



Analytical review

Review of Quantitative Structure – Activity Relationships for Acute Mammalian Toxicity

Ivanka Tsakovska^{1*}, Igljika Lessigiarska², Tatiana Netzeva¹, Ilza Pajeva³, Andrew Worth¹

¹Joint Research Centre, Institute for Health and Consumer Protection,
European Chemicals Bureau
I-21020, Ispra (VA), Italy
E-mail: ivanka.tsakovska@jrc.it

²Liverpool John Moores University, School of Pharmacy and Chemistry
Byrom Street, Liverpool, L3 3AF England

³Centre of Biomedical Engineering “Prof. Ivan Daskalov” - Bulgarian Academy of Sciences
105 Acad. G. Bonchev Str., Sofia 1113, Bulgaria

*Corresponding author

Received: September 11, 2006

Accepted: November 22, 2006

Published: December 8, 2006

Abstract: This paper reviews Quantitative Structure-Activity Relationship (QSAR) models for acute mammalian toxicity published in the last decade. A number of QSAR models based on cytotoxicity data from mammalian cell lines are also included because of their possible use as a surrogate system for predicting acute toxicity to mammals. On the basis of the review, the following conclusions can be made: i) a relatively small number of models for in vivo toxicity are published in the literature. This is due to the nature of the endpoint – acute systemic toxicity is usually related to whole body phenomena and therefore is very complex. The complexity of the mechanisms involved leads to difficulties in the QSAR modelling; ii) most QSAR models identify hydrophobicity as a parameter of high importance for the modelled toxicity. In addition, many models indicate the role of the electronic and steric effects; iii) most of the literature-based models are restricted to single chemical classes. Models based on more heterogeneous data sets are those incorporated in expert systems. In general, the QSAR models for mammalian toxicity identified in this review are considered useful for investigating the mechanisms of toxicity of defined chemical classes. However, for predictive purposes in the regulatory assessment of chemicals most of the models require additional information to satisfy internationally agreed validation principles. In addition, the development of new models covering larger chemical domains would be useful for the regulatory assessment of chemicals.

Keywords: QSAR, Acute mammalian toxicity, Expert system, Review.

Introduction

In October 2003 a new chemicals regulation was proposed by the European Commission [6]. It aims to address the existing data gaps and to obtain the necessary information for all substances imported or manufactured in the European market at volumes greater than 1 tonne per year [8]. In order to achieve economic and animal savings the future REACH



(Registration, Evaluation and Authorisation of Chemicals) legislation envisages development of intelligent testing strategies based on (Q)SAR (Quantitative Structure-Activity Relationship), read-across, and grouping approaches as well as other alternative approaches to animal testing.

The use of QSAR in ecotoxicology is well established, and predictions can be made with sufficient accuracy for a number of endpoints and wide variety of chemicals. The situation in mammalian toxicology is rather different. There are a number of reasons for this, namely the wide variations in the quality and source of experimental data, in the organisms used, combined with a limited understanding of the biological mechanisms involved. Thus, although a considerable amount of data is available, the modelling can be problematic [11]. Often the problem is related to the different laboratories and different protocols used. Despite this, there have been numerous efforts for developing QSAR models for acute mammalian toxicity [9, 11, 26, 46].

The aim of this paper was to review QSAR models for acute mammalian toxicity that have been published in the last decade and to provide a snapshot on the availability and coverage of such models for use under the new chemical legislation in the European Union (EU). Taking into account that cytotoxicity data may be helpful in predicting *in vivo* toxicity [43], the review also includes QSAR models based on mammalian cell line data. Analysis of the state-of-the-art leads to the conclusion that further development in the field is necessary in order to meet the needs of the REACH legislation.

Testing methods for acute systemic toxicity

Acute toxicity studies are based on a single administration of the chemical or several administrations given within 24 hours. Most acute toxicity studies aim to determine the median lethal dose (LD50) of the chemical. The LD50 is defined as an expression of a single dose of a chemical that can be expected to kill 50% of animals in the experimental group [29]. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg). When the route of exposure is inhalation, the endpoint is either the median lethal concentration (LC50) or the median lethal time (LT50).

The principle of the classical LD50 test is to dose groups of animals with a single dose of a test substance at concentrations expected to cause death in at least a fraction of the animals dosed. Results of the test enable the calculation of the LD50 value, i.e. the dose expected to kill 50% of the animals within 14 days after a single exposure.

Since the end of the 1970s, the conventional acute toxicity test has been widely criticised on both scientific and animal welfare grounds [3, 23]. At their November meeting in 2001, the OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology, agreed that OECD Test Guideline (TG) 401 [32], based on the LD50 test should be deleted from the OECD manual of internationally accepted TGs. Alternatives to the traditional procedure include the Fixed Dose Procedure (OECD TG 420; [33]), the Acute Toxic Class method (OECD TG 423; [34]), and the Up-and-Down Procedure (OECD TG 425; [35]).

Within the EU the standardised testing methods to determine acute toxicity of chemicals are included in Annex V of Directive 67/548/EEC [7]. The methods are closely linked with the OECD Test Guidelines [44].



Non-testing methods

Non-testing data can be provided by the following approaches: a) structure-activity relationships (SARs) and quantitative structure-activity relationships (QSARs), collectively called “(Q)SARs”; b) expert systems incorporating (Q)SARs and/or expert rules; and c) grouping methods (categories and read-across). These approaches can be used to assess acute toxicity if they provide relevant and adequate data for the chemical of interest [47]. Non-testing methods should be documented according to the appropriate reporting formats. In the case of (Q)SARs and expert systems, a detailed description of available models is provided in the ECB (European Chemicals Bureau) Inventory of (Q)SAR Models (<http://ecb.jrc.it/qsar>).

At the moment there is little information about the formal use of (Q)SARs in support of regulatory decisions related to acute toxicity. Read-across has been used to a limited extent, and on a case by case basis [37]. Nevertheless, the spirit of REACH for increased safety of chemicals by more comprehensive use of existing information and utilization of non-testing methods where possible is driving the development of reliable (Q)SAR models and grouping approaches.

QSARs for mammalian toxicity *in vitro*

The idea of using cytotoxicity assays to predict *in vivo* toxicity arises from the concept of “basal cell cytotoxicity” proposed by Ekwall [13]. He suggested that for most chemicals, toxicity is a consequence of non-specific alterations in cellular functions. Evaluating the toxic potential of compounds *in vitro* (cytotoxicity) may therefore give an indication of their toxic potential *in vivo* [15]. A number of studies have analysed the correlation between cytotoxicity and *in vivo* toxicity [21, 39, 43].

A DEFRA (The Department of Environment, Food and Rural Affairs) – funded research project carried out by Liverpool John Moores University and FRAME (Fund for the Replacement of Animals in Medical Experiments) aimed to review the status of alternatives to animal testing and to recommend areas for further research with respect to the REACH proposal [24]. The project considered 12 acute toxicity tests. Among them there is one acute toxicity test being validated.

In vitro – *in vivo* correlations

The Multicenter Evaluation of *in vitro* Cytotoxicity (MEIC) programme was set up to evaluate the relevance for human acute toxicity of *in vitro* of cytotoxicity tests [14]. For this purpose, 29 laboratories tested 50 reference chemicals in 61 cytotoxicity assays. Comparisons performed between IC₅₀ values from the 61 assays and the human lethal dosage demonstrated that human cell line tests gave better average results ($R^2 = 0.64$), than mammalian ones ($R^2 = 0.52$).

Lessigiarska et al. used the toxicity data collected in the MEIC programme in order to develop QSAAR (Quantitative Structure-Activity-Relationship) and QSAR models and to evaluate their potential application as alternatives to animal testing [27]. In fact, QSAAR models combine both data for biological endpoints and structural descriptors to predict other biological especially *in vivo* endpoints. Data for rat, mouse, and human toxicity of the same chemicals were taken from the MEMO programme (MEIC monographs on time-related human lethal blood concentrations). Rat and mouse toxicities (rat LD₅₀ and mouse LD₅₀ values) were collected from the NIOSH (National Institute for Occupational Safety and Health) / RTECS (Register of Toxicology Effects of Chemical Substances). Human toxicity data were collected from publications of clinical and forensic studies. For the QSAR development,



octanol-water partition coefficient ($\log P$), aqueous solubility, and approximately 250 different quantum-chemical, topological, and charge descriptors were calculated. Human *in vivo* toxicity data were correlated with *in vivo* rodent toxicity or *in vitro* human liver cell toxicity in combination with structural descriptors, accounting for the H-bond donor ability, molecular aromaticity, and electronic properties. It was concluded that the models for HAP (human toxicity), represented as acute blood/serum peak LC50 values could be useful alternatives to animal testing for hazard and risk assessment. In addition, QSAR models have been obtained for HAP, which had slightly better statistical parameters than the QSAAR model and these models are also considered as a possible tool to partially replace animal testing. Considering HLD (human toxicity represented as the acute oral lethal dose) QSAAR analysis revealed that it correlated best with the mouse and rat *in vivo* toxicity ($R^2 = 0.74$). When the number of H-bond donors and Kier benzene-likeness index (measure of molecular aromaticity) were added, the accuracy of the results was improved. Rat LD50 values correlated best with the toxicity to rat hepatocytes ($R^2 = 0.66$). Adding the number of six-membered rings or molecular polarisability improved the correlation. The first parameter may be related to the size and/or shape of the molecule. The second one might influence the transport or distribution of the chemicals across the cell membranes. The QSAR for rat LD50 included the hydrophobicity factor, the electrotopological state descriptor and the number of six-membered rings. The same descriptors also appeared to describe well the mouse LD50, although the statistical parameters were slightly worse. Mouse and rat LD50 values intercorrelated with $R^2 = 0.71$. The results showed that in the case of QSAARs for *in vivo* human and rodent toxicity, including *in vitro* toxicity endpoints in combination with structural descriptors, the addition of the structural parameters increases only slightly the goodness-of-fit (R^2). In comparison, QSARs for the *in vivo* toxicity endpoints had better statistical parameters.

Analysis of QSAAR models relating *in vivo* to *in vitro* endpoints can give an insight into the factors that determine differences between the *in vivo* and *in vitro* toxicity effects. Generally, the descriptors encoded electronic/reactivity properties, the presence of oxygen atoms, and size/shape properties, which are probably related to toxicokinetic factors. In general the QSAAR and QSAR models for *in vivo* toxicity developed in the study were regarded as suitable priority-setting methods or means of providing supplementary information in a weight-of-evidence approach to hazard and risk assessment.

QSAR models for different chemical classes

There is considerable human exposure to phenols as they are found in tea, fruits and vegetables. In recent years, the effects of different substituents on phenol toxicity *in vitro* have been investigated by the Hansch group [20, 25, 41]. The researchers aimed to explain how the parameters used in the QSAR models can help in understanding the reasons behind the various types of toxicity. The QSAR models obtained for electron-releasing phenols are described by Eq. (1) and Eq. (2) [41].

$$\log 1/ID50 = (-1.98 \pm 0.15) \sigma^+ + (0.18 \pm 0.04) \log P + (3.31 \pm 0.11) \quad (1)$$

n = 51 R² = 0.895 s = 0.227 R²_{cv} = 0.882

$$\log 1/ID50 = (-0.19 \pm 0.02) BDE + (0.21 \pm 0.03) \log P + (3.11 \pm 0.10) \quad (2)$$

n = 52 R² = 0.920 s = 0.202 R²_{cv} = 0.909

In these models, ID50 represents the molar concentration of phenol that induces 50% growth inhibition in murine leukemia L1210 cells, σ^+ is the Brown variation of the Hammett σ



constant, and $\log P$ is calculated octanol-water partition coefficient. BDE (the bond dissociation energy) is the energy associated with the abstraction of a hydrogen atom from the hydroxy moiety. This term in the equation is related to the formation of the phenoxyl radical, a reactive oxygen species.

In both models, the low coefficient with the hydrophobic term ($\log P$) suggests that the radical may be interacting with a receptor such as DNA or it may represent the slightly enhanced transport of the phenoxy radical in the cellular environment.

