

# **Review of QSAR Models and Software Tools for predicting Acute and Chronic Systemic Toxicity**

**Silvia Lapenna, Mojca Fuart-Gatnik and Andrew Worth**

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European Commission  
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**Contact information**

Address: Via E. Fermi 2749, 21027 Ispra (VA), Italy  
E-mail: andrew.worth@ec.europa.eu  
Tel.: +39 0332 789566  
Fax: +39 0332 786717

<http://ihcp.jrc.ec.europa.eu/>  
<http://www.jrc.ec.europa.eu/>

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## **ABSTRACT**

Information on acute and chronic systemic toxicity, arising from the oral, dermal and inhalation routes of exposure, are key elements in the regulatory assessment of chemicals. Traditionally, this information is obtained from animal studies. However, due to animal welfare and cost considerations, alternatives to animal experiments are being sought, and regulatory frameworks are providing an increasing opportunity or obligation to use such methods. This report provides a review of different computational estimation methods for predicting acute and chronic systemic toxicity. It provides an overview of Quantitative Structure-Activity Relationship (QSAR) models published in the literature, commonly used software tools, and available databases suitable for QSAR analysis. It also briefly explains the Threshold of Toxicological Concern (TTC) concept and how this is used in prioritising chemicals for further assessment and preliminary risk characterisation.

## LIST OF ABBREVIATIONS

ANN	Artificial Neural Network
BfR	German Federal Institute for Risk Assessment
CEBS	Chemical Effects in Biological Systems
CoMFA	Comparative Molecular Field Analysis
CPDB	Carcinogenic Potency Database
CVS	Cross-Validation Set
DART	Developmental and Reproductive Toxicity
DIMDI	German Institute for Medical Documentation and Information
EFSA	European Food Safety Authority
EMA	European Medicines Agency (formerly known as EMEA)
ES	Expert System
EU	European Union
FAO	Food and Agriculture Organization
FDA	United States Food and Drug Administration
GHS	Globally Harmonised Classification System (United Nations)
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods (USA)
ILSI	International Life Sciences Institute
IRIS	Integrated Risk Information System (USA)
ISS	Istituto di Sanità (Italy)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JRC	Joint Research Centre
kNN	k Nearest Neighbour
LD50	Lethal Dose that kills 50% of the test animals in an experiment
NIPALS	Nonlinear estimation by Iterative Partial Least Squares
NTP	National Toxicology Program
QSAR	Quantitative Structure-Activity Relationship
RA	Regression Analysis
RF	Random Forest
RTECS	Registry of Toxic Effects of Chemical Substances
NLM	National Library of Medicine (USA)
NIEHS	National Institute of Environmental Health Sciences (USA)
NIOSH	National Institute of Occupational Safety and Health (USA)
TCDD	2,3,7,8-dibenzo-p-dioxin and its analogues
ToR	Threshold of Regulation
TTC	Threshold of Toxicological Concern
WHO	World Health Organization
ZEBET	Centre for Documentation and Evaluation of Alternatives to Animal Experiments (Germany)

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## **1. Introduction**

Systemic toxicity studies aim at investigating the effects of chemicals in laboratory animals exposed to various dosage regiments for different durations. Exposure is generally through the oral, dermal or inhalation routes. The information from systems toxicity studies is used in hazard and risk assessment of chemicals occurring in food, industrial chemicals, biocides, and cosmetics. In this report, we give an overview of the (Q)SAR models and software packages used in the assessment of acute systemic toxicity, chronic systemic toxicity and organ- and system-specific toxicity, as well as the databases available for obtaining such data.

## **2. Acute systemic toxicity**

Acute toxicity describes the adverse effects caused by either a single exposure to a chemical substance or multiple exposures within 24 hours.. The acute lethal dose to 50% of the treated animals (LD<sub>50</sub> value) is the basis for the hazard assessment and classification of chemicals and is widely used for regulatory purposes. However, the LD<sub>50</sub> value presents some drawbacks when used for QSAR modelling. First, acute toxicity effects may result from a wide spectrum of biokinetic, cellular and molecular events. Converting the complex, whole-body phenomena related to acute toxicity into a simple number necessarily leads to a loss of information. Second, available data are highly variable, having been generated by different laboratories, protocols, animal species and strains. This undermines the reliability and repeatability of acute toxicity measurements. These facts complicate the modelling process and may explain why there are relatively few (Q)SAR models and expert systems for predicting oral acute toxicity, in comparison with other endpoints.

### **2.1 Literature models**

Several reviews have been published on available models for rodent (rat and mouse) systemic toxicity (Cronin *et al.*, 2003; Tsakovska *et al.*, 2008; Devillers & Devillers, 2009). In comparison with other *in vivo* endpoints for which extensive datasets with diverse compounds have been used to construct QSARs, most literature-based QSAR models of rodent oral acute toxicity are specific to one or a small number of classes of chemicals. The examined classes and a summary of model characteristics for these local models are given in Table 1. Usually, hydrophobicity and the electronic and steric effects have been identified as parameters of high importance for the modelled toxicity. These models are generally accompanied by a statement of applicability domain, which is defined on the basis on inclusion/exclusion rules regarding the chemical class(es) comprised in the training set.

Among the local QSAR model for rodent oral acute toxicity, organophosphates have received particular attention. For example, Devillers (Devillers, 2004) used partial least squares (PLS) regression and artificial neural network (ANN) analysis to predict rat LD<sub>50</sub> values for 51 organophosphorus pesticides. This study highlighted the usefulness of descriptors such as lipophilicity, molar refractivity, H-bonding acceptor ability (HBA) and H-bonding donor ability (HBD). On the other hand, Garcia-Domenech and coworkers (García-Domenech *et al.*, 2007) have proposed a model using solely topological indices to predict rat acute toxicity of 62 organophosphorous pesticides by multiple regression analysis. Neither of these models is available in a form suitable for practical use, but they do identify useful descriptors for further research and QSAR development.

A number of QSAR models for predicting *in vitro* cytotoxicity data are available (e.g. Chan *et al.*, 2007); and these have been reviewed by Tsakovska *et al.* (2008). While such models are not directly relevant to the regulatory assessment of acute toxicity, they may provide insights into the mechanisms of cytotoxic action, and may provide useful supporting information, for example when performing a

read-across of LD<sub>50</sub> values between analogues. As with other models, they would need to be implemented in the form of a user-friendly tool.

*In vitro* endpoints, and in particular cytotoxicities, have also been used as descriptors in combination with structural parameters. This approach is called quantitative structure-activity-activity analysis (QSAAR). An example of this approach, reported by Lessigiarska *et al.* (2006), used rat hepatocyte toxicity data for QSAAR modelling of an heterogeneous set of 48 compounds, comprising pesticides (malathion, warfarin, lindane, chloroform), simple organics, alkaloids and drugs. Interestingly, for this particular dataset, models for rat and mouse acute toxicity showed a better fit when based solely on structural descriptors (i.e. hydrophobicity factor, the electrotopological state descriptor and the number of six-member rings), than when *in vitro* endpoints were included. QSAAR modelling could be a promising approach for acute toxicity prediction, particularly in cases when a significant correlation exists between *in vivo* data (LD<sub>50</sub>) and *in vitro* cytotoxicity (IC<sub>50</sub>), and the additional inclusion of physicochemical parameters serves to improve the correlation. It has been observed that direct *in vitro*-*in vivo* correlations are generally poor, even within the same species, especially for large chemical datasets (ICCVAM *et al.*, 2001). In these *in vitro* studies, most of the compounds showing a bad linear fit are underpredicted on the basis of the *in vitro* data. In practical terms, QSAAR could be particularly useful if high-throughput screening methods are used to generate the *in vitro* data.

Zhu *et al.* (Zhu *et al.* 2009a) have recently reported a novel modelling approach for predicting rodent acute toxicity. In this study, the authors divided the dataset (ZEBET) into two groups, i.e. compounds with a good or a bad IC<sub>50</sub>/LD<sub>50</sub> correlation. Then by using exclusively chemical descriptors (DRAGON) and the *k*-nearest neighbour method, the authors developed specific QSAR models as follows: a) binary classification models to partition external compounds into two groups, i.e. i) compounds having a good IC<sub>50</sub>/LD<sub>50</sub> linear correlation within a defined band, and ii) those falling outside the band, and b) class-specific models to predict LD<sub>50</sub> values for each subclass. The LD<sub>50</sub> prediction accuracy of the resulting models proved superior to TOPKAT models applied to the same external test set of rodent acute toxicity data (RTECS chemicals). The innovative aspect of this hierarchical two-step modelling approach is that it used the relationship between *in vitro* and *in vivo* acute toxicity data as the initial information for the construction of QSARs for predicting LD<sub>50</sub> values which are based solely on chemical descriptors. Although no mechanistic rationalisation of the modelling approach was provided by the authors, it could be useful to discriminate between chemicals belonging to class i) vs. class ii), as described above, which may reflect biotransformation or other *in vivo* effects which cannot be represented by *in vitro* toxicity data alone. Nevertheless, a disadvantage of this modelling approach is that it requires highly specialised expertise and tools, which may not be transferable to other users and computational platforms. Due to its complexity, it is difficult to judge whether the models are reproducible, as neither the algorithm used is transparent, nor the exact compositions of the training and test sets are available.

In addition to the above-mentioned local models, a number of QSAR models for rat oral acute toxicity have been developed using large datasets (global models) have been recently reported by Zhu and coworkers (Zhu *et al.*, 2009b). These models were built by using a combinatorial QSAR modelling approach, including several sets of descriptors and employing several statistical modelling methods (e.g. nearest neighbour methods, the Random Forest predictor, and the FDA MDL QSAR method). Ultimately, consensus models were developed by averaging the predicted LD<sub>50</sub> for every compound using all five models, which afforded higher prediction accuracy as compared to individual models. However, as a result of using a large number of descriptors, which are often sparsely populated, the multidimensional space defined by each of these models is complex and fragmented. As a result of the high complexity of the modelling procedure, these models are difficult to reproduce, even by a specialist, and thus they are not easily transferable and practically useful. The consensus modelling developed in this study is nevertheless interesting as the basis for further research in computational methods to predict acute oral toxicity.

A recent study by Raevsky and coworkers (Raevsky *et al.*, 2010) proposed the so-called Arithmetic Mean Toxicity (AMT) modelling approach, which produces local models based on a *k*-nearest neighbours approach. The authors showed that LD<sub>50</sub> values could be predicted with r<sup>2</sup> values up to 0.78, depending on the selection of nearest neighbours (analogues), which is significantly better than the statistics associated with in vitro-in vivo correlation (typically r<sup>2</sup> values less than 0.5). The error of prediction was in the range 0.30 to 0.52 log units, which is comparable with the experimental error associated LD<sub>50</sub> determination. These predictions are based on the following general formula, in which LD<sub>50</sub> AMT refers to the average LD<sub>50</sub> values of one or more pairs of analogues (nearest neighbours), and the coefficients depend on the specific settings (how many pairs of analogues are included in the calculation):

$$\log(1/\text{LD}50)\exp = a_0 + a_1 * \log(1/\text{LD}50) \text{ AMT}$$

This approach is transparent and reproducible, but would need to be implemented in a software tool for ease of application. It can be thought of as an automated read-across approach.

## 2.2 Software

Software tools capable of predicting endpoints related to systemic toxicity are listed in Table 2.

The commercial software ACD/Tox Suite (now developed and marketed by Advanced Chemistry Development [ACD/Labs] and formerly by Pharma Algorithms as ToxBoxes) predicts toxicity in both the mouse and rat for various administration routes, including oral, as either quantitative LD<sub>50</sub> values or classification into the five GHS categories.

The statistically-based programs TOPKAT and MCASE use multiple QSARs on small and homogenous sets of data. The rat oral LD<sub>50</sub> 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<sub>50</sub> module in MCASE (named A56) is based on and comprises data for 7920 chemicals from the FDA, WHO and NTP datasets. Tunkel and coworkers (Tunkel *et al.*, 2005) compared the performance of the TOPKAT and MCASE rat LD<sub>50</sub> 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. Overall, these results support the usefulness of the TOPKAT and MCASE tools when used for hazard classification.

Other software tools available for predicting acute toxicity (LD<sub>50</sub>) to rat/mouse, are also available, such as MDL QSAR and TerraQSAR. The TerraQSAR software models, based on neural networks, include modules for predicting both oral and intravenous LD<sub>50</sub> values in mice and rats (<http://www.terrabase-inc.com/>).

## 2.3 Databases

Sources of rat LD<sub>50</sub> values which may be suitable for the development of QSARs, the application of read-across, and the evaluation of high-throughput in vitro methods, are listed in Table 3. In particular, Acutoxbase (Kinsner-Ovaskainen *et al.*, 2009) is being database developed in the context of the EU FP6 project ‘A-Cute-Tox’ ([www.acutetox.org](http://www.acutetox.org)), which aims to optimise and “pre-validate” an *in vitro* testing strategy for predicting acute human toxicity. At present, Acutoxbase is accessible only to the partners of the A-Cute-Tox project, but it is foreseen that (part of) the database will be placed in the public domain after the project is completed (July 2010). It is not yet decided when, and to what extent, the A-Cute-Tox data will be made available by publication of this database. However, parts of the data have been published in the literature (Kinsner-Ovaskainen *et al.*, 2009).

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. In addition, inorganic and organometallic compounds, salts, and compound mixtures are often removed from the analysis. For the development of QSARs, LD<sub>50</sub> values should be converted to log[1/(mol/kg)] (if originally expressed as mol/kg). Finally, approximate LD<sub>50</sub> values should be converted to discrete values, and multiple LD<sub>50</sub> 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 (Zhu *et al.*, 2009a,b).

## 2.4 Conclusions

On the basis of the literature review, it is concluded that 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). However, these tools should be further investigated in relation to apparently high degree of false negatives generated, since this would be undesirable in the regulatory assessment of pesticides. The performance of other software tools in predicting acute toxicity should also be investigated. It is recommended that targeted studies are carried out to explore the usefulness of these software tools not only for classifying chemicals but also for making quantitative predictions of LD<sub>50</sub> values for chemical inventories of regulatory importance (e.g. pesticides).

In the scientific literature, QSAR models have been generated for sets of congeneric compounds (organophosphates, aromatic amines, anilines, etc.) and are scattered over many original publications (see Table 1). Despite their limited applicability when taken individually, these local models might be usefully combined into an expert system for toxicity predictions. Further research and development in this area is therefore encouraged. In addition, several recent research studies (Zhu *et al.*, 2009; Raevsky *et al.*, 2009) have demonstrated the ability to make reasonable quantitative predictions for structurally diverse datasets, especially when high throughput bioactivity data are used in combination with traditional QSAR descriptors. These approaches should be explored further with a view to practical implementation. In this respect, the future availability of the models developed by Zhu *et al.* for use as LD<sub>50</sub> predictors via the EPA website and the ChemBench web portal (Zhu *et al.*, 2009a) are promising initiatives.

### **3. Chronic systemic toxicity**

Chronic (repeated dose) toxicity refers to the general toxicological effects in mammals occurring as a result of prolonged and repeated (oral, dermal or inhalation) exposure to a substance. The general toxicity includes a wide range of possible adverse effects including changes in morphology, physiology, growth, development or life span which result in impaired functional capacity, impaired capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences.

