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RESEARCH ARTICLE

Prediction of acute toxicity in fish by using QSAR methods and chemical modes of action

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Abstract

Three quantitative structure–activity relationship (QSAR) models were evaluated for their power to predict the toxicity of chemicals in two datasets: (1) EPAFHM (US Environmental Protection Agency—Fathead Minnow) and (2) derivatives having a high production volume (HPV), as compiled by the European Chemical Bureau. For all three QSAR models, the quality of the predictions was found to be highly dependent on the mode of action of the chemicals. An analysis of outliers from the three models gives some clues for improving the QSAR models. Two classification methods, Toxtree and a Bayesian approach with fingerprints as descriptors, were also analyzed. Predictions following the Toxtree classification for narcosis were good, especially for the HPV set. The learning model (Bayesian approach) produced interesting results for the EPAFHM dataset but gave lower quality predictions for the HPV set.

Keywords: *QSAR; mode of action; LC_{50}; fathead minnow; classifications*

Introduction

Achieving a high level of environmental protection against the adverse effects of chemicals requires an effective chemical regulatory policy. Approximately 100,000 different chemical substances are registered in the European Union (EU), of which about 30,000 are manufactured or imported in quantities greater than one metric ton per year[1]. While experimental physicochemical properties and toxicity data are available for "new chemicals" (developed since 1981), there are no such provisions for "existing chemicals" (developed before 1981), even though they make up almost 99% of the total volume in the EU market. Therefore, adequate toxicological and ecotoxicological data are available only for a very small proportion of chemicals. To address this lack of health and safety data, the European Union recently adopted a new regulation, called REACH[2,3] (Registration, Evaluation, Authorization and Restriction of Chemicals). In REACH, all compounds manufactured or imported into the EU in quantities greater than one metric ton per year are required to be registered in a central database. Registration includes providing information concerning the effects of the chemical on human health and the environment. Under REACH, the requirements in terms of aquatic toxicity call for data from short-term toxicity testing on invertebrates and growth inhibition studies on aquatic plants. In addition, short-term toxicity data on fish are required for chemicals manufactured/imported in quantities greater than 10 metric tons per year. Traditionally, such information has been obtained through *in vivo* animal testing; however, under REACH legislation, quantitative structure–activity relationship (QSAR) models and *in vitro* methods as alternatives to animal experiments are expected to play a significant role. (Q)SARs will be used more extensively, in the interests of time- and cost-effectiveness and animal welfare. In particular, (Q)SARs are likely to play an important role in the assessment of chemicals produced or imported in quantities between 1 and 10 metric tons per year, for which minimal animal testing is foreseen[3,4]. In aquatic

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ecotoxicology, QSARs allow, for example, an estimation of toxic concentrations based on physical and/or chemical properties, without the use of experiments.

A number of conditions still need to be met in order that (Q)SAR results can provide an acceptable alternative to experimental data. These are: (1) a defined endpoint; (2) an unambiguous algorithm; (3) a defined domain of applicability; (4) appropriate measures of goodness-of-fit, robustness, and predictivity; and (5) a mechanistic interpretation, if possible[5]. No single QSAR model yet fulfills all of these criteria, and incorrect selection of a QSAR model can result in 10- to 1000-fold errors in toxic potency estimates[6]. Therefore, a key factor in the use of QSARs for predicting chemical toxicity is the choice of QSAR model.

Traditionally, the selection of structural analogs or QSARs has been based on the assumption that compounds from the same 'chemical class' should behave in a toxicologically similar manner. However, identification of chemical classes could be problematic because chemicals often carry different chemical moieties, thereby confounding efforts to achieve a meaningful classification. Moreover, within the QSAR programs themselves, new chemicals are per definition not included in the training set, so it is difficult to assign a good QSAR model to such chemicals. As a result, another approach consists of defining a general model based on a maximum number of chemicals and/or focusing on the notion of mode of toxic action[7,8]. Various modes of toxic action for chemicals in fish have been identified and extended to other aquatic organisms. Currently, the Verharr scheme seems to be the most widely used, recognizing modes of action associated with different structural classes: class 1—inert chemicals associated with non polar narcosis; class 2—relatively inert chemicals associated with polar narcosis; class 3—reactive chemicals associated with enhanced toxicity; and class 4—active chemicals associated with a central nervous system effect[7]. In the context of acute toxicity to fish, three groups of QSAR models have been reported in the literature: models for narcoses, global QSARs (developed without respect to chemical class and mode of action (MOA)), and QSARs for specific chemical classes.

Considering the volume of measurements to be undertaken, the lack of experience with implementation of such QSAR test policies, and the need for making informed choices about the multiple available QSAR models (which should eventually be available to all stakeholders in the REACH process), it is necessary to evaluate the accuracy of prediction of various models when they are used as a "black box," without clear guidance on when to use which QSAR. The present article compares the predictive quality of three QSAR models applied to a database of 617 compounds, the Environmental Protection Agency (EPA) Fathead Minnow Acute Toxicity database. This database was generated by the US Environmental Protection Agency with the goal of developing an expert system to predict acute toxicity from chemical structure based on a chemical's MOA. The 617 industrial chemicals were expressly chosen to serve as a useful training set for the development of predictive QSARs. The three QSAR models investigated here are: (1) a model developed in our laboratory, referred to as CERMN[9]; (2) the TOPKAT expert system[10]; and (3) a model developed by the European Chemicals Bureau (ECB)[11].

Materials and methods

Data set

A set of 617 chemicals was extracted from the EPA site[12]. The record for each chemical contains its name, mode of action (MOA)[6], molecular weight (MW), hydrophobicity ($\log P_{OW}$), and biological data corresponding to 96-h LC_{50} bioassays for the fathead minnow (*Pimephales promelas*). Chemicals without $\log P_{OW}$ and/or LC_{50} values were discarded, leading to a set of 566 chemical derivatives. Hereafter, we refer to this set as the EPAFHM set. This dataset was the basis for the Bayesian categorizations (see "Learning molecular categories" below).

A second set was created with ECB-high production volume (HPV)[11] chemicals. This set was used as a test set after the classification as baseline narcosis (Bayesian categorizations). In total, 1749 substances from over 2782 structures described in the ECB-HPV dataset were downloaded from the ECB site. Of these 1749 derivatives, 225 derivatives had 96-h LC_{50} value(s) for the fathead minnow recorded and were considered in this study (the lowest LC_{50} value was taken if several values were given for one derivative).

QSAR models

For the prediction of the biological response (96-h LC_{50} (mol/L)) toward the fathead minnow, four QSAR models were first tested.

TOPKAT[10] is a QSAR-based system which generates assessments of chemical toxicity solely from a chemical's molecular structure. TOPKAT uses cross-validated models based on experimental data.

The ECB model[13] is a model (Equation (1)) associated with narcosis as the MOA based on one hydrophobic descriptor ($\log P_{OW}$):

$$\log LC_{50} = -0.81 \log P_{OW} - 1.74 \quad (1)$$

In addition to the ECB model, Netzeva *et al.*[14] published a general model (regardless of MOA) for toxicity in the fathead minnow (Equation (2)) using the same previous descriptor but with a lower intercept value (higher basic toxicity) and a slight decrease of the slope relative to $\log P_{OW}$:

$$\log LC_{50} = -0.70 \log P_{OW} - 2.28 \quad (2)$$

Our QSAR model[9], referred to below as CERMN, is also a general model (Equation (3)), incorporating $\log P_{OW}$ and

two other descriptors corresponding to steric (MW) and electronic descriptors (E_{LUMO}):

$$\log LC_{50} = -0.509\log P_{OW} - 0.0045MW + 0.067E_{LUMO} - 1.98 \quad (3)$$

Calculation of descriptors and regressions

Hydrophobicity or octanol–water partition coefficient ($logP_{OW}$)

In EPAFHM, the values of $logP_{OW}$ correspond to experimental values or to calculated (ClogP[15]) values when experimental ones are missing. For the testing set, $logP_{OW}$ values were calculated by KOWWIN[16]. This latter software gave either experimental $logP_{OW}$ values if they were recorded in KOWWIN's internal database or estimated values in other cases. For EPAFHM, the correlation between ClogP and KOWWIN values was very good ($r^2 = 0.96$).

Quantum descriptors

LUMO (lowest unoccupied molecular orbital energy) values were calculated for each chemical with Pipeline Pilot[17] using VAMP, a semi-empirical molecular orbital package, and AM1 for Hamiltonian.

Pipeline Pilot fingerprints (ECFP_12)

Extended Chemical Fingerprint 12, called ECFP_12, is a class of circular substructural fingerprint[18]. The ECFP_12 fingerprint encodes each atom and its molecular environment within a circle with a maximal diameter of 12 chemical bonds. The fingerprint was recorded into a fixed length of 1024 bits.

Regression methods

All the regressions were done in Microsoft Excel. The correlation coefficient r^2 represents the quality of the regression between two variables (how much the variance of one variable is accounted for by the predictive power of the other variables). The standard deviation *s* characterizes the width of the distribution of the predicted values by the regression. The greater is the standard deviation, the more widely distributed are the values and the worse is the predictive power. The limit for outliers was defined from the *s* value (data normally distributed). The *n* values correspond to the number of chemicals used to calculate the correlation.

Classification tools

Toxtree[19]

Toxtree is an open-source application that places chemicals into categories and predicts various kinds of toxic effects by applying decision tree approaches. Verhaar's scheme[7], for aquatic MOAs, classifies chemicals into five classes: (1) narcosis or baseline toxicity; (2) less inert compounds; (3) nonspecific reactivity; (4) compounds acting by a specific mechanism; and (5) unclassified compounds.

Learning molecular categories[17]

This protocol was built using multiple Bayesian categorization equations within a single model, using a property that lists the categories. The EPAFHM set is divided into nine clear MOAs (see Table 1). For each MOA, the confidence degree toward this classification is expressed from "High" to "Low." Compounds for which the MOA confidence had "High" and "High-Moderate" probabilities for each MOA were used as the training set. AlogP, molecular weight, the number of H donors and acceptors, the number of rotatable bonds, the molecular polar surface area, and ECFP_12 fingerprint were used for descriptors[17].

Results and discussion

Choice of ECB model

Two different QSARs with $logP_{OW}$ as the single descriptor could be used in principle (Equation (1) for narcosis and Equation (2) for a general model). The $logLC_{50}$ values predicted by the two methods are completely correlated. However, as expected, the toxicity values predicted by the general model (Equation (2)) are in most cases higher than those predicted by Equation (1), although the observed differences are smaller than the level of accuracy. Therefore, we arbitrarily chose the narcosis model (Equation (1)) for the following studies.

Table 1. Classification of EPAFHM derivatives based on mode of action (MOA). Compounds with "High" or "High-Moderate" probabilities for each MOA were used as the training set for defining the categories.

MOA	Initial set	Training set	Classification of the remaining set
Baseline narcosis	241	142	99
Arylate and ester narcosis	26	11	15
Polar narcosis	38	29	9
Electrophile or proelectrophile reactivity	97	44	53
Acetylcholinesterase inhibition	17	17	0
Central nervous system seizure or stimulant	9	9	0
Neurodepressant	6	6	0
Respiratory blocker or inhibitor	4	2	2
Uncoupler of oxidative phosphorylation	12	11	1
MOA not determined	116	0	116

Table 2. Statistical results for the studies.

	TOPKAT	ECB	CERMN
$r^2 =$	0.64	0.61	0.65
$s =$	0.81	0.86	0.81
$n =$	492	566	566

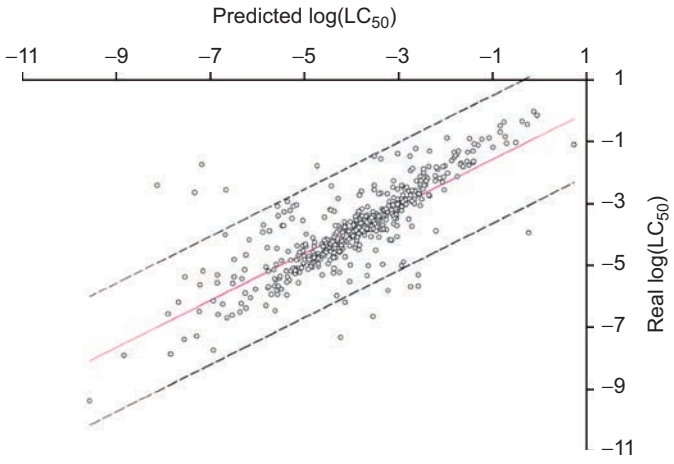


Figure 1. Predicted $logLC_{50}$ vs. real $logLC50_{50}$ for TOPKAT.

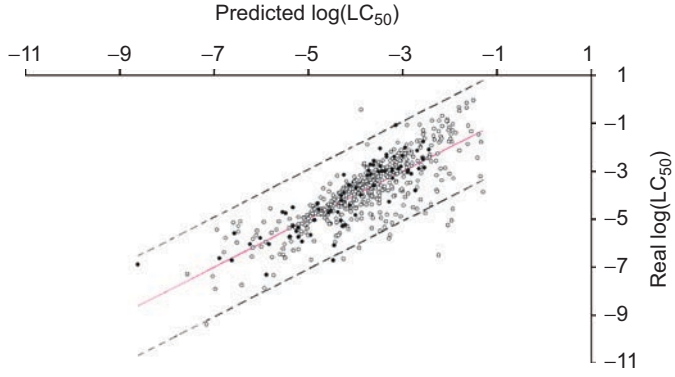


Figure 2. Predicted $logLC_{50}$ vs. real $logLC_{50}$ for CERMN. Black full circles represent the chemicals not predicted with TOPKAT.

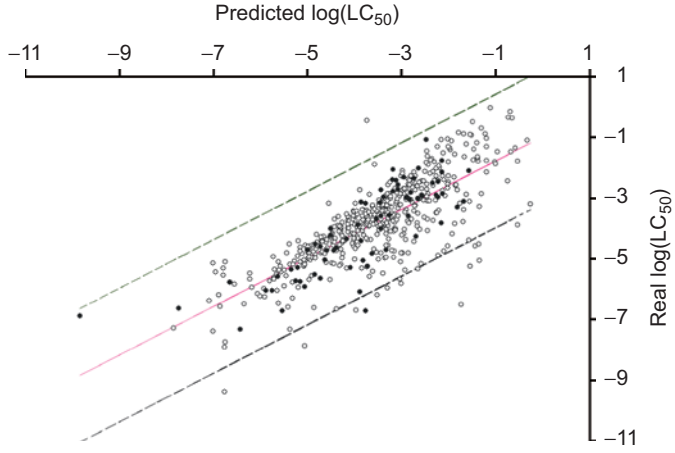


Figure 3. Predicted $logLC_{50}$ vs. real $logLC_{50}$ for ECB. Black full circles represent the chemicals not predicted with TOPKAT.

Quality of the predictions

The statistical results for the three studies are presented in Table 2. The ECB model is slightly inferior to the TOPKAT and CERMN models. However, TOPKAT (Figure 1) could predict only 492 chemicals out of 566 derivatives (EPAFHM). Also, the graphical representations of the relationships between the real and predicted values show some discrepancies between the models. Moreover, we note that the compounds excluded by TOPKAT are generally well predicted by the two other models (black full circles, Figures 2 and 3).

Outliers

Several compounds with high under- and overestimated values appeared (outliers). We selected a cutoff (dashed line) at 2.56*s* (see Table 2 for *s* values) to point out these outliers. For the ECB and CERMN models, only one derivative was overestimated for its toxicity (Figures 2 and 3); in contrast, TOPKAT (Figure 1) overestimated five derivatives. Concerning the underestimated outliers, TOPKAT generated the fewest outliers with eight derivatives; CERMN generated 12 outliers and ECB 15. Three of the underestimated outliers (**4**, **6**, and **7**) are common to all models (Table 3). Eleven of the underestimated outliers are common (Table 3) to the CERMN and ECB models.

Outliers with overestimated values. $LogP_{OW}$ is the main descriptor in the CERMN and ECB models. An incorrect prediction of $logP_{OW}$ could be one explanation for the observed overestimates. This could be the case for **22**. Indeed, this compound has a calculated value (ClogP) of 2.46 in EPAFHM but with KOWWIN the prediction is–4.15. The experimentally determined $logLC_{50}$ value for **22** is–0.45 ($logLC_{50}$ in mol/L). Starting from the KOWWIN prediction, the predicted LC_{50} value would be 1.62 for ECB (–3.73 initially with ClogP) and–0.51 for CERMN (–3.88 with ClogP). Thus, the prediction of the CERMN model is very good for this derivative using the KOWWIN calculation for $logP_{OW}$. Experimental determination of $logP_{OW}$ should be done to clarify this point. At this stage, we presume that the KOWWIN value is closer to the real $logP_{OW}$ value for **22**. The other overestimated values generated by TOPKAT correspond to nitriles (**9**, **11**, and **12**). TOPKAT must consider these derivatives to be very toxic, presumably as a result of release of the cyanide group (we note that **18** and **19** are not outliers for TOPKAT). However, in our opinion, without activation of one proton of the alpha carbon of the nitrile group, the formation of cyanide is less probable (see below for a discussion on this point). For the two other derivatives (**10**, **13**) we have no explanation. TOPKAT could estimate a toxicity based on the action of amine oxidase on **10** as described for some derivatives[20]. The toxicity assigned to **13** is more surprising in light of the interest of propargyl amine in neuroprotection, for instance[21].

Outliers with underestimated values. Several of the outliers correspond to electrophile or proelectrophile reactants. Among them, aldehydes such as **14** (acrolein) are systematic outliers in several QSAR studies. The toxic potency of acrolein, mediated by a Michael addition toward nucleophiles, is very high and often underestimated. Compounds **4**, **6**, **7**, **15**, and **16** are also outliers, and their mechanism

Table 3. Descriptions of the outliers produced by the models.

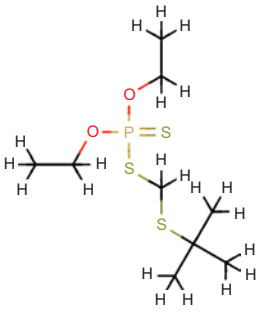
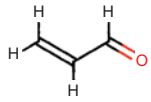
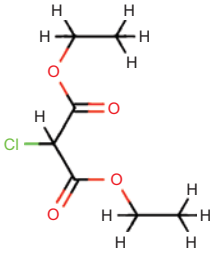
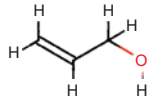
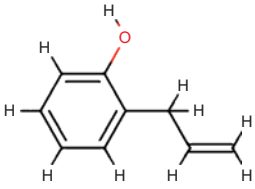
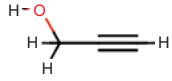
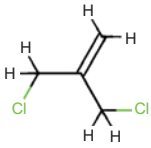
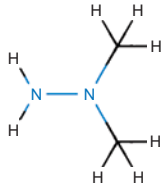
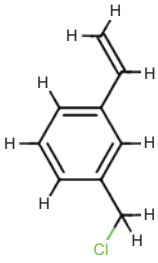
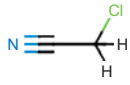
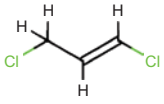
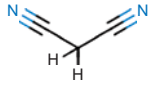
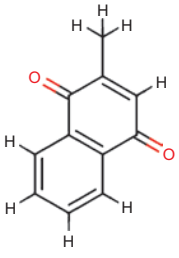
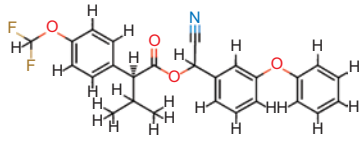
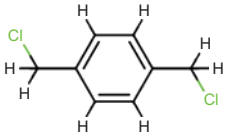
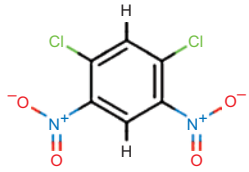
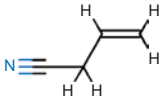
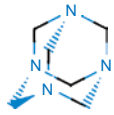
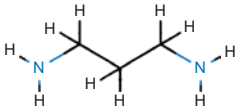
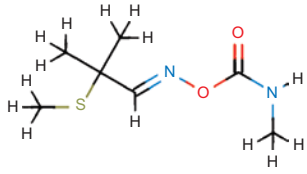
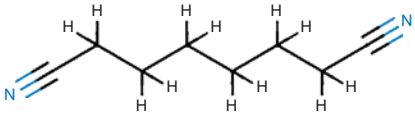
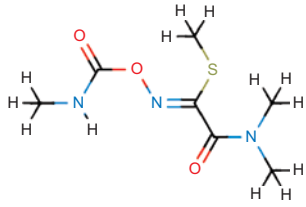
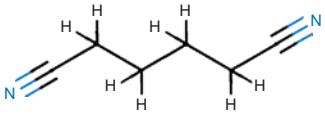
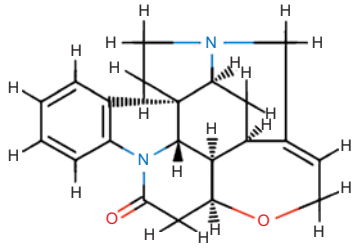
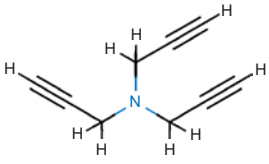
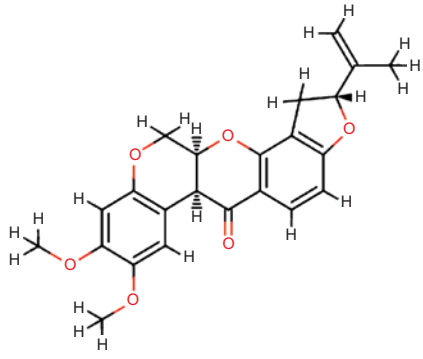
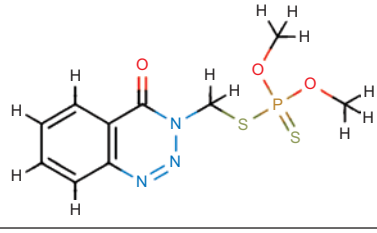
Structure	N° (CAS number)	Structure	N° (CAS number)
	**1**(13071-79-9) – (TOPKAT) Bio:–7.34		**14**(107-02-8) – (ECB) – (CERMN) Bio:–6.52
	**2** (14064-10-9) – (TOPKAT) Bio:–5.31		**15** (107-18-6) – (ECB) – (CERMN) Bio:–5.26
	**3** (1745-81-9) – (TOPKAT) Bio:–3.95		**16** (107-19-7) – (ECB) – (CERMN) Bio:–4.58
	**4** (1871-57-4) – (TOPKAT) – (ECB) – (CERMN) Bio:–5.82		**17** (57-14-7) – (ECB) – (CERMN) Bio:–3.88
	**5** (30030-25-2) – (TOPKAT) Bio:–5.7		**18** (107-14-2) – (CERMN) Bio:–4.75
	**6** (542-75-6) – (TOPKAT) – (ECB) – (CERMN) Bio:–5.67		**19** (109-77-3) – (ECB) – (CERMN) Bio:–5.07
	**7** (58-27-5) – (TOPKAT) – (ECB) – (CERMN) Bio:–6.19		**20** (70124-77-5) – (ECB) – (CERMN) Bio:–9.38

Table 3. continued on next page.

Table 3. Continued.

Structure	N° (CAS number)	Structure	N° (CAS number)
	**8** (623-25-6) – (TOPKAT) – (CERMN) Bio:–6.65		**21** (3698-83-7) – (ECB) – (CERMN) Bio:–6.72
	**9** (109-75-1) + (TOPKAT) Bio:–2.57		**22** (100-97-0) + (ECB) + (CERMN) Bio:–0.45
	**10** (109-76-2) + (TOPKAT) Bio:–1.79		**23** (116-06-3) – (ECB) Bio:–5.34
	**11** (629-40-3) + (TOPKAT) Bio:–2.41		**24** (23135-22-0) – (ECB) Bio:–4.51
	**12** (111-69-3) + (TOPKAT) Bio:–1.75		**25** (60-41-3) – (ECB) Bio:–5.95
	**13** (6921-29-5) + (TOPKAT) Bio:–2.65		**26** (83-79-4) – (ECB) Bio:–7.88
			**27** (86-50-0) – (ECB) Bio:–6.69

Note. Minus symbol (–), underestimated compounds; plus symbol (+), overestimated compounds; Bio, real biological data ($logLC_{50}$ in mol/L).

of toxicity could be similar to that of α,β unsaturated aldehyde. Indeed, the metabolites of **16** have been studied[22], and they result from oxidation of the alcohol leading to aldehyde and carboxylic acid. Thus, the metabolites of **16** should have the same chemical properties as acrolein. The alcohol function of **15** could be oxidized leading straight to acrolein. The metabolites of **6** (1,3-dichloropropene) were described by the US EPA[23]. The formation of an alcohol

followed by oxidation led to 3-chloroacrolein. We can suppose that compound **4** has the same type of metabolites, with easy substitution of one chlorine atom by a hydroxyl group followed by oxidation. Compound **7** has the chemical features of an α,β unsaturated aldehyde. Compound **8** could lead to 1,4-dibenzaldehyde. We have no information about whether the toxicity of this last derivative is related to α,β unsaturated aldehyde, with an activation of the phenyl group by the two electron-withdrawing groups. However, the graphic representation of the relationships between these derivatives and $\log P_{OW}$ shows a good statistical relationship if we discard acrolein (because of its underestimated toxicity):

$$\log LC_{50} = -0.49 \log P_{OW} - 5.04$$
$$n = 6, r^2 = 0.91, s = 0.23 \quad (4)$$

We observed that four other derivatives (see Table 4) are close to the cutoff value. With these derivatives, the following equation was obtained:

$$\log LC_{50} = -0.61 \log P_{OW} - 4.66$$
$$n = 10, r^2 = 0.88, s = 0.36 \quad (5)$$

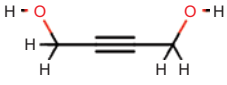
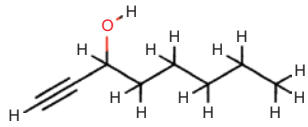
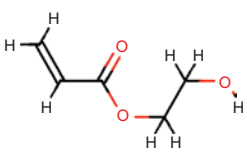
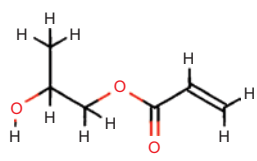
With this class of derivatives, we have a uniformly high toxicity (value of intercept) and the toxicity increases with their ADME (adsorption, distribution, metabolism, excretion) properties ($\log P_{OW}$ and metabolism).

As for the other outliers, **17**, a hydrazine derivative, is described as being very reactive[24], although the mechanism of its toxicity is not actually known. For halonitrile, **18**, and dicyanomethane, **19**, their high toxicities should derive from the oxidation of a CH bond (activation with chlorine or nitrile group) followed by the release of HCN[25], a potent inhibitor of cytochrome c oxidase. For **21**, the rapid formation of phenol must be the first step (substitution of the chlorines by hydroxyl groups). Oxidation of one of the two phenol groups could lead to a very reactive derivative. The toxicities of nitrobenzene are also higher than either the baseline or polar narcosis MOA would suggest[26]. These two factors could explain the toxicity of **21**. Compounds **23**, **24**, **27**, and **1** correspond to acetyl cholinesterase inhibitors. Therefore, their predicted toxicities could be improved by considering a QSAR model specific for this class of derivatives. Compounds **20**, **25**, and **26** are flucythrinate, strychnine, and rotenone, respectively, well-known poisons used as pesticides. Their MOAs are very specific and their toxicities cannot be reliably estimated by QSAR models such as ECB or CERMN. An expert system such as TOPKAT, with a specific database, is more powerful. The toxicity of **5** was correctly predicted starting from Equation (5). We have no clear explanation for the mechanism of toxicity of diethyl chloromalonate, **2**. For 2-allyl phenol, **3**, used as a fungicide[27], its action was reported to be mediated by glutathione *S*-transferase interactions (GSTP1, GSTM1, GSTA1)[28].

Quality of the prediction in relation to mode of action

The derivatives from the EPAFHM dataset were classified into nine MOAs (Table 1). For this part of the study we focused on the following MOAs: baseline narcosis; arylate/ester narcosis; polar narcosis; electrophile or proelectrophile reactivity (reactants). Arylate/ester narcosis and polar narcosis MOAs were combined into a single group called "multiple narcosis." The statistical results from the same predictive models (TOPKAT, ECB, and CERMN) are summarized in Table 5. The results of the TOPKAT model for baseline narcosis in relation to overestimation (see Figure 4) of the toxicity are described above for three derivatives (**11**, **12**, and **13**). The CERMN model produced a very good correlation; however, we observed a slight overestimation of toxicity of the compounds (Figure 5). The quality of the correlation decreased in all three models for the MOAs multiple narcosis and reactants. TOPKAT predicted fewer derivatives without giving better predictions. From these initial data, a very high correlation was obtained for derivatives with an MOA of baseline narcosis. Therefore, we sought to determine whether we could classify a derivative as belonging to a specific MOA, and particularly to baseline narcosis, before a prediction of its toxicity was made. For this study, two different datasets were used: (1) EPAFHM, to analyze relationships between the described and predicted classifications; and (2) the HPV dataset, to analyze relationships between the predicted and real biological data starting from the supposed classification.

Table 4. Description of chemicals used to create the model with Equation (5).

Structure	N° (CAS number)	Structure	N° (CAS number)
	**28** (110-65-6) Bio:–3.21		**30** (818-72-4) Bio:–5.49
	**29** (818-61-1) Bio:–4.38		**31** (999-61-1) Bio:–4.59

Note. CAS number and real biological data ($\log LC_{50}$ in mol/L).

Table 5. Quality of the prediction models for the three principal MOAs.

MOA	TOPKAT	ECB	CERMN
Baseline narcosis	$r^2=0.69$ $s=0.72$ $n=215$	$r^2=0.91$ $s=0.39$ $n=240$	$r^2=0.93$ $s=0.35$ $n=240$
Multiple narcosis	$r^2=0.31$ $s=0.65$ $n=51$	$r^2=0.74$ $s=0.43$ $n=64$	$r^2=0.76$ $s=0.42$ $n=64$
Reactants	$r^2=0.27$ $s=0.88$ $n=79$	$r^2=0.40$ $s=0.82$ $n=94$	$r^2=0.41$ $s=0.82$ $n=94$

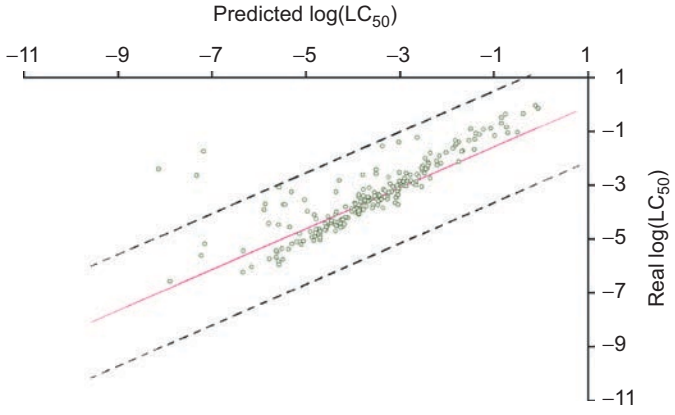


Figure 4. Real vs. predicted logLC50 values (baseline narcosis for MOA) with TOPKAT.

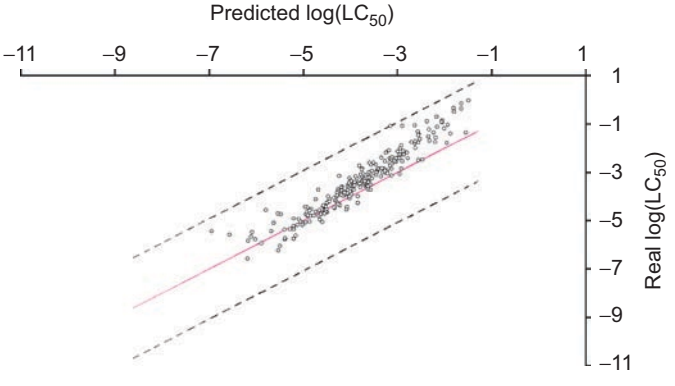


Figure 5. Real vs. predicted $\log LC_{50}$ values (baseline narcosis for MOA) with the CERMN model.

Table 6. Results of classification with Toxtree.

	Dataset	
MOA	EPAFHM	ECB-HPV
Narcosis baseline	99	57
Less inert	77	34
Nonspecific reactivity	89	26
Specific mechanism	4	0
Unclassified	297	108
Total	566	225

Classification with Toxtree software

Toxtree (Verhaar scheme) classified the chemicals into five groups (Table 6). We were particularly interested in the narcosis group. The initial correlation between the real and predicted values (generated by the CERMN model) for the HPV set of 225 derivatives, without classification, was 0.37 (r^2). Ninety-nine EPAFHM derivatives and 57 HPV derivatives were classified as having a narcosis MOA. From these derivatives, the correlation between real and predicted values with the CERMN model was 0.87 (r^2) for EPAFHM and 0.72 for HPV. Therefore, Toxtree is able to extract and correctly predict (starting from the CERMN model) derivatives with a potential baseline narcosis for the MOA, but the statistical relationship is lower than expected (Table 5).

Table 7. Reclassification of MOA for chemicals in the initial set and prediction by the learning model.

	EPAFHM		
MOA	Initial set	Prediction	HPV prediction
Baseline narcosis	241	358	136
Arylate and ester narcosis	26	29	13
Polar narcosis	38	61	31
Electrophile or proelectrophile reactivity	97	39	20
Acetylcholinesterