Chapter 10

In Silico Models for Acute Systemic Toxicity

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Abstract

In this chapter, we give an overview of the regulatory requirements for acute systemic toxicity information in the European Union, and we review the availability of structure-based computational models that are available and potentially useful in the assessment of acute systemic toxicity. The most recently published literature models for acute systemic toxicity are also discussed, and perspectives for future developments in this field are offered.

Key words Acute systemic toxicity, Regulation, Organ-specific toxicity, In silico model

1 Introduction

Acute systemic toxicity comprises the general adverse effects that occur after a single or multiple exposure of an animal to a substance within 24 h and during an observation period of at least 14 days. The substance may be administered orally, by inhalation, or dermally.

Acute mammalian toxicity tests are often the first in vivo toxicity tests to be performed on a chemical. In recent years there have been considerable efforts to replace, reduce, or refine these animal tests by applying alternative approaches, including both in vitro and in silico models. An increasing number of models are available to predict acute mammalian toxicity. This is partly due to the fact that a reasonable number of datasets are openly available for modeling. However, the reliability of the in vivo data can be highly variable, and the metadata provided is often insufficient to determine the suitability of the data for modeling purposes. Another challenge is related to the multiple mechanisms leading to this complex effect, which is typically expressed as a single numerical value (LD_{50} for oral and dermal toxicity; LC_{50} for inhalational toxicity). In addition there are also differences between

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the routes of administration and species, and different data should be modeled separately [1].

Target organs, such as the liver, kidneys, heart, lungs, and brain, can be affected by exogenous chemicals to the extent that they cease to function. Thus, the use of QSAR models for organ/system specific toxicity would be extremely helpful when predicting acute systemic toxicity. A limited number of QSAR models for specific target organ and tissue effects are available.

The information obtained from acute systemic toxicity studies is used in the hazard assessment of chemicals occurring in food, industrial chemicals, biocides, pesticides, and cosmetics. In this chapter, we give an overview of the regulatory requirements for acute systemic toxicity information in the European Union, the software packages available for assessment of acute systemic toxicity and organ- and system-specific toxicity, as well as the databases available for obtaining such data. Since comprehensive reviews of literature QSAR studies are available elsewhere [2–5], we focus here on some of the more recently published literature models for acute systemic toxicity. Some of these software and literature models are documented in the JRC's QSAR Model Database (http://qsardb.jrc.ec.europa.eu/qmrf/).

2 Regulatory Context in the European Union

For the assessment of acute systemic toxicity, only in vivo tests are currently accepted by regulatory bodies (Table 1). However, in vivo acute systemic toxicity studies are prohibited for cosmetic substances and products [14].

The endpoint measured in the majority of the standard assays is animal morbidity or death. Evident signs of toxicity (i.e., clear signs of toxicity indicating that exposure to the next highest concentration would cause severe toxicity in most animals within the observation period) are only used in the oral fixed dose procedure (FDP), which causes less suffering and is, therefore, more humane.

Table 1
In vivo methods currently available for acute systemic toxicity

Exposure route	OECD	EU test method
Oral	TG 420: fixed dose procedure [6]	B.1 bis [7]
	TG 423: acute toxic class method [8]	
	TG 425: up and down procedure [9]	B.1 tris [7]
Dermal	TG 402 [10]	B.3 [7]
Inhalation	TG 403 [11]	B.2 [12]
	TG 436 (acute toxic class method) [13]	B.52 [12]

The assessment of acute systemic toxicity is one component in the safety evaluation of substances and represents a standard information requirement within several pieces of EU chemicals legislation, including the Regulation on Classification, Labelling and Packaging (CLP) of substances and mixtures [15], the Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [16], the Biocidal Products Regulation [17], the Plant Protection Products Regulation [18], and the Cosmetic Products Regulation [14]. In preclinical drug development [19], however, these studies are no longer required to support first clinical trials in man. The information needed can be obtained from appropriately conducted dose-escalation studies or short-duration dose ranging studies that define a maximum tolerated dose in the general toxicity test species [20, 21]. Further information on the regulatory requirements in the EU is given in Prieto et al. [22].

3 Software for Predicting Acute Systemic Toxicity

Several software tools capable of predicting endpoints related to systemic toxicity are available, as reviewed previously [23]. An updated list is given in Table 2 and some updates on the programs are described below.

Among the commercial software programs covering a broad spectrum of systemic toxicological effects is ACD/Labs Percepta, which is developed and marketed by Advanced Chemistry development Inc. (http://www.acdlabs.com/). The platform has two modules related to systemic toxicity prediction—Acute Toxicity Prediction Module and Health Effects Prediction Module. The Acute Toxicity predictor has been built using experimental data for more than 100,000 compounds extracted from the Registry of Toxic Effects of Chemical Substances (RTECS) and former European Chemical Substances Information System (ESIS) databases. It provides three different software components related to acute mammalian toxicity:

- LD_{50}—Provides predictions of LD_{50} values for rats and mice according to various routes of administration. Prior to modeling, the original experimental data were converted to logarithmic form (pLD_{50}) in order to maintain linear relationship with used descriptors. The final prediction results returned to the user are converted back to LD_{50} values (mg/kg). The predictive model for pLD_{50} has been derived using GALAS (Global, Adjusted Locally According to Similarity) modeling methodology.
- Hazards—A knowledge-based expert system that identifies and visualizes hazardous structural fragments.

Table 2
Software tools for systemic toxicity endpoints

Software (and developer)	Availability	Endpoint							
		Acute (oral) toxicity	Chronic (oral) toxicity	Hepatotoxicity	Nephrotoxicity (+ urinary tract toxicity)	Neurotoxicity	Cardiotoxicity	Immunotoxicity[a]	Cytotoxicity
Accelrys Discovery Studio, including TOPKAT (BIOVIA)	Commercial	●	●	●	●		●		
ACD/Percepta (Advanced Chemistry Development, Inc.)	Commercial	●		●	●		●		
ADMET Predictor (Simulations Plus Inc.)	Commercial	●		●			●	●	
admetSAR (Laboratory of Molecular Modeling and Design, East China University of Science and Technology)	Freely available	●							
CASE Ultra (MultiCASE Inc.)	Commercial	●	●	●	●				
Derek Nexus (Lhasa Ltd)	Commercial			●	●	●	●	●	
Lazar (In silico toxicology GmbH)	Freely available		●						
Leadscope (Leadscope)	Commercial			●	●	●	●		
MetaDrug/ToxHunter™ (Thomson Reuters)	Commercial			●	●				
Molcode Toolbox (Molcode Ltd)	Commercial	●	●				●		●

OpenVirtualToxLab (Biographics Laboratory 3R)	Freely available for academic organizations		●			●		
Pallas System including ToxAlert and HazardExpert Pro (CompuDrug Inc.)	Commercial				●		●	●
PASS (geneXplain GmbH)	Commercial	●	●					
Pred-hERG 2.0 (Laboratory for Molecular Modeling and Drug Design, Federal University of Goiás.)	Freely available					●		
PROTOX (Charite University of Medicine Institute for Physiology)	Freely available	●						
TerraQSAR (TerraBase)	Commercial	●						
T.E.S.T. (US EPA)	Freely available	●						
TIMES (Laboratory of Mathematical Chemistry, University "Prof. Dr. Asen Zlatarov")	Commercial	●	●					
Tox-Comp.net (Faculty of Pharmacy, Jagiellonian University Medical College)	Freely available					●		

[a]Immunotoxicity other than skin sensitization

- Categories—Classifies compounds into one of five Globally Harmonised System (GHS) categories for acute oral toxicity.

The Health Effects module predicts the probability of a compound having a health effect on a particular organ or organ system (blood, cardiovascular system, gastrointestinal system, kidney, liver, and lungs). The models are based on data collected from chronic, sub-chronic, acute toxicity and carcinogenicity studies with adverse effects reported in particular organs or organ systems.

A common goal of toxicity prediction is to distinguish between toxicologically active and inactive compounds. Since multiple mechanisms are involved in systemic toxicity, this requires the availability of predictive tools that are able to cover a wide region of the activity space. This is the main feature of the expert systems that make assessments on the basis of structural alerts covering a spectrum of structural properties associated with the complex endpoint. One commonly used expert system, developed and marketed by Lhasa Ltd (http://www.lhasalimited.org/), is Derek Nexus which is a development of the former Derek for Windows (DfW). This contains knowledge rules derived from the known relationship between a given substructure and a toxicological effect of the molecule and applies these rules to predict potential toxicological effects of compounds. Derek Nexus generates a prediction by comparing the structural features of the target compound with a toxicophore encoded as structural pattern(s) in its knowledge base. The final predictions are derived from a reasoning scheme which takes into account the presence of a toxicophore in the query structure ('structural alert') and a limited number of calculated molecular properties, which, taken together, return an "uncertainty term" for the prediction itself. For some alerts, supporting examples are provided and the system states whether the query compound already exists as an example in the knowledge base. Literature references are also included to enable the user to assess the applicability of the structural alert to the predicted structure and to allow for an expert knowledge assessment. Derek Nexus covers multiple endpoints, including hepatotoxicity, nephrotoxicity, and cardiotoxicity.

CASE Ultra (http://www.multicase.com/) is further development of MCASE methodology and falls in the range of fragment based QSAR expert systems [24]. The CASE Ultra model mainly consists of a set of "positive alerts" (biophores), and "deactivating alerts" (biophobes), i.e., those fragments that are identified as statistically significant for increasing/decreasing the activity. The improvement of CASE Ultra over its predecessor is related to the identified alerts that are no longer limited to linear paths of limited size or limited branching pattern. In addition the training sets can be larger than 8000 molecules. The applicability domains

of individual toxicity alerts within the models quantitatively define the necessary structural environment of the toxicity alerts.

The statistically based program TOPKAT (http://accelrys.com/) uses multiple QSARs on small and homogenous sets of data. It is now a part of QSAR, ADMET and Predictive Toxicology module within Biovia Discovery Studio platform. The rat oral LD_{50} module in TOPKAT comprises 19 regression analyses developed using experimental values of approx. 4000 chemicals from RTECS, including pesticides and industrial chemicals. The rat oral LD_{50} module in MCASE (named A56) is based on and comprises data for 7920 chemicals from the FDA, WHO, and NTP datasets. Tunkel and coworkers [25] compared the performance of the TOPKAT and MCASE rat LD_{50} modules against an external test set of 73 organic compounds covering 32 chemical categories retrieved from submissions to the EPA High Production Volume (HPV) Challenge Program (http://www.epa.gov/chemrtk/). The predictive accuracy of each software tool was assessed by applying the EPA's New Chemical classification approach (http://www.epa.gov/oppt/newchems/index.htm), from the low-concern class (>2000 mg/kg) to the high-concern class (<15 mg/kg). While neither model was able to classify all 73 compounds, TOPKAT correctly classified 67 % of the chemicals, while MCASE classified 70 % correctly. However, it should be noted that the test set used was significantly skewed toward "low concern" chemicals, which both models predicted correctly with a high degree of accuracy (82 % and 100 % correct for TOPKAT and MCASE, respectively). Moreover, a high degree of false negatives was found for moderate and high concern HPV chemicals (TOPKAT, 72 %; MCASE, 100 %), suggesting that these programs are less reliable for the identification of more toxic compounds. The authors also compared the model outputs against the GHS five-tier scheme for classification of rat oral acute toxicants (<5, 5–50, 50–300, 300–2000, and 2000–5000 mg/kg), which is similar to the one adopted by EPA (<15, 15–50, 50–500, 500–2000, >2000 mg/kg). When compared against the GHS scheme, the ability of TOPKAT and MCASE to produce correct classifications was 73 % and 70 %, respectively, for the HPV test set chemicals, thereby changing slightly with respect to the EPA scheme, albeit enough to invert the rank order of these models.

VirtualToxLab is an in silico technology for estimating the toxic potential of chemicals [26] based on an automated protocol that simulates and quantifies the binding of small molecules towards a series of proteins, known or suspected to trigger adverse effects. The interface to the technology allows building and uploading molecular structures, viewing and downloading results and rationalizing any prediction at the atomic level by interactively analyzing the 3D binding mode of a compound with its target protein(s) in real-time. The VirtualToxLab has been used to

predict the toxic potential for over 2500 compounds and the free platform, OpenVirtualToxLab, is accessible (in client-server mode) over the Internet. It is free of charge for universities, governmental agencies, regulatory bodies, and nonprofit organizations.

The LeadScope software (http://www.leadscope.com) links chemical and biological data that allows exploration of large sets of chemical compounds, their properties, and biological activities. Chemical structures are organized in a taxonomy of familiar structural features each combined with common substituents—the common building blocks of medicinal chemistry [27]. LeadScope provides QSAR models for diverse physiological adverse effects including cardiological, hepatobiliary, and urinary endpoints.

Other software tools available for predicting acute toxicity (LD_{50}) to rat/mouse are also available, such as TerraQSAR (http://www.terrabase-inc.com/), ADMET Predictor (http://www.simulations-plus.com), Molcode Toolbox (http://molcode.com/). The TerraQSAR software is based on neural network methodology and includes models for predicting both oral and intravenous LD_{50} values in mice and rats. ADMET Predictor includes a number of in-built models for ADMET, and allows new predictive models to be built from the user's data. ADMET Predictor's Toxicity Module provides predictions of various toxicity endpoints including hepatotoxicity, carcinogenicity, acute rat toxicity, and cardiotoxicity. Molcode Toolbox has a range of modules for predicting toxicological endpoints, including intravenous acute LD_{50} values and in vitro cytotoxicity (IC_{50} values) (from the Registry of Cytotoxicity). The models are well documented and the underlying experimental data is made available with references and structure files (MDL molfiles).

4 Databases Containing Information on Acute Systemic Toxicity

Sources of rat LD_{50} values which may be suitable for the development of QSARs are listed in Table 3. Some recent updates are discussed in the section below.

In particular, Acutoxbase [29] was developed in the context of the EU FP6 project 'A-Cute-Tox' (http://www.acutetox.eu/), which aimed to optimize and "pre-validate" an in vitro testing strategy for predicting acute human toxicity ([30, 31]; Prieto and Kinsner-Ovaskainen 2015). While the database is not available, the in vitro and animal data are published in several publications [30–32].

Recently the COSMOS database has been developed as a part of the COSMOS project (http://www.cosmostox.eu/), one of seven projects forming the Seurat-1 research cluster (http://www.seurat-1.eu/). Version 1 of the COSMOS database (http://cosmosdb.cosmostox.eu/) contains 12,538 toxicity studies for

1660 compounds across 27 endpoints, including acute toxicity data for 1697 compounds tested on different animal species, as well as in vitro data.

The Hazardous Substances Data Bank (HSDB) is a part of NLM's Toxicology Data Network (TOXNET) [33]. It contains chemical substance information with one record for each specific chemical or substance, or for category of chemicals or substances. HSDB has approximately 5600 chemicals and substances, with information for toxicity and human exposure. All data comes from public scientific sources. HSDB's content is peer-reviewed by a group of experts.

The Registry of Toxic Effects of Chemical Substances (RTECS) database includes basic toxicity information for: prescription and non-prescription drugs, food additives, pesticides, fungicides, herbicides, solvents, diluents, chemical wastes, reaction products of chemical waste, and substances used in industrial and household situations. It covers six categories of toxicity data including acute toxicity data. In vitro toxicology data has been added as well. Accelrys now produces the RTECS files using existing data selection criteria and rules established by NIOSH (http://accelrys.com/products/databases).

In order to be useful for QSAR development, datasets should be first curated, i.e., the accuracy of the structures should be verified and the quality of biological data should be reviewed. It is useful to provide a reference to the source of the experimental data. In addition, inorganic and organometallic compounds, salts, and compound mixtures are often removed from the analysis. For the development of QSARs, LD_{50} values should be converted to log[1/(mol/kg)] (if originally expressed as mol/kg or mg/kg). Finally, approximate LD_{50} values should be converted to discrete values, and multiple LD_{50} values from different labs/experiments should be converted to a single value. The ChemIDplus and ZEBET databases have been recently employed as data sources for QSAR analyses [34, 35].

5 Prediction of Organ-Specific and System-Specific Toxicity

5.1 Ability to Predict In Vivo Toxicity

Some currently available software tools (e.g., TOPKAT and MCASE) are useful for predicting acute toxicity in categorical terms (e.g., in terms of GHS classifications). The performance of different software tools in predicting acute toxicity has been investigated [36, 37]. In these studies, ACD and T.E.S.T. have performed well.

In the scientific literature, local QSAR models have been generated for sets of congeneric compounds (organophosphates, aromatic amines, anilines, etc.) and are scattered over many original publications. Some of these studies have also explored the use of

in vitro data as additional descriptors in the derivation of so-called quantitative structure activity-activity relationships, QSAAR [38]. QSAAR modeling revealed good potential for acute toxicity prediction, particularly in cases when a significant correlation exists between in vivo data (LD_{50}) and in vitro cytotoxicity (IC_{50}), and the additional inclusion of physicochemical parameters serves to improve the correlation. In practical terms, QSAAR could be particularly useful if high-throughput screening methods are used to generate the in vitro data.

Despite their limited applicability when taken individually, local QSAR models might be usefully combined into an expert system for toxicity predictions. As a part of the efforts to develop global QSAR models for acute toxicity Raevsky and coworkers [39] proposed the so-called Arithmetic Mean Toxicity (AMT) modeling approach, which produces local models based on a k-nearest neighbors approach. Arithmetic mean toxicity values of one or more pairs of analogues (nearest neighbors) are considered as the toxicity of the chemical of interest. Recently a classification model based on 436 Munro database chemicals and developed using Dragon descriptors has been proposed as a tool for chemical screening [40]. Kleandrova et al. [3] have developed a multitasking (mtk) QSTR model based on ANN (artificial neural networks) for simultaneous prediction of acute toxicity by considering different routes of administration, different breeds of laboratory animals, and the reliability of the experimental conditions. The model is based on a diverse dataset comprising 1494 chemicals retrieved from CHEMBL (http://www.ebi.ac.uk/chembldb).

A consensus approach has been exploited in some studies where the models are built by using a combinatorial QSAR modeling approach, including multiple descriptors and employing several statistical modeling methods. It has been claimed that the predictive accuracy of consensus QSAR models is superior to the individual ones [34, 41]. In addition, several research studies [35, 42, 43] have demonstrated the ability to improve quantitative predictions for structurally diverse datasets when high throughput bioactivity data are used in combination with traditional molecular descriptors. This can also be regarded as an example of the QSAAR approach. These hybrid approaches and their underlying datasets are publicly available via the ChemBench web portal (https://chembench.mml.unc.edu/).

5.2 Ability to Predict Non-apical Toxicities

The feasibility of using in vitro cytotoxicity data for the prediction of in vivo acute toxicity has been investigated in a number of research programs [28, 44, 45]. Over 70 % correlation has been established between in vitro basal cytotoxicity and rodent LD_{50} values [46]. The applicability of 3T3 Neutral Red Uptake Cytotoxicity Assay for the identification of substances with an $LD_{50} > 2000$ mg/kg has been evaluated by the EURL ECVAM Scientific Advisory

Table 3
Databases containing acute toxicity information

Database	Availability	Information
Acutoxbase, linked to the EU FP6 project 'A-Cute-Tox'; http://www.acutetox.eu/	Database not available, but the data are included in several publications (see text)	The following data are available for 97 reference chemicals (i.e., 52 % drugs, 31 % industrial chemicals, 12 % pesticides, 5 % others): • In vitro: approx. 100 in vitro assays including general acute cytotoxicity, metabolism-mediated toxicity, biokinetics, and organ-specific toxicity. • In vivo: Over 2200 LD_{50} values in rodents (rat and mouse) and other animals (e.g., guinea pig, dog) with various administration routes (oral, intravenous, etc.) compiled from published literature. For 97 reference chemicals, nearly 2800 human acute poisoning cases from clinical/forensic reports are also available.
COSMOS Database; http://cosmosdb.cosmostox.eu/	Freely available through the Internet after registration	Includes US FDA PAFA acute toxicity data.
CEBS, developed by the US NIEHS; http://cebs.niehs.nih.gov/	Freely available through the Internet	Includes in vivo study data and acute dose of a small number of known hepatotoxicants to rat.
ChemIDplus, developed by the US NLM; http://chem.sis.nlm.nih.gov/chemidplus/	Freely available through the Internet, structure-searchable	Toxicity data is available for over 400,000 chemical records, of which over 300,000 include chemical structures that are retrieved from TOXNET® (TOXicology Data NETwork; http://toxnet.nlm.nih.gov). It includes HSDB (Hazardous Substances Data Bank, an older subset of the RTECS database). A search for rat and mouse oral LD_{50} values found 15,866 and 33,009 records, respectively.
Food Safety Acute Toxicity Database; https://www.leadscope.com/toxicity_databases/regulatory_databases/	Commercial	Contains acute oral toxicity (LD_{50}) data from US FDA CFSAN PAFA database for1070 food additives and 1633 tests. Test systems include mainly • Rats: 950 chemicals • Mice: 366 chemicals Other test systems include rabbits, guinea pigs, dogs, and monkey.

(continued)

Table 3
(continued)

Database	Availability	Information
RTECS, originally compiled and maintained (until 2001) by the US NIOSH and currently maintained by Accelrys Technologies. Structure-searchable through the Accelrys Toxicity Database; http://accelrys.com/products/databases/bioactivity/toxicity.html Also searchable via other databases including the Leadscope Toxicity Database; http://www.leadscope.com/databases/	Commercial	Rat acute oral toxicity (LD_{50}) and acute inhalation toxicity (LC_{50}) data are compiled from the open scientific literature for approx. 7000 compounds (organic, inorganic and mixtures), including approx. 4000 organic compounds.
HSDB—TOXNET database; http://toxnet.nlm.nih.gov	Freely available through the internet	Toxicology database that focuses on potentially hazardous chemicals. Contains nonhuman toxicity values for almost 3000 chemicals.
Registry of Cytotoxicity (RC) database	Freely available on request from BfR ZEBET (zebet@bfr.bund.de)	Based on the publication by Halle [28], this comprises rodent acute oral LD50 values and published IC50 values from diverse in vitro cytotoxicity assays on approximately 550 chemicals

CEBS chemical effects in biological systems, *HSDB* Hazardous Substances Data Bank, *RTECS* registry of toxic effects of chemical substances; *TOXNET NLM's* Toxicology Data Network, *US NLM* US National Library of Medicine, *US NIEHS* US National Institute of Environmental Health Sciences, *US NIOSH* US National Institute of Occupational Safety and Health, *BfR ZEBET* Centre for Documentation and Evaluation of Alternatives to Animal Experiments of the German Federal Institute for Risk Assessment

Committee (ESAC). It was recommended however that the results should always be used in combination with other information sources. For instance, the assay is recommended as a component of an Integrated Approach to Testing and Assessment (IATA) [47]. A reason for the absence of a clear relationship between basal cytotoxicity and in vivo acute toxicity could be that specific organ toxicity is the most sensitive parameter for acute toxicity. Common sensitive systems and organs include nervous, cardiovascular, immune system, kidneys and liver, lungs and blood. IATA proposed for acute systemic toxicity are a combination of complementary approaches (in vitro, ex vivo, in silico, in chemico) that address functional mechanistic endpoints tied to adverse outcomes of regulatory concern [48].

As summarized in Table 4, there is a limited number of literature models for predicting toxicities at tissue and organ levels. A list of software applications is provided in Table 2. They are based on expert system or regression/categorical QSAR models. In the case of ligand–protein interactions, molecular modeling approaches are mainly used. Among the commonly used software tools, Derek Nexus provides over 500 structural alerts for a range of organ and system-specific toxicities, and other miscellaneous endpoints. Models for predicting liver toxicity are further covered in Chapter 11 (Hewitt et al.).

Some of these models are based on the concept of reactivity-based toxicity. The covalent binding of reactive electrophiles to cellular targets (i.e., nucleophilic sites of macromolecules) has the potential to initiate a chain of biological effects (e.g., depletion of glutathione and protein thiols) resulting in specific organ and system toxicities.

Among the few comprehensive studies covering a range of organ toxicities and relying on a broad structural space in the training set are the models published by Matthews et al. [49]. These models were developed for urinary tract toxicities of drugs. For each organ, a number of toxicity endpoints were considered in the QSAR analysis. The investigation utilizes four software programs: MC4PC (versions 1.5 and 1.7); BioEpisteme (version 2.0); MDL-QSAR (version 2.2); Leadscope Predictive Data Miner (LPDM version 2.4). The four QSAR programs were demonstrated to be complementary and enhanced performance was obtained by combining predictions from two programs. The best QSAR models exhibited an overall average 92 % coverage, 87 % specificity, and 39 % sensitivity. These results support the view that a consensus prediction strategy provides a means of optimizing predictive ability.

In the work of Myshkin et al. [51], a detailed ontology of toxic pathologies for 19 organs was created from the literature in a consistent way to capture precise organ toxicity associations of drugs, industrial, environmental, and other compounds. Models for nephrotoxicity and for more specific endpoints related to these organ injuries were developed using a recursive partitioning algorithm. The models performed better at the prediction of distinct organ toxicity subcategories than general organ toxicity, reflecting the well-known tendency of QSAR models to have a better predictive performance for more specific endpoints.

In a more recent study, Lee et al. [50] present QSAR models for three common patterns of drug-induced kidney injury, i.e., tubular necrosis, interstitial nephritis, and tubulo-interstitial nephritis. Binary classification models of nephrotoxin versus non-nephrotoxin with eight fingerprint descriptors were developed based on heterogeneous pharmacological compounds data. Two types of data sets were used for construction of the training set, i.e., parent compounds of pharmaceuticals (251 nephrotoxins and

Table 4
Overview of published organ and system-specific toxicity QSAR models

Model, reference	Endpoint	Statistical method/software	Statistical parameters	Training set	Test set	Class(es) studied	Significant parameters	Note
Urinary tract toxicity [49]	Six types of urinary tract injury (acute renal disorders, nephropathies, bladder disorders, kidney function tests, blood in urine, urolithiases)	Software programs: MC4PC, BioEpisteme, MDL-QSAR Leadscope Predictive Data Miner	Best consensus models: sensitivity 56 %, specificity 78 %	≈1600	n/r	Multiple	n/r	Consensus models based on two programs increased sensitivity to 56 %
Nephrotoxicity [50]	Tubular necrosis (TN), interstitial nephritis (IN), and tubulointerstitial nephritis (TIN)	SVM	Best parent compounds-based TIN Model: CA = 0.80 and MCC = 0.32 Best Metabolite-based Models: CAs—0.84, 0.85, and 0.83; MCCs—0.69, 0.69, and 0.62 for TN, NI, and TIN models, respectively	487 parent compounds/624 metabolites	338 parent compounds/156 metabolites	Multiple	Topological fingerprints implemented in PaDEL-Descriptor	Metabolite sets consist of major urinary metabolites of pharmaceuticals in parent compound sets
Nephrotoxicity [51]	Nephrotoxicity, kidney necrosis, kidney relative weight gain, nephron injury	Recursive partitioning algorithm, as (ChemTree™ software)	Sensitivity and specificity above 90 %	172–847 depending on the model	42–154 depending on the model	Multiple	Two-dimensional structural descriptors	Models are available in the MetaDrug/ ToxHunterTM systems pharmacology suite.
Nephrotoxicity, hematotoxicity [52]	Renal tubular necrosis, hemolytic anemia			16		Derivatives of 1,2-and 1,4-naphthoquinone	Structural alerts	SAR analysis, outlining important structural alerts
Nephrotoxicity [53]	cGST and MGST1 enzyme activity	LR	r^2 (specific activities for MGST1-catalyzed reactions) = 0.943	9	n/r	Haloalkenes	E_{LUMO}	The relation between nephrotoxicity of the haloalkanes and E_{LUMO}, reflect their propensity for conjugation reactions catalyzed by glutathione transferase enzymes
Acute and delayed neurotoxicity [54]	Inhibitory activity and pairwise selectivity toward serine esterases including acetylcholinesterase and neuropathy target esterase	MLR (Hansch analysis), MFTA	Hansch analysis best models: r = 0.699–0.993 MFTA best models: r^2 = 0.57–0.96; q^2 = 0.47–0.91	Hansch analysis: 7/9 MFTA: 18–52	n/r	Organophosphorus compounds	Hansch analysis: hydrophobicity of substituent R in the general formula (RO)2P(O)X MFTA: effective charge, Q, on an atom; effective van der Waals radius, Re; group lipophilicity, Lg	

Acute and delayed neurotoxicity [55]	Inhibitory activity and pairwise selectivity toward serine esterases including acetylcholinesterase and neuropathy target esterase	FSMLR, BPNN, MFTA, CoMSIA	FSMLR models: Q^2_{DCV} range 0.180–0.778. BPNN models: Q^2_{DCV} range 0.601–0.800 MFTA models: r^2 range 0.62–0.96 CoMSIA models: r^2 range 0.8–0.93, q^2 range 0.62–0.80	30–58	n/r	*O*-Phosphorylated oximes	FSMLR: fragmental descriptors of up to eight non-hydrogen atoms, Log *P* MFTA: effective charge, *Q*, on an atom; effective van der Waals radius, Re; group lipophilicity, Lg	
Neurotoxicity [56]	Seven endpoints related to neurotoxicity: including effects on vesicular and membrane transporter-mediated uptake of dopamine, glutamate and gamma-aminobutyric acid	PCA; PLS	Two significant principal components (t1 and t2) explaining 51 % of the variation in the in vitro screening data (t1 = 37 % and t2 = 14 %) The PLS model (response formation of the chemically reactive oxygen containing species): $Q^2 = 0.63$	20	n/r	Non-dioxin-like polychlorinated biphenyls	67 chemical descriptors including partial atomic charges and parameters describing size and substitution pattern; Significant parameters (PLS model): molecular size, expressed as number of substituents and total surface area	PCA used to derive general toxicity profiles from the in vitro screening data
Neurotoxicity [57]	EC_{30}*rat*, EC_{30}*mouse*	TOPS-MODE approach	Model (rat): $r = 0.902$, $s = 0.252$, $F(6,38) = 27.5$, $r_{cv} = 0.902$, RMSECV = 0.273 Model (mice): $r = 0.901$, $s = 0.250$, $F(7,38) = 23.4$, $r_{cv} = 0.881$, RMSECV = 0.264	45/46	n/r	Non-congeneric series of solvents	Spectral moments, multiplication of moments, indicator of Hydrogen bond capacity of groups, experimental values of BP	Structural fragments, responsible for difference in neurotoxicity are analyzed
Neurotoxicity (in vitro [58])	Acetylcholinesterase inhibition, IC_{50}; in vitro	Pharmacophore modeling, using Catalist software	$r > 0.98$	8	n/r	Organophosphorous compounds	Pharmacophore models include at least one hydrogen bond acceptor site and 2–3 hydrophobic sites	
Lung toxicity [59]	IL-8 gene expression; in vitro	MLR Software: ADMEWORKS	Not reported statistics of the MLR model; 75 % accuracy of external validation (classification: upregulation class/downregulation class)	54	8	Multiple	WTPT3, MOLC4, V5CH, SYMM2, S3C, CRB_LEADL, and OPERA RULEI	The 54 chemicals are classified into two groups based on the gene expression of IL-8, namely upregulation class and downregulation class
Lung toxicity [60]	Cellular membrane damage of immortalized human lung epithelial cells (via lactate dehydrogenase release); in vitro	MLR, PCA, LDA classification	Models for TiO_2: $r^2 = 0.70–0.77$ Models for ZnO: $r^2 = 0.19–0.49$	24 measurements from five different TiO_2 features and 18 measurements from six different ZnO features	n/r	TiO_2 and ZnO nanoparticles	TiO_2: size in ultrapure water, concentration in ultrapure water, and zeta potential in ultrapure water. ZnO: size in ultrapure water, size in CCM, and concentration	The goodness of the classification measured by the resubstitution error

(continued)

Table 4
(continued)

Model, reference	Endpoint	Statistical method/software	Statistical parameters	Training set	Test set	Class(es) studied	Significant parameters	Note
Lung toxicity [61]	Activity of phospholipases A2 (PLA2), C (PLC), and D (PLD); in vitro	LR	r = 0.97, 0.98, 0.99, for PLA2, PLC, and PLD, respectively	4	n/r	Lipid ozonation products	E_{HOMO}, E_{LUMO}, and the net charge on the H49 atom	
Immunotoxicity [62]	Each endpoint corresponds to one out of 1418 assays, 36 molecular and cellular targets, 46 standard type measures related to immunotoxicity	TOPS-MODE approach	Accuracy = 91.76 %	6747	1156	Multiple	spectral moments	Multi-target QSAR (mt-QSAR)
Immunotoxicity [63]	Binding to the aryl hydrocarbon receptor (AhR)	CoMFA	Best model: r^2 = 0.858 q^2 = 0.684; r^2 (test set) = 0.851	59	19	Polychlorinated dibenzo-dioxins, polychlorinated dibenzo-furans, and polychlorinated biphenyls	Steric and electrostatic fields	
Immunotoxicity [64]	Log ED_{50}	MLR	r = 0.964 r_{cv} = 0.884	209	n/r	Polychlorinated diphenyl ethers	A parameter of electrostatic equilibrium on molecular surface	
Myelotoxicity [65]	pIC50 for human CFC-GEMM cells (colony-forming cell granulocyte, erythroid, megakaryocyte, macrophage) and GM-CFC (granulocytes monocytes colony-forming cell)	PCA, PLS Pentacle software: GRIND toxicophore-based descriptors calculated and PLS-DA performed	Best PLS model: r^2 = 0.79 q^2 = 0.72 r^2 (test set) = 0.67 RMSEP = 0.69 PLS-DA: Accuracy (test set prediction) = 86 %	14	21	Multiple	Volsurf descriptors, 2D structural and electrotopological E-states descriptors	Pentacle software used to calculate GRIND toxicophore-based descriptors
Cardiotoxicity [66]	hERG channel blocking							Review
Cardiotoxicity [67]	hERG channel blocking							Review

AE adverse effect, *BP* boiling point, *BPNN* Backpropagation Neural Networks, *CA* classification accuracy, *cGST* cytosolic glutathione transferases, *Clog* calculated partition coefficient *P*, CoMFA comparative molecular field analysis, *CoMSIA* Comparative Molecular Similarity Index Analysis, *CRB LEADL* count of rotatable bonds, *ELUMO* energy of the lowest unoccupied molecular orbital, *FSMLR* Fast Stepwise Multiple Linear Regression, *hERG* human Ether-a-go-go Related-Gene, *IL-8* interleukin-8, *LDA* linear discriminant analysis, *LR* linear regression, *MCC* Matthews correlation coefficient, *MFTA MLR* multiple linear regression, Molecular Field Topology Analysis, *MGST1* microsomal glutathione transferase 1, *MOLC4* path-2 molecular connectivity, *n/r* not reported, *OPERA RULEI* the rule based on Lipinski's rule, *RMSECV* root mean square error of cross validation, *RMSEP* root-mean-squares error of prediction, *SVM* support vector machine, *q* cross-validation parameter, *Q2DCV* determination coefficient for double cross-validation, *r* correlation coefficient, *S3C* third order cluster MC Simple, *SYMM2* geometrical symmetry, *TOPS-MODE* topological sub-structural molecular design, *V5CH* fifth order chain MC Valence, *WTPT3* sum of atom indexes for all heteroatoms

387 non-nephrotoxins) and their major urinary metabolites (307 nephrotoxins and 233 non-nephrotoxins). Thus the study reflects the fact that the nephrotoxicity of a pharmacological compound is induced by the parent compound as well as its metabolites. The results of a tenfold cross-validation and external validation procedures showed a high accuracy of the models (better than 83 % for external validation sets).

For kidney toxicity, local QSARs have been developed for specific chemical classes, such as the haloalkenes. These high-volume chemicals used in industrial, synthetic, and pharmaceutical applications are common environmental pollutants. Many haloalkenes are known to be nephrotoxic in rodents after bioactivation via the cysteine conjugate beta-lyase pathway, which is triggered by formation of hepatic glutathione S-conjugates, a reaction catalyzed by cytosolic and microsomal glutathione transferases [68]. The study by Jolivette and Anders [53] relates the nephrotoxicity of nine haloalkenes to their lowest unoccupied molecular orbital energies, E_{LUMO}, reflecting their propensity for conjugation reactions catalyzed by glutathione transferase enzymes.

Very few QSAR studies of neurotoxicity have been published. An example is the work of Estrada et al. [57]. Their models are based on the TOPS-MODE approach, which provides a means of estimating the contributions to neurotoxicity in rats and mice of a series of structural fragments.

Organophosphorus (OP) compounds are well-known neurotoxic agents. These chemicals are potent inhibitors of serine esterases, the most critical of which is the widely distributed nervous system enzyme acetylcholinesterase (AChE). This well established mechanism of action underlies the usefulness of molecular modeling approaches like 3D QSAR and pharmacophore modeling to predict the inhibition potency of OPs. Several published models are based on these approaches [54, 55, 58, 63].

Among the commonly used software tools, Derek Nexus estimates neurotoxicity using a number of structural alerts: gamma-diketone or precursor, acrylamide or glycidamide, nitroimidazole, carbon disulfide or precursor, pyrethroid, 1-methyl-1, 2,3,6-tetrahydropyridine, lead or lead compound, and organophosphorus ester.

Few studies have been published in relation to other organs/systems. Immunotoxicity can refer to immunosuppression in humans (caused, for example, by benzene and halogenated aromatic hydrocarbons), autoimmune disease (for example the pesticide dieldrin induces an autoimmune response against red blood cells, resulting in hemolytic anemia), and allergenicity (chemicals which stimulate the immune system can cause allergies or hypersensitivity reactions such as anaphylactic shock). Thus, immunotoxicity refers to a wide variety of biological effects, many of which involve complex biochemical networks. Tenorio-Borroto

et al. [62] have trained and validated a multi target-QSAR model for high-throughput screening of drug immunotoxicity using TOPS-MODE approach. Yuan et al. [63] have studied the key molecular features of polychlorinated dibenzodioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls for determining binding affinity to the aryl hydrocarbon receptor (AhR)—an intracellular receptor which has been correlated to immunotoxicity, thymic atrophy, weight loss and acute lethality. CoMFA (Comparative Molecular Field Analysis) was applied to generate 3D QSAR models. In a study by Hui-Ying et al. [64], linear relationships between immunotoxicity values (log ED_{50}) and other biological activities of polychlorinated diphenyl ethers and their structural descriptors were established by multiple linear regression. It was shown that the structural descriptors derived from molecular electrostatic potentials together with the number of the substituting chlorine atoms on the two phenyl rings can be used to express the quantitative structure–property relationships of polychlorinated diphenyl ethers.

Evaluation of hematotoxicity is important step in early drug design. Particularly it is a common dose-limiting toxicity associated with anticancer drugs. The first attempt to build in silico models to predict the myelosuppressive activity of drugs from their chemical structure was made by Crivori et al. [65]. Two sets of potentially relevant descriptors for modeling myelotoxicity (i.e., 3D Volsurf and 2D structural and electrotopological E-states descriptors) were selected and PCA (Principal Component Analysis) was carried out on the entire set of data (38 drugs). The first two principal components discriminated the highest from the least myelotoxic compounds with a total accuracy of 95 %. In addition, a highly predictive PLS (Partial Least Squares) model was developed by correlating a selected subset of in vitro hematotoxicity data with Volsurf descriptors. After variable selection, the PLS analysis resulted in a one-latent-variable model with r^2 of 0.79 and q^2 of 0.72.

In contrast to other organ-specific effects, the in silico modeling of cardiotoxicity has been a rather productive field. This is because drug cardiotoxicity is one of the main reasons for drug related fatalities and subsequent drug withdrawals. In recent years, the hERG channel has been extensively investigated in the field of cardiotoxicity prediction as it has been found to play a major role in both cardiac electrophysiology and drug safety. Because hERG assays and QT animal studies are expensive and time consuming, numerous in silico models have been developed for use in early drug discovery. The earliest attempts to identify whether a molecule is a hERG blocker include a set of simple rules based on structural and functional features, but these rules are not always reliable predictors for identifying hERG blockers. In order to give more accurate predictions of hERG blockage, a wide range of QSAR models have been developed based on a variety of statistical

techniques and machine learning methods, including multiple linear regression, partial least square (PLS), k-nearest neighbor algorithm (kNN), linear discriminant analysis (LDA), artificial neural networks (ANN), support vector machine (SVM), self-organizing mapping (SOM), recursive partitioning (RP), random forest, genetic algorithm, and naive Bayesian classification (NBC). Most of these QSAR models are classifiers and only a few regression models have been reported.

Pharmacophore modeling has also been employed to develop ligand-based prediction models of hERG channel blockers. Since the crystal structure of the hERG channel is not available, all structure-based studies on its blockage are performed on homology models and are more qualitative and descriptive rather than predictive. For example they have been used for molecular docking, molecular dynamics simulations and free energy calculations to explore the hERG-blocker interactions.

Reviews by [66] and Villoutreix and Taboureau [67] summarize the advances and challenges in computational studies of hERG channel blockage. It is expected that the development of in silico models for hERG-related cardiotoxicity will stay active in the coming years in order to design drugs without undesirable side effects.

6 Conclusions

The modeling of acute systemic toxicity has largely focused on QSARs for predicting LD_{50} values and for categorizing chemicals according to ranges of LD_{50} values. For these purposes, which are potentially useful in the regulatory assessment of chemicals, the in silico models seem to perform as well as in vitro cytotoxicity methods. The developments in this field can be attributed to the availability of extensive LD_{50} datasets and a wide range of machine learning techniques. Many of these datasets, and software tools derived from the datasets, are in the public domain.

The emergence of mechanism-based toxicology (e.g., adverse outcome pathways) is a tremendous opportunity to improve current models with better biological knowledge. Indeed, the time of global (and scientifically dubious) QSARs predicting LD_{50} based on chemical properties for the whole chemical space is probably coming to an end. Future models should target specific toxicity mechanisms on the basis of current biological knowledge. Historically, this was actually done implicitly by focusing model building on very limited chemical classes (supposedly acting via the same mechanism). According to this approach, global LD_{50} models would be the sum of a multitude of accurate predictors dedicated to describe well-defined mechanisms of action. In this context, the use of biological (in vitro) descriptors in combination

with traditional molecular descriptors provides a promising means of building local QSAARs based on mechanistically based chemical classes.

In general, the modeling of organ-specific and system-specific effects represents an underdeveloped field, ripe for future research but far from regulatory applications, which typically rely on the assessment of lethality. A notable exception concerns the modeling of receptors and ion channels implicated in specific organ pathologies, such as the hERG channel in relation to cardiotoxicity. The development of models for upstream (molecular and cellular) effects represents a more scientifically meaningful exercise which also promises to unify the traditional regulatory distinction between the acute and repeat dose toxicity.

A future research initiative could include, for example, reexamination of the datasets for hepatobiliary and urinary tract toxicities of drugs with a view to developing more accessible models and assessing their applicability to chemicals other than pharmaceuticals. In addition, the concept of reactivity-based toxicity, now established as a plausible mechanism for hepatocyte toxicity, could be further exploited using data from hepatocyte cultures and cell lines. In some areas, such as immunotoxicity, short-term progress seems unlikely. The complexity of such effects probably means that systems biology approaches will be more appropriate.

In general, the development of models for organ-specific and system-specific effects will depend on a new generation of databases, such as the COSMOS database, which contain high quality data that are structured and annotated according to meaningful chemical and biological ontologies.

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