Phenols with substituents of an electron-attracting nature have non-specific cytotoxicity which is modelled by hydrophobicity according to Eq. (3) [40]:

$$\log 1/ID50 = (0.62 \pm 0.16) \log P + (2.35 \pm 0.31) \quad (3)$$

n = 15 R² = 0.845 s = 0.232 R²_{cv} = 0.800

Recently the same authors examined the activation of caspases by phenols and subsequent apoptosis in a murine leukaemia cell line [42]. The results were then compared with their corresponding cytotoxicities in the same cell line to determine if apoptosis plays a major role in the overall cytotoxicity of monophenolic compounds. The following QSAR equation was derived (Eq. (4)):

$$\log 1/I50 = (1.06 \pm 0.12)B52 + (0.33 \pm 0.20)B53 - (0.18 \pm 0.09)\pi_{2,4} - (0.92 \pm 0.46) \quad (4)$$

n = 51 R² = 0.886 s = 0.349 R²_{cv} = 0.866

In Eq. (4), I50 is the concentration of a substituted phenol that induces caspase-mediated apoptosis by 50%. B52 is Verloop's sterimol descriptor and is a measure of the width of the larger substituent in the ortho position. B53 represents the width of the larger substituent in the meta position. The hydrophobic parameter $\pi_{2,4}$ represents the sum of the hydrophobicity of substituents in the para position and the bulkier ortho position. In the model, 81% of the variance in the data is explained by the steric parameter B52, which led the authors to suggest a receptor-mediated interaction of the phenols, with caspases or mitochondrial proteins being the likely targets.

The study of structure-cytotoxicity relationships of 65 electron-releasing phenols in the same cell line led to the development of the following model (Eq. (5)):

$$\log 1/ID50 = (-1.39 \pm 0.19) \sigma^+ - (0.28 \pm 0.05) B5_{2,6} + (0.16 \pm 0.05) \log P - (0.58 \pm 0.24)I_2 - (1.04 \pm 0.25)I_1 + (3.90 \pm 0.19) \quad (5)$$

n = 65 R² = 0.840 s = 0.271 R²_{cv} = 0.808

In Eq. (5) B5_{2,6} represents the sum of the width of the substituents in the ortho position. Cytotoxicity decreases as the width of these substituents increase. The negative coefficient with σ^+ implies that highly electron-releasing substituents enhance stabilisation of the phenoxy radical and increase cytotoxicity. I₁ and I₂ are indicator variables for methyl and methoxy substituents respectively. They both decrease the cytotoxicity as evident from the equation.



The following QSAR for 27 electron-attracting phenols was derived (Eq. (6)):

$$\log 1/ID50 = (0.56 \pm 0.11)\log P - (0.30 \pm 0.18) B52 + (2.79 \pm 0.22) \quad (6)$$

n = 27 R² = 0.848 s = 0.233 R²_{cv} = 0.812

In Eq. (6), logP was of critical importance in describing the cytotoxicity, since it accounts for 85% of the variance in the data.

The significant differences between the cytotoxicity and apoptosis QSAR models suggest that apoptosis contributes little to the observed cytotoxicity.

Recently Loader et al. [28] investigated a dataset of ortho alkyl-substituted phenols previously studied by the Hansch group as described above. The method of quantum topological molecular similarity (QTMS) was used [31]. This method uses electronic descriptors drawn from *ab initio* wavefunctions of geometry-optimised molecules. The results did not support the hypothesis that the steric factor is important for the cytotoxicity of the investigated compounds. The authors concluded that the cytotoxicity of these phenols is dependent primarily on electronic and radical effects.

Argese et al. [2] investigated the toxicity of eighteen substituted anilines by means of a short-term *in vitro* assay, using SMPs (SubMitochondrial Particles) as biosensors. The test with phosphorylating SMPs was carried out by determining the effect of toxicants on the process of reverse electron transfer, where exogenous NAD⁺ is reduced to NADH, which strongly absorbs light at 340 nm. The toxicant concentration at which the rate of NADH production was diminished by 50% (EC50 values in mol/l) was used as the endpoint for developing QSARs.

The investigated anilines had substituents with a wide range of the electron donor/acceptor capabilities, whereas hydrophobicity varied in a narrow range (logP values varied from 0.04 to 1.89). Thus, the study aimed to assess the influence on the toxicity of the electronic properties of the substituents only. This is reasonable taking into account that the investigated anilines with comparable logP values exhibited different toxic effects. A small correlation between logP and compound toxicity was found (R² = 0.22), probably due to the low variation in compound hydrophobicity.

The EC50 values were correlated with the Hammett sigma constants (σ), LUMO (Lowest Unoccupied Molecular Orbital), HOMO (Highest Occupied Molecular Orbital), qH⁺ (the largest positive partial charge on any hydrogen atom) and q⁻ (the largest negative partial charge on any atom). For strong electron-withdrawing substituents (COCH₃, CN, NO₂), the nucleophilic σ_p was used, which takes into account the resonance effect, present when these groups are conjugated with an electron-donating group, such as NH₂ in anilines. For the disubstituted anilines, a summation of the single substituent constants was used.

The QSARs derived by Argese et al. [2] are given in Table 1. The electronic Hammett parameter σ gave the best fit. It showed that toxicity increases by increasing the electron-withdrawing effects of the substituents. Two compounds, 3,5-dinitroaniline and 4'-aminoacetophenone, were found to be outliers and were excluded from the regression. According to the authors, the excess toxicity exhibited by these compounds could be related to the possibility of the NO₂ and COCH₃ groups to form further H-bonds by acting as H-atom acceptors.



Table 1. Results of the regression analysis [2]

Descriptors	n	R ²	R ² _{cv}	s	F	a ¹	b ¹
σ	18	0.81	0.76	0.25	69	3.20 ± 0.08	0.57 ± 0.07
σ (excluding outliers)	16	0.91	0.88	0.16	136	3.16 ± 0.05	0.53 ± 0.05
HOMO	18	0.71	0.65	0.31	39	-0.83 ± 0.71	-13.4 ± 2.1
HOMO (excluding outliers)	15	0.84	0.74	0.20	66	-0.30 ± 0.47	-11.7 ± 1.4
LUMO	18	0.82	0.78	0.25	72	4.62 ± 0.13	-11.0 ± 1.3
LUMO (excluding outliers)	17	0.86	0.83	0.22	90	4.58 ± 0.12	-11.0 ± 1.2
qH+	18	0.67	0.59	0.39	33	-12.2 ± 2.8	44.0 ± 7.7
qH+ (excluding outliers)	17	0.77	0.70	0.28	50	-13.1 ± 2.4	46.2 ± 6.5

¹ a and b – the regression coefficients in the general equation: $\log 1/EC50 = a + b \cdot X$;
X – the molecular descriptor.

The following two-variable QSARs was obtained (Eqs. (7), (8)):

$$\log 1/EC50 = 0.68 \sigma - 8.4 q^- - 4.0 \quad (7)$$

n = 18 R² = 0.89 s = 0.20 F = 59

The exclusion of the identified statistical outliers (3,5-dinitroaniline and 4'-aminoacetophenone) resulted in Eq. (8):

$$\log 1/EC50 = 0.61 \sigma - 5.9 q^- - 1.9 \quad (8)$$

n = 16 R² = 0.95 s = 0.12 F = 130

According to the authors, the QSARs indicate the importance of electronic interactions and H-bonding donor capacity in the toxicity of anilines. These findings support a mechanism of toxic action based on H-bonding between the NH₂ group of substituted anilines and polar groups at the membrane/water interface of the SMPs, leading to a disruption in the membrane structure and disturbance of its functioning.

Polybrominated diphenyl ethers (PBDEs) have become widely distributed as environmental contaminants due to their use as flame retardants. Their structural similarity to other halogenated aromatic pollutants has led to speculation that they might share toxicological properties such as hepatic enzyme induction [5]. In order to develop predictive models for the toxicity of PBDEs congeners, Wang et al. used 3D-QSAR approaches [48]. They used a data set of 18 PBDEs taken from [5]. The affinity to the rat hepatic Ah receptor (aryl hydrocarbon receptor), as derived from competitive binding assays was used as the endpoint. For the molecular modelling study, CoMFA (Comparative Molecular Field Analysis) and CoMSIA (Comparative Molecular Similarity Indices Analysis) approaches were applied. In the alignment of the structures a set of rules were defined for selecting a template molecule, namely: (i) the most active compound; (ii) the lead and/or commercial compound; and (iii) the compound containing the greatest number of functional groups. The alignment of the structures was carried out by flexible fitting. CoMFA and CoMSIA regression models were built by PLS (partial least squares) regression and the LOO (leave-one-out) cross-validation procedure was used to check the internal consistency and to estimate the predictive ability of



the resulting QSAR models. The statistical parameters of the obtained models were the following:

CoMFA (steric+electrostatic field): $R^2_{cv} = 0.580$; $R^2 = 0.995$; $F = 337.627$
CoMSIA (steric+electrostatic+hydrophobic field): $R^2_{cv} = 0.680$; $R^2 = 0.982$; $F = 98.049$

Both models identified the electrostatic field as the most important determinant of the relative binding affinity of PBDEs. On the basis of the models, 3D contour plots were built. They indicated the regions with the highest influence of the steric, electrostatic and hydrophobic fields on the relative binding affinity values. The results also showed that non-planar conformations of PBDEs resulted in the lowest energy level.

The two 3D QSAR models were used to predict the RBA value of 46 PBDEs not included in the training set. However, since the experimental values were unavailable, it was not possible to make conclusions about the external predictive power of the models.

QSARs for acute mammalian toxicity *in vivo*

QSARs for inhalational toxicity

Some simple regression models have been developed for predicting the inhalational toxicity of volatile substances. Typically, parameters such as vapour pressure (VP) and boiling point (BP) have been found to be useful predictors of the acute toxic effect. These models are based on the assumption that toxicity occurs by the non-specific mechanism of narcosis, and that the LC50 data are based on tests in which a steady-state concentration has been reached in the blood.

For instance acute (non-lethal) neurotoxicity toxicity data for the neurotropic effects of some common solvents on both rats and mice were subjected to QSAR analysis by Cronin [10], using data taken from Frantik et al. [16]. In the study, 44 chemicals were included and the logarithm of the micromolar toxicity was used as the endpoint in the QSAR analysis. After removing four outliers identified as being much less toxic relative to the other compounds and three other considered atypical of the data set, the stepwise regression analysis of the 4h toxicity data causing the 30% depression in response ($\log 1/ECR_{30}$) in rats gave the following equation (Eq. (9)):

$$\log 1/ECR_{30} = 0.361 \text{ ClogP} - 0.117 \text{ }^0\chi - 1.76 \quad (9)$$

$n = 37$ $R^2 = 0.817$ $s = 0.280$ $F = 35.2$

This relationship demonstrated a partial dependence of toxicity on $\log P$. In addition, the negative correlation with the zero-order molecular connectivity suggests that the membrane permeability of large molecules may be reduced.

Stepwise regression for mouse neurotoxicity gave the following equation (Eq. (10)):

$$\log 1/ECM_{30} = 0.212 \text{ ClogP} + 0.00767 \text{ BP} - 0.176 \text{ }^0\chi - 2.03 \quad (10)$$

$n = 39$ $R^2 = 0.811$ $s = 0.271$ $F = 22.4$

Principal Component Analysis (PCA) was performed on all 17 physiochemical parameters included in the study. The analysis identified six physicochemical descriptors as most successful at separating out compounds of high neurotoxicity from those of low neurotoxicity: ClogP , $(\text{ClogP})^2$, boiling point, melting point, CMR and $^0\chi$. When any of these parameters



(or any combination) were omitted from the analysis, the separation between groups was poorer. On this basis, the author suggested that parameters describing hydrophobicity, molecular volume and size, melting and boiling point were important for toxicity, despite the fact that some were not found to be important by stepwise regression. Overall the analysis suggested that in addition to partitioning through a membrane, aqueous solubility and volatility are also important factors governing toxicity.

QSARs for predicting LD50

The study of Amaral et al. aimed at obtaining a better understanding of the structural features contributing to the lethal toxicity of local anesthetics [1]. For this purpose a set of sixteen para-substituted N,N-[(dimethylamino)ethyl] benzoate hydrochlorides structurally related to procaine was used. The median lethal doses (LD50, mM/kg) of the compounds were assessed in the mouse. Log 1/LD50 was taken in the QSAR analysis as the biological parameter, indicating the lethal potency of the compounds. The apparent partition coefficients were determined experimentally (shake-flask or HPLC methods). A number of physicochemical parameters were taken from the literature: hydrophobic parameter π (Hansch-Fujita substituent constant), electronic parameters σ , \mathfrak{S} Swain-Lupton substituent constant, \mathfrak{R} substituent constant, and polarizability-related parameter MR₄ (molar refractivity of the substituent at the *para*-position). The IR stretching frequencies of the carbonyl group ($\nu_{C=O}$) were determined in chloroform and taken as one of the electronic parameters. Its use was justified by the significant correlation with σ , and \mathfrak{S} and \mathfrak{R} . In order to evaluate the nature and relative contribution of the physicochemical parameters significantly involved in lethal potency in the set of investigated compounds a Hansch analysis was performed. First the correlations of each physicochemical parameter and the lethal potency were analysed. The results indicated that the lipophilic term explained a larger portion of the observed variation in the lethal potency. Further, the relative contributions to lethal toxicity of both lipophilic and electronic terms were evaluated in a subset of 15 compounds:

$$\log 1/LD50 = 0.24 (\pm 0.06) \log P_{app} - 0.32 (\pm 0.32) \mathfrak{R} + 2.19 (\pm 0.12) \quad (11)$$

N = 15 R = 0.952 s = 0.116 F = 57.518 R²_{cv} = 0.859

$$\log 1/LD50 = 0.23 (\pm 0.07) \log P_{app} - 0.017 (\pm 0.015) \nu_{C=O} + 31.41 (\pm 25.2) \quad (12)$$

N = 15 R = 0.956 s = 0.111 F = 64.433 R²_{cv} = 0.869

The simultaneous use of logP_{app} and MR₄ did not improve the statistical significance of the models. The authors concluded that hydrophobicity has major contribution to the lethal toxicity of the studied anesthetics. Borderline contribution of electronic properties was outlined.

QSAR models were derived for the acute oral toxicity of organophosphorus pesticides to male and female rats [12]. The training set included 51 chemicals. Additionally nine chemicals were used for external validation. The toxicity data (LD50 for adult male and female Sherman rats) were extracted from papers by Gaines [17, 18, 19]. The autocorrelation method was used to describe the molecules. First, from the fragmental constants of Rekker and Mannhold for each molecule, an autocorrelation vector H representing lipophilicity was derived. Second, an autocorrelation vector MR, encoding molar refractivity was designed from the fragmental constants of Hansch and Leo or directly from the classical Lorentz-Lorentz equation. Third, autocorrelation vectors encoding the H-bonding acceptor ability (HBA) and H-bonding donor ability (HBD) of the molecules were calculated by means of Boolean contributions. In order to relate the LD50 values to the autocorrelation descriptors, the PLS regression method was



used. The model was constructed by using the NIPALS (Nonlinear estimation by Iterative Partial Least Squares) algorithm.

As a second step, attempts were made to derive a non-linear QSAR model from a three-layer feed forward neural network trained by different algorithms – back-propagation, conjugate gradient descent, quasi-Newton, Levenberg-Marquardt, quick propagation, and delta-bar-delta algorithm. During the design of the neural network model, four LD50 values were randomly selected from the testing set to constitute a cross-validation set to correctly monitor the learning phase.

The best results were obtained with an 8/4/1 ANN (Artificial Neural Network) model designed from the autocorrelation descriptors and trained with the back-propagation and conjugate gradient descent algorithms. The root mean square residual for the training and test set was equal to 0.29 and 0.26 respectively. This model allows simultaneous calculation of LD50 for males and females.

Expert Systems

The complexity of the mammalian toxicity endpoint as well as the lack of large and consistent databases with measured data are the main reasons for the limited number of models, more of them restricted to a given chemical class. Historically the complexity of the endpoints is one of the reasons for the development of so called expert systems [22]. Knowledge based expert systems, such as HazardExpert, use expert knowledge rules about generalised relationships between structure and toxicity and these rules are derived from human expert opinion. Statistically based expert systems, such as TOPKAT and Multicase, use structural descriptors and apply statistical methods to derive QSAR models. In the section below some of the expert systems predicting acute mammalian toxicity are shortly described.

The TOPKAT software package computes the toxic and environmental effects of chemicals solely from their molecular structure [45]. It employs cross-validated quantitative structure – toxicity relationship (QSTR) models for assessing various measures of toxicity. The descriptors used in the TOPKAT models quantify the electronic, shape, and symmetry attributes of a molecular structure. The electronic attributes are expressed by the electrotopological state (E-state) values of specially designed 1-atom and 2-atom fragments of non-hydrogen atoms in different hybridization states.

In conjunction with a prediction of toxicity, TOPKAT provides an assessment of its reliability. For this purpose, the program performs an analysis of whether all of the structural fragments of the query chemical are well represented in the training set and also whether the query structure fits within or near the periphery of the Optimum Prediction Space (OPS) of the model. The OPS is a multi-dimensional space in which the number of dimensions is more than the number of model parameters. If a query structure is inside all dimensions of the model's OPS, the computed toxicity value is considered "acceptable". If the query structure is outside one or more dimensions, the computed toxicity value may or may not be "acceptable", depending on the query chemical's distance from the OPS. Every TOPKAT model has a permissible limit of distance from OPS and above this value the assigned toxicity is considered "unacceptable".

The Rat Oral LD50 module of the TOPKAT includes 19 QSAR regression models. The accuracy of the models as estimated by LOO procedure is presented in Table 2.



Table 2. Accuracy of the 19 submodels determined by the LOO procedure [45]

Class	No of chemicals	% of chemicals predicted within a factor of				95% of chemicals predicted within a factor of
		2	3	4	5	
Organophosphates (P=O)	230	48	67	80	86	9
Organophosphates (P=S)	285	58	81	90	95	5
Carbamates	205	63	84	91	96	5
Heteroaromatics	429	63	83	92	97	5
Multiple Benzenes	367	70	85	92	95	5
Fused Benzenes	75	84	100			3
Single Benzenes (1 substituent)	196	80	96	99	100	3
Single Benzenes (2 substituents)	274	76	93	98	100	3
Single Benzenes (3 substituents)	162	80	92	97	100	4
Single Benzenes (> 3 substituents)	101	74	92	99	100	4
Alicyclic	361	65	85	93	97	4
Acyclic Amines	225	68	87	93	96	4
Acyclic	63	73	88	98	100	4
Halo/Hydrocarbons						
Acyclic	138	67	89	98	100	3
Acids/Esters						
Acyclic Alcohols	74	90	98	100		3
Acyclic Carbonyls	60	81	94	100		3
Acyclic Ethers	47	93	100			2
Acyclic C, O, H	108	90	100			2
Miscellaneous						
Acyclic (others)	224	59	81	89	93	6

The models are based on a number of structural, topological and electrotopological indices, and make predictions of the oral acute median lethal dose in the rat (LD50). The models report results in units of chemical weight/body weight.

The TOPKAT rat oral LD50 models are based on experimental values of 4000 chemicals from the RTECS (Register of Toxicology Effects of Chemical Substances). Since RTECS lists the most toxic value when multiple values exist, the TOPKAT model tends to overestimate the toxicity of query structures.

The Rat Inhalation LC50 module of TOPKAT contains five submodels related to different chemical classes (Table 3). For the model development only exposure times in the range of 0.5 to 14 hours were accepted. Endpoints were modelled as $\log_{10}(1/C) - \log_{10}(\text{hours of exposure})$, where C is the concentration in mols/m^3 .



Table 3. Inhalation LC50 submodels in TOPKAT [45]

Class	No of chemicals in the model	R ²	adj R ²	SEE
Single benzenes	133	0.849	0.772	0.408
Heteroaromatics and multiple benzenes	134	0.864	0.806	0.36
Alicyclics	71	0.874	0.837	0.399
Acyclics (without halogens)	187	0.851	0.812	0.517
Acyclics (with halogens)	118	0.849	0.810	0.571

The MultiCASE software uses a fragment based technology [30]. It is based on a hierarchical statistical analysis of a database composed of a number of chemicals associated with their toxicity data. The program discovers substructures that appear mostly in active molecules and therefore most likely to be responsible for the observed activity. At the beginning it identifies the statistically most significant substructure within the training set. This fragment, labelled the top biophore, is considered responsible for the activity of the largest possible number of active molecules. The active molecules containing this biophore are then removed from the database, and the rest of them are submitted to a new analysis for identification of a new biophore. The procedure is repeated until either the activity of all the molecules in the training set has been accounted for or no additional statistically significant substructure can be found. Then for each set of molecules containing a specific biophore, the program identifies additional parameters called modulators. They consist of certain substructures or physicochemical parameters, such as HOMO/LUMO energies, logP, water solubility, location of hydrogen donors/acceptors, lipophilic centers with respect to biophore, that significantly enhance or diminish the activity attributable to the biophore. QSARs are then derived by using these modulators. The knowledge that the program gains during the training process can then be used to predict the biological activity of new chemicals not included in the training set. Multicase mammalian toxicity modules are presented in the Table 4.

Table 4. Multicase mammalian toxicity modules [30]

Module	Number of compounds in the training set
FDA NTP WHO Rat LD50	7920
NTP Maximum Tolerated Dose - Mice	321
NTP Maximum Tolerated Dose - Rats	321
FDA MRTD humans	1169
FDA Maximum tolerated dose Male Rat -nontoxic dose	1014
FDA Maximum tolerated dose Female Rat -nontoxic dose	1020
FDA Maximum tolerated dose Male Mouse -nontoxic dose	939
FDA Maximum tolerated dose Female Mouse-nontoxic dose	951
FDA Maximum tolerated dose Male Rat -lethal dose	1015
FDA Maximum tolerated dose Female Rat -lethal dose	1020
FDA Maximum tolerated dose Male Mouse -lethal dose	939
FDA Maximum tolerated dose Female Mouse -lethal dose	951



HazardExpert is a module of the Pallas software developed by CompuDrug Limited [38]. The program works by searching the query structure for known toxicophores, stored in the “Toxic Fragments Knowledge Base” and including substructures that exert both positive and negative modulator effects. Once a toxicophore has been identified, this triggers estimates for a number of toxicity endpoints, including neurotoxicity. The default knowledge base of the system is based on a US EPA report [4] and scientific information collected by CompuDrug Limited. The rule-based system of the program has open architecture, allowing the user to understand, expand or modify the data on which the toxicity estimation relies. A further application of the program is prediction the toxicity of the parent compound and its metabolites by linking with the MetabolExpert system (another module of Pallas). Further development of the toxicity predictions is the HazardExpert Pro module. It relies on an ANN based approach using atomic descriptors to categorise compounds according to their *in vitro* human cytotoxicity.

Conclusions

In this review, a number of QSAR models for acute mammalian toxicity published in the last ten years have been summarised. The relatively small number of models identified for *in vivo* toxicity is related mainly to the nature of the endpoint. The mammalian toxicity measurements are usually related to whole body phenomena. They include processes of absorption, distribution, bioaccumulation, metabolism, and excretion [9]. The complexity and multiplicity of the mechanisms involved leads to inherent difficulties in the QSAR modelling process. In addition, difficulties arise from the lack of high quality data suitable for modelling purposes.

Most of the QSAR studies are restricted to single classes of chemicals, such as alcohols, phenols, anilines. The models identify hydrophobicity as a parameter of high importance for the modelled toxicity. In addition, many of the models indicate the role of electronic and steric effects. Thus they give a deeper insight into the mechanisms involved in the toxicity of the investigated substances. The only models based on more heterogeneous data are those incorporated into commercially developed expert systems.

In general, the QSAR models for acute mammalian toxicity identified in the review may be useful for investigating the mechanisms of toxicity of defined chemical classes. However, for predictive purposes in the regulatory assessment of chemicals, they are still far from meeting all the requirements of the OECD validation principles [36]. Therefore, more work is needed in this field to develop QSAR models useful for the assessment of chemicals under the future REACH legislation.

References

1. Amaral A. T, A. C. Oliveira, R. Neidlein, M. Gallacci, L. Caprara, Y. Miyazaki (1997). Physicochemical Parameters Involved in the Lethal Toxicity of N,N-[(dimethylamino)ethyl]-4-substituted Benzoate Hydrochlorides: A QSAR Study, *Eur. J. Med. Chem.*, 32, 433-443.
2. Argese E., C. Bettiol, M. Fasolo, A. Zambon, F. Agnoli (2002). Substituted Aniline Interaction with Submitochondrial Particles and Quantitative Structure-activity Relationships, *Biochimica et Biophysica Acta*, 1558, 151-160.
3. Balls M. (1991). Why Modification of the LD50 Test will not be Enough, *Lab. Anim.*, 25, 198-206.
4. Brink R. H, J. D. Walker (1987). EPA TSCA ITC Interim Report, Dynamic Corporation, Rockville, 6(4).



5. Chen G., A. D. Konstantinov, B. G. Chittim, E. M. Joyce, N. C. Bols, N. J. Bunce (2001). Synthesis of Polybrominated Diphenyl Ethers and Their Capacity to Induce CYP1A by the Ah Receptor Mediated Pathway, *Environ. Sci. Technol.*, 35, 3749-3756.
6. Commission of the European Communities (2003). Proposal for a Regulation of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Establishing a European Chemicals Agency and Amending Directive 1999/45/EC and Regulation (EC) {on Persistent Organic Pollutants}, <http://europa.eu.int/comm/enterprise/chemicals/chempol/whitepaper/reach.htm>
7. Council Directive 67/548/EEC of 27 June 1967 on the Approximation of Laws, Regulations and Administrative Provisions Relating to the Classification, Packaging and Labelling of Dangerous Substance.
8. Council of the European Union (2006). Common Position Adopted by the Council with a View to the Adoption of a Regulation of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Establishing a European Chemicals Agency, Amending Directive 1999/45/EC of the European Parliament and of the Council and Repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. Text Agreed by the Council of Ministers on 12 June 2006, http://europa.eu.int/comm/enterprise/reach/index_en.htm
9. Cronin M. T., J. C. Dearden (1995). QSAR in toxicology. 2. Prediction of Acute Mammalian Toxicity and Interspecies Correlations, *Quant. Struct.-Act. Relat.*, 14, 117-120.
10. Cronin M. T. D. (1996). Quantitative Structure-Activity Relationship (QSAR) Analysis of the Acute Sublethal Neurotoxicity of Solvents, *Toxicology in Vitro*, 10, 103-110.
11. Cronin M. T., J. C. Dearden, J. D. Walker, A. P. Worth (2003). Quantitative Structure-Activity Relationships for Human Health Effects: Commonalities with Other Endpoints, *Environ. Toxicol. Chem.*, 22, 1829-1843.
12. Devillers J. (2004). Prediction of Mammalian Toxicity of Organophosphorus Pesticides from QSTR Modeling, *SAR QSAR Environ. Res.*, 15, 501-510.
13. Ekwall B. (1983). Screening of Toxic Compounds in Mammalian Cell Cultures, *Ann. N. Y. Acad. Sci.*, 407, 64-77.
14. Ekwall B., C. Clemedson, F. A. Barile, A. Castano, R. H. Clothier, P. Dierickx, B. Ekwall, M. Ferro, G. Fiskesjo, L. Garza-Ocanas, M. J. Gomez, M. Gulden, T. Hall, B. Isomaa, A. Kahru, G. Kerszman, U. Kristen, M. Kunimoto, S. Karenlampi, L. Lewan, A. Loukianov, T. Ohno, G. Persoone, L. Romert, T. W. Sawyer, R. Shrivastava, H. Segner, A. L. Stamatii, N. Tanaka, M. Valentino, E. Pierdominici, E. Walum (1998). MEIC Evaluation of Acute Systemic Toxicity. Part 6. Prediction of Human Toxicity by Rodent LD50 Values and Results from 61 in Vitro Tests, *ATLA*, 26, 617-658.
15. Evans S. M., A. Casartelli, E. Herreros, D. T. Minnick, C. Day, E. George, C. Westmoreland (2001). Development of a High Throughput in Vitro Toxicity Screen Predictive of High Acute in Vivo Toxic Potential, *Toxicol. in Vitro*, 15, 579-584.
16. Frantik E., M. Hornychova, M. Horvath (1994). Relative Acute Neurotoxicity of Solvents: Isoeffective Air Concentrations of 48 Compounds Evaluated in Rats and Mice, *Environ. Res.*, 66, 173-185.
17. Gaines T. B. (1960). The Acute Toxicity of Pesticides to Rats, *Toxicol. Appl. Pharmacol.*, 2, 88-99.
18. Gaines T. B. (1969). Acute Toxicity of Pesticides, *Toxicol. Appl. Pharmacol.*, 14, 515-534.



19. Gaines T. B., R. E. Linder (1986). Acute Toxicity of Pesticides in Adult and Weanling Rats, *Fundam. Appl. Toxicol.*, 7, 299-308.
20. Garg R., A. Kurup, C. Hansch (2001). Comparative QSAR: On the Toxicology of the Phenolic OH Moiety, *Crit. Rev. Toxicol.*, 31, 223-245.
21. Garle M. J., J. H. Fentem, J. R. Fry (1994). In Vitro Cytotoxicity Tests for the Prediction of Acute Toxicity in Vivo, *Toxicol. in Vitro*, 8, 1303-1312.
22. Greene N. (2002). Computer Systems for the Prediction of Toxicity: An Update, *Adv. Drug. Deliv. Rev.*, 54, 417-431.
23. Gribaldo L., A. Gennari, K. Blackburn, C. Clemedson, A. Deguercy, A. Meneguz, W. Pfaller, I. Ruhdel (2005). Acute Toxicity, *ATLA*, 33(1), 27-34.
24. Grindon C., R. Combes, M. T. Cronin, D. W. Roberts, J. Garrod (2006). A review of the Status of Alternative Approaches to Animal Testing and the Development of Integrated Testing Strategies for Assessing the Toxicity of Chemicals under REACH-a Summary of a DEFRA-funded Project Conducted by Liverpool John Moores University and FRAME, *ATLA*, 34(1), 149-158.
25. Hansch C., S. C. McKarns, C. J. Smith, D. J. Doolittle (2000). Comparative QSAR Evidence for a Free-radical Mechanism of Phenol-induced Toxicity, *Chem. Biol. Interact.*, 127, 61-72.
26. Lessigiarska I., A. P. Worth, T. I. Netzeva (2005). Comparative Review of QSARs for Acute Toxicity, JRC report EUR 21559 EN, Ispra, Italy, European Commission, Joint Research Centre.
27. Lessigiarska I., A. P. Worth, T. I. Netzeva, J. C. Dearden, M. T. D. Cronin (2006). Quantitative Structure-activity-Activity and Quantitative Structure-activity Investigations of Human and Rodent Toxicity, *Chemosphere*, 65, 1878-1887.
28. Loader R. J., N. Singh, P. J. O'Malley, P. L. Popelier (2006). The Cytotoxicity of Ortho Alkyl Substituted 4-X-phenols: A QSAR Based on Theoretical Bond Lengths and Electron Densities, *Bioorg. Med. Chem. Lett.*, 16, 1249-1254.
29. Lu C. F. (1991). *Basic Toxicology. Fundamentals, Target Organs, and Risk Assessment*, Taylor & Francis.
30. Multicase, MULTICASE Inc., 23811 Chagrin Blvd., Ste 305 Beachwood, OH 44122, www.multicase.com
31. O'Brien S. E., P. L. A. Popelier (2001). Quantum Molecular Similarity. 3. QTMS Descriptors, *J. Chem. Inf. Comput. Sci.*, 41, 764-775.
32. OECD (1987). OECD Guidelines for the Testing of Chemicals No. 401: Acute Oral Toxicity, 7 pp., Paris, France, Organisation for Economic Cooperation and Development.
33. OECD (2001). OECD Guidelines for the Testing of Chemicals No. 420: Acute Oral Toxicity – Fixed Dose Procedure, 14 pp., Paris, France, Organisation for Economic Cooperation and Development.
34. OECD (2001). OECD Guidelines for the Testing of Chemicals No. 423: Acute Oral Toxicity – Acute Toxic Class Method, 14 pp., Paris, France, Organisation for Economic Cooperation and Development.
35. OECD (2001). OECD Guidelines for the Testing of Chemicals No. 425: Acute Oral Toxicity – Up-and-down Procedure, 26 pp., Paris, France, Organisation for Economic Cooperation and Development.
36. OECD (2004). The Report from the Expert Group on (Quantitative) Structure Activity Relationship ([Q]SARs) on the Principles for the Validation of (Q)SARs, OECD Series on Testing and Assessment No. 49, ENV/JM/MONO(2004)24, OECD, Paris, France, 206 pp.
37. OECD (2006). Report on the Regulatory Uses and Applications in OECD Member Countries of (Q)SAR Models in the Assessment of New and Existing Chemicals.



38. Pallas, CompuDrug International Inc., 115 Morgan Drive, Sedona, AZ 86351, USA, www.compuDrug.com
39. Peloux A. F., C. Fédérici, N. Bichet, D. Gouy, J. P. Cano (1992). Hepatocytes in Primary Culture: An Alternative to LD50 Testing? Validation of a Predictive Model by Multivariate Analysis, *ATLA*, 20, 8-26.
40. Selassie C. D., T. V. DeSoyza, M. Rosario, H. Gao, C. Hansch (1998). Phenol Toxicity in Leukemia Cells: A Radical Process? *Chem. Biol. Interact.*, 113, 175-190.
41. Selassie C. D., A. J. Shusterman, S. Kapur, R. P. Verma, L. Zhang, C. Hansch (1999). On the Toxicity of Phenols to Fast Growing Cells. A QSAR Model for a Radical-Based Toxicity, *Perkin Transactions*, 2, 2729-2733.
42. Selassie C. D., S. Kapur, R. P. Verma, M. Rosario (2005). Cellular Apoptosis and Cytotoxicity of Phenolic Compounds: A Quantitative Structure-activity Relationship Study, *J. Med. Chem.*, 48, 7234-7242.
43. Spielmann H., E. Genschow, M. Liebsch, W. Halle (1999). Determination of the Starting Dose for Acute Oral Toxicity (LD50) Testing in the Up and Down Procedure (UDP) from Cytotoxicity Data, *ATLA*, 27, 957-966.
44. The EU Testing Methods Adoption Process, <http://ecb.jrc.it/>
45. TOPKAT, Accelrys Ltd., 334 Cambridge Science Park Cambridge, <http://www.accelrys.com/products/topkat>
46. Tsakovska I., T. Netzeva, A. Worth (2006). Review of (Q)SARs for Mammalian Toxicity, JRC report EUR 22486 EN, Ispra, Italy, European Commission, Joint Research Centre.
47. Worth A. P., A. Bassan, A. Gallegos, T. I. Netzeva, G. Patlewicz, M. Pavan, I. Tsakovska, M. Vracko (2005). The Characterisation of (Quantitative) Structure-Activity Relationships: Preliminary Guidance, JRC report EUR 21866 EN, European Chemicals Bureau, Joint Research Centre, European Commission, Ispra, Italy, http://ecb.jrc.it/DOCUMENTS/QSAR/QSAR_characterisation_EUR_21866_EN.pdf
48. Wang Y., H. Liu, C. Zhao, H. Liu, Z. Cai, G. Jiang (2005). Quantitative Structure-activity Relationship Models for Prediction of the Toxicity of Polybrominated Diphenyl ether Congeners, *Environ. Sci. Technol.*, 39, 4961-4966.