusion, as well. In general, the NF membrane retained pharmaceutical residues greater than the UF membrane. But even with the NF membrane, more polar, less volatile, and less hydrophobic compounds had only relatively low retention down to retention rates of less than 40%.

The potential of different NF membranes for the separation of pharmaceutical and estrogenic compounds (propranolol, ethinyl estradiol, ibuprofen, diclofenac and carbamazepine) from salts in urine was investigated by Pronk et al. (2006). Their aim was the production of a micro pollutant-free nutrient solution that could be used as a fertilizer. Among all membranes tested, the NF270 membrane showed the highest retention for the investigated micropollutants. Under optimum pH conditions at a pH of 5, the retention of all compounds in non-hydrolyzed urine was above 92%, while the corresponding value for a synthetic urine solution was above 73%. The authors concluded that the retention mechanism is determined by steric and electrostatic effects as well as by the partitioning of the micropollutants in the membrane. Phosphate and sulfate were also almost completely retained, whereas the nutrients urea and ammonia were well permeated and ready for use as a fertilizer.

Kimura et al. (2003a, 2004) investigated the rejection of disinfection by-products, endocrine disrupting compounds and PhACs by polyamide NF/RO membranes based on a protocol established for the determination of the rejection efficiency of NF/RO membranes (Kimura et al. 2003b). Their results showed that negatively charged compounds such as the analgesic drug diclofenac can be rejected to a great extent (i.e., >90%) regardless of other physical/chemical properties of the tested compounds due to electrostatic repulsion. Otherwise, rejection of non-charged compounds was influenced mainly by the size of the compounds. However, solute affinity for the mem-

brane also influenced the rejection efficiency. Especially NF was unable to remove non-charged compounds such as the antipyretic drug phenacetine used as a model compound (rejection less than 20%). On the other hand, the antiepileptic drug primidone, another non-charged compound, was always rejected by more than 70%, suggesting that additional processes are responsible for its good rejection. Kimura et al. (2004) also studied the retention behavior of pharmaceutical residues by RO membranes by applying two different materials (cellulose acetate and polyamide). In conclusion, the polyamide membrane exhibited a better performance in terms of rejection, but often retention was incomplete (57-91%). Kimura et al. (2004) stated that the molecular weight of the compounds can generally be used as an indicator of their tendency for rejection at polyamide membranes as size exclusion dominates the retention by the polyamide membrane. In contrast, polarity may be used to describe retention trends of the individual compound by the cellulose acetate membrane. Kimura et al. (2004) also pointed out that salt rejection or molecular weight cut-off (MWCO) often used to characterize membrane rejection properties does not provide quantitative information on the rejection of drug residues by NF/RO membranes. Kimura et al. (2003a) also observed another very interesting phenomenon when conducting experiments with drug residues spiked at varying feed water concentrations. Thus, experiments conducted at concentrations of 100 ng l^{-1} resulted in a statistically significantly lower rejection efficiency (between 14 and 72% for NF and 50 and 78% for RO, respectively) as compared to experiments conducted at 100 µg l^{-1} (rejection was between 19 and 93% for NF and 71 and 95% for RO, respectively).

The retention mechanisms of pharmaceuticals by nanofiltration membranes were also studied by Nghiem et al. (2005a). They found that speciation of drug residues may lead to a dramatic change in retention being substantially influenced by the pH. They generally observed much greater retention for ionized, negatively charged compounds. But they also found that ibuprofen considerably adsorbs to the membrane in its neutral form due to its relatively high hydrophobicity. A high dipole moment was also identified as an important intrinsic physicochemical property of polar organic compounds, which can substantially affect their retention. In a more general statement, Nghiem et al. (2005a) concluded "that retention of pharmaceuticals by a tight NF membrane is dominated by steric (size) exclusion, whereas both electrostatic repulsion and steric exclusion govern the retention of ionizable pharmaceuticals by a loose NF membrane."

The rejection of pharmaceutical residues at a variety of commercially available RO, NF, and ultra-low-pressure RO (ULPRO) membranes was investigated by Xu et al. (2005). In their experiments, they simulated the operational conditions for drinking-water treatment and wastewater reclamation. Rejection rates obtained for ionic drug residues exceeded 95% applying NF-90, XLE, and TFC-HR membranes and were around 90% for the NF-200 membrane. The presence of effluent organic matter improved the rejection of ionic organics by tight NF or by RO membranes. This was seen as a result of a decreased negatively charged membrane surface. The presence of effluent organic matter could, however, suspend the effect of the hydrodynamic operating condition on rejection performance.

Urase and Sato (2007) investigated the effect of deterioration of NF membranes on the retention of pharmaceutical residues. They studied the retention of eight acidic and two neutral pharmaceuticals by a loose and a tight NF membrane and the change in retention caused by the exposure of the membranes to chlorine. For the loose NF

membrane, an increase of the retention of acidic pharmaceuticals was observed at increasing pH of the solution. This was explained by their dissociation into ions. In the case of the loose type NF membrane, retention of drug residues was more influenced by membrane exposure to chlorine than salt retention. According to Urase and Sato (2007), this was caused by the increase in pore size and additionally by the decreasing electric repulsion effect. In the case of the tight NF membrane, the influence of chlorine exposure and pH on the retention of acidic pharmaceuticals was less distinctive, apparently resulting primarily from increasing pore size.

Nghiem and Schaefer (2006) identified critical risk points of NF and RO processes in water recycling applications. The results of their investigations indicate that membranes can serve as a large reservoir for endocrine disrupting contaminants, and their release may be possible during membrane cleaning or erratic pH variation during operation. Thus, they suggest that this should be carefully considered as such residues are amongst the target contaminants in NF/RO membrane filtration.

In a study reported by Drewes et al. (2002), different wastewater treatment technologies (activated sludge, trickling filter, soil-aquifer treatment (SAT), NF, and RO) were investigated for their capability to remove pharmaceuticals at full-scale facilities used for indirect potable reuse in Arizona and California. In contrast to SAT, none of the investigated drugs were detected in tertiary treated effluents after NF or RO. Even the highly refractory residues of the antiepileptic drugs carbamazepine and primidone not affected by SAT were completely removable by high-pressure membrane filtration with NF or RO membranes (Drewes et al. 2002).

Snyder et al. (2007) tested the performance of membrane filtration for the removal of endocrine disruptors, pharmaceuticals, and personal care products at pilot- and/or full-scale facilities. They applied feed water (raw sewage, primary, secondary or tertiary effluents, spiked secondary effluent or spiked saline groundwater) to several membrane types and applications including microfiltration, ultrafiltration, NF, RO, electrodialysis reversal, MBRs (please refer to the previous section) and combinations of membranes in series. In these trials, only a few target compounds were rejected by micro- and ultrafiltration. Some loss of steroidal-type compounds was observed and supposed to be a function of their relatively lower water solubility. But Snyder et al. (2007) also state that other compounds did not follow this pattern. In contrast, NF and RO were capable of significantly rejecting nearly all investigated compounds. Nevertheless, a few compounds such as iopromide and pentoxifylline were still detectable at trace levels in the permeates.

In summary, the above cited investigations have shown that high-pressure-driven membrane filtration using NF and especially RO membranes is in general capable of removing residues of PhACs efficiently from contaminated raw water sources. In general, tighter NF and RO membranes retain residues of trace pollutants more effectively than loose NF membranes. Operational conditions (pH, chlorine exposure and charge of membranes), the individual concentrations of PhAC residues in the raw water and the presence of effluent organic matter have been identified as important factors potentially influencing the removal efficacy of the individual compound. MBRs and other low-pressure-driven membrane techniques such as microfiltration or ultrafiltration are also capable of removing, retaining or decreasing the concentrations of selected residues. Thus, such techniques might be applied to address a defined problem or to complement an existing treatment train. An effective and sustainable retention or re-

moval of residues of PhAC residues does, however, not seem to be possible and should in general not be expected when applying low-pressure membrane techniques.

27.4 Mobile Drinking Water Purification Units (MDWPUs)

In two field trials, Heberer et al. (2002b, 2004) investigated the performance of membrane-based mobile drinking water purification units (MDWPUs) for the removal of PhACs from highly contaminated raw-water sources. This chapter presents selected results from these "worst-case" scenario field trials carried out for testing the functionality of such devices and their efficacy in removing residues such as PhACs. In September 2000, a commercially available MDWPU was tested at the Teltowkanal in Berlin, Germany. This canal was chosen because of its high shares of municipal sewage effluents. In September 2001, the performance of the prototype was tested directly at the sewer of a municipal sewage treatment plant in Berlin using municipal sewage effluents as raw water for the generation of drinking water.

In civil disaster operations, in military out-of-area missions or after terrorist attacks, one of the main objectives is to guarantee a secure drinking water supply independent of the sources and the quality of the raw water which will often be unknown. Thus, it might be necessary to generate drinking water even from highly polluted surface waters containing a variety of organic, inorganic, and microbial pollutants. Modern MDWPUs have to deal with all different kinds of contaminants to generate a drinking water that meets the strong national, European, and military regulations such as the German drinking water regulation (TrinkwV 2001), the European directive for drinking water or water for human use (ECD 1998) or the NATO Standardization Agreement (STANAG 2002). Concerning their operational costs, MDWPUs are not comparable to stationery full-scale facilities. Their applicability and the purpose of their use are also fundamentally different from those of stationery devices. MDWPUs have to guarantee an immediate and highly reliable low-scale drinking water supply for an almost limited number of consumers under field-operational conditions. For security reasons, an adjustment and a previous testing of operational membrane-filtration conditions is not applicable. Thus, for membrane-based MDWPUs, the use and inclusion of RO as the final treatment process are inevitable and operational costs are less important compared to aspects of human health security.

The ability and performance of the new membrane-based MDWPUs to remove residues originating from municipal sewage has recently been investigated in two field trials carried out in terms of a research project funded by the German Ministry of Defense (BMVg). These field trials were part of an extended one-year testing of these devices. At first, a commercially available MDWPU with bag prefiltration and final purification by RO was tested in a field trial carried out in September 2000 (Heberer et al. 2002b). In this field trial, a canal was used as the raw water source. This canal is prone to high amounts of solid particulate matter and also carries high shares of municipal sewage effluents. Meanwhile, this and several other devices of this type are successfully operated in out-of-area missions in Afghanistan, Djibouti (Fig. 27.2), and in Kosovo. In September 2001, a prototype of a second MDWPU was tested in a field-trial using sewage effluent as raw water source for the generation of drinking water (Heberer et al. 2004). The field trial was carried out at the sewage treatment plant (STP)

Fig. 27.2. Kärcher WTC 1600 currently stationed by the Bundeswehr at the harbor in Djibouti (Republic of Djibouti, Africa). Reproduced with kind permission from Alfred Karcher GmbH & Co (Winnenden, Germany)

in Ruhleben (Berlin, Germany). This prototype applies slit prefiltration, ultrafiltration, and RO for water purification. In both cases (commercial and prototype device), RO is an essential part of the MDWPU and might be applied either in single-pass or in double-pass mode to remove even nuclear contaminations. The results of the removal of PhAC residues obtained from both field trials will be presented and discussed in the following sections.

27.5 Experimental Details

27.5.1 Specifications of the Tested MDWPUs

Kärcher WTC 1600

The field study at the Teltowkanal (Heberer et al. 2002b) was carried out using the mobile drinking water purification unit WTC 1600 GT (Fig. 27.2), commercially available since 1999 from Alfred Kärcher GmbH & Co., Winnenden, Germany. It has been designed to generate drinking water from surface, river, sea, and brackish water. The capacity of the unit has been calculated to produce up to 1 600 liters of drinking water per hour (only in single pass mode) meeting the requirements of the European and the German drinking water directives (ECD 1998, TrinkwV 2001).

The tested system (Fig. 27.2) consisted of the following components: A power supply generator, a raw water supply pump (including a connection hose and a floating device for the pump), a pre-filtration unit (duplex bag filters with a particle separation <0.5 µm), two water-cooled, high-pressure pumps, and eight reverse-osmosis units using the cross-flow technique (Fig. 27.3), an UV-disinfection unit, a post chlorination unit, a remineralization unit, a heating unit (to operate the system at very low temperatures), an automatic cleaning device, measurement technology (e.g., for conductivity measurements), a microprocessor-operated control device (SPS) for automated operation of the unit, and an one-axis trailer. Detailed specifications have been described by Heberer et al. (2002b). Usually, the unit is operated in single-pass mode. In this mode, the concentrate obtained from the first six membrane modules is again applied to the last two modules to increase the raw water extraction yields (Fig. 27.4). In case of nuclear or critical chemical contamination, the device has to be run in double-pass mode, as shown in Fig. 27.5.

The Prototype

The second field trial in September 2001 was carried out using a prototype of a membrane-based MDWPU planned and constructed by Sterling-Berkefeld (Celle, Germany) as commissioned by the Bundeswehr (Heberer et al. 2004). It was designed to produce drinking water meeting the requirements of the German, European and military drinking water directives (TrinkwV 2001; ECD 1998; STANAG 2002) from surface, river, sea, and brackish water. As also shown in Fig. 27.6, the purification unit is positioned in a

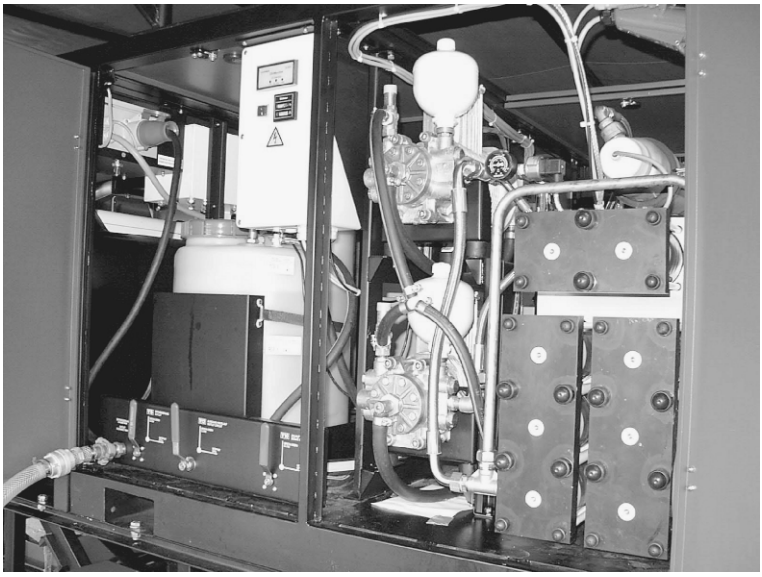

Fig. 27.3. Design of the reverse osmosis units

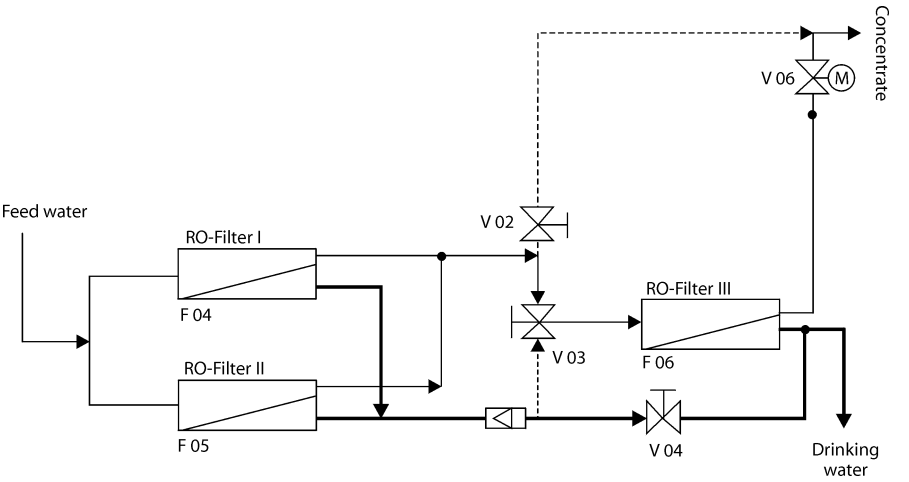

Fig. 27.4. Flow scheme of the MDWPU WTC 1600 operated in single-pass mode. (*Feed water* = raw water after bag filtration)

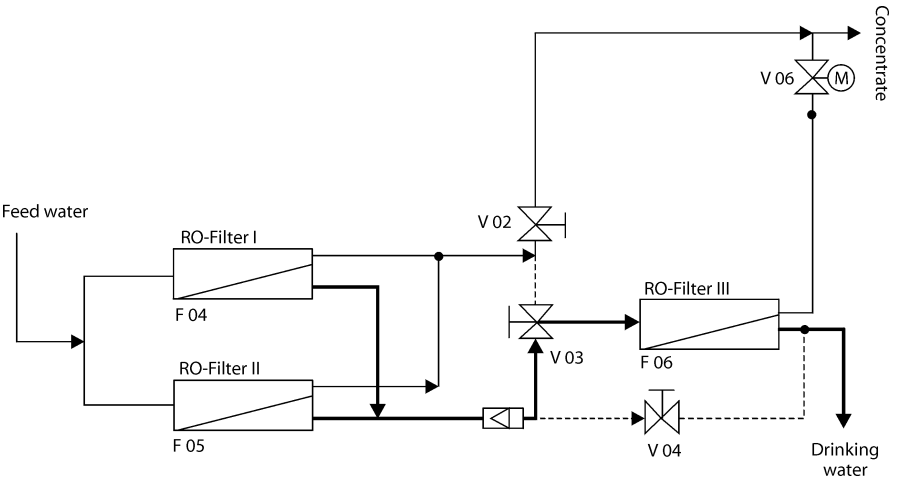

Fig. 27.5. Flow scheme of the MDWPU WTC 1600 operated in double-pass mode. (*Feed water* = raw water after bag filtration)

twenty-foot container right behind another ten-foot container that encloses the power supply unit run by diesel fuel. Both containers are placed on a cross-country truck-tractor train but can also be used for stationary operation. These containers are also ready for air transportation. The whole system is protected against mechanical pressure and electro-magnetic pulses (EMPs). A combination of different filtration techniques (slit filtration, ultrafiltration and RO) is applied for the generation of up to 10000 liters of drinking water per hour.

Fig. 27.6. Prototype of the MDWPU at the field site in Berlin-Ruhleben in September 2001

The tested system consists of the following components: A diesel fuel power supply generator, three high-pressure pumps and an additional centrifugal pump used for double-pass operation, a submerged pump (to pump the raw water into the system), a prefiltration unit (mechanical separation of large particles ≥100 μm by slit filtration), ten ultrafiltration modules (for bacteria removal and protection of the RO membranes from clogging), eight RO modules, a post chlorination unit, a remineralization unit, a heating unit (to operate the system at very low temperatures), an automatic cleaning device, measurement technology (e.g., for conductivity measurements), and a microprocessor-operated control device for automated operation of the unit. Specifications for the ultrafiltration unit are as follows: Membrane system Taga V 80 48-43-PM 100 from Koch (Wilmington, USA) with ten modules (nominal active surface area: 23.9 square meters each). Exclusion size: 100 000 Dalton equal to 0.01 μm. Detailed specifications are described by Heberer et al. (2004).

In single-pass mode, the concentrate obtained from the first six membrane modules is again applied to the last two modules to increase the raw water extraction yields. In case of nuclear or other critical chemical contamination, the device needs to be run in double-pass mode passing the permeate from the first six RO modules again through the remaining two RO modules. A scheme showing the operation of the system in double-pass mode is shown in Fig. 27.7 (dotted lines show flow paths only used in single-pass operation). A minimum of 20 000 liters of raw water per hour are needed to run the MDWPU. The device might be run using only ultrafiltration in single-pass mode, or in double-pass mode generating approximately 10, 5, and 3 cubic meters of drinking water per hour, respectively.

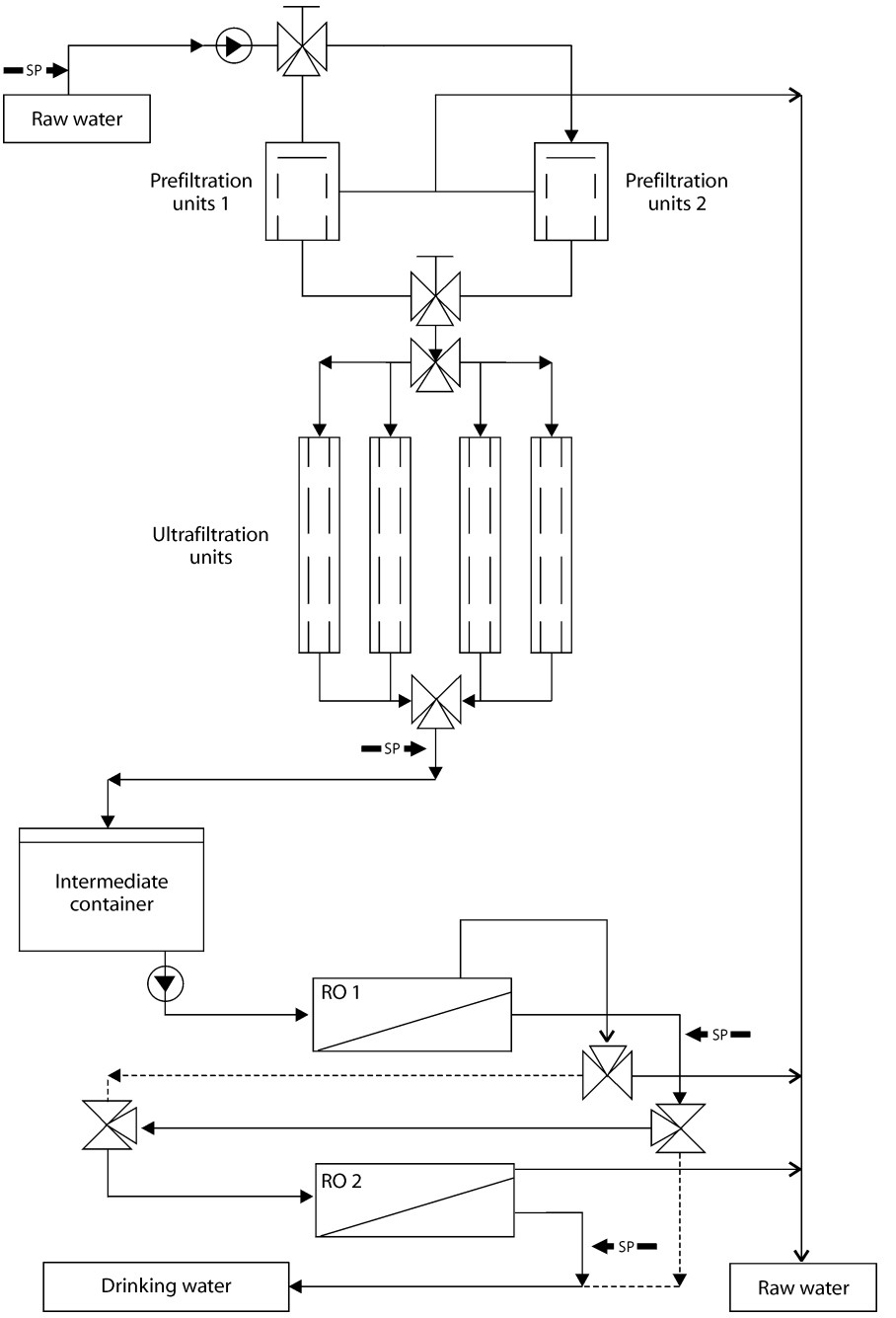

Fig. 27.7. Flow scheme of the prototype operated in single- or double-pass mode. *Dotted lines* show flow paths only used in single-pass operation (*SP* = sampling point)

27.5.2 Description of the First Field Site at the Teltowkanal

The performance of the two MDWPUs was tested for more than one year each under different (extreme) environmental conditions with all possibly expected kinds of contaminants being expected to occur in military missions in (unknown) contaminated waters including nuclear, biological, and chemical warfare agents (NBC). The purpose of the additional field trial, carried out at the Teltowkanal (Berlin, Germany) in September of 2000, was to check the performance of the commercially available MDWPU under realistic but "worst-case" conditions. Various organic, inorganic, and microbial contaminants were investigated in this field trial (Heberer et al. 2002b). However, the main target compounds were PhACs, which had never before been tested as possible contaminants in performance studies of membrane filtration devices (Heberer et al. 2002b). Native surface water containing municipal sewage discharges, contaminants from other sources, and many algae and particulates was needed for the field trial to test the practical performance of the drinking water purification unit. The Teltowkanal, a shallow canal located in the southern districts of Berlin was selected for the field study because it carries the highest loads of sewage discharges of all Berlin waterways.

Additionally, it also contains many algae and much particulate organic matter that may easily block the prefiltration unit of the device. In the shallow canal, the particulates are also often circulated by the shipping traffic. The Teltowkanal is characterized by high proportions of sewage effluents being discharged into the canal by Berlin's two largest STPs in Ruhleben (only from April to October) and Waßmannsdorf and by the municipal STP in Stahnsdorf. In several sections of the canal, the municipal sewage effluents account for up to 40% of the average surface water flow, but under extreme conditions (dry periods with low surface water flows) the proportions of municipal sewage may also reach up to 84% (Heberer et al. 2002b). As far as contaminations from municipal sewage discharges are concerned, the surface water of the Teltowkanal represents some kind of "worst-case scenario." The field study was carried out downstream from the sewers of the STPs in Waßmannsdorf and Ruhleben to guarantee a high degree of contamination by municipal sewage effluents in the raw water.

27.5.3 Description of the Second Field Site and Experimental Design

The purpose of the field trial carried out in September of 2001 at the STP in Ruhleben (Berlin, Germany) was to check the performance of a prototype of a MDWPU in removing PhACs and other sewage-borne contaminants under realistic but "worst-case" conditions. Various organic, inorganic, and microbial contaminants were investigated in this field trial (Heberer et al. 2004). However, the main target compounds were PhACs, which had only in this field trial been tested within the one year fatigue test studies of this membrane filtration device. The investigated STP in Ruhleben located in the northwestern districts of Berlin, Germany, treats both household (including rain runoff from the streets) and industrial sewage. The sewage is purified by mechanical and biological sewage treatment also applying biological phosphorus and nitrogen removal. The sludge is dewatered in centrifuges and incinerated in fluidized bed fur-

naces, followed by waste heat utilization and flue gas cleaning. More than 200 000 cubic meters of sewage per day are processed in this STP during dry weather conditions.

The field trial was carried out from September 17 to 19, 2001, using freshly purified municipal sewage effluents from a sewer basin as raw water source. On Tuesday at 9:30 a.m. it was beginning to rain heavily, resulting in dilution of the sewage effluents and decreasing conductivity values (from approximately 1100 down to less than 700 μSi). The raw water was pumped via a fire hose from the sewage basin into the MDWPU using a submerged pump. Samples were collected every three hours from the raw water (municipal sewage effluents) entering the system, after passing pre- and ultrafiltration, after passing the first six RO modules (equal to single-pass mode purification), after passing the remaining two RO modules (double-pass mode purification), and after chlorination (only for microbiological testing) (Fig. 27.7). For technical reasons it was not possible to take samples between the prefiltration and the ultrafiltration unit. In total, eighty-five water samples were collected and analyzed in this field trial (Heberer et al. 2004).

27.5.4
Sample Storage and Analysis

All samples analyzed for PhAC residues were collected in brown one-liter glass bottles, acidified (pH < 2), cooled down to 4 °C, and immediately transported into the laboratories for analysis. The samples were analyzed for thirty-one environmentally important organic contaminants including twenty-one PhACs. Until now, no standard methods are available for the analysis of these contaminants. Thus, two multi-methods applying solid-phase extraction and gas chromatography with mass spectrometric detection were used in these studies (Heberer et al. 1998, Reddersen and Heberer 2003).

27.6
Results and Discussion

27.6.1
Results from the First Field-Trial at the Teltowkanal in Berlin, Germany

The commercially-available MDWPU from Kärcher (WTC 1600 GT) tested in September of 2000 at the Teltowkanal (Berlin, Germany) was operated in single-pass and double-pass mode without additional disinfection of the permeate (optional UV-disinfection or chlorination was turned off) (Heberer et al. 2002b). Due to the huge amount of organic matter such as algae and solid particles, the lifetime of the bag filters used for prefiltration varied between only 1.5 and 2.5 hours depending on the shipping traffic in the Teltowkanal. Whenever the bag filter was blocked, the system switched automatically to the second filter without interrupting the purification process. The prefiltration unit and the whole system were working continuously and with high reliability. In total, more than 5000 individual analytical results were obtained and evaluated from this field trial to check the ability of the MDWPU to remove a variety of microbial, organic, and inorganic pollutants from the contaminated raw water (Heberer et al. 2002b).

Table 27.1 compiles the average concentrations ($N = 11$) of different organic and inorganic residues measured during the field trial in the surface water of the Teltowkanal. Several organic residues were detected at average individual concentrations up to 945 ng l^{-1} (for the flame retardant TCIPP: tris-(chloroisopropyl)-phosphate). In the permeate of the drinking water purification unit, none of the investigated PhACs or any other organic contaminant was detected at significant concentrations, neither when the system was operated in the single-pass mode nor when it was operated in the double-pass mode (Heberer et al. 2002b). The concentrations of all contaminants detected in the raw water obtained from the Teltowkanal were decreased to concentrations below their analytical limits of detection of only 1 ng l^{-1} for the individual PhACs (Reddersen and Heberer 2003). Table 27.1 compiles the average removal rates measured for the individual PhACs and several other contaminants applying single-pass mode purification.

27.6.2 Results from the Second Field Trial at the Sewage Treatment Plant in Ruhleben (Berlin, Germany)

The prototype of a MDWPU was tested in September 2001 in a continuous 48-h field trial at the sewer of Berlin's largest STP in Ruhleben (Germany). It was continuously operated in double-pass mode including disinfection of the permeate by chlorination (Heberer et al. 2004). In contrast to the commercially-available MDWPU, samples could not only be collected from the raw water entering the system and the final permeate but also after passing the pre- and ultrafiltration units, after passing the first six RO modules (equal to single-pass mode purification), after passing the remaining two RO modules (double-pass mode purification), and after chlorination. In total, more than 7 000 individual analytical results were obtained and evaluated from this field trial, checking the prototype device's ability to remove a variety of microbial, organic, and inorganic pollutants from the municipal sewage effluents in order to generate potable water that has to meet drinking water regulations (Heberer et al. 2004). The following two sections will focus on the presentation of the results for the removal of PhACs and related contaminants.

Raw Water Quality (Municipal Sewage Effluents)

In total, seventeen raw water samples were collected and analyzed for a broad spectrum of inorganic and organic contaminants during the 48-h field trial (Heberer et al. 2004). The results from these investigations did not only show the composition and quantities of sewage-borne contaminants such as PhACs or flame retardants but they also provided some information concerning the variation of the concentrations in the municipal sewage effluents. Some interesting results have also been obtained from the changes in the composition of the sewage effluents observed after a heavy rain event on the second day of the field trial (Heberer et al. 2004). Table 27.2 compiles the results for some organic contaminants detected in the municipal sewage effluents of the STP in Berlin-Ruhleben (Germany). Fifteen different PhACs were detected at average concentrations up to 2.3 µg l^{-1} in the sewage effluents used as raw water for the MDWPU. Additionally, two chlorinated flame retardants TCEP (tris-(chloroethyl)-

Table 27.1. Average concentrations of selected contaminants in the raw water from the Teltowkanal and in the permeate after water purification using single-pass mode

Compound	Average raw water concentration	Standard deviation	Rel. standard deviation (%)	Average permeate concentration	Removal rate[c] (%)
Pharmaceuticals					
AMDOPH	290 ng l^{-1}	56 ng l^{-1}	19	<1 ng l^{-1}	>99
Caffeine	429 ng l^{-1}	58 ng l^{-1}	13	<1 ng l^{-1}	>99
Clofibric acid	155 ng l^{-1}	6 ng l^{-1}	4	<1 ng l^{-1}	>99
Diclofenac	330 ng l^{-1}	53 ng l^{-1}	16	<1 ng l^{-1}	>99
Ketoprofen	17 ng l^{-1}	7 ng l^{-1}	41	<1 ng l^{-1}	>94
Naproxen	38 ng l^{-1}	13 ng l^{-1}	34	<1 ng l^{-1}	>97
Propyphenazone	177 ng l^{-1}	60 ng l^{-1}	34	<1 ng l^{-1}	>99
Pesticides					
Diurone	0.10 µg l^{-1}	0.03 µg l^{-1}	30	<0.05 µg l^{-1}	
Mecoprop	93 ng l^{-1}	11 ng l^{-1}	12	<1 ng l^{-1}	>98
Flame retardants					
TCEP[a]	359 ng l^{-1}	171 ng l^{-1}	48	<10 ng l^{-1}	>97
TCIPP[b]	945 ng l^{-1}	338 ng l^{-1}	36	<10 ng l^{-1}	>98
Inorganics					
Aluminium	0.39 µg l^{-1}	0.35 µg l^{-1}	90	<0.04 µg l^{-1}	>89
Ammonium	0.34 mg l^{-1}	0.05 mg l^{-1}	15	<0.05 mg l^{-1}	>85
Borate	0.26 mg l^{-1}	0.05 mg l^{-1}	19	<0.05 mg l^{-1}	>80
Iron	0.77 µg l^{-1}	0.16 mg l^{-1}	21	<0.05 µg l^{-1}	>93
Nitrate	18.84 mg l^{-1}	3.10 mg l^{-1}	16	<1.0 mg l^{-1}	>94
Nitrite	0.39 mg l^{-1}	0.04 mg l^{-1}	10	<0.01 mg l^{-1}	>97
Phosphate	0.96 mg l^{-1}	0.10 mg l^{-1}	11	<0.2 mg l^{-1}	>79

[a] Tris(chloroethyl)phosphate.
[b] Tris(2-chloroisopropyl)phosphate.
[c] Removal rates were calculated from the average raw water concentrations and the average permeate concentrations. In all of the shown cases, analytes were not detected in the permeate samples. Thus, removal rates were calculated by using the individual limit of detection.

phosphate) and TCIPP (tris-(chloroisopropyl)-phosphate) were also found at concentrations up to the µg/l-level.

Table 27.2. Average concentrations of pharmaceuticals and their absolute and relative standard deviations in the municipal sewage effluents ($N = 17$)

Compound	Average concentration (ng l^{-1})	Standard deviation (ng l^{-1})	Relative standard deviation (%)
AMDOPH	811	277	34
Bezafibrate	257	217	84
Carbamazepine	2 282	536	23
Clofibric acid	178	52	29
Diclofenac	869	155	18
Fenofibric acid	705	736	104
Gemfibrozil	16	25	156
Ibuprofen	88	178	202
Indomethacin	46	20	43
Ketoprofen	99	49	49
Mefenamic acid	16	5	31
Naproxen	224	69	31
Oxazepam	153	24	16
Primidone	734	220	30
Propyphenazone	309	114	37

For most of the compounds, the concentrations decreased during and after the rain event. This is also shown in Fig. 27.8 for the anti-epileptic drugs carbamazepine and primidone. Apart from this dilution effect, most of the PhACs were detected at almost constant concentrations. Thus, the relative standard deviations of the concentrations of the PhACs were almost less than 50% except for the blood-lipid regulating PhACs bezafibrate, fenofibric acid, and gemfibrozil, and for the analgesic drug ibuprofen (Table 27.2). After rainfall events, Ternes (1998) observed lower removal rates for several PhACs during sewage treatment. This phenomenon was also observed during this field trial. Thus, increasing concentrations were observed after the rain event especially for bezafibrate, fenofibric acid, and ibuprofen but less pronounced also for the analgesics diclofenac and naproxen and for gemfibrozil. For bezafibrate, fenofibric acid, and ibuprofen, a more than ten-fold increase of the concentrations was observed after the rain event (examples given in Fig. 27.9). Similar peak concentrations were also observed for several inorganic parameters such as phosphate, ammonia, and nitrite (Fig. 27.10), whereas nitrate only showed a slight increase and then the concentrations decreased to less than 40% of the pre-rainfall values.

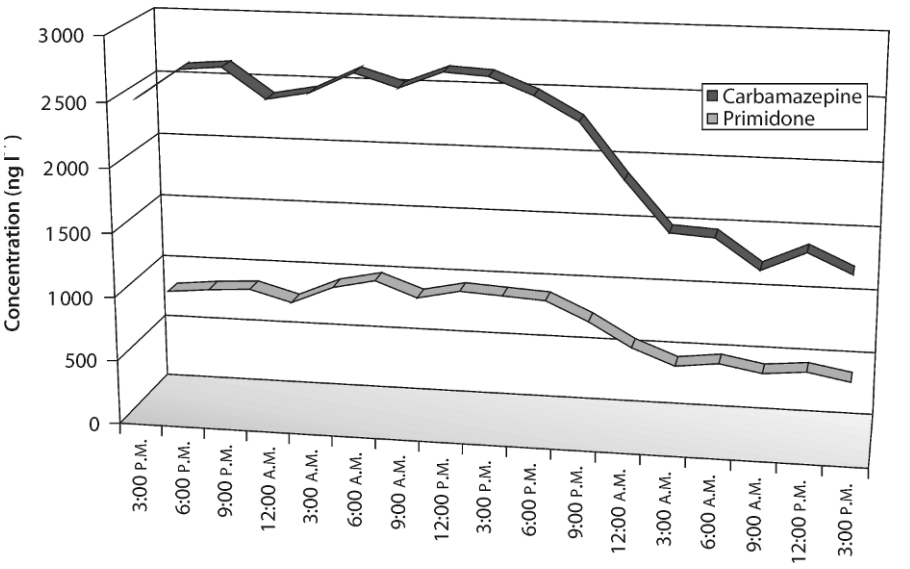

Fig. 27.8. Concentrations of carbamazepine and primidone in the raw water

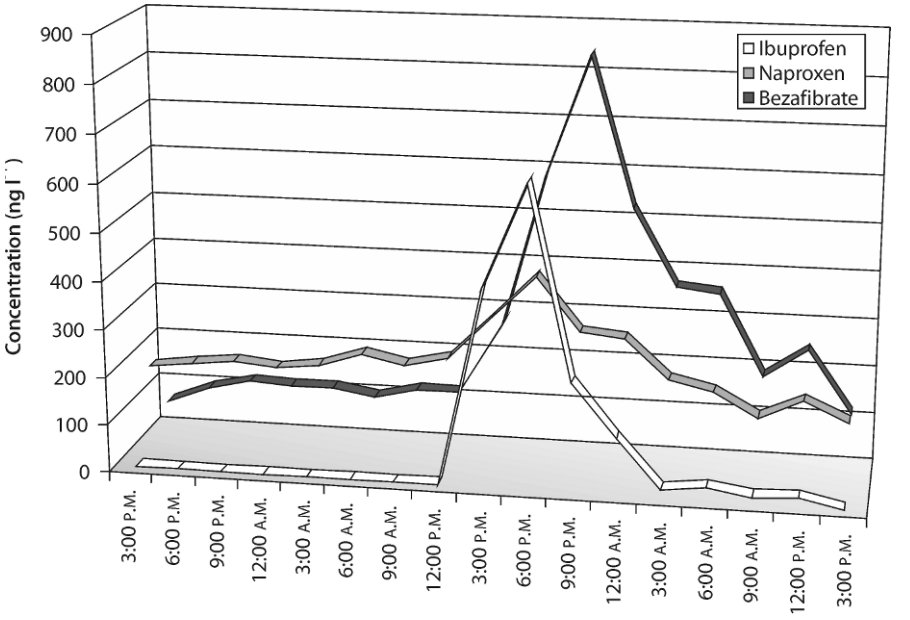

Fig. 27.9. Concentrations of bezafibrate, naproxen and ibuprofen in the raw water

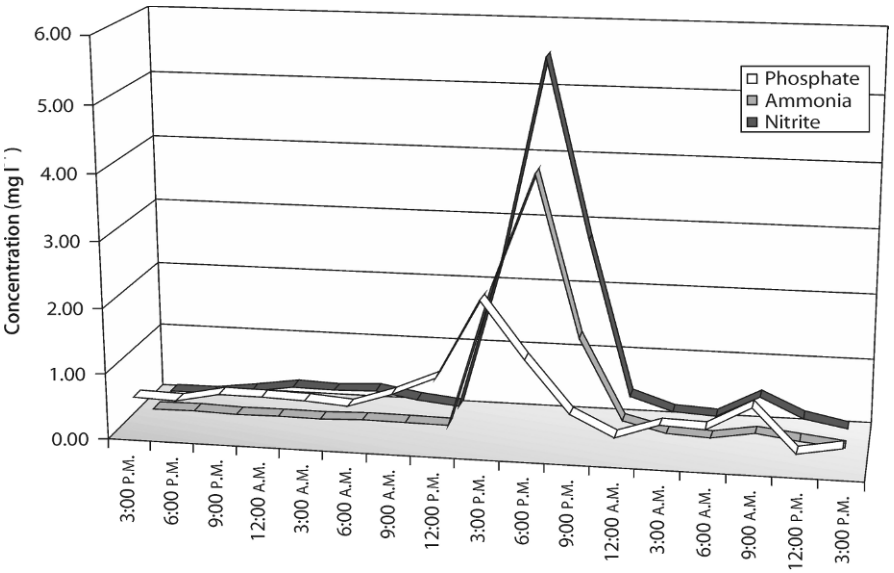

Fig. 27.10. Concentrations of ammonium, nitrite and phosphate in the raw water

Removal of PhACs and Related Polar Organics

The removal rates determined for the pharmaceutical residues, the chlorinated flame retardants, and several related organics found in the raw water and the potable water processed by the MDWPU are compiled in Table 27.3 together with their concentrations in the final permeate after double-pass RO purification. In the permeate of the drinking water purification unit, none of the investigated PhACs or any other related organic contaminant was detected at concentrations above 10 ng l^{-1}, neither in single-pass nor in double-pass operation. The concentrations of all contaminants detected in the municipal sewage effluents could be decreased to concentrations below the analytical limits of detection. For several compounds, a decrease of their concentrations up to 62% was already observed after pre- and ultrafiltration (Table 27.3). PhACs such as the analgesics diclofenac and propyphenazone found in the raw water at average concentrations of 870 and 310 ng l^{-1} were already removed by 44 and 46% after ultrafiltration. These compounds may be adsorbed to organic particulates or membrane surfaces and thus be removed by the pre- or the ultrafiltration unit. Both compounds are also known to be significantly adsorbed to soil or by particular organic matter (Preuß et al. 2001; Mersmann et al. 2002; Ternes et al. 2002; Verstraeten et al. 2002). On the other hand, the concentrations of bezafibrate, an even more hydrophobic compound, were only slightly decreased (7%) after ultrafiltration. Therefore, other additional mechanisms than mere hydrophobic adsorption must have been responsible for the decrease of the concentrations of some selected PhACs. Several of the contaminants significantly removed by pre- and/or ultrafiltration, e.g., TCEP or TCIPP, have prominent ionic properties. It can be supposed that at the given pH these and other

Table 27.3. Removal rates of PhACs and related organic contaminants calculated from raw water and permeate concentrations measured after passing the different membrane units ($N = 17$ for each sampling point)

Compound name	Average raw water concentration (ng l^{-1})	Removal rate (%) (after pre- and ultra-filtration)	Removal rate (%) (single-pass RO)	Removal rate (%) (double-pass RO)	Average permeate concentration (double pass) (ng l^{-1})
Pharmaceuticals					
AMDOPH	811	32	>99.9	>99.9	<1
Bezafibrate	257	7	96.0	>99.9	<5
Carbamazepine	2282	13	>99.9	>99.9	<1
Clofibric acid	178	20	>99.4	>99.4	<1
Diclofenac	869	44	>99.9	>99.9	<1
Fenofibric acid	705	22	97.0	>99.9	<1
Gemfibrozil	16	38	>93.3	>93.3	<1
Ibuprofen	87	12	98.5	>98.9	<1
Indometacin	46	0	92.0	>97.8	<1
Ketoprofen	99	20	>99.0	>99.0	<1
Naproxen	224	0	98.2	>99.5	<1
Oxazepam	153	0	>99.3	>99.3	<5
Primidone	734	0	>99.9	>99.9	<1
Propyphenazone	309	46	99.3	>99.7	<1
Pesticides					
Bentazone	13	>93.3	>93.3	>93.3	<1
Mecoprop	69	0	>98.6	>98.6	<1
Flame retardants					
TCEP[a]	851	34	98.5	99.2	7
TCIPP[b]	3621	62	99.7	>99.9	<5

[a] Tris-(chloroethyl)phosphate.
[b] Tris-(2-chloroisopropyl)phosphate.
The removal rates were calculated from the average raw water concentrations ($N = 17$) and the average concentrations ($N = 17$ for each sampling point) of the water samples taken after ultrafiltration or reverse osmosis, respectively. When substances were not detected in the samples, the removal rates were calculated from the individual limits of detection.

contaminants (including diclofenac) were partly removed by electrostatic repulsion at the surface of the ultrafiltration membranes. AMDOPH (1-acetyl-1-methyl-2-dimethyl-oxamoyl-2-phenylhydrazide) and propyphenazone, which are also rejected at

considerable amounts, also have ionic properties due to their hybrid betaine structures. The concentrations of several other PhACs such as indomethacin, naproxen, oxazepam, and primidone were not at all influenced by pre- and/or ultrafiltration. Nevertheless, these contaminants like all the other PhACs were efficiently removed by RO treatment (Table 27.3).

27.7 Conclusions and Future Applications of MDWPUs

The commercially-available MDWPU and the prototype were successfully operated in both field trials (Heberer et al. 2002b, 2004) as well as in some other laboratory studies and field trials which have also been carried out in extreme climates (high and low ambient temperatures or high humidity) or with extreme contaminations (e.g., NBC). The laboratory and the field studies proved the high efficiency and reliability of modern purification units applying membrane filtration. In both cases, the pre-filtration device was very effective in rejecting algae and/or solid particles protecting the ultrafiltration (only prototype) and the reverse osmosis (RO) membranes from clogging to enable an almost maintenance-free operation. In the field trials at the Teltowkanal and at the sewer of the STP in Ruhleben, all contaminants could efficiently be removed from the contaminated surface water and the municipal sewage effluents used as raw water sources for the production of potable water. After remineralization, the generated permeate meets all requirements for drinking water set by the different regulations (ECD 1998; TrinkwV 2001; STANAG 2002). Although no maximum tolerance levels have been set for most of the sewage-borne contaminants such as PhACs, the German drinking water regulation (TrinkwV 2001) also requires minimizing anthropogenic contaminants as far as technically possible and economically reasonable (§6(3): "precautionary principle"). Thus, the field trial has also shown that, from a technical point of view, that it is possible to reduce the concentrations of PhACs and related sewage-borne contaminants below the detection limits even when using municipal sewage effluents as raw water sources. Several units of the commercially available MDWPU (Heberer et al. 2002b) are already successfully operated under extreme ambient conditions in several military out-of-area missions not only by the Bundeswehr in Afghanistan, Djibouti, and Kosovo but also by the Australian Army in Eastern Timor. The prototype is much larger and able to produce enough drinking water to serve a whole field hospital. Additionally, this device is also protected against electromagnetic pulses (EMPs). Although a precise calculation of the actual operational costs is not yet possible, first estimations already indicate that the total costs of the generation of drinking water will be much lower than those of the transport of bottled drinking water or of the production of drinking water using conventional MDWPUs applying chemicals and active charcoal filtration for drinking water purification.

27.8 Summary and Outlook

Membrane filtration techniques, especially those using nanofiltration (NF) or reverse osmosis (RO) membranes, are among the most efficient and promising procedures for the removal of pharmaceutically active compounds (PhACs) from contaminated

raw water sources. Despite higher operational costs, an increasing number of sewage or drinking water facilities are using membrane filtration as their final purification method. Several studies have investigated the rejection capacities of membrane filtration for PhAC residues. Some of the results obtained from laboratory experiments, with full-scale facilities or with mobile drinking water purification units (MDWPUs) are very promising.

Investigations of municipal sewage treatment plants (STPs) equipped with integrated membrane bioreactors (MBRs) applying micro- or ultrafiltration membranes showed that removal of trace organic contaminants was comparable to those observed for conventional STPs. On first sight, the results confirmed the expectation that the micro- and ultrafiltration membranes are inappropriate for removing micropollutants directly by sieving (the molecular size is at least 100 times smaller than the pore size of the membranes). On the other hand, results from other studies indicate that extended sludge ages and contact times often reached in MBRs can improve the microbial degradation and removal of certain pharmaceutical residues and/or endocrine disrupting compounds (EDCs). Much better removal rates and in several cases a complete removal of PhACs and EDCs below their limits of detection were achieved by facilities or units using NF or RO membranes. The rejection of the PhACs by RO or NF membranes is influenced by the physical-chemical properties of the individual molecules (molecular size, polarity, dipole moment and charge), the operating conditions of the membrane filtration process (especially by the pH of the raw water), and the properties of the individual membranes such as pore size, hydrophobicity, permeability, and charge. For micro- and ultrafiltration membranes, only adsorptive effects such as hydrophobic adsorption and electrostatic repulsion (for the retention of ionizable pharmaceuticals) appeared to be instrumental in maintaining high retention. For NF membranes, exhibiting low retention of ions, both size exclusion and adsorptive effects were important, whereas size exclusion dominates retention with the tighter NF or with RO membranes.

MDWPUs are used in civil disaster operations or military out-of-area missions for the generation of drinking water from surface waters which are often highly contaminated. Two novel membrane-based MDWPUs, a commercially available device and a prototype, have been tested for their ability to remove microbes, as well as inorganic and organic pollutants from contaminated raw water sources. Both devices were able to remove residues of PhACs from the raw water, decreasing their concentrations in the generated drinking water below the individual detection limits of between 1 and 5 ng l^{-1}. Thus, the results from the fatigue tests confirmed the ability of the membrane-based MDWPU to remove PhACs from raw-water sources contaminated with municipal sewage discharges. When using MDWPUs in military out-of-area missions or in civil disaster operations, the main objective is to ensure a secure drinking water supply independent from the composition and the sources of the raw water that in advance are usually unknown. For such kinds of applications, economical considerations are also important but only in second place compared to security aspects. Thus, the per cubic meter costs of producing drinking water through the application of MDWPU will always be much higher than those of large-scale operations. Both from an economical and an ecological perspective, it will neither be reasonable nor possible to apply exactly the same technique and similar high security standards. Nevertheless, the field trials have shown that it is technically possible to efficiently reduce sewage

contaminant concentrations using membrane filtration. And the membrane techniques used by the MDWPUs might also be applied and adapted by large-scale purification units. For a known and almost constant contamination problem, membrane filtration can be adjusted and optimized to a particular situation. Techniques that demand less energy such as nanofiltration as opposed to RO might be used to lower operational costs while still reducing or removing all undesired contaminants. Thus, investigations of large-scale operations in the U.S. conducted by Drewes et al. (2002) and Snyder et al. (2007) have already shown that NF and RO are also able to remove PhACs efficiently from contaminated raw water sources such as municipal sewage effluents.

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Photooxidation as Advanced Oxidation Treatment of Hospital Effluents

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28.1 Introduction

"Pharmaceuticals in the environment" is an issue of growing interest. Since the 1990s, there have been a large number of studies into the occurrence, effects and risks of these xenobiotic species (Halling-Sørensen et al. 1998; Kümmerer 2001; Heberer 2002). This has been extremely useful in helping one understand that, after consumption and excretion, in the case of hospitals as well as domestic use, pharmaceuticals are able to reach the municipal wastewater treatment plants (MWTPs). Owing to the low biodegradability of many pharmaceuticals, the commonly employed treatment processes are not effective enough for a complete removal of such species, and the discharge of the treated effluents into receiving waters can lead to contamination with these micropollutants. As a result, there are surface waters containing trace concentrations of pharmaceuticals. These concentrations, when found not only in surface waters, but also in MWTPs effluents, have proven to be high enough to cause toxic effects to environmental organisms (Kümmerer et al. 2000; Halling-Sørensen et al. 2000). This picture is even serious when one takes into account the complete lack of MWTPs (in less developed countries) and eventual consumption of contaminated and untreated surface waters. The removal capacity of MWTPs for pharmaceuticals is currently being investigated in more detail. Technologies such as nanofiltration, active coal adsorption and ozonation have proven to have a high potential for the elimination of pharmaceuticals (Ternes et al. 2004).

Photochemical natural processes in environmental compartments have been investigated for the last few years (Zeep et al. 1992). On the basis of this, there has been an increasing interest in the use of technologies based on simple UV irradiation or a combination with auxiliary oxidants or photocatalysts (photochemical advanced oxidation processes, AOPs) for the treatment of urban and industrial effluents, potable water, as well as process water.

28.2 Pharmaceutical Photooxidation

Photochemical processes applied to the degradation of organic compounds in an aqueous medium are also being investigated (Glaze et al. 1995; Martins et al. 2006a; Martins et al. 2006b). Despite the large gaps in knowledge regarding the chemistry involved in these processes, its use for wastewater treatment as well as for potable water has received a good deal of attention in the last few years.

In general, these processes are based on (*a*) induced photoionization of the substrate by the absorption of light and generating radical cations, as a consequence; (*b*) direct reaction of the substrate with oxygen by the influence of UV/Vis irradiation; and (*c*) a reaction of the substrate with transient active species formed by the excitation of a precursor. This last condition gives rise to the so-called Advanced Oxidation Processes (AOPs) that generate hydroxyl radicals (HO·) in a thermodynamically and kinetically favorable way for the degradation of organic and inorganic species in aqueous and gaseous media.

Studies regarding the fate of pharmaceuticals in the environment have demonstrated that photolysis is involved in the removal of a large number of pharmaceuticals in the aquatic environment (Andreozzi et al. 2003; Cardoza et al. 2005; Poiger et al. 2001). A series of studies reporting the photodegradability of compounds has been published and classifies the photoprocesses as an alternative for the treatment of effluents containing pharmaceuticals (Ahmad et al. 2006; Gómez-Taylor et al. 2006; Uwai et al. 2005). However, in most of the cases direct photolysis is a slow process, which can make it impracticable to employ it for effluent treatment. Thus, the use of photochemical AOPs can offer satisfactory levels of micropollutant degradation by reducing the time required for treatment. Studies involving the use of O_3-based AOPs in the degradation of pharmaceuticals on a bench and pilot scale are much more developed and strengthen the applicability of advanced oxidation in this field (Huber et al. 2003; Ternes et al. 2003; Huber et al. 2004; Huber et al. 2005). The reaction rate constants between pharmaceuticals and HO· (Table 28.1) corroborate with this assumption.

The generation of photo-initiated HO· occurs normally by electronic excitation of auxiliary chemical oxidants, in general, O_2, H_2O_2 or O_3, starting a complex chain of radical reactions, which provides suitable conditions for the micropollutants' degradation (Buxton et al. 1988; Payton and Glaze 1988; Liao and Gurol 1995). A schematic representation (adapted from Oppenländer 2003) shows Fig. 28.1. Photo-Fenton reactions, where H_2O_2 and UV/Vis radiation maintain the Fe^{2+}/Fe^{3+} redox cycle with formation of HO· as the principal intermediary, are also an excellent example of photochemical AOP that has been extensively used by water and effluent treatment (Zepp et al. 1992; Martins et al. 2006a). Good yields have been obtained by the degradation

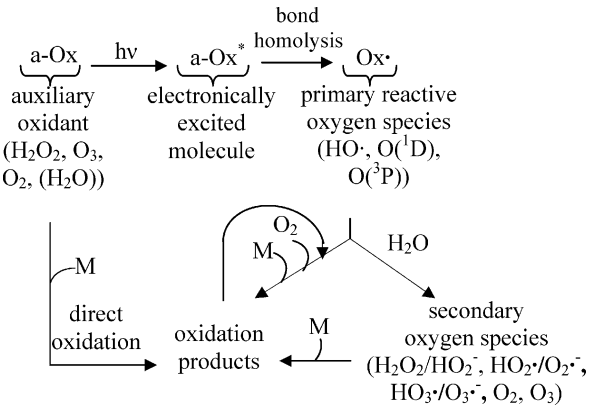

Fig. 28.1. Scheme of photo-initiated oxidation reactions based on the presence of auxiliary oxidants and the corresponding nomenclature (Oppenländer 2003)

of pharmaceuticals by means of this kind of technology (Arslan-Alaton and Dogruel 2004; Arslan-Alaton and Gurses 2004; Bautitz and Nogueira 2007; Sirés et al. 2007).

Another photochemical AOP of great practical utility is photoperoxidation (H_2O_2/UV), where HO· is generated by photo-induced homolytic cleavage of the O–O bond from H_2O_2 (Liao and Gurol 1995; Martins et al. 2006b). Kinetic degradation studies from 5-methyl-1,3,4-thiadiazole-2-methylthio (MMTD-Me) and its precursor, 5-methyl-1,3,4-thiadiazole-2-thiol (MMTD) demonstrate satisfactory degradation at concentration levels near to the real ones (Lopez et al. 2003). The MMTD is an intermediate used for the synthesis of cefazolin and is also a human metabolite of this antibiotic. Both com-

Table 28.1. Rate constants for the reaction of HO· with some pharmaceuticals

Compound	k_{HO} ($\times 10^9$ M^{-1} s^{-1}) [a]
Roxithromycin	5.4 (±0.3)[b]
Azithromycin	2.9 (±0.6)[b]
Tylosin	8.2 (±0.1)[b]
N(4)-acetyl-sulfamethoxazole	6.8 (±0.1)[b]
Ciprofloxacin	4.1 (±0.3)[b]
Enrofloxacin	4.5 (±0.4)[b]
Trimethoprim	6.9 (±0.2)[b]
Lincomycin	8.5 (±0.2)[b]
Penicillin G	7.3 (±0.3)[b]
Cephalexin	8.5 (±0.7)[b]
Tetracycline	7.7 (±1.2)[b]
Vancomycin	8.1 (±0.3)[b]
Amikacin	7.2 (±0.3)[b]
Bezafibrate	7.4 (±1.2)[c]
Carbamazepine	8.8 (±1.2)[c]
Diazepam	7.2 (±1.0)[c]
Diclofenac	7.5 (±1.5)[c]
17α-ethinylestradiol	9.8 (±1.2)[c]
Ibuprofen	7.4 (±1.2)[c]
Iopomide	3.3 (±0.6)[c]
Sulfamethoxazole	5.5 (±0.7)[c]
Naproxen	5.5 (±0.7)[d]

[a] 25 °C, pH 7.
[b] Apparent second-order rate constant (Dodd et al. 2006).
[c] Huber et al. 2003.
[d] Packer et al. 2003.

pounds have already been detected in groundwater (Guardini et al. 1999). The degradation by means of direct photolysis of MMTD-Me as well as of MMTD was also confirmed.

Among the AOPs, in general, one of the most promising technologies is the heterogeneous photocatalysis. It is based on the irradiation of a catalyst, generally, an inorganic semiconductor like TiO_2, ZnO or CdS, and aims to promote electrons from the valence to the conduction band, forming oxidant and reductive sites capable of creating the conditions for the degradation of pollutants (Eqs. 28.1 and 28.2, and Fig. 28.2). During the process, water molecules or hydroxyl groups adsorbed to the catalyst surface are oxidized under the formation of HO·, which thus opens up another pathway of degradation of organic compounds (Eqs. 28.3 and 28.4; Hoffmann et al. 1995; Serpone et al. 1997; Diebold 2003).

$$TiO_2 + h\nu \rightarrow TiO_2\ (e^-, h^+) \tag{28.1}$$

$$TiO_2\ (h^+) + RX_{ads} \rightarrow TiO_2 + RX\cdot^+_{ads} \tag{28.2}$$

$$TiO_2\ (h^+) + H_2O_{ads} \rightarrow TiO_2 + OH\cdot_{ads} + H^+ \tag{28.3}$$

$$TiO_2\ (h^+) + OH^-_{ads} \rightarrow TiO_2 + OH\cdot_{ads} \tag{28.4}$$

Regarding the degradation of pharmaceuticals, heterogeneous photocatalysis is, without doubt, the most investigated photochemical AOP. In the literature, there is a series of investigations regarding the relevance of process variables, different types of catalysts, or simply, better ways of knowing the response of pharmaceuticals facing these processes. Studies involving the photocatalytic degradation of furosemide, ranitidine, ofloxacine, phenazone, naproxen, carbamazepine, clofibric acid, iomeprol and iopromide have proven the efficiency of the process and confirmed that it is a suitable alternative to, for example, biological treatment of contaminated waters with pharmaceuticals (Molinari et al. 2006; Doll and Frimmel 2004; Doll and Frimmel 2005a–c).

During the treatment by heterogeneous photocatalysis, the degradation grade depends on the time of contact of the substrate with the photogenerated gap, the formed reductive zone and the active species that relies on this process. For this to happen, the heterogeneous photocatalysis also depends to a great extent on the substrate ad-

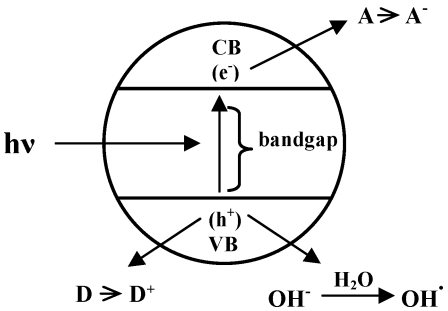

Fig. 28.2. Schematic representation of the photochemical activation of a semiconductor and of the formation of hydroxyl radical. *VB*: valence band; *CB*: conduction band: *A*; electronic acceptor compound; *D*: electronic donating compound; h^+: electron hole.

sorption at the catalyst surface. Doll and Frimmel (2005a) provided evidence of this trend through the photocatalytic treatment of carbamazepine, clofibric acid and iomeprol, where the degradation grade was, in general, proportional to the adsorption of the compounds at the catalyst surface.

However TiO_2 has been the most largely used photocatalyst, other semiconductors can also lead to satisfactory results. During the photocatalytic treatment, the use of ZnO has been more effective than TiO_2, both in the removal of sulfamethazine and in the mineralization of the organic matter (OM), sulfur- and nitrogen-compounds (Kaniou et al. 2005). As in the case of all other AOPs, one of the major drawbacks of the heterogeneous photocatalysis continues to be its high operational costs. Thus, the state-of-the-art of this technology is the search for alternatives that can provide a maximum degree of efficiency in the process. The use of transition metals as modifiers of the catalyst surface has been investigated as a tool for the increment of the photocatalytic activity (Linsbigler et al. 1994). The addition of species like H_2O_2, O_2 and O_3 are also being tested. The use of H_2O_2 increases the photocatalytic degradation of sulfamethazine, sulfonamide and norfloxain (Kaniou et al. 2005; Haque and Muneer 2007).

As the presence of other compounds in the reaction medium can inhibit the degradation process, the matrix complexity can represent another limitation during the heterogeneous photocatalysis. One of the major problems is the dissolved organic natural matter (DOM), as it causes attenuation of the radiation (competitive absorption), competition for active sites and reactive species, and also deactivation of the catalyst surface due to its adsorption (Doll and Frimmel 2005a).

Other limitations that must be overcome are the difficulties in removing the catalyst from the treated effluent for obtaining a high relation surface/volume, and the scaling-up. In this way, the use of heterogeneous photocatalysis in connection with nanomembranes, the so-called hybrid systems, have demonstrated a high degree of efficiency. This guarantees the catalyst and/or the pollutant species confinement (at the molecular level) in the reactional environment. In addition, it permits a continuous operation without the need of an additional step to separate the catalyst from the treated effluent. Some of these systems have been already tested for the pharmaceuticals' degradation and obtained variable yields (Augugliaro et al. 2005; Doll and Frimmel 2005b; Molinari et al. 2006).

The employment of photoprocesses is a suitable alternative for the degradation of the anti-inflammatory diclofenac, since its photosensitivity is well known (Moore et al. 1990). Investigations carried out into protecting the environment have found that this species is partially degraded by photolysis (Vogna et al. 2004). Comparative studies with clofibric acid and ibuprofen have shown the complete destruction of diclofenac by O_3/H_2O_2, even in river water, whilst the other two compounds showed around 50% degradation in distilled water, and 70–90% in river water (Zwiener and Frimmel 2000). Among the photochemical AOPs, photo-Fenton processes demonstrated the complete mineralization of diclofenac even at higher concentrations (Ravina et al. 2002). At an initial concentration of 1.0×10^{-3} mol l^{-1}, diclofenac was completely destroyed after 90 min of H_2O_2/UV treatment (5.0×10^{-3} mol l^{-1} H_2O_2 and pH 7; Vogna et al. 2004). By using a heterogeneous system that corresponded to the conditions found at MWTPs, Hofmann et al. (2007) confirmed the efficiency of different catalysts for the diclofenac degradation.

During the diclofenac degradation, liberation of chloride occurs resulting in hydrochloric acid formation, which causes a lowering of the pH (Pérez-Estrada et al. 2005). This, allied to the low solubility of the pharmaceutical in acidic pH, set the precipitation up to the level of a degradation auxiliary when the waters containing the pollutant were decontaminated. A description of the products formed during the degradation of diclofenac by means of AOPs is given below.

A special case involving the photodegradation of pharmaceuticals is that of antimicrobial fluoroquinolones. A series of studies based on the interest in the photooxidation of these species have demonstrated its photosensitivity (Tiefenbacher et al. 1994; Fasani et al. 1999, 2001). In general, by absorbing radiation, molecules containing 4-quinolone or 4-naftipiridone are converted to the triplet state, which has a greater electrophylic character than the molecules in the ground state (Mella et al. 2001). The formation of this triplet state governs the photoreactivity of fluoroquinolones. The occurrence of reactions after photoexcitation is due to processes involving substitution that acts photochemically in unstable sites (e.g., carbon-fluorine bond, piperazine ring and carboxyl group). Environmentally, photolysis is involved with the removal of fluoroquinolones in aquatic compartments (Golet et al. 2002; Cardoza et al. 2005; Lam et al. 2003; Belden et al. 2007). Studies simulating real conditions have demonstrated that one of the first steps is the rupture of the piperazine ring, followed by the formation of CO_2 and other compounds of greater polarity (Burhenne et al. 1997a,b, 1999). When account is taken of the photoproducts of fluoroquinolones, investigations suggest that the primary products from the photodegradation of ciprofloxacin do not show ready-biodegradability (Vasconcelos 2006). On the other hand, photolysis causes a loss of biological activity, which is a relevant factor in its impact on environmental microorganisms (Phillips et al. 1990).

Ofloxacine was efficiently degraded by heterogeneous photocatalysis at different pH values (Molinari et al. 2006). Good degradation rates for norfloxacin were obtained when different types of TiO_2 were employed during heterogeneous photocatalysis (Haque and Muneer 2007). The addition of H_2O_2, although not significantly accelerating the compound degradation, increases the rate of mineralization to a considerable extent. Photoperoxidation was also investigated and yielded levels of mineralization and norfloxacin destruction similar to those obtained through heterogeneous photocatalysis.

In spite of the growing interest in the applicability of photochemical AOPs to the degradation of pharmaceuticals in effluents, very few studies applied these processes to real samples. This kind of investigation provides a real evaluation of the capability of these processes to destroy the compounds of interest (analytes) in the middle of the concomitants, which compete for the incident radiation, catalysts active sites, oxidants and active intermediaries formed in the medium. Photo-induced oxidation and heterogeneous photocatalysis have been used for ciprofloxacin degradation in hospital effluents (Vasconcelos 2006). The effluents had a concentration of 200 $\mu g\ l^{-1}$ ciprofloxacin and a chemical oxygen demand (CQD) of 658 $mg\ l^{-1}$. In both processes, a 125 W medium pressure mercury lamp and pH 3 were used in a batch helicoidal tube reactor with recirculation (suspended 400 mg TiO_2). Ciprofloxacin was completely destroyed after approximately 2.5 and 1 h of photo-induced oxidation and heterogeneous photocatalysis, respectively. Despite the high capacity to destroy ciprofloxacin, the complexity of the matrix retarded the processes. Photo-induced oxidation of ciprofloxacin in synthetic solution, at conditions not far from those referred above

(150 W medium pressure mercury lamp, 2.5 l batch reactor, 30 °C, pH 9, 100 μg l^{-1} ciprofloxacin) provide ciprofloxacin elimination at a rate that is fifty times higher. This result shows the strong inhibitory effect caused by the effluent matrix. In addition, the mineralization of the constitutive organic matter of the effluent measured through COD did not reach 50%, which is one more proof of the inhibitory effect of the matrix. What was stated above is well suited to the trend of photochemical AOPs: they should not be employed in mineralization or even in the simple removal of biodegradable organic pollutants, since this would greatly increase the time of the processes and make them uneconomical. Their application envisages the conversion (rather than mineralization) of specific micropollutants (with high toxicity and low biodegradability) to less toxic and more susceptible species to conventional treatments. In this way, AOPs should be used as either pre- or post-treatments for microbiological processes, and thus allow a more favorable cost/benefit relation.

28.2.1 Photodegradation of Pharmaceuticals during Photochemical AOPs

The large number of compounds detected during the degradation of pharmaceuticals through photochemical AOPs demonstrates the complexity of the involved reactions and suggests several degradation routes that can result in multi step and interconnected pathways (Doll and Frimmel 2005). The identified primary subproducts are hydroxylated species, in general, such as hydroquinones and catechols. The secondary subproducts are short chain carboxylic acids (Doll and Frimell 2004; Sirés et al. 2007).

The subproducts of the diclofenac degradation during the photochemical AOPs have been extensively studied and can serve as a model for the general behavior of pharmaceuticals (Vogna et al. 2004; Pérez-Estrada et al. 2005; Calza et al. 2006; Hofmann et al. 2007). After reaction with HO·, diclofenac undergoes hydroxylation and rupture of the C–N bond, forming species like phenols, anilines, quinones and hydroquinones. The subsequent cleavage of the aromatic structures leads to the formation of low molecular mass aliphatic compounds like oxalic, formic and malonic acids. The identification of the accumulated chloride and ammonium in the medium corroborates this behavior. Some identified compounds are shown in Fig.28.3. Prolonged irradiation times and suitable conditions can provide complete substrate mineralization (Ravina et al. 2002; Doll and Frimmel 2004; Calza et al. 2006). However, it must borne in mind that this should not be the required target, under real conditions, where the treated water shows an extremely complex matrix and the constitutives have a high scavenge capacity of HO·.

The structural nature of the subproducts formed during the photochemical AOPs, in general, differ from that of the mother compound, in so far as it changes its toxicological, chemical and biodegradable profile (Arslan-Alaton and Gurses 2004; Doll and Frimmel 2005; Calza et al. 2006). Baran et al. (2006) verified that the photoproducts that were obtained by means of heterogeneous photocatalytic degradation of sulfonamides show greater biodegradability and lower toxicity than the mother compounds. The same behavior was observed by applying photo-Fenton-like oxidation ($Fe^{3+}/H_2O_2/UV$) to the decomposition of procaine penicillin G formulation (Arslan-Alaton and Gurses 2004). It is very common that in some cases, a suitable period of treatment must be achieved in order to obtain satisfactory results. Inhibition effects on bacteria *Vibrio fischeri* lu-

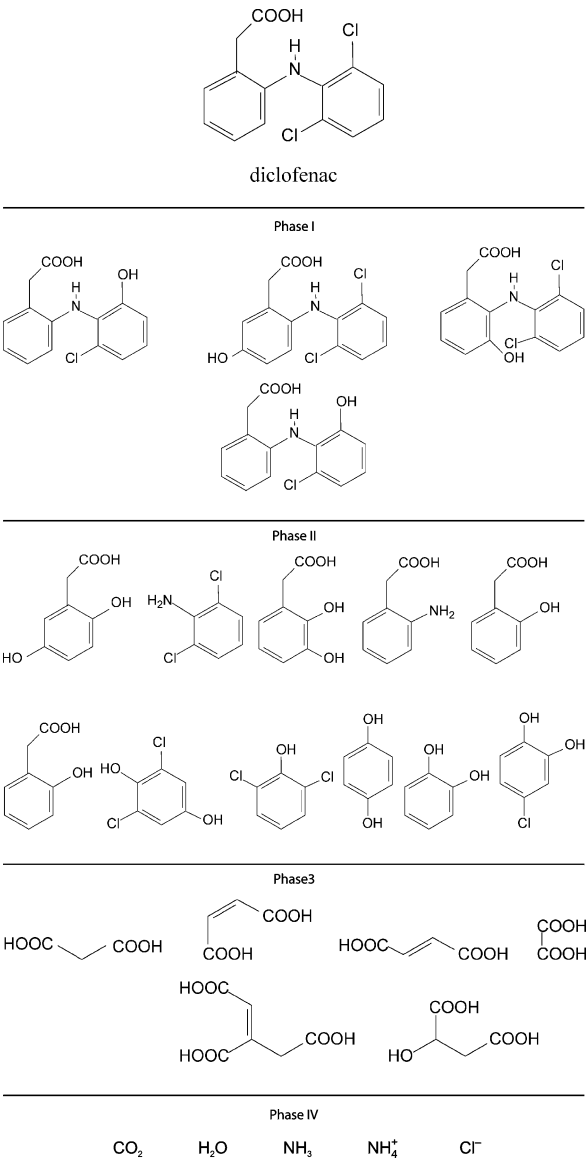

Fig. 28.3. Degradation of diclofenac

minescence suggest that some diclofenac degradation subproducts formed during the photochemical AOPs show more accentuated toxicity than the parent compound (Calza et al. 2006). However, prolonged irradiation times tend to bring toxicity down to zero. These subproducts were mentioned as possible chlorderivatives. The ability of the photochemical AOPs to degrade the target compounds as well as the byproducts has already been reported in the literature (Doll and Frimmel 2004). As mentioned above, the knowledge of the final characteristics of the treated effluent is of fundamental impor-

tance to guarantee the projected levels of destruction for specific micropollutants, reduction of toxicity and increase of biodegradability.

28.2.2 Costs Related to the Use of Photoprocesses

The question of costs of the photoprocesses is critical, and their practical application depends on factors like the nature and concentration of the substrate, the composition of the water to be treated, the reactor design and the desired objectives of the treatment. Studies have already been carried out on the influence of variables of the processes in the degradation of pharmaceuticals (Ravina et al. 2002; Arslan-Alaton and Gurses 2004; Calza et al. 2006). In recent years, there has been a research challenge involving AOPs to create systems that provide a cost/benefit ratio that can allow the use of this kind of technology on a large scale. Thus, several companies have already developed systems using AOPs for varied applications, not only for water and effluent treatment, but also for soils and air.

The potential use of solar radiation by photochemical AOPs offers an excellent alternative for the reduction of operational costs. Efforts have been made in order to try to develop photoreactors which can maximize the use of solar energy (Malato et al. 1999; Krutzler et al. 1999; Augugliano et al. 1999), and the current challenge is how to minimize problems related to the transfer of the processes from the bench and pilot to full scale. Studies involving degradation of pharmaceuticals, based on solar energy, by means of simulating lamps as well as solar energy itself, are now being undertaken and show a great potential (Doll and Frimmel 2004; Pérez-Estrada et al. 2005; Bautitz et al. 2007; Augugliaro et al. 2005).

28.3 Conclusions

The use of technologies based on photoprocesses, especially, photochemical AOPs, has proved to be an excellent tool for the decontamination of waters and effluents that contain micropollutants in general, and, especially, pharmaceuticals. However, despite the large number of studies carried out in this field, a number of knowledge gaps still have to be filled. A much greater understanding is required of the reactions involved by the treatment, chemical and ecotoxicological characteristics, and degree of biodegradability of the formed byproducts. Advances in reactor technology and photocatalysts and related areas can ensure this kind of technology will be more extensively employed in the future, although this will still involve high operational costs. In this way, the use of photoprocesses based on solar radiation, or the combination with other technologies, in particular, to the microbiological treatment, can both be regarded as promising alternatives to reduce costs and thus make the full-scale use of these processes more widespread.

Although there remains a long way to go, there are already plants in operation based on photoprocesses and a considerable number of commercial systems for the treatment of waters, air and soils available on the market. This is a very clear indication that photochemical processes are in effect tools of great potential for the degradation of pharmaceuticals in effluents.

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Pharmaceuticals and Environment: Role of Community Pharmacies

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29.1 Introduction

Over the last several years, many new medications have been launched on the market and more medications have come to be consumed worldwide. At the same time, the importance of minimizing their impact on the environment has become more obvious. In fact, human and environmental health are potentially affected by each step from the production of medicines to their utilization. In this context, what are the duties of the community pharmacist?

In this chapter, the magnitude of the problem of unwanted medicines and the role of community pharmacies in the waste disposal process will be discussed. This will include the collection of medicines, the education of patients regarding this collection and the appropriate use of medicines, as well as the prevention of hoarding and prescription of unwanted medicines.

29.2 Unwanted Medicines

Among the many reasons medicines may become unwanted are

- Death of the patient;
- Change in prescription;
- Poor adherence to medication;
- Poor adaptation of packaging size;
- Repeat filling of prescriptions without assessing the amount on hand.

Patients often stock medicines for short or long periods of time in their medicine cabinets and eventually discard them before or after their expiration date. However, keeping unused medicines at home or discarding them in an insecure fashion can increase the risks of misuse, which may lead to poisoning and suicides.

In addition, the improper disposal of unwanted medicines represents a source of pharmaceutical pollution. The way people choose to dispose of their unwanted medicines is influenced by the household hazardous products management policy in their locality. The habits in disposing of medicines identified in three studies (Kuspis and Krenzelok 1996; Slack et al. 2005; Abahussain and Ball 2007) are presented in Table 29.1. Although their settings are not comparable, all three studies show that returning medicines to pharmacies is clearly not the first option used. It should be noted that in the area where the second study was conducted, there were campaigns promoting the re-

Table 29.1. Disposal habits for unwanted medicines

	Kupsis and Krenzelok (1996)	Slack et al. (2005)	Abahussain and Ball (2007)
Number of respondents	500	400	200
Returned at pharmacy	1.4%	~24%	Included in "others"
Thrown in the trash	54.0%	~43%	97%
Flushed down the sewages	35.4%	~29%	1%
Others	9.2%	~4%	2%

turn of unwanted medicines to pharmacies. According to another recent study carried out in an outpatient pharmacy in the USA (Seehusen and Edwards 2006), more than 35% of patients believe that it is acceptable to flush medicines down the toilet and 21% believe it is acceptable to rinse them down the sink.

Most studies on unwanted medicines are based on what is returned to pharmacies, and hence provide a limited view of the topic. Even if the studies are performed in different settings, they provide an estimate of the amounts and types of medicines wasted. More than two-thirds of returned medicines, mostly capsules and tablets, are prescription drugs; the remainder consists of OTC products and a few samples (Garey et al. 2004; Isacson and Olofsson 1999). The proportion of each drug class returned is similar for classes of prescribed medications (Garey et al. 2004), although chronic treatment medications, in particular drugs to treat cardiovascular, respiratory and central nervous system disorders, are more often returned than those for acute treatments (Langley et al. 2005). Most returned drug classes, however, are not necessarily those that cost the most. Numerous studies have found that 20% to 53% of returned medicines were unopened, with many of the remainder being almost complete (Bronder and Klimpel 2001; Ekedahl 2006; Mackridge and Marriott 2007).

These figures illustrate the difficulties encountered by patients in managing their medications. In a Swedish study conducted by Ekedahl (2006), 19% of returned packs were current treatments that had passed their expiration date. This study also found that 3% of patients returned 24% of all returned medicines, with each patient returning twenty-three or more different packs. The majority of patients who returned their medications to their pharmacy were sixty-five years of age or older. A cross-sectional study amongst this population estimated that drug wastage accounted for 2.3% of all drug costs (Morgan 2001). These results confirm that elderly people should be the principal targets for patient empowerment and medication review. It is also important to note that about 30% of the packs were returned from deceased patients (Ekedahl 2006). Treatment at the end of a patient's life is tricky to manage, and many changes can occur. For these patients, a nurse or pharmacist using a weekly delivery system may better manage medication use.

Published studies, however, do not provide a comprehensive overview of the determinants of medicine returns. Patients often mention that they returned medicines to the pharmacy because they were cleaning out their medicine cabinets or the medicines were out of date. Only the reasons medicines remained unused can help in un-

derstanding and preventing wastage. In addition, when a patient returns several medicines, there may be different reasons for returning each one, a factor often not considered in studies. Furthermore, the person who returns medicines to the pharmacy may not be the person for whom these medicines were prescribed. Little is known about unused medicines directly collected from patients' homes, with only a fraction brought back to pharmacies.

The economic value of wasted medications has also been studied. In each country where studies have been performed, the wastage represents tens of millions of euros, and even this amount is probably an underestimate because these figures represent only medicines returned to pharmacies (Bronder and Klimpel 2001; Ekedahl 2006; Isacson and Olofsson 1999). In addition to the costs of the unused drugs, their economic value also includes the time needed to prescribe and dispense these medications. Furthermore, poor adherence leads to increased health care costs through additional hospital admissions, doctor visits, tests and supplementary prescriptions. Finally, the cost of destruction by high temperature incineration further adds to the financial burden.

Another important factor to consider is the impact of drug reimbursement plans. Some countries have economic incentives to discourage patients from obtaining drugs they will never use. In fact, a German study, consisting of two periods of evaluation in the same setting separated by a ten-year interval, showed that the quantity of wasted prescribed medicines was not reduced by higher charges (Bronder and Klimpel 2001).

29.3 Collection of Unwanted Medicines in Community Pharmacies

The distribution network used in reverse is the most effective solution to the problem of disposing of unwanted medicines, thus minimizing the negative effects on the environment and promoting public safety. Hence, in many countries, community pharmacies often are the principal collection points. The wholesaler plays the role of the waste carrier from the pharmacies to the nearest hazardous waste disposal facility. There, the medicines are incinerated at high temperatures by a regulated incinerator. Pharmaceutical industries also have a responsibility, which is why they often pay for part of the disposal costs.

Community pharmacies are an ideal location to establish collection programs. By definition, they are accessible places, visited by the consumers of medicines. The pharmacy staff is qualified to properly handle medicinal waste, and appropriate and safe storage areas, using special containers, are available. To ensure the safety of the staff, handling of medicines should be minimized and performed using protective equipment according to proper procedures. Pharmacists should be aware that a returned bag of mixed medicines may contain unexpected products, including mercury, iodized products, pesticides, cytotoxic products, sharps, needles and so on. These products must be identified by opening the bag or emptying it in front of the patient. Each must then be disposed of separately from ordinary solid forms using the proper precautions.

Generally, this return service is free of charge for customers but not for pharmacies. Indirect costs of sorting and storage, working time and infrastructure are not compensated. In some cases, pharmacists even have to assume the elimination ex-

penses. Moreover, pharmacies often dispose of medicines that customers obtained from other sales channels. On the other hand, this service can generate positive advertising for pharmacies, because customers are generally pleased to have the ability to safely discard their unused drugs. However, public and health authorities should better recognize the added value of this pharmaceutical service.

Over the past twenty years, various waste medicine disposal programs have been set up in many countries around the world. In Europe, pharmaceutical return programs generally encourage residents to bring unwanted medicines to pharmacies. In the UK, for example, the disposal of unwanted medicines has constituted essential service n° 3 of the NHS community pharmacy contract. The whole process is regulated, in particular the conditions of sorting and storage for each kind of pharmaceutical product (*www.psnc.org.uk/index.php?type=more_news&id=1572*, October 2007). In France, a reverse distribution network called Cyclamed has operated for several years. It is currently being completely reorganized, due to the application of a recent law preventing drug donation (*www.unpf.org/cyclamed/index.htm*, October 2007). In Portugal, the return medicine disposal network called Valormed was created in 2001 by pharmacists, pharmaceutical companies and wholesalers' associations, and nearly all Portuguese pharmacies are members of this network (*www.valormed.pt*, October 2007). In Switzerland, there is no nationally harmonized system, but pharmacies generally accept unwanted medicines for free. In some areas, however, Swiss pharmacists have to pay for incineration (Dommer Schwaller 2004). In Germany, a private service provider, mandated by 75% of community pharmacies, collects and destroys expired medicines in addition to the collection of packaging. Waste is sorted by pharmacists into three categories: primary packing, packages and leaflets, and unused medicines (*www.ctm.at/vfw/index.html*, November 2007).

In Australia, the National Return and Disposal of Unwanted Medicines Project, launched in 1998, is financed by the government and the pharmaceutical industry. In addition, pharmaceutical wholesalers have agreed to discount charges for the delivery and collection of containers from pharmacies. Protocols for returning unwanted medicines have been written for each step of the disposal. Furthermore, the program promotes consumer awareness (*www.ctm.at/vfw/index.html*, November 2007).

In the USA, there are many different return programs available to the public, including ongoing collection at pharmacies or household waste facilities, single-day collection events, mail-back programs and public education. Nevertheless, it is important to note that some states have laws preventing pharmacies from accepting returned medications. Because no regulations are in place at the national level, guidelines for disposal were issued by the American Pharmaceutical Association and the White House encouraging the public to dispose of unused drugs through collection programs, if available. In areas where no such program was available, they recommended taking unwanted medicines out of their original containers, mixing them with an undesirable substance and putting them in opaque containers that are thrown in the trash just before the next garbage collection (*www.returnmed.com.au*, October 2007).

In Canada, the pharmaceutical industry voluntarily established the Medications Return Program. Although this program has been implemented on a national level, each province has taken a slightly different approach. With the support of industry, volunteer pharmacists have established a collaborative program to return and dispose of unwanted medicines, inform patients about this service and maintain return sta-

tistics (*www.whitehousedrugpolicy.gov/drugfact/factsht/proper%5Fdisposal.html*, October 2007).

29.4 Patient Information and Drug Donation Issue

Patients need clear guidance on how to dispose appropriately of their unwanted medicines. The potential dangers in retaining unusable medicines at home and the ecologic consequences of using wrong disposal routes must also be communicated to the public; a call to avoid the oversupply and hoarding of medicines is needed as well.

In countries with national disposal systems, funds are often allocated to consumer awareness campaigns, using TV advertisements, posters, newspaper and magazine articles, websites, and brochures. Every time a patient comes to the pharmacy, however, the pharmacy staff should consider using the opportunity to explain to the patient what to do with remaining medicines. In a recent study, previous counseling about disposal was positively associated with increased return rates of medicines to pharmacies or other care providers (Seehusen and Edwards 2006).

To prevent people from donating drugs, the dangers of this practice must also be communicated to the public. Drug donations are considered controversial. Obviously, returned medicines should not be recycled, on safety and ethical grounds, because it is not possible to guarantee that they were stored under appropriate conditions. On the other hand, however, many people cannot afford to buy medicines they need, and some medicines are simply not available in some areas.

Although donors have good intentions, many do not realize the possible unintended consequences to people who receive these medications. They often have the mistaken belief that in acute emergency situations, any type of drug is better than none at all. Many problems related to donated drugs are due to a lack of communication between the donor and the recipient. For example, these medicines may not be appropriate to the disease pattern of the recipient, not be labeled in the local language or in English or be unfamiliar to local healthcare practitioners. In addition, the quantities of drugs may be too large relative to the expiration date. Thus considerable amounts of donated medicines are not usable and must be disposed of at the emergency area. When shipment charges and customs taxes are included, drug donation is clearly not a cost-effective solution to disposing of unwanted medicines. For example, following the Southeast Asia Tsunami in 2004, 4 000 tons of medicines were received for fewer than two million people, according to figures published by Pharmaciens Sans Frontières (*www.medicationsreturn.ca*, October 2007). These medicines were labeled in more than sixteen foreign languages. Of the amount donated, 600 tons have been identified for destruction, which will cost an estimated 2.4 million Euros.

To coordinate drug donation practices, guidelines were established in 1999 by the World Health Organization, according statements of the Federation International of Pharmacists adopted in 1997 (*www.psfci.org/new/fr/Medias/synthese.pdf*, October 2007; *www.euro.who.int/document/EHA/PAR_Donate_Guidelines.pdf*, October 2007). Several countries have enacted their own laws, often based on these guidelines. Pharmaciens Sans Frontières has applied these guidelines since 1999 for acute emergencies and focused their actions on development aid in non-emergency situations (*www.fip.org/www2/uploads/database_file.php?id=196&table_id=*, October 2007).

29.5 Prevention of the Waste of Medicines

Visits to the pharmacy can provide opportunities for educating patients about their medicines, including preventing the delivery of medicines that will not be consumed. It is important to determine the effectiveness and tolerability of medications for each patient before prescribing full quantities. Indeed, the supply of test quantities during therapy changes and initiation is an effective practice and, according to a Canadian survey, has been accepted by patients (Paterson and Anderson 2002).

Specific actions can also be organized, such as "brown-bag events" (*www.psfci.org/new/indexuk.htm*, October 2007), in which patients are encouraged to gather all their prescriptions and over-the-counter medications and supplements and return them to their pharmacist. This type of event enables pharmacists to identify possible problems, including improper dosing, lapsed expiration dates, drug mismatches, compliance issues, duplicate medications and poorly stored medications. The pharmacist can then follow up by counseling the patient on appropriate medication usage and, when necessary, can refer the patient to his physician. Implementation of regular, timely medication review, including patient interviews, may contribute to the reduction of drug wastage. This is especially true for the elderly population, which generates a large quantity of unused medicines (Ekedahl 2006; Morgan 2001). Promotion of therapeutic adherence and good prescription practices can also have an indirect effect on preventing drug wastage.

The systems by which patients request prescription refills have been found to contribute to wastage of medicine. Among the measures that may reduce this problem are setting maximum dispensed dosages per year and not allowing the next refill until two-thirds of the anticipated treatment period delivered has passed, even if, as in Sweden, this last measure does not prevent a minority of patients from hoarding medicines by obtaining several prescriptions for the same drug (Ekedahl 2006). In all cases, however, it is important to make patients aware that once the medicine is delivered, it should not be reused, even if the package or the vial is unopened and not expired, and it should not be donated to patients in a developing country.

Better coordination between health care professionals during the hospital discharge of a patient should lead to better outpatient care, including the management of medications. This will be clinically beneficial to the patient and enhance the rational use of medicines.

29.6 Conclusion

The best way to keep the environment free of the impact of unwanted medicines is to advise customers to bring back their unused medicines to the pharmacy, which can then dispose of them properly. The community pharmacist, however, should also be involved upstream of the waste disposal problem. The pharmacist should promote good prescription practices, optimal dispensation, drug use and patient adherence. In addition, every returned prescription medicine represents wasted health care expenditure. Careful analysis of medicines not taken by patients may provide important clues about ways to decrease waste in health care funds.

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Mitigation of the Pharmaceutical Outlet into the Environment – Experiences from Sweden

B. Gunnarsson · Å. Wennmalm

30.1 Background

In 2001, two major actors in the Swedish health care system – Apoteket AB and Stockholm County Council (SCC) – independently identified the outlet of human medicines or breakdown products thereof as a potential hazard to the environment. Apoteket, public owner of all pharmacy shops in Sweden, arranged a national conference on pharmaceuticals and the environment with leading international speakers in May, 2001. SCC, the provider of all public health care in the greater Stockholm region, had identified pharmaceutical leakage into the environment as one of its five most important environmental aspects. The conference was the starting point for the subsequent environmental collaboration between Apoteket and SCC. This cooperation has involved contacts with universities and other research organizations, EU bodies as well as national governmental authorities, international drug companies, NGOs, doctors and other health care staff, water authorities, analytical laboratories, and media.

The guiding principle in this work has been to protect freshwater, our most important ingested provision, from pollution with medicines and their metabolites. At the same time, it has been explicitly understood that pharmaceuticals exert an indispensable factor in modern healthcare and that the general public should have access to the best available medicines. The challenge has been, and will continue to be, to establish pharmaceutical management that combines the best possible treatment of the patient with a sustainable environmental cautiousness.

30.2 EU and National Authorities

30.2.1 The New EU Directives for Pharmaceuticals

In November 2001, the European Commission launched proposals to change the directives on the evaluation and authorization of human and veterinary medicinal products within the community. The responsible directorate behind the proposals in the Commission was DG Enterprise. The proposals contained very few, if any, measures to guarantee protection of the environment. The DG Environment, which participated in the Commission's adoption of the new proposals, had obviously not realized their environmental potential. The proposals should, according to the rules of codecision in the EU, be processed twice in the Parliament and twice in the Council before being adopted.

Apoteket and SCC decided to lobby in the European Parliament for inclusion of more environmental caution in the proposals. The response from the Parliament was positive, and in the amendments sent to the Council and Commission in early 2003 all the suggestions for environmental improvements put forward by Apoteket/SCC were included. Unfortunately, the Council, in its decision from June 2003, turned down all the amendments for environmental improvement from the Parliament.

The second reading of the Commission proposals in Parliament occurred during the fall of 2003. During the second reading, Parliament agreed on the same environmental amendments as in the first reading. After an intensive COREPER (an EU body preparing documents from Parliament to Council) process in November between Parliament and Council, it was eventually agreed that a major part of the environmental amendments should be included and on top of that also some additional environmental measures not suggested by Parliament. The final text of the new legislation was taken on March 31, 2004, thereby being operative in all EU member countries.

The environmental paragraphs included in the final legislation were as follows:

1. In the introduction to the new directive (2004/27/EC), a new rectical was included: "*The environmental impact should be assessed and, on a case-by-case basis, specific arrangements to limit it should be envisaged. In any event this impact should not constitute a criterion for refusal of a marketing authorisation.*"
2. In Article 1, point 28, definitions of risk and risk-benefit balance connected to the use of a medicinal product were included: "*Risks related to use of the medicinal product: – any risk relating to the quality, safety or efficacy of the medicinal product as regards patients' health or public health; – any risk of undesirable effects on the environment; Risk-benefit balance: An evaluation of the positive therapeutic effects of the medicinal product in relation to the risks as defined in point 28, first indent.*"
3. In Article 8(3), a point on compulsory documentation in the authorization application was inserted: "*Evaluation of the potential environmental risks posed by the medicinal product. This impact shall be assessed and, on a case-by-case basis, specific arrangements to limit it shall be envisaged.*"
4. In Article 54, point (j), prescribing compulsory warning text on the medicine package was replaced with the following: "*Specific precautions relating to the disposal of unused medicinal products or waste derived from medicinal products, where appropriate, as well as reference to any appropriate collection system in place.*"
5. Finally, the following new rule (Article 127b) was introduced: "*Member States shall ensure that appropriate collection systems are in place for medicinal products that are unused or have expired.*"

According to EU legislation the new directive should be incorporated into the national legislation of the member states by the end of October 2005.

The main environmental achievements in the new legislation were that

a The environmental impact of pharmaceuticals was officially recognized;
b More detailed risk assessments were required; and
c Disposal of unused medicinal products was highlighted in the instructions on the package for medicine consumers, and member states were required to establish collection systems for unused medicines.

30.2.2 Initiatives by the Swedish Government

In December 2002, the Swedish Government commissioned an official report from the Medical Products Agency (MPA) on the environmental effects of pharmaceuticals and related products. The report should include a risk assessment of environmental effects based on the occurrence of the products in the environment. A study of the possibilities to introduce environmental classification of pharmaceutical products was also part of the commission.

In their report presented in August 2004, the MPA proposed a number of measures to be taken to increase the knowledge of environmental risk connected to the use of pharmaceuticals, and how to use such knowledge to mitigate this risk (Medical Products Agency Sweden 2004). As to the question of environmental classification of pharmaceuticals, the MPA considered that the most suitable approach would be to work actively at the EU level. The agency also noted that while awaiting a possible EU system for environmental classification, a voluntary national system might be introduced. Reluctance was, however, expressed as to the benefit of such a national system due to the lack of proper scientific data.

Shortly after the presentation of the MPA report, the Swedish Minister of the Environment invited a number of stakeholders to a hearing aimed at identifying a possible pathway to improve environmental cautiousness in the management of medicines in the health care system. As a result of this hearing, the Swedish Association of the Pharmaceutical Industry (LIF) invited the MPA, Apoteket, SCC and the Swedish Federation of Local Authorities and Regions to jointly develop a system of classification of pharmaceuticals for human use. To achieve acceptance of the system among the international drug companies, LIF established an international reference group who followed and interacted with the Swedish working group. In June of 2005, agreement was reached within the working group on a first version of the system. Since then, some modifications of the system have been adopted, but the general principles have been maintained.

30.3 Assessment of Environmental Hazard and Risk of Pharmaceuticals

30.3.1 Environmental Classification of Drugs

The idea behind a classification system was to allow more environmentally friendly drug management decisions on several levels: (*i*) the drug producer in the developing process of new drugs, (*ii*) the doctor when prescribing drugs, and (*iii*) the patient/customer when buying and using drugs.

Various methods are available to evaluate the environmental impact of chemical products in general. These methods can be also applied to human medicines. Two different means of evaluation are available: a *hazard* identification and a *risk* assessment.

In the hazard identification, two inherent chemical and physical properties of the substance, i.e., its persistence and its potential to bioaccumulation, are identified. On the basis of these data, together with information about the ecotoxicological properties of the substance, the degree of inherent danger posed by the substance is expressed.

In the risk assessment, a model is applied that compares the theoretical concentration of the substance in the aquatic environment (its PEC or Predicted Environmental Concentration) to the highest concentration that can be considered harmless (the PNEC or Predicted No Effect Concentration).

30.3.2 Hazard Assessment of Drugs

There is currently no official standard for how drugs should be classified or undergo a hazard assessment from an environmental standpoint, either internationally or nationally. The SCC and Apoteket AB, following consultation with the Swedish Chemicals Inspectorate and other ecotoxicology experts, have produced a working model (the "Stockholm model") for the hazard classification of drugs, which is described below. By using and developing this model, it may become a refined instrument for hazard classification. As such, it will provide prescribers, patients and other interested parties valuable environmental information. Furthermore, it may encourage drug manufacturers to develop future drugs with less environmental impact.

At the initiative of LIF and in collaboration with MPA, the Swedish Association of Local Authorities and Regions, Apoteket and SCC, the model has been developed in regard to a number of important points. The hope is that the developed model can be established as a Swedish standard ("the Swedish model") and also be used internationally.

30.3.3 Risk Assessment of Drugs

The model described below is, in principle, similar to that described in the EMEA guidelines for the risk assessment of drugs (CHMP 2006). The assessment relates to the aquatic environment and is carried out in three stages:

30.3.3.1 *Exposure Assessment – Calculation of PEC*

The sales of the substance in a particular region are calculated as the mass amount of the active substance (AS). In the case of drugs in Sweden, this information is available in statistics from Apoteket AB. The entire sold quantity of AS in the product is assumed to be consumed, excreted and diluted in sewage (using a default value of 200 l person^{-1} day^{-1}) and the effluent from the treatment plant to the receiving body of water is assumed to bring about further dilution (using a default value of 10).

If the calculated concentration does not exceed 0.01 µg l^{-1} and if there are no special reasons for assuming that the substance produces a negative environmental impact, the environmental risk is considered to be so small that no further investigation is required.

30.3.3.2 *Effect Assessment – Calculation of PNEC*

The NOEC (No Observed Effect Concentration) is the highest concentration of the active substance, which in the aquatic environment has been shown not to have any

adverse effect on any of three aquatic organisms: normally a fish, *Daphnia magna* and algae. If these organisms differ in their sensitivity to the active substance, the most sensitive species is chosen in the evaluation. An assessment factor is introduced to compensate for the fact that (*i*) the NOEC is likely to be higher in acute tests than in chronic exposure, (*ii*) that there may be differences in sensitivity among different populations of the organisms tested, (*iii*) that higher sensitivity may be found in species not tested, and (iv) that laboratory tests can give different results from those found in nature. The assessment factor, depending on the quality of the data available, test conditions etc., is set at a value of between 1 and 10 000. In the effect assessment of drugs, a common value for the assessment factor is 100. This means that the highest concentration of the active substance that can be assumed to have any effect on aquatic organisms is

$$PNEC = NOEC / 100$$

30.3.3.3
Risk Assessment through an Overall Appraisal of the Exposure and Effect Assessments.

If the exposure assessment (see also above) shows that PEC > 0.01 $\mu g\, l^{-1}$, or if special conditions prevail, a risk assessment is made by calculating the ratio PEC/PNEC. If the ratio >1 (i.e., if PEC is greater than PNEC), adverse effects from the active substance may be expected in the aquatic environment, whereas if the ratio <1 (i.e., if PEC is smaller than PNEC), adverse effects from the active substance should theoretically not be expected in the aquatic environment.

This type of rather rough assessment can only be used to give an approximate idea of the risk of adverse environmental effects. The method contains several approximations which both over- and underestimate the risk of an adverse environmental impact.

30.3.4
Risk or Hazard Assessment as a Basis for Environmental Classification?

A risk assessment of a drug or a chemical, as can be seen from above, is often associated with several uncertainty factors. Both the PEC and the PNEC are based on approximations and assumptions, and these quantities vary in value geographically and over time. The substance may turn out to be used in entirely different amounts than assumed and several manufacturers of the same substance may enter the market, which means that an overall assessment in all likelihood is required.

The basis for a hazard assessment is knowledge of the inherent adverse properties of the substance, i.e., how dangerous it is. It is therefore important that these properties (which determine whether it is environmentally hazardous) are determined with as much accuracy as is reasonable. The hazard assessment is independent of exposure, the amount of drug sold by different manufacturers, water flows, etc. On the basis of how dangerous they are, drugs (in common with other chemicals) are placed in a hazard class.

The hazard assessment and risk assessment cannot replace one another, but complement one another well.

30.3.5 "The Stockholm Model"

30.3.5.1 *Assessment Criteria*

Inherent environmental hazard is assessed on the basis of the criteria (*i*) biodegradability, (*ii*) potential to bioaccumulation and (*iii*) toxicity to aquatic organisms, as follows.

Biodegradability is assessed according to the OECD's test guidelines or other equivalent degradation tests.

Potential to bioaccumulation is assessed from the *n*-octanol/water partition coefficient, P_{ow}, where substances with $\log P_{ow} > 3$ are judged to be potentially bio-accumulating. If actual data on bioaccumulation in the fatty tissue of a test organism are available they are preferable.

Toxicity to aquatic organisms is assessed from ecotoxicological basic data comprising the three trophic levels fish, *Daphnia* (crustaceans) and algae. The tests used are

- Acute toxicity test of fish: A short-term test primarily aimed at determining the LC_{50} or lethal concentration, the test concentration at which fifty per cent of the fish are expected to die after ninety-six hours of exposure;
- Acute toxicity test of *Daphnia* sp.: A short-term test aimed at determining the EC_{50} or effect concentration, the test concentration at which fifty per cent of the test animals are expected to become immobilized after twenty-four or forty-eight hours of exposure;
- Growth inhibition test of algae: A short-term test aimed at determining the IC_{50} or inhibition concentration, the test concentration which is expected to cause fifty per cent inhibition of growth or rate of growth of the algae after seventy hours of exposure.

If the three species tested differ in the sensitivity they show to the test substance, the value for the most sensitive organism is used in the assessment. The toxicity is divided into four categories:

- $LC/EC/IC_{50} < 1\ mg\ l^{-1}$ very high toxicity;
- $LC/EC/IC_{50}$ 1–10 $mg\ l^{-1}$ high toxicity;
- $LC/EC/IC_{50}$ 10–100 $mg\ l^{-1}$ moderate toxicity;
- $LC/EC/IC_{50} > 100\ mg\ l^{-1}$ low toxicity.

30.3.5.2 *Overall Appraisal and Evaluation*

Weighting of the three assessment criteria mentioned above takes place as follows:

i for biodegradability:
  – Readily biodegradable 0
  – Not readily biodegradable 3

ii for potential to bioaccumulation:
- Yes 3
- No 0

ii for toxicity:
- Very high toxicity 3
- High toxicity 2
- Moderate toxicity 1
- Low toxicity 0

Overall appraisal implies that the sum of the weights for a drug's biodegradability (0 or 3), its potential for bioaccumulation (0 or 3) and toxicity (0–3) are added. A drug that is readily biodegradable, lacks bioaccumulation potential and is of low toxicity thus receives the total value zero (0+0+0), while a drug that is not readily biodegradable, is potentially bioaccumulating and is of high toxicity receives the value 9 (3+3+3). The total weighted value should be regarded as an indication of the inherent environmental hazard of the active substance and can point to substances that may be of interest to study further from the standpoint of environmental hazards.

The above classification model does not take into account the drug metabolites, which may be more or less environmentally hazardous than the parent substance. Furthermore, the classification is based on acute effects, i.e., it does not take into account long-term exposure of aquatic organisms to low concentrations. For additional information about the model, see Wennmalm and Gunnarsson (2005). The results of the classification of about 350 active drug substances can be found on the Stockholm County Council's website for pharmaceutical information (*www.janusinfo.se*).

30.3.6 "The Swedish Model"

This classification system differs in two important respects from the Stockholm model. Firstly, it is based on a combination of risk and hazard assessments. Secondly, assessments are presented at three different target group levels, directed towards patients, prescribers and specialists, respectively. The option of assessing drug metabolites is also introduced, together with PBT (Persistent, Bioaccumulative and Toxic) and vPvB (very Persistent and very Bioaccumulative) assessments.

30.3.6.1 *Patient Level*

Environmental information is given through a single verbal risk assessment consisting of one sentence, as follows:

- If PEC/PNEC < 0.1,
  - Use of the medicine has been considered to result in insignificant environmental risk.
- If 0.1 < PEC/PNEC < 1,
  - Use of the medicine has been considered to result in low environmental risk.

- If 1 < PEC/PNEC < 10,
  - Use of the medicine has been considered to result in moderate environmental risk.
- If PEC/PNEC > 10,
  - Use of the medicine has been considered to result in high environmental risk.
- If there is not sufficient data to calculate the PEC/PNEC, either of the following two statements will be used:
  - Risk of an environmental impact cannot be excluded since no ecotoxicity data are available; *or*
  - Risk of the environmental impact cannot be excluded; however some ecotoxicity data are available.
- If PEC/PNEC < 1 but the drug meets the EU criteria for PBT,
  - Hazardous environmental properties.

30.3.6.2 *Prescriber Level*

Environmental risk information presented at the patient level is repeated here. Details are also given of the PBT classification for the active substance of the drug.

- Degradation:
  - The medicine is degraded in the environment *or*
  - The medicine is slowly degraded in the environment *or*
  - The medicine is potentially persistent
- Bioaccumulation:
  - No significant bioaccumulation potential *or*
  - Potential to bioaccumulate in aquatic organisms
- If the drug fulfils the criteria for PBT and/or vPvB
  - According to the established EU criteria, the compound should be regarded as a PBT/vPvB substance.
- If there is insufficient data to characterize the potential for degradation, the following statement will be used:
  - The potential for persistence cannot be excluded due to lack of data.
- If there is insufficient data to characterize the potential for bioaccumulation, the following statement will be used:
  - The potential for bioaccumulation cannot be excluded due to lack of data.

30.3.6.3 *Specialist Level*

This level presents available environmental data for the product in detail, e.g.:

- Results from ecotoxicological tests;
- Results from degradation tests;
- Partition coefficient, e.g., octanol/water, or other suitable indicator of bioaccumulation;
- Information about the form in which the drug is excreted (as parent substance or metabolites, together with details of their relative proportions);

- Results of CMT test (carcinogens, mutagens, teratogens), together with details of the potential for endocrinal disturbance;
- Pharmacological activity of the metabolites;
- Total quantity (kg) of sales in Sweden of the active substance (to include all products with the same active substance). This information must relate to the most recently available data;
- Integrated interpretation of data from the risk and hazard assessments;
- Calculation of risk assessment.

Additional information on the system can be obtained from the LIF website (*www.fass.se*).

30.3.7 Comments on the Classification Systems

Drug residues released into the environment have not yet been shown to have any direct effects on people's health. Thus, the work done in Sweden on the environmental assessment and environmental classification of drugs can be considered preventive and in accordance with the precautionary principle.

The development of models for environmental risk and hazard assessments has revealed limitations in the existence of test data for the majority of drugs. These limitations must be eliminated. Furthermore, the risk assessment should preferably be based on chronic toxicity data instead of acute data. Unfortunately, most data available today are acute. In addition, the pharmacological effect of the drug in the test organisms should be assessed instead of its ecotoxic, i.e., lethal, effects. At present non-lethal pharmacological effects of the drug in aquatic organisms are not included in the risk assessment. This is an obvious drawback. Greater attention to excreted metabolites of the drug would also be desirable. To make this possible, the pharmacological and ecotoxicological effects of the excretion products must be determined.

In view of these comments, it becomes evident that extremely thorough work is needed before adequate risk and hazard assessments can be made for the entire range of drugs. It is likely that more extensive tests will be carried out on new drugs in the future, while existing drugs will scarcely be the object of new and comprehensive evaluations. In this way, the natural turnover of the range of drugs will primarily serve as the basis for better environmental assessments in the future.

30.4 Return of Unused Drugs

Unused or expired drugs are a source of the pharmaceutical residues that often find their way into the soil and water. According to international agreements, drugs may not be recycled for reasons of safety. Apoteket therefore collects the public's drugs for destruction, and all pharmacies all over the country take part in this duty. In 2005 as well as in 2006, about 900 tons of discarded drugs, including packages, were sent for disposal in approved and safe incinerators.

Surveys reveal that about sixty-five percent of the population hands in leftover drugs at a pharmacy shop. About sixty-five percent of the packages returned to the pharmacy had at least 2/3 of the original contents remaining, and about forty percent were unbroken. About fifty percent of the unbroken packages had passed the expiration date. These findings indicate that the doctors may prescribe volumes of drugs that are too large, and the patient may buy too many packages. Apoteket has established informational activities directed towards prescribers as well as patients concerning the importance of prescribing, purchasing and using the proper volume of drugs.

Ideally there would be no leftover drugs. One way of reducing this quantity is to use a "starter package," with medication for no more than about one week, at the initiation of the treatment. If the patient does not tolerate the drug, only a small amount of it will be discarded. Apoteket therefore advocates more frequent use of starter packs. Follow-up surveys indicate a substantial increase in the proportion of starter packs in recent years. One contributing factor to this increase is the greater use of electronic (web-based) prescriptions, in which the system utilized automatically proposes starter packs.

30.5 Personal Care Products

Beside pharmaceuticals, Apoteket also distributes a substantial amount of personal care products related to drugs. These products may also contain environmentally hazardous substances. Apoteket makes an environmental classification of all substances into three colored classes:

- *Red substances* are classified as environmentally hazardous in accordance with OECD rules;
- *Yellow substances* have environmental properties that call for attention, regardless of the fact that they do not meet the criteria for environmentally hazardous substances;
- *Green substances* are environmentally acceptable substances.

As for drugs, characteristics as good function and safety are the most important factors also for personal care products. Therefore, yellow and red substances may have to be accepted in some products. For example, in sun protection products, the substance with the sun shielding effect is harmful to the environment. Since no environmentally friendly alternative is present, the product has to be used, despite its harmful effect. Preservatives are also examples of substances which have to be accepted despite their negative environmental effect.

Apoteket's color declaration may guide producers in their development of new products and consumers who prefer greener purchasing. At present (2007), five percent of Apoteket's assortment of personal care products contains red substances. The target is to reduce it to 2.5% by 2010.

30.6 Communication

Once the decision to approach the issue of pharmaceuticals in the environment had been taken by Apoteket and SCC, there was a need to consider how and to whom the

working plans and achievements should be communicated. Target groups needed to be identified, as well as the means to establish and maintain contact with them. The message needed to be specifically formulated to each target group; yet these messages were never supposed to be in conflict with each other.

The initial step by Apoteket/SCC in their communication was to increase the awareness of the general public about the fact that pharmaceuticals may pose a hazard to the environment. In doing so, it was necessary to clearly express whether the environmental hazard related to the outlet of pharmaceutical residues was assumed to be directed towards aquatic organisms and plants only, to the flora and fauna in general, or to human health. Analyses of the occurrence of pharmaceutical residues in drinking water and in surface water turned out to be helpful to verify the significance of the target problem. Observations on hermaphroditic fish supported the concept of environmental threat from drug residues. The main message was, however, the risk of polluting drinking water for future generations of humans.

Several successful attempts to put media on the pharma track were made in Sweden from 2002 to 2004, and may have contributed to the initiative from the producers to develop a classification system. Major TV news channels covered the topic in studio interviews several times. The first report on drug residues in Stockholm drinking water was top news in Sweden's largest morning paper in May 2005.

With the media increasingly reporting on the issue, health care staff have demonstrated an interest to learn more. Environmental coordinators in the regional hospitals arranged seminars and lectures on pharmacology, initially mostly for colleagues with a similar interest, but eventually also for other categories of hospital staff. Apoteket/SCC published in 2005 a small book about drugs and the environment to be used in order to increase the knowledge of all stakeholders. This book was also translated into English (Environment and Pharmaceuticals).

It turned out that doctors were the group most difficult to attract with this message, partly because of the time constraints they have in their daily work. The interest among doctors did however grow, in particular when the information was strictly scientific, and linked to firm advice on how to manage the problem with drug residues. Today, most of the pharmacological experts in the Swedish regional pharmaceutical committees who have the task to select the most appropriate medicines for different diseases have been informed and are taking action to reduce the emission of pharmaceutical residues.

In SCC, a running program informing at least 30% of all doctors annually about pharmacological cautiousness has been adopted. This information may be given in general meetings, seminars, workshops or personal meetings taking place in hospitals or in open care units. Other regions in Sweden are preparing to take on the same steps.

The environmental classification of pharmaceuticals described above is presented on the web (*www.janusinfo.se*; *www.fass.se*) and in a printed version; both versions are presented in Swedish as well as in English. The English web version has about 300 visits per month and the Swedish version about 1 000 visits per month (early 2007).

A growing international interest in the Swedish experiences on pharmacology is emerging. Representatives from Apoteket/SCC have reported about it in lectures given in Denmark, Germany, Italy, UK, Austria, Switzerland, Spain, United States and China.

30.7 Levels of Pharmaceuticals in Drinking Water and Surface Water in Stockholm

Follow-up of pharmaceutical levels in regional water sources was started in 2002, and has since then been conducted annually. The number of pharmaceuticals analyzed has increased from six antibiotics at the start to more than 70 substances from seven different ATC groups in 2007. During the period from 2002 to 2004, only outlet samples from sewage treatment plants and their recipients were taken, and from 2005 on, both drinking water and wastewater have been analyzed. Selected results are shown Table 30.1 (drinking water) and in Fig. 30.1 (outlet from sewage treatment plants).

Table 30.1. Levels of pharmaceuticals in three major water treatment plants (*N*, *L*, and *G*) in the Stockholm region. "*Inlet*" represents raw water from the source, and "*outlet*" represents water going to the customers (tap water)

Generic name	Plant N inlet	Plant L inlet	Plant G inlet	Plant N outlet	Plant L outlet	Plant G outlet
Citalopram	0.1	0.4	1.1	<0.1	0.3	<0.1
Etinyl estradiol	<0.3	0.7	<0.3	<0.3	<0.3	0.4
Metoprolol	1.0	1.0	1.0	0.8	0.8	0.3
Naproxen	0.9	0.8	0.9	0.4	0.3	0.6
Oxazepam	1.3	1.7	1.2	1.5	1.4	0.8
Propoxyfen	0.2	0.3	0.7	0.1	0.2	0.1
Trimetoprim	0.2	0.2	0.6	<0.1	0.1	<0.1

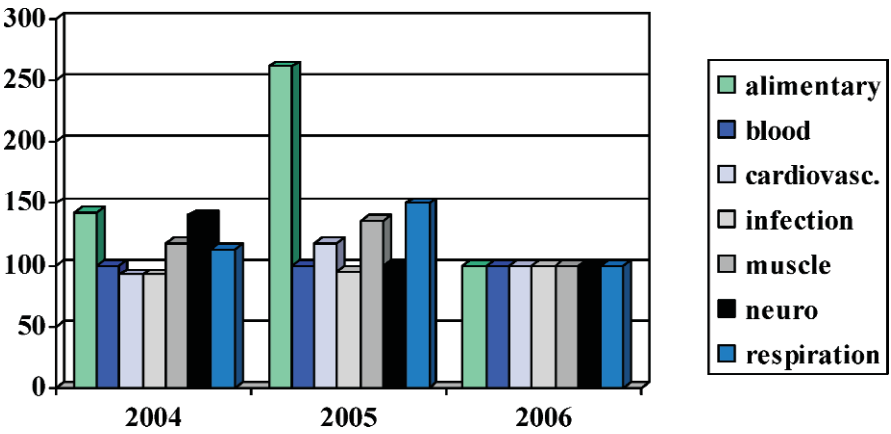

Fig. 30.1 Mean outlet of groups of pharmaceuticals from the three major sewage treatment plants in the Stockholm region for three consecutive years. Values are presented as a percentage of the outlet during the year 2006

In 2006 it was adopted as a target to lower the levels of environmentally disturbing pharmaceuticals in the drinking water and in the outlet from the sewage treatment plants by the end of 2011 in comparison to 2005. Whether this target is possible to reach only through improved pharmacological management in the health care system is still an open question. The levels of drugs in the outlets from the sewage treatment plants do not display any definite trend during the period 2002 to 2006 (see Fig. 30.1). It may well be necessary to install additional cleaning procedures (ozonization, UV treatment and active carbon filters) both in water and sewage treatment plants to fulfill this object. At present, only a few Swedish water treatment plants have carbon filters to eliminate natural organic material. No Swedish sewage treatment plant has installed equipment for permanent ozonization or UV treatment.

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Pharmaceutical Waste

S. Castensson

31.1 Introduction

Pharmaceuticals form a group of substances that are of considerable importance for society as healthcare tools. A variety of pharmaceuticals can now be detected in surface, ground, and drinking waters (Kümmerer 2004, 2008). This raises concerns about the potentially adverse environmental consequences of this contamination. The risk is directly proportional to the active concentration of the chemical substances in various environmental compartments, and pharmaceutical waste adds to that risk if it is not managed properly.

Pharmaceuticals are widely distributed, and there is a consistent global increase in the use of potent pharmaceuticals driven by drug development, an aging population in Western countries and the efforts to improve health in developing countries. Following this use is a corresponding increase in the generation of pharmaceutical waste. The reduction of waste generation and risk of any leakage of toxic drug substances into the environment from waste is an important task.

The waste and disposal problem starts with the production of the active pharmaceutical ingredient (API) and finishes with the final disposal of a pharmaceutical product. During the manufacture and use of pharmaceuticals, lots of materials become contaminated with an API increasing the waste volume.

Pharmaceutical waste is not only an environmental issue. Like other waste management, it is part of many peoples' working conditions in respect to how it is handled, contained and disposed of. When the material entails a serious hazard, it requires special handling to ensure safety. Where there are issues with higher risk products, e.g., controlled drugs, increased security in handling pharmaceutical waste is also required.

Proper pharmaceutical waste management is a new and highly complex frontier in environmental management. Occupational health and safety is a highly integrated issue in the management of certain pharmaceuticals like chemotherapeutics, and the waste generated from these involve a significant hazard. This chapter focuses on the handling and disposal problems of finished pharmaceutical products in the EU and USA, irrespective of being used or unused, and some related contamination problems.

31.2 Planning Waste Management

Pharmaceutical waste may be present in any of the common physical forms like solids, liquids and gases. The waste can be categorized in several ways, e.g., depending on source, physical state, hazard, security, handling and disposal.

31.2.1 Legislation

Management of pharmaceutical waste is especially challenging given the complexity of the governing regulations.

European Union

In the European Union (EU), a legislative framework (outlined in Fig. 31.1) forms the base for practice. Waste management planning is based on three directives that describe the obligations: the Directive on Waste (75/442/EEC), the Directive on Hazardous Waste (91/689/EEC) and the Directive on Packaging and Packaging Waste (94/62/EC). The Directive on Waste (75/442/EEC) lays down requirements for all types of waste, unless they are specifically regulated by other directives. Pursuant to the two first directives mentioned, a list of wastes is included in a Commission Decision (2000/532/EC). Pharmaceutical waste categories are coded and fully defined by six-digit codes in the list of wastes in Chapter 18 (Wastes from human or animal health care and/or related research, except kitchen and restaurant wastes not arising from immediate health care) and in Chapter 20 (Municipal wastes; household waste and similar commercial, industrial and institutional wastes, including separately collected fractions). Cytotoxic and cytostatic medicines are considered hazardous waste and consequently subject to special provisions. Cytotoxicity means that the drug will harm or kill cells, and cytostatics are drugs used to block the growth of cancer cells. Cytotoxic drugs do not

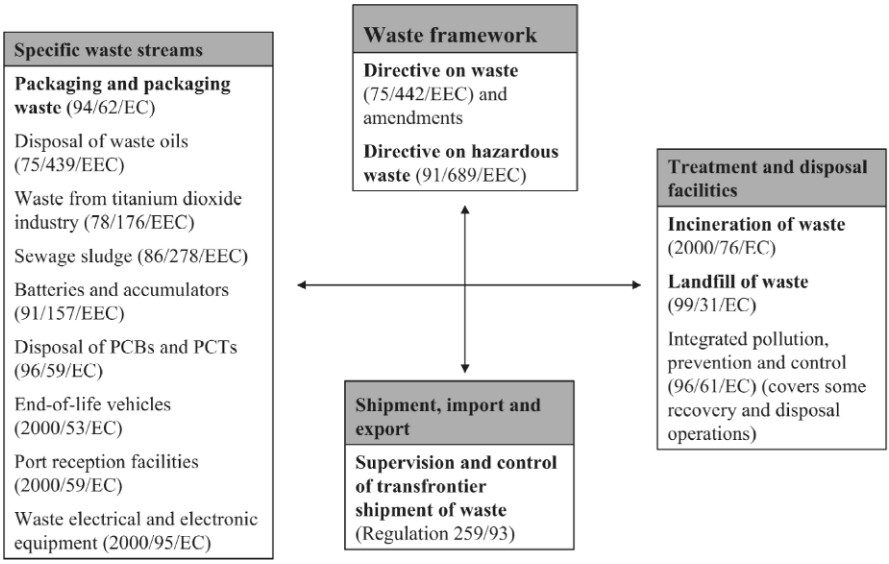

Fig. 31.1. Overview of EU directives on waste (European Topic Centre on Waste and Materials Flows 2003). Regulations pertinent to pharmaceutical waste are shown in *bold*

specifically affect cancer cells, but all dividing cells. This makes cytotoxic drugs extremely hazardous to living organisms.

The EU strategy for waste management includes the prevention and precautionary principle to secure a reduction in the impacts of waste on human health and the environment and especially to reduce the hazardous substances in waste. Dir. 2004/27/EC amending Dir. 2001/83/EC on the Community code relating to medicinal products for human use and Dir. 2004/28/EC amending Dir. 2001/82/EC on the Community code relating to veterinary medicinal products both consider the precautionary and safety measures to be taken for the disposal of waste products, together with an indication of potential risks presented by the product to the environment. Member States are obliged to ensure that appropriate collection systems are in place for medicinal products that are unused or have expired.

United States of America

Laws governing pharmaceutical waste have existed for many years, and the goal of the regulations is to prevent harm to human health and environment. The following framework can be identified:

- The Resource Conservation and Recovery Act (RCRA) of 1976;
- Clean Water Act;
- Clean Air Act;
- Environmental Protection Agency's Audit Policy of 12/1995; updated 4/2000;
- Community Right to Know Act;
- Hospital/Medical/Infectious Waste Incineration Rule;
- Land Disposal Restrictions Regulations.

Federal RCRA regulations apply to hazardous pharmaceutical waste. Hazardous wastes are divided into two categories: (1) listed wastes that appear on one of four lists of hazardous waste (F, K, P, and U), and (2) characteristic wastes exhibiting certain hazardous properties – ignitability, corrosiveness, reactivity, and toxicity. Pharmaceuticals are found on the P and U lists. Because of toxicity, some chemicals and heavy metals used in drug formulations appear on the D-list. Drug formulations containing these chemicals and heavy metals are of concern in solid waste landfill environment above certain concentrations.

For non-regulated hazardous pharmaceutical waste, the best management practice is applicable. This practice encourages avoiding the drain disposal of any waste pharmaceuticals, with emphasis on those that are hazardous. It is always appropriate to manage drug waste at a higher level of care than required by regulation.

When it comes to the proper disposal of prescription drugs at home the present federal guideline recommends unused, unneeded, or expired drugs are to be taken out of their original containers and thrown in the refuse. Where pharmaceutical return programs or community solid-waste programs exist, which allow the public to bring unused drugs to a central location, these are advocated as a proper disposal. A few medicines, mainly controlled drugs, are recommended to be flushed down the toilet for security reasons (Office of National Drug Control Policy 2007).

31.2.2 Waste Sources

Spills/breakage, partially used vials and bottles, discontinued and unused preparations, and outdated pharmaceuticals are all scenarios where pharmaceutical waste is generated. In addition, the devices used to administer drugs and general compounding in hospitals and pharmacies add to the waste volume.

A significant waste source is pharmaceutical dosage forms that contain large residues of API after normal use (see below). A transdermal patch is a dosage form designed to maintain a consistent release rate during the time of application to the skin. An excess of twenty times the amount of drug that will be absorbed is commonly loaded into the product. The surplus is needed to achieve the stable concentration gradient to provide a consistent drug release. When the API is an estrogen, the environmental concern of proper disposal of used transdermal patches is imperative as minute concentrations of endocrine disruptors are known to have detrimental effects on aquatic species and possibly on human health and development (Sumpter et al. 2005). Table 31.1 reviews transdermal patches marketed in Sweden 2007 that contain estrogens and the residual amounts after use.

31.2.3 Waste Streams

Pharmaceutical waste is not one, but many distinct waste streams that reflect the handling and usage of pharmaceutical products. Two main streams can be identified. The first is connected to the use of pharmaceuticals by people in primary care either for themselves or for their pets. This stream may also include the use of drugs in livestock farming. The second main stream is generated by hospital care and other care institutions and also establishments in which research activities may be included. Healthcare establishments for humans or animals produce a range of various wastes, which according to regulation belongs to five basic groups: municipal, medical, infectious medical, hazardous, and low level radioactive waste.

Unused Medicine

When a patient has finished a drug treatment, there may be some unused drug left. The unwanted or leftover drug can be disposed of by the patient at a pharmacy, another designated place, or it is disposed of in some other way. Drugs returned to pharmacies have been studied in Sweden since the 1970s in order to understand what drugs are returned and how much is left in the packs in relation to age and sex of the patients. A few studies have been published from other countries (Socialstyrelsen 2004).

A comprehensive study in Sweden by Ekedahl et al. (2003) showed a return to pharmacies of 4.6% of the total sale in Defined Daily Doses (DDD) and 3.8% of totally sold packs. 51% of the packs had expired and 65% had more than two thirds left (38% were unopened). Data published from other countries generally report lower proportion of unopened packs; Great Britain 20% (Hawksworth et al. 1996), Germany 24% (Bronder and Klimpel 2001).

Several international studies report that a larger proportion of unused medicines are returned by the elderly (Braybrook et al. 1999, Hawksworth et al. 1996). A

Table 31.1. Transdermal patches containing estrogens authorized on the Swedish market 2007

Product name	*API*	Initial *API* content (µg)	Dose (µg/24 h)	Application time (d)	Residue (%)
Climara®	*ED*	3800	50	7	91
Estalis®	*ED* *NE*	510 4800	50 250	3.5	66 82
Estalis®Sekvens (I)	*ED*	4330	50	3.5	96
Estalis®Sekvens (II)	*ED* *NE*	510 4800	50 250	3.5	66 82
Estradot®	*ED*	390	25	3.5	78
Estradot®	*ED*	585	37.5	3.5	78
Estradot®	*ED*	780	50	3.5	78
Estradot®	*ED*	1170	75	3.5	78
Estradot®	*ED*	1560	100	3.5	78
Evorel®	*ED*	1550	25	3.5	94
Evorel®	*ED*	3100	50	3.5	94
Evorel®	*ED*	4650	75	3.5	94
Evorel®	*ED*	6200	100	3.5	94
Evorel®Micronor	*ED*	3100	50	3.5	94
Evra®	*EE* *NG*	600 6000	20 150	7	77 82
FemSeven	*ED*	1453	50	7	76
FemSeven	*ED*	2180	75	7	76
Oesclim®	*ED*	5000	25	3.5	98
Oesclim®	*ED*	7500	37.5	3.5	98
Oesclim®	*ED*	10000	50	3.5	98

API: Active pharmaceutical ingredient; *ED:* Estradiol: *EE:* Ethinylestradiol; *NE:* Noretisteron; *NG:* Norelgestromin.

similar skew is described by Ekedahl (2003) reporting 60% of the patients are ≥65 years old and return 64% of all packs. Figure 31.2 is adapted from the same study and shows how few patients return the majority of unused prescription drugs to pharmacies.

Used Medicine with Residue

All used packs will contain some pharmaceutical substance residues. Packs with visible residues should be classified as pharmaceutical waste, demanding handling and

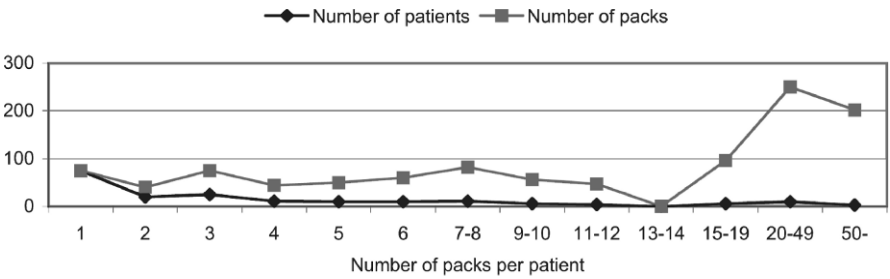

Fig. 31.2. Number of returned packs of prescribed drugs and number of patients returning divided on number of packs per patient. 5% of the patients are returning 36% (≥24 packs/patient); 10% are returning 50% (15–101 packs/patient); 69% are returning 22% (1–4 packs/patient)

disposal procedures that give proper protection to people and the environment. An empty pack that has not contained hazardous API can be discarded as municipal waste if truly empty. However, a lot of pharmaceutical products are designed with a packaging that is not transparent. Here visual inspection is impossible and some of these, like tubes and aerosol cans, may contain substantial residues when used.

It can be seen in Table 31.2 that several pharmaceutical dosage forms contain a significant residual amount even when used correctly. The controlled collection and disposal of these products after use will lessen the environmental risks.

Packages

Packaging materials and administration accessories consume a small, though significant proportion of natural resources. They can impact the environment through pollution caused by incineration and landfills. No pharmaceutical packaging is reused, but the outer packaging of pharmaceuticals is usually a non-contaminated material that can undergo recovery or recycling together with empty non-hazardous pharmaceutical packs by segregation as cardboard, paper, plastic, glass, and metals.

Plastic is the most common packaging material, followed by cardboard and paper. The plastic material is dominated by polyethylene and polypropylene without notable environmental pollution problems. Other environmentally accepted plastic materials are acetal plastic, ethyl-vinyl-acetal plastic, polyamides, polyesters, polycarbonates, polyurethanes, and styrene plastics. Polyvinyl chloride (PVC) is questioned environmentally because of potential pollution of the environment during manufacturing and disposal. PVC is the dominant plastic in blisters.

Contaminated Material

Products such as personal protective equipment, materials used to perform routine cleaning and decontamination as well as spill clean up materials may become contaminated. When contamination with a hazardous material is suspected or known, the resulting waste will normally receive the same regulatory status as the original classified API component.

Table 31.2. Pharmaceutical dosage forms with significant residues after normal use (adapted from Castensson and Riemsdijk, to be published)

Pharmaceutical dosage form	Residual reason	Residue[a] (%)
Eye drops	Design, *Dose prec.*, *Shelf life*	0 – 91
Eye drops, prolonged release	Design, *Dose prec.*, *Shelf life*	50 – 72
Implant	Design, *Dose prec.*	8 – 27
Inhalation gas	Design, *Dose prec.*	–
Inhalation powder	Design, *Dose prec.*	20 – 83
Intrauterine delivery system (*vet.*)	Design, *Dose prec.*	51
Medicated chewing-gum	Design, *Dose prec.*	10 – 30
Medicated plaster	Design, *Dose prec.*	42 – 81
Nasal spray	Design, *Dose prec.*	2 – 29
Transdermal patch	Design, *Dose prec.*	28 – 98
Vaginal delivery system	Design, *Dose prec.*	28 – 88

[a] Products on the Swedish market 2006.
Dose prec.: Dose precision; *Shelf life:* Shelf life after first opening or following reconstitution; *vet.:* veterinary.

Sharps

Sharps, needles and syringes with needles can be collected in containers made of hard plastic. There are also several devices available for cutting and collecting the needle, making it possible to segregate the syringe and the needle for separate disposal.

31.2.4 Collection and Segregation

Hospitals and Care Centers

In order for waste not to pose a threat to human health and the environment, they have to be properly identified, segregated and disposed of. Unfortunately, due to imperfect procedures, the waste from different groups becomes mixed together. As a result, the waste has to be managed at a higher standard according to regulation and thus requires costly methods of treatment. In reality, most waste produced by the healthcare sector is simply non-hazardous municipal waste that can be recovered and recycled. While the first priority has to be identifying, segregating and properly managing hazardous pharmaceutical waste, all other pharmaceutical waste should be collected and segregated in a systematic way for correct disposal.

With proper segregation in place, the amount of infectious medical waste, hazardous waste and radioactive waste can be reduced to 2–25%, depending on the type of

establishment and the scope of services it provides. The hazardous waste segregation system must be adapted to the waste treatment technology applied (Health Care Without Harm Europe 2005).

Non-Institutional Care

The results from a questionnaire sent to the associations for pharmacies in Europe in 2003 are presented in Fig. 31.3. In the Netherlands, Spain and Sweden, all pharmacies were active in the collection of leftover medicine. In nine countries, all pharmacies accepted taking back leftover drugs from the public, in five others some pharmacies would do this, and in one country no leftover drugs were accepted. In Belgium and France, some pharmaceuticals were segregated for recycling as donations, which is disparate to WHO Guidelines for drug donations from 1999 (Socialstyrelsen 2004). A European survey of twenty-eight countries performed by the European Federation of Pharmaceutical Industries and Associations (2007) indicated a formal product return scheme of leftover drugs in twenty countries.

In Sweden (population 9 million) approximately five per cent of the drugs counted as DDDs collected from pharmacies will never get used. About seventy-five per cent of the leftover drugs are returned to pharmacies by the public for controlled incineration, which amounts to more than 900 tons yearly including packaging.

Country code		ES	CS	PT	IE	IT	DK	AT	SK	NL	GB	NO	FR	HU	GR	FI	DE	SE	BE
Pharmacy commitment	All active	■								■								■	
	All on request		■	■			■	■	■			b		■	■	■			
	Some on request					■					a		■				■		■
	None				■														
Recycling	Resold																		
	Aid program												c						c
	None	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	
Disposal	Incineration	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
	At pharmacy							■				d							
	Other																e		
Data on how much and which drugs	Yes				f		■	■		f	■	■	■					■	■
	No	■	■	■		■			■					■	■	■	■		
Reason data	Yes							■	f	f	■	■						■	
	No	■	■	■	■	■	■						■	■	■	■	■		■

Fig. 31.3. Results of a European survey 2003 on the collection of unwanted medicine at pharmacies from the public. *Shaded boxes* confirm the questions presented in the questionnaire (adapted to Socialstyrelsen 2004); *a*: most on request, *b*: one chain active, *c*: unopened, essential and listed drugs with remaining shelf-life, *d*: narcotics, *e*: "normal" pharmaceutical waste is disposed of with other waste for incineration, *f*: no references

In the USA, there is no national program or regulation for the collection of unused and expired drugs, but local programs are encouraging people to dispose of them at designated drop sites.

31.2.5 Treatment and Disposal

According to the European Waste Catalogue, pharmaceutical waste is classified by six-digit codes:

- 18 01 01 (18 02 01) Sharps (non-infectious);
- 18 01 03* (18 02 02*) Special requirements in order to prevent infection;
- 18 01 08* (18 02 07*) Cytotoxic and cytostatic medicines;
- 18 01 09 (18 02 08) All other medicines;
- 20 01 31* Cytotoxic and cytostatic medicines;
- 20 01 32 All other medicines.

The codes within brackets represent waste involving animals, and the codes marked with an asterisk are considered hazardous waste.

The use of incineration as a disposal method is regulated in the EU by the directive Incineration of waste (2000/76/EC). The method has the drawback that it may cause pollutants. This is handled by regulations stipulating limit values on the emission to the atmosphere. Also, the temperature of the gases in the process should be at stipulated values depending on the waste content. In the EU, the incineration technology is widespread for disposal of the solid waste (see Fig. 31.3). However, liquid waste may be disposed of down the drain, as environmentally adapted procedures just recently have come into focus.

In the USA, hospital pharmaceutical waste is generally discarded down the drain or in landfill sites, except chemotherapy agents, which are often sent to a regulated medical waste incinerator. The statutory definition of hazardous waste provides sound reasoning for broadening the range of chemotherapeutic drugs that should be managed as hazardous waste. The statute defines the term "hazardous waste" to mean a solid waste or combination of solid wastes that because of its quantity, concentration, physical, chemical, or infectious characteristics may (1) cause, or significantly contribute to an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness; or (2) pose a substantial present or potential hazard to human health or the environment when improperly treated, stored, transported, or disposed of, or otherwise managed (Hospitals for a Healthy Environment 2006).

Pharmaceuticals are designed to be resistant to biological degradation. Wastewater treatment plants are designed to remove conventional pollutants such as suspended solids and biodegradable organic material, but they are not designed to remove low concentrations of synthetic pollutants such as pharmaceuticals. The removal efficiency of various wastewater treatment technologies appear to be chemical-specific.

Land filling pharmaceutical waste should be avoided, both for environmental and security reasons. Drugs added to a landfill will eventually leach into groundwater or be deliberately pumped out from its leaching beds.

Thermal destruction of discarded drugs provides the closest match to best management practice at this time. Non-incineration medical waste treatment technologies may sometimes be an alternative for pharmaceutical waste (Health Care Without Harm Europe 2004).

31.3 Minimizing Pharmaceutical Waste

There are inherent limitations in the practice of substituting a hazardous drug since this hazardous nature of the API often provides the therapeutic effect. However, an environmentally preferable product choice can sometimes be made. This concerns firstly those products that may contain heavy metals or persistent, bioaccumulating and highly toxic ingredients or very persistent and very bioaccumulating chemicals. Source reduction is an important key to reducing the amount and toxicity of waste.

One way to decrease the volume of unused medicines is to have a greater choice of packaging sizes. Smaller packs, especially when initiating a new longer treatment and individualized dispensing have great potential to decrease the amount of leftover drugs.

Working groups in medical establishments monitoring and enhancing a waste reduction program will greatly influence the amount of waste that is destined for ultimate disposal. Detailed procedures in performing a program can be found in Hospitals for a Healthy Environment (2006) and Health Care Without Harm (2005).

The potential benefits of waste minimization for society are environmental risk reduction and cost reductions. Healthcare establishments win better compliance with regulatory standards, enhanced occupational health and safety and improved community relations.

31.4 Conclusion

Pharmaceutical waste management is especially challenging, given the complexity of the problem. Proper management of hazardous pharmaceutical waste is first priority, but careful consideration should be given to manage all pharmaceutical waste. As research data accumulate on the adverse impacts of pharmaceutical waste on human health and the environment, application of the precautionary principle becomes increasingly relevant.

A rewarding challenge is to find all possible ways to minimize pharmaceutical waste and reduce its harmful nature. Over the long term it would be possible to tackle the problem with reference to where the problem starts, i.e., the use of pharmaceuticals and the product itself. A correct diagnosis and intervention with pharmaceuticals combined with increased surveillance and follow-up will optimize the outcome of the treatment and reduce the chance for leftover medicine. Many elderly people use more than ten prescription drugs simultaneously, and this polypharmacy is an important reason why drugs become unused.

With more personalized drug therapies and medicine interventions, and with better characteristics of the API it is possible that less adverse effects may be observed, leading both to better compliance and less unused medicines. Personalized dosing and package size are product design features effectively reducing waste generation.

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