The most commonly performed animal tests are the subacute (28-day) and subchronic (90-day) oral toxicity tests in rodents. Testing is sometimes performed with a longer testing period (12 months or more), and sometimes with in a non-rodent species (e.g. dogs, primates). The studies are used to identify adverse effects on various organs and tissues (e.g. liver, kidney, central nervous system, reproductive organs, immune system, and the endocrine system), and to establish a dose metric for risk assessment - the lowest dose that induces an adverse effect (Lowest Observed Adverse Effect Level; LOAEL) or the highest dose with no biologically or statistically significant adverse effects (No Observed Effect Level; NOEL). In this assessment, all toxicological responses are taken into account and the critical (most sensitive) effect is identified. The results of repeated-dose testing can also be used to classify chemicals on the basis of systemic toxicity. Within the Globally Harmonised Classification System (GHS) for chemicals, the results of repeated dose studies can be used, in a weight-of-evidence approach, to place systemic toxicants in two hazard categories.

Thus, chronic toxicity is not really a single endpoint, but a common term for a multitude of biological effects that have different mechanisms, occur in different tissues and organs and over different time scales. This presents a challenge for QSAR modelling, which should ideally focus on groups of chemicals with a common mode of action. Perhaps for this reason, there have been few attempts to develop QSAR models for chronic toxicity in mammalian. Some of these studies have been reviewed in Tsakovska *et al.* (2008).

#### **3.1 Literature models**

A summary of literature-based QSARs for repeated dose toxicity are given in Table 4.

Garcia-Domenech and colleagues (de Julian-Ortiz *et al.*, 2005; Garcia-Domenech *et al.*, 2006) used the same data used in the TOPKAT training set (EPA and NTP reports) to develop multilinear regression models for predicting the chronic LOAEL and linear discriminant analysis models for classifying chemicals into two or three groups based on LOAEL ranges. They showed that models based on the EPA pesticides database were better than models based on the NTP database. The models are transparent and have the advantage of being based on molecular connectivity indices, which are easily computed, invariant molecular descriptors. The error of the regression models was equivalent to the variance in the underlying experimental data. These models could be useful if implemented in the form of software that also generates the necessary descriptors,

Mazzatorta *et al.* (2008) reported a regression-based QSAR for rat chronic toxicity (180 or more days). The model was developed by applying multivariate analysis to LOAELs for 445 diverse compounds selected from multiple sources, including the dataset of Munro *et al.* (1996) and various chemical assessment reports (JECFA, JMPR, NCI and NIH). The training set, which was not provided in the paper, included pesticides, drugs and natural products. The resulting model, based on 19 easily computed DRAGON descriptors, is explicitly defined. Since the prediction error of 0.70 was found to be close to the experimental error of 0.64 log units, the model was considered to have reasonable predictive ability. The model was analysed in terms of the mechanistic significance of the descriptors, and it was argued that the chronic toxicity is driven by the bioavailability of the compounds, which constitutes a baseline effect, plus excess toxicity possibly described by a few chemical moieties.

A model for predicting oral MRTD values and NOELs in humans was developed by Matthews *et al.* (2004a), who used MRTD data for 1309 pharmaceuticals and defined NOEL as MRTD/10. MultiCase was used to develop a classification model to distinguish between high-toxicity chemicals (having low MRTDs) and low-toxicity chemicals (having high MRTDs) on the basis of structural alerts. While most of the training set is made available (Matthews *et al.* (2004b), the algorithm is not provided, so it would be necessary to have a MultiCase license in order to reproduce and use the model. The model is reported to have a high positive predictivity (93%) at the same time as a low false positive rate (5%), which implies the model can be used to reliably identify toxic chemicals. Examples are given of five structural alerts for high toxicity, out of a total of 134 derived by the MultiCase algorithm. Thus, little of knowledge obtained is transferable to another platform. Interestingly, an additional analysis showed a poor correlation ( $R^2$  of 0.2) between the MTD in rodents and the MRTD in humans, on the basis of a dataset of 326 pharmaceuticals for which the human and rodent data were available. The implications of this finding were not discussed. Overall, this study presents an interesting approach to the estimation of chronic toxicity in humans, but due to a lack of transparency in the algorithm, it would be necessary to purchase a MultiCase license in order to apply the model. A cheaper and more transparent alternative would be to use alternative modelling approaches to explore the dataset and relate the experimental results to rodent data.

The first reanalysis of the FDA MRTD database to be published was performed by Maunz & Helma (2008). They applied Support Vector Regression (SVR), a pattern recognition technique, to predict MRTD on the basis of local clusters of similar molecules. This is an example of an instance-based model (or lazy learning) since a local model for each query compound is built from structurally similar compounds in a dataset. It can be thought of an automated read-across. On the basis of the paper alone, this modelling approach is not transferable and the applicability domain is not defined in an easily interpretable manner. However, the authors have promised to implement the approach in the freely available Lazar software (see above), which could make it useful to the non-specialist.

An example of the application of “manual” read-across to predict the 28-day NOAELs of substituted anilines has been illustrated by Sakuratani *et al.* (2008). This can be performed without any computational tools but is facilitated by using a tool such as the OECD QSAR Toolbox. This process requires a fair degree of computational and toxicological expertise.

### 3.2 Software

Software tools capable of predicting repeated dose toxicity are given in Table 2. At present, the best known is probably TOPKAT, which predicts oral rat chronic LOAEL values. The model includes five regression-based models for five classes of chemicals (acyclics, alicyclics, heteroaromatics, single benzenes and multiple benzenes), developed on the basis of 393 chemicals from various sources (EPA and National Cancer Institute/National Toxicology Program (NCI/NTP) databases; FDA drug applications reports; and the open literature). The paper describing the original model development (Mumtaz *et al.*, 1995), based on 234 structurally-diverse chemicals for which chronic data (12 months or more) were available from the above-mentioned sources, provides a transparent description of the model – it is multilinear regression QSAR based on 44 structural descriptors. In contrast, the algorithm for the updated TOPKAT model, based on five regression models and an extended dataset of 393 chemicals, has not been published.

In a model assessment study by Venkatapathy *et al.* (2004), the predictive performance of TOPKAT was tested against 343 chemicals from the EPA’s Office of Pesticide Programs (OPP) database. After removal of compounds that TOPKAT could not recognise or which generated various types of warnings, the percentages of chemicals in TOPKAT’s database that had a LOAEL predicted within a factor of 2, 5 and 10 of the experimental LOAEL were 65%, 83%, and 91%, respectively. When testing against chemicals not already in TOPKAT’s database (i.e. an external validation), the corresponding percentages were 34%, 57% and 72%. Similar statistics were obtained when the TOPKAT predictions were compared against 313 chemical in the “IHP database”, so-called because it

was derived the Integrated Risk Information System (IRIS), Health Effects Assessment Summary Tables (HEAST), and Provisional Toxicity Value (PTV) databases. If prediction within a factor of 2 is taken as the criterion for “correct classification”, this implies a misclassification rate of 35-66%; and if a factor of 10 is adopted, the corresponding misclassification rate would be 9-28%.

In another assessment, Tilaoui *et al.* (2007) investigated the ability of TOPKAT to predict the LOAELs of substances typically occurring in food, on the basis of 607 substances taken from Munro *et al.* (1996). After excluding the 267 substances in the TOPKAT training set, the number of validation substances was reduced to 340. Of those 340 molecules, 287 had predicted LOAELs with the model applicability domain (OPS), of which 86% were predicted within a factor of 2.

In addition to providing point estimates of chronic toxicity, the similarity search capacity of TOPKAT can be used to identify analogues in the TOPKAT database for use in read-across assessments. For example, in order to predict the LOAEL of dichlorobenzophenone (DCBP), which is a metabolite of chlorobenzilate, dichlorodiphenyltrichloroethane, and dicofol, Mougdal *et al.* (2003) identified 47 potential analogues in the TOPKAT database, of which five were selected on the basis that there were toxicity data in an EPA database (IRIS, HEAST or PTV). Among the five potential surrogates, chlorobenzilate was chosen as a surrogate for DCBP, since it had the most conservative chronic oral reference dose (RfD). The RfD is the US EPA’s maximum acceptable oral dose of a toxic substance, obtained by dividing the NOEL or LOAEL by various uncertainty factors.

The other main software tool capable of predicting LOAELs, is a module of the recently developed MolCode Toolbox. A QMRF for this model is available in the JRC QSAR Model Database.

### 3.3 Databases

There are two main databases suitable for the development and assessment of (Q)SARs for repeat-dose toxicity (Table 5). The RepDose database developed by the Fraunhofer Institute (Bitsch *et al.*, 2006) contains NOELs and LOAELs for over 650 industrial chemicals, but is not made publicly available. A database of human Maximum Recommended Therapeutic Dose (MRTD) values has been compiled and made publicly available by the US FDA (Matthews *et al.*, 2004b).

In addition to these databases, there are several datasets in the published literature. Munro *et al.* (1996) developed a database of 612 structurally well-defined organic chemicals, divided into the three structural Cramer classes (Cramer *et al.*, 1978) and associated with 2944 (subchronic and chronic) NOELs derived from non-carcinogenic endpoints in oral rodent or rabbit studies. This database has provided the basis of the TTC concept. Oral NOELs for 45 consumer product ingredients (not in the Munro database) have been published by Blackburn *et al.* (2005).

### 3.4 Conclusions

The availability of (Q)SAR models for chronic toxicity endpoints is currently very limited. Since a large number of potential targets and mechanisms are associated with repeated dose effects, it is unlikely that any single model or software tool will be capable of making reliable predictions for all chemicals of interest to dietary risk assessment. The most commonly used software tool at present is TOPKAT, and despite the lack of transparency in its predictions, several studies have shown that it gives reasonable predictions for a range of chemicals (including pesticides, industrial chemicals). Another more recently developed tool is a module of MolCode Toolboxes. Predictions from such tools could be used in a weight-of-evidence approach along with additional data. Additional research investigations into the applicability of TOPKAT and MolCode Toolboxes across a wide range of food chemicals would be worthwhile. In addition, a transparent expert system or battery of (Q)SAR models needs to be developed for this endpoint. The studies performed by Garcia-Domenech and co-workers, using the same data as used for TOPKAT, have shown that simple, transparent regression and classification models can be developed, with an equivalent performance to TOPKAT. Thus, it is

recommended that the predictive abilities of these models are compared, and refinements of the literature models explored.

A useful alternative to QSAR when limited data are available is to estimate the toxicity of a chemical of interest by reading across from the corresponding data for suitable analogues. Thus, read across provides an alternative or additional approach to the use QSAR in the estimation of chronic toxicity. Several studies have demonstrated the usefulness of reading across chronic toxicity data, and at least one freely available software tool is available to automate the task in the case of human MRTDs (Lazar). In view of the limited availability of QSARs and predictive software for chronic toxicity effects, the read-across approach merits further investigation, and automated software should be developed further.

## 4. Organ-specific and system-specific toxicity

In addition to models for acute and repeated dose toxicity at the *in vivo* level, a number of models have been developed for predicting toxicities at the cellular, tissue and organ levels. 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 resulting in adverse events in specific organ and system toxicities. Electrophilic chemicals could, for example, deplete glutathione (GSH) and protein thiols. A number of (Q)SAR studies have focused on modelling toxicity to some of these systems, sometimes by modelling *in vitro* data.

### 4.1 Hepatic and urinary tract toxicities

Matthews and co-workers (Matthews *et al.* 2009b) have recently conducted an evaluation study to compare the performances of in-house models built using four QSAR software tools, CASE/MC4PC, MDL-QSAR, BioEpisteme, and LeadsScope Predictive Data Miner), in predicting serious hepatobiliary and urinary tract toxicities of drugs. Models were constructed for five types of liver injury (liver enzyme disorders, cytotoxic injury, cholestasis and jaundice, bile duct disorders, gall bladder disorders) and 6 types of urinary tract injury (acute renal disorders, nephropathies, bladder disorders, kidney function tests, blood in urine, urolithiases). The training set comprised approximately 1600 pharmaceuticals based on observations made in humans in pharmaceutical clinical trials and/or post-market surveillance by the FDA (Ursem *et al.* 2009). For model construction, the toxicities of the training set drugs (continuous values) were classified either as being of low risk (0, negative) or as high risk (1, positive) by identification of an optimised breakpoint activity value (BP) distinguishing active from inactive drugs. The best QSAR models exhibited an overall average 92% coverage, 87% specificity and 39% sensitivity. Furthermore, the sensitivity could be increased to 56% by combining any two of these programs, or 68% by calling a chemical positive if predicted to be positive in at least one of four programs. It was thus argued that, collectively, the four methods offer a high confidence method for predicting serious drug hepatobiliary and urinary tract toxicity. This provides evidence that a consensus prediction strategy provides a means of optimising predictive ability. However, while the models reported in this study are applicable to drugs, it is not clear to what extent they are applicable to other types of chemicals. Furthermore, the models are not transferable – it would be necessary to purchase the four software tools and rebuild the models from the same dataset.

A number of studies have developed local QSAR models for hepatocyte toxicity. For example, Chan and coworkers have used linear regression analysis to develop several correlations between physicochemical parameters and hepatocyte toxicity of a few chemical classes: alpha,beta-unsaturated esters, : alpha,beta-unsaturated aldehydes, *p*-benzoquinones and halobenzenes (Chan *et al.*, 2007, 2008a,b,c). Such models are not directly useful in the regulatory assessment of chemicals, but they have helped to establish mechanisms of hepatotoxicity, and they have identified useful descriptors for

further model development. For example, a QSAR for hepatocyte toxicity of alpha,beta-unsaturated esters, consisting of acrylates and methacrylates, indicates that toxicity is correlated with electronic parameters ( $E_{LUMO}$  and partial charges of the carbon atoms in the reactive centre) as expected for a mechanism based on electrophilic reactivity.

In a more recent study, building upon previous work by (Moridani *et al.*, 2003), Roy and Popelier describe the advantages of quantum-chemical descriptors (quantum topological molecular similarity indices) over physicochemical electronic descriptors (pKa and Hammett electronic constant) for predicting the hepatocyte toxicity of phenols (Roy *et al.*, 2008). Phenols are widely distributed in edible plants, and are used by the chemical industry as chemical intermediates or biocides. While it is well known that polyphenols can protect cells from the oxidative stress, there is also an increasing evidence of their prooxidant cytotoxicity (Nemeikaite-Ceniene *et al.*, 2005). Because of the large human and environmental exposure to phenols, there is much interest in assessing their potential hazard.

#### 4.2 Local models of nephrotoxicity

Very few (Q)SAR studies of nephrotoxicity have been published, and those identified focus on very small groups of compounds, which limits the applicability of the resulting models. For example, structural alerts have been derived for the rat nephrotoxicity of 1,2- and 1,4-naphthoquinones (Munday *et al.*, 2007), and a QSAR has been developed for the nephrotoxicity of haloalkenes (Jolivette *et al.*, 2002). Haloalkenes are high-volume chemicals used in industrial, synthetic, and pharmaceutical applications and are common environmental pollutants. Many haloalkenes are known to be nephrotoxic in rodents after bioactivation via the cysteine conjugate •-lyase pathway, which is triggered by formation of hepatic glutathione S-conjugates, a reaction catalysed by cytosolic and microsomal glutathione transferases (Anders *et al.*, 1998). The study by Jolivette and Anders (Jolivette *et al.*, 2002) related the nephrotoxicity of nine haloalkenes to their lowest unoccupied molecular orbital energies,  $ELUMO$ , reflecting their propensity for conjugation reactions catalysed by glutathione transferase enzymes.

#### 4.3 Neurotoxicity

Neurotoxicity is an adverse effect on the functioning of the nervous system resulting from exposure to a chemical substance. The standard oral 28-day and 90-day toxicity studies include endpoints capable of detecting neurotoxic effects (e.g. clinical observations, behavioural changes, motor activity assessment, neurochemical changes and histopathological changes in the central or peripheral nervous system).

Only a few (Q)SAR studies have focused on the effects of chemicals on the central nervous system, in some cases through the modelling of in vivo toxicity (Table 6). For example, Crofton (1996) described a SAR study of 14 different triazole fungicides which cause hyperactivity in rats. A QSAR for PCB neurotoxicity (Pessah *et al.*, 2005), based on data for 28 ortho-substituted PCBs, and building on earlier work (Nevalainen *et al.*, 1994), revealed a relationship between electronic descriptors ( $ELUMO$ ,  $EHOMO$ , the  $ELUMO \cdot EHOMO$  gap, and molecular polarisability) and the binding affinity of PCBs to the aryl hydrocarbon (Ah) receptor. In particular, impairment of the developing nervous system by PCBs has been linked to their ability to alter the spatial and temporal fidelity of  $Ca^{2+}$  signalling in muscle and nerve cells through one or more receptor-mediated processes (Pessah *et al.*, 2009).

Among the commonly used software tools, Derek for Windows v.12 estimates neurotoxicity using the following structural alerts: •-diketone or precursor, acrylamide or glycidamide, nitroimidazole, carbon disulphide or precursor, pyrethroid, 1-methyl-1,2,3,6-tetrahydropyridine, lead or lead compound and organophosphorus ester.

#### **4.4 Immunotoxicity**

Immunotoxicity is an adverse effect on the functioning of the immune system resulting from exposure to a chemical substance. The standard oral 28-day and 90-day toxicity studies include endpoints capable of detecting immunotoxic effects (e.g. haematological parameters, alterations in immune system organ weights such as spleen and thymus, and histopathological changes in immune organs such as spleen, thymus, lymph nodes and bone marrow).

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 haemolytic anaemia), 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.

No (Q)SARs for predicting immunotoxicity specifically were identified in the literature review, which is perhaps not surprising given the biological complexity of immunotoxic effects.

#### **4.5 Conclusions**

In general, the modelling of organ-specific and system-specific effects represents an underdeveloped field, ripe for future research but far from regulatory applications. Future research initiative could include, for example, re-examination 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 alternative (e.g. systems biology) approaches will need to be investigated in the longer term. Ultimately, it seems unlikely that QSAR models for organ-specific and system-specific effects will be used directly for regulatory purposes, where the focus is on the assessment of apical endpoints. However, these models could become a useful contribution to priority setting exercises, and provide means of providing supporting information, such as on the mechanisms of toxicity.

### **5. The Threshold of Toxicological Concern (TTC) approach**

Chronic systemic toxicity studies after oral exposure have been used to develop the Threshold of Toxicological Concern (TTC) concept. The TTC is a generic human exposure level for chemicals below which there is low probability of risk to human health, assuming lifetime exposure. The principle of TTC is built on the premise that a safe level of exposure can be identified for chemicals present at low concentrations in the diet, even for those with unknown toxicity, on the basis of their chemical structure (Kroes *et al.*, 2004). As such it can be used to support preliminary hazard characterisation and to set priorities in toxicity testing (Barlow, 2005).

The idea that toxicologically insignificant exposure levels to chemicals exist was proposed by Frawley due to an increasing demand for toxicity testing (Frawley, 1967). Although his estimation of a threshold level was based on limited systemic toxicity studies, the concept became broadly accepted (Safford, 2008).

The first toxicological threshold level for chemicals migrating from food packaging, was developed by a probabilistic assessment of the distribution of carcinogenic potency data, from rodent lifetime studies (Rulis, 1992). Rulis proposed a level of exposure of 0.5 ppb equivalent to an intake of 1.5 $\mu$ g/day/adult (Safford, 2008) which would be protective for known and unknown carcinogens. The cut-off value was then accepted by the US Food and Drug Administration (FDA) as a Threshold of Regulation

(ToR), which meant that no further testing was required for substances migrating from packaging into food below this level of exposure. This was the first use of TTC concept for regulatory purposes.

Further development of the TTC concept was carried out by Cheeseman and colleagues, who confirmed that the threshold level of 1.5 $\mu$ g/day, proposed by Rulis, is valid for most carcinogens, and that the dose would be protective also against other toxic endpoints (Cheeseman *et al.*, 1999). They also proposed higher exposure threshold levels for chemicals lacking structural alerts for carcinogenicity, chemicals that were negative in genotoxicity testing and having acute toxicity (LD50) values above 1000mg/kg.

The TTC approach was subsequently refined by different authors with the aim of providing a tiered approach based mostly on chemical structure and oral systemic toxicity data. Munro and colleagues developed a generic threshold for chemicals where non-carcinogenic toxic effects are expected, by evaluating the impact of chemical structure on toxicity. For this purpose they applied the Cramer decision tree, which places chemicals into three structural classes according to the level of concern based on systemic toxicity. The Cramer decision tree approach uses the knowledge on structure activity relationships, metabolism, chemical reactivity, human exposure levels and other relevant information (Cramer *et al.*, 1978). The decision tree consists of 33 questions. Each question can be answered as yes or no, leading to the final classification of a chemical into one of three classes, reflecting the presumption of low, moderate and high toxicity. As a result substances are classified into one of three classes.

- Class I (Low) contains substances of simple chemical structure with known metabolic pathways and innocuous end products which suggest a low order of oral toxicity.
- Class II (Intermediate) contains substances that are intermediate. They possess structures that are less innocuous than those in Class 1 but they do not contain structural features that are suggestive of toxicity like those in Class 3.
- Class III (High) contains substances with a chemical structure that permits no strong initial impression of safety and may even suggest a significant toxicity

The Cramer scheme (and its Toxtree implementation, see 5.1) is applicable to organic molecules and their salts. Polymers, oligomers and inorganics cannot be classified by the decision tree.

Munro *et al.* (1996) proposed human exposure thresholds of 1800, 540 and 90 $\cdot$ g/person/day for classes II and II, respectively. To further evaluate the thresholds proposed by Munro, an expert group was established by International Life Sciences Institute (ILSI) Europe. The group concluded that adverse effects on the nervous system, immune system, endocrine system and development were covered by the thresholds previously proposed by Munro for the three Cramer classes. An exception was identified for organophosphates, which are more toxic. For this group of substances, a specific TTC of 18 $\cdot$ g/person/day was derived (Kroes *et al.*, 2004).

The so-called “cohort of concern” was identified. This includes aflatoxin-like, azoxy- and nitroso-compounds, which are genotoxic, and TCDD (2,3,7,8-dibenzo-p-dioxin and its analogues) and steroids, which are endocrine disruptors. Since these groups of compounds were considered to result in the highest risks if present at very low concentrations in the diet, they were excluded from the TTC approach. Other exclusions from the TTC approach include polyhalogenated dibenzodioxins/dibenzofurans/biphenyls and heavy metals, all of which are known to accumulate in the body; and proteins, because of their allergenic potential. For chemicals having structural alerts for genotoxicity but which do not belong to the cohort of concern, a TTC of 0.15 $\cdot$ g/day was recommended (Kroes *et al.*, 2004).

The ILSI expert group also proposed a decision tree to act as guidance on how and when the TTC principle could be applied as a preliminary step in safety evaluation of chemicals (Kroes *et al.*, 2004). The decision tree is intended for use on chemicals with known structure and low molecular mass. Data on total human exposure are relevant for the successful application of the TTC approach.

So far, the TTC approach has been successfully applied in the safety assessment of food contaminants migrating from packaging by the US FDA, as well as flavouring agents by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The European Food Safety Authority (EFSA) uses the TTC approach to evaluate flavouring substances, and the European Medicines Agency (EMA) uses it in support of marketing applications for genotoxic impurities in pharmaceutical preparations, and recommends a TTC of 1.5 • g per day for all but highly potent subset of compounds (EMEA, 2006). Although at a draft stage, the US FDA also issued a draft guidance on recommended approaches for genotoxic and carcinogenic impurities in drug products enumerating acceptable TTC values, e.g. 1.5 • g per day for both marketing applications and greater than 1-year clinical trials (FDA, 2008). The application of the TTC approach has also been explored for its applicability to consumer products (Safford 2008; Felter *et al.*, 2009). It has also been proposed that the TTC could be adapted for environmental risk assessment (Barlow, 2005).

The scientific basis of the Cramer TTC scheme and its applicability in different regulatory areas has been assessed by various researchers (Phillips *et al.*, 1987) and institutions. For example, an EFSA opinion on the applicability of TTC in the food and feed areas is currently being developed and will be published in 2011.

## 5.1 Databases underlying the derivation of TTC values

The main databases that have been used to develop the TTC concept and to derive structure-based threshold values (as described in the above-mentioned studies) are summarised in Table 7.

## 5.2 Software for TTC estimation

One of the best known software tools for supporting TTC estimations is the JRC's Toxtree software. Toxtree is a freely available open source software tool that estimates toxic hazard by applying a decision tree approach. It was developed by Ideaconsult Ltd (Bulgaria) under the terms of a JRC contract. It is designed to be user-friendly and flexible, being capable of extensions and revisions to its rulebases (plug-ins). Since it is licensed under the General Public License (GPL), any user has the right to modify and redistribute the software in accordance with the GPL licensing conditions. Toxtree can be downloaded from the JRC (<http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE>) and from Sourceforge (<https://sourceforge.net/projects/toxtree/>)

The current version of Toxtree (v2.1.0, June 2010), includes the following plug-ins (rulebases) related to TTC assessment

- 1) the *original Cramer rulebase* (Cramer *et al.*, 1978; Figure 1)

The Toxtree implementation of the original Cramer decision has been evaluated by Patlewicz *et al.* (2008).

- 2) the *Cramer rulebase with extensions* (Figure 2)

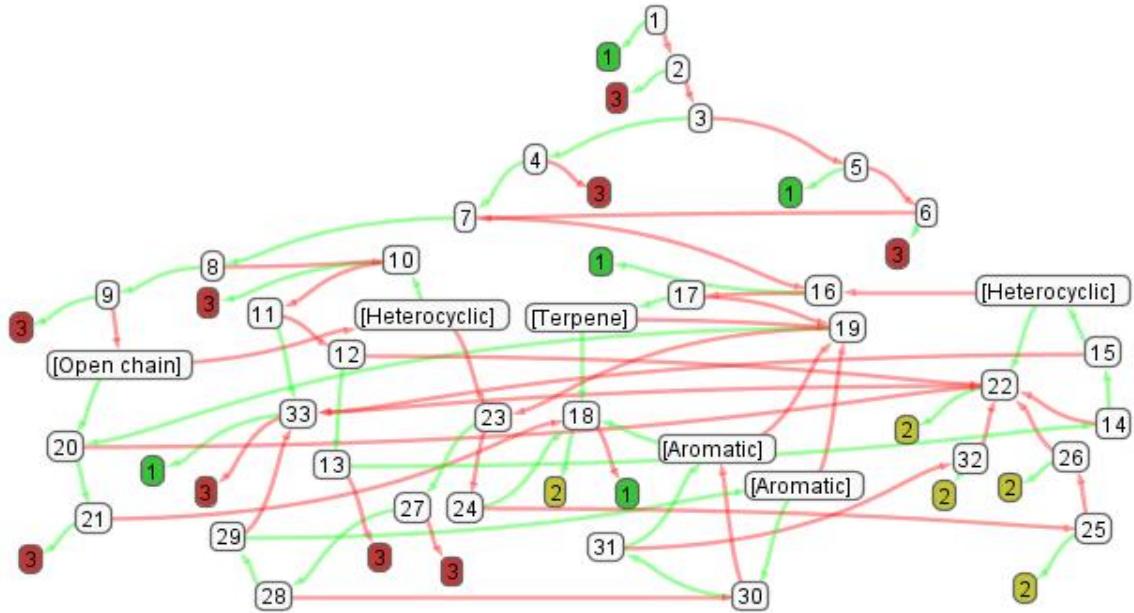
This rulebase (first available in v1.60, July 2009) works by assigning compounds to Class I, II, or III, according to the rules from Cramer, and some extra ones. Several compounds were classified by Munro in 1996 as Class I or Class II compounds according to the Cramer rules, even though Munro reported low NOEL values upon oral administration (indicating relatively high toxicity). To overcome such misclassifications, five rules were introduced to capture the possible toxicity of these compounds. This plug-in was developed by Curious-IT, The Netherlands, on behalf of JRC.

- 3) the *TTC decision tree of Kroes et al.* (2004).

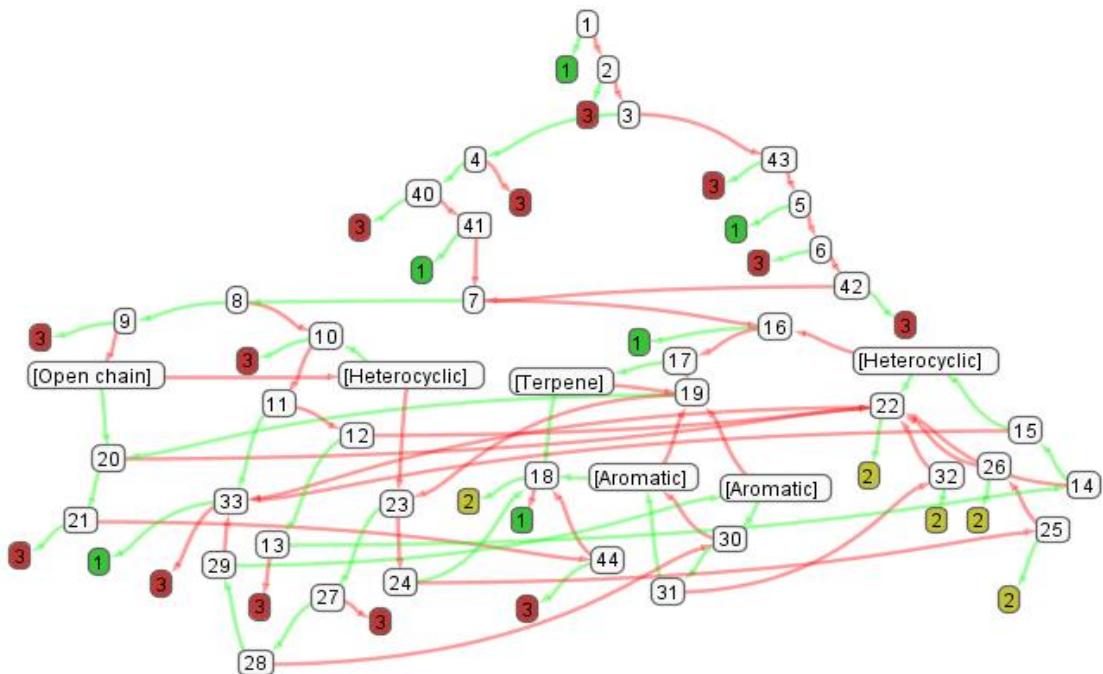
This rulebase (first available in v2.1.0) results in three possible outcomes: a) substance would not be expected to be a safety concern; b) negligible risk (low probability of a life-time cancer risk greater than 1 in  $10^6$ ); and c) risk assessment requires compound-specific data. It incorporates the Benigni/Bossa rules for the identification of genotoxic carcinogens (developed earlier by ISS, Rome on behalf of the JRC), and requires the user to input the estimated daily intake.

## **6. Disclaimer**

Any conclusions and opinions expressed in this document are those of the authors as individual scientists and do not constitute an official position by the JRC or the European Commission.



**Figure 1.** Cramer scheme (original). Yes branch in green. No branch in red. Terminal nodes (labelled 1, 2 & 3) refer to Cramer classifications I, II and III.



**Figure 2.** Cramer scheme with extensions decision tree. Yes branch in green. No branch in red. Terminal nodes (labelled 1, 2 & 3) refer to Cramer classifications I, II and III.

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**Table 1. Literature-based QSAR models for acute toxicity in rodents**

Reference	Exposure route	Endpoint predicted	QSAR method	Compound class(es)	Training set size	Test set size
Zhu <i>et al.</i> (2009a)	oral	LD50	kNN	multiple	230 (rat) and 211 (mouse)	23 (rat) and 24 (mouse)
Zhu <i>et al.</i> (2009b)	oral	LD50	kNN, RF	multiple	3,472	3,913
Fratev (2007)	oral		GRID	allelochemicals	52	21
Garcia-Domenech <i>et al.</i> (2007)	oral		RA	organophosphorus	39	23
Toporov <i>et al.</i> (2007)	oral		RA	substituted benzenes (mostly nitrobenzenes)	14	14
Juranić <i>et al.</i> (2006)	oral		RA	<i>N</i> -alkyl and <i>N</i> -cycloalkyl fluoroacetamides	19	-
Lessigiarska <i>et al.</i> (2006)	oral		RA	multiple	26	5
Guo <i>et al.</i> (2006)	oral		CoMFA	organophosphorus	30	-
Devillers (2004)	oral		NIPALS, ANN	organophosphorus	51	9
Zahouily (2002)	oral		RA	organophosphorus	47	20
Gough and Hall (1999)	oral		RA	amide herbicides	50	9
Eldred and Jurs (1999)	oral		RA	organophosphorus	49	5
			ANN		44 (+5 CVS)	5
Wang and Bai (1998)	oral		Decision tree based on twenty-seven rules	alcohols	95	25
Johnson and Jurs (1997)	oral		RA	substituted anilines	103	12
			ANN		87 (+11 CVS)	11
Zakarya <i>et al.</i> (1996)	oral		RA	amide herbicides	44	-
Jäckel and Klein (1991)	oral		RA	amines and anilines	29	-
Nendza (1991)	oral		RA	phenylurea herbicides	12	-
Enslein, (1978)	oral		RA	multiple	425	100
Durden (1973)	oral		RA	2-alkyl- and 2,6-dialkyl-anilines	8	-

Abbreviations. ANN, artificial neural network; CoMFA, Comparative Molecular Field Analysis; CVS, cross-validation set; ES, expert system; kNN, k nearest neighbour; NIPALS, nonlinear estimation by iterative partial least squares; RF, random forest; RA, regression analysis.

**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	Cytotoxicity
ACD/Tox Suite (ToxBoxes)	Commercial	•					
ADMET Predictor (Simulations Plus Inc.)	Commercial			•			
ADME/Tox WEB	Freely available	•					
BioEpisteme	Commercial			•	•		
Caesar project models (Mario Negri Institute)	Freely available						
Derek (Lhasa Ltd)	Commercial			•	•	•	•
HazardExpert (CompuDrug)	Commercial					•	•
Lazar (In Silico Toxicology; Freiburg university)	Freely available		•	•			
Leadscope (Leadscope)	Commercial			•	•	•	
MCASE/MC4PC (MultiCASE)	Commercial	•		•	•		•
MDL QSAR (MDL)	Commercial	•		•	•		
MolCode Toolbox	Commercial		•				
OASIS-TIMES (Laboratory of Mathematical Chemistry, Bourgas University)	Commercial						
OncoLogic (US EPA)	Freely available						
Pallas Suite including ToxAlert, Cytotoxicity (CompuDrug)	Commercial					•	•
TerraQSAR (TerraBase)	Commercial	•					
TOPKAT (Accelrys)	Commercial	•	•				
Toxtree (JRC)	Freely available						
Molcode Toolbox ( Molcode Ltd)	Commercial					•	

(1) immunotoxicity other than skin sensitisation.

**Table 3. Databases containing acute toxicity information**

Database	Availability	Information
Acutoxbase, linked to the EU FP6 project ‘A-Cute-Tox’; <a href="https://acubase.amwaw.edu.pl">https://acubase.amwaw.edu.pl</a>	Access through the internet, currently restricted to project partners	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 <sub>50</sub> 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 86 reference chemicals, human acute poisoning cases from clinical/forensic reports are also available.
ChemIDplus, developed by the US NLM; <a href="http://chem.sis.nlm.nih.gov/chemidplus/">http://chem.sis.nlm.nih.gov/chemidplus/</a>	Freely available through the Internet, structure-searchable	Toxicity data for over 139,000 records, retrieved from TOXNET® (TOXicology Data NETwork; <a href="http://toxnet.nlm.nih.gov">http://toxnet.nlm.nih.gov</a> ) which includes HSDB (Hazardous Substances Data Bank). The HSDB is an older subset of the RTECS database. A search for rat and mouse oral LD <sub>50</sub> values found 13,548 and 28,033 records, respectively.
CEBS, developed by the US NIEHS; <a href="http://cebs.niehs.nih.gov/">http://cebs.niehs.nih.gov/</a>	Freely available through the Internet	<i>In vivo</i> study data and acute dose of a small number of known hepatotoxicants to rat.
RTECS, originally compiled and maintained (until 2001) by the US NIOSH and currently maintained by Symyx Technologies. Structure-searchable through the Symyx Toxicity Database:  <a href="http://www.symyx.com/products/databases/bioactivity/rtecs/index.jsp">http://www.symyx.com/products/databases/bioactivity/rtecs/index.jsp</a>  Also searchable via the LeadsScope Toxicity Database ( <a href="http://www.leadscope.com/databases/">http://www.leadscope.com/databases/</a> )	Commercial	Rat acute oral toxicity (LD <sub>50</sub> ) and acute inhalation toxicity (LC <sub>50</sub> ) data compiled from the open scientific literature for approx. 7,000 compounds (organic, inorganic and mixtures), including approx. 4000 organic compounds.
TerraBase databases <a href="http://www.terrabase-inc.com/">http://www.terrabase-inc.com/</a>	Commercial	Several databases include rat and mouse LD <sub>50</sub> values for different product types (natural compounds, drugs, pesticides)
ZEBET, compiled by BfR ZEBET; <a href="http://www.dimdi.de">http://www.dimdi.de</a>	Freely searchable through the DIMDI website. Published in a report by ICCVAM (ICCVAM <i>et al.</i> , 2001)	Includes rat or mouse LD <sub>50</sub> values (from the RTECS database) and cytotoxicity (IC <sub>50</sub> ) data for 347 compounds compiled from the open literature.

Abbreviations: CEBS, Chemical Effects in Biological Systems; DIMDI, German Institute for Medical Documentation and Information; ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods; RTECS, Registry of Toxic Effects of Chemical Substances; 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.

**Table 4. Literature-based QSAR models for chronic toxicity in rodents**

Reference	Exposure route	Endpoint predicted	QSAR method	Compound class(es)	Training set size	Test set size
de Julian-Ortiz <i>et al.</i> (2005)	Oral	Rat chronic LOAEL	MLR, LDA	multiple	234	17
Garcia-Domenech <i>et al.</i> (2006)	Oral	Rat chronic LOAEL	MLR, LDA	multiple	86	16
Mazzatorta <i>et al.</i> (2008)	Oral	Rat chronic LOAEL	GA and PLS for descriptors selection; LOO-SMLR for model generation	multiple	445	none
Matthews <i>et al.</i> (2004a and 2004b)	Oral	Human MRTD and NOEL	Classification model based on SAs	pharmaceuticals	1309	none
Maunz & Helma (2008)	Oral	Human MRTD	SVR	pharmaceuticals	1215	None (internal cross-validation)

Abbreviations. LDA, linear discriminant analysis; LOO-SMLR, leave-one-out stepwise multiple linear regression; MLR, multiple linear regression; SA, structural alerts; SVR, support vector regression.

**Table 5. Databases containing repeated dose toxicity information**

<b>Database</b>	<b>Availability</b>	<b>Information</b>
US FDA Maximum Recommended Therapeutic Dose (MRTD) Database  <a href="http://www.fda.gov/AboutFDA/CentersOffices/CDER/cm092199.htm">http://www.fda.gov/AboutFDA/CentersOffices/CDER/cm092199.htm</a> <a href="http://www.epa.gov/ncct/dsstox/sdf_fdamdd.html">http://www.epa.gov/ncct/dsstox/sdf_fdamdd.html</a>	Freely available	MRTD values for 1215 pharmaceuticals from clinical trials, mostly by oral administration and daily treatments, usually for 3-12 months. (with 5% of the pharmaceuticals being administered intravenously and/or intramuscularly). Includes structures. Available from FDA and EPA DSSTOX
RepDose database developed by Fraunhofer Institute of Toxicology and Experimental Medicine  <a href="http://www.fraunhofer-repdose.de/">http://www.fraunhofer-repdose.de/</a>	Freely available for online searching	Subacute to chronic, oral and inhalation NOELs and LOAELs and for 655 industrial chemicals (version 2009); publicly available rat, mouse and dog studies; includes structures, physicochemical properties and study designs
Mazzatorta <i>et al.</i> (2008) <a href="http://pubs.acs.org/doi/suppl/10.1021/ci8001974">http://pubs.acs.org/doi/suppl/10.1021/ci8001974</a>	Freely available as MS Excel file	molecular structures (encoded as canonical SMILES strings) with LOAEL values for 445 unique chemicals
The Munro and Cramer datasets: <a href="http://apps.ideaconsult.net:8080/ambit2/dataset/26538?max=100">http://apps.ideaconsult.net:8080/ambit2/dataset/26538?max=100</a> <a href="http://apps.ideaconsult.net:8080/ambit2/dataset?search=Cramer">http://apps.ideaconsult.net:8080/ambit2/dataset?search=Cramer</a>  <a href="http://www.efsa.europa.eu/en/scdocs.htm">http://www.efsa.europa.eu/en/scdocs.htm</a>	Available from AMBIT website.  Also expected to be available from EFSA in 2011	Munro database contains 612 structurally well-defined organic chemicals and associated NOELs  Cramer dataset contains 83 structures (no toxicological data)

**Table 6. Literature-based (Q)SAR models for organ- and system-specific toxicity**

Reference	Endpoint	Compound class(es)	Dataset Size (1)
Gu et al. (2009)	Neurotoxicity, immunotoxicity (i.e., AHH and EROD induction potency)	PADDs	13
Gu et al. (2009)	Neurotoxicity, immunotoxicity (i.e., AhR binding affinity)	PADDs	25
Tichý et al. (2009)	rat hepatocyte toxicity	Aliphatic alcohols, ketones, esters	15
Matthews et al. (2009b)	Human clinical hepatobiliary and renal tract toxicities	pharmaceuticals	~1600
Roy et al. (2008)	rat hepatocyte toxicity	phenols	31
Chan et al. (2008a)	human and rat hepatocyte toxicity	p-benzoquinones	10
Chan et al. (2008b)	rat hepatocyte toxicity	acrylates and methacrylates	10
Chan et al. (2008c)	rat hepatocyte toxicity	•,•-unsaturated aldehydes	11
Chan et al. (2007)	human and rat hepatocyte toxicity	halobenzenes	12
Pessah et al. (2005)	developmental neurotoxicity (ryanodine receptor type 1-binding affinity) - SAR	Ortho-substituted PCBs	28
Jenkins et al. (2004)	developmental neurotoxicity (neurite outgrowth and cell death) - SAR	Organotin	4
Moridani et al. (2003)	rat hepatocyte toxicity	phenols	31
Jolivette et al. (2002)	Rat <i>in vivo</i> nephrotoxicity	1,2- and 1,4-naphthoquinones	9
Yazal et al. (2001)	<i>in vitro</i> neurotoxicity (AChE inhibition, IC <sub>50</sub> )	organophosphorous	8
Crofton (1996)	rat <i>in vivo</i> neurotoxicity (hyperactivity) - SAR	triazoles	16
Nevalainen et al. (1994)	Neurotoxicity, immunotoxicity (aryl hydrocarbon receptor binding affinity)	PCDDs	14
Nevalainen et al. (1994)	Neurotoxicity, immunotoxicity (aryl hydrocarbon receptor binding affinity)	PCDFs	35
Nevalainen et al. (1994)	Neurotoxicity, immunotoxicity (aryl hydrocarbon receptor binding affinity)	PCBs	14
Nevalainen et al. (1994)	Neurotoxicity, immunotoxicity (aryl hydrocarbon receptor binding affinity)	PCDEs	12
Mager (1982)	rat <i>in vivo</i> neurotoxicity (ataxia)	organophosphorous	22

(1) Training set + test set (when applicable)

Abbreviations. Aryl hydrocarbon hydroxylase (AHH); Aryl hydrocarbon receptor (AhR); 7-ethoxyresorufin O-deethylase (EROD); Polyhalogenated dibenz-p-dioxins (PADDs); polychlorinated dibenz-p-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and diphenylethers (PCDEs). 3.6 Genotoxicity (including mutagenicity) and carcinogenicity.

**Table 7. Summary of Threshold of Toxicological Concern datasets**

<b>Author</b>	<b>Database (number of substances)</b>	<b>Evaluated experimental data (count of data)</b>	<b>Conclusions</b>
Rulis (1986)	CPDB carcinogens (343)	Chronic long term exposure	Proposed ToR of 0.5 ppb equivalent to 1.5µg/day adult intake
Munro (1996)	JECFA, US EPA IRIS, non tumour from NTP, DART, literature (611)	Oral toxicity data from chronic, sub-chronic, reproductive, teratology studies	Proposed TTC for the three Cramer classes: 1880 µg/day for Class I; 540 µg/day for Class II; 90 µg/day for Class III
Cheeseman (1999)	CPDB carcinogens (709)	Short-term toxicity data, genotoxicity testing	Confirmation of the validity of 1.5µg/day for subsets of potent and non potent carcinogens
	RTECS (3306) RTECS (2542)	Oral reproductive toxicity data Data from other repeat-dose toxicity tests	Confirmation of the validity of 1.5µg/day for other toxic effects
ILSI working group (2000, 2004)	Munro DB  JECFA, US EPA IRIS, non tumour from NTP, DART, literature (611)	Subchronic neurotoxicity data (45) Acute neurotoxicity/toxicity data (37) Developmental neurotoxicity (52) Immunotoxicity (37) Developmental (81)	Confirmation of TTC proposed for the three Cramer classes, also for other toxic endpoints  Lower TTC of 18 µg/day for organophosphates
	Cheesman's CPDB carcinogens (709) extended (730)		Identified 5 groups of chemicals of highest concern “cohort of concern”: 3 groups of genotoxic compounds (aflatoxin-like compounds, azoxy-compounds, nitroso-compounds) and 2 groups of endocrine disruptors (TCDD, steroids)



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**Abstract**

Information on acute and chronic systemic toxicity, arising from the oral, dermal and inhalation routes of exposure, are key elements in the regulatory assessment of chemicals. Traditionally, this information is obtained from animal studies. However, due to animal welfare and cost considerations, alternatives to animal experiments are being sought, and regulatory frameworks are providing an increasing opportunity or obligation to use such methods. This report provides a review of different computational estimation methods for predicting acute and chronic systemic toxicity. It provides an overview of Quantitative Structure-Activity Relationship (QSAR) models published in the literature, commonly used software tools, and available databases suitable for QSAR analysis. It also briefly explains the Threshold of Toxicological Concern (TTC) concept and how this is used in prioritising chemicals for further assessment and preliminary risk characterisation.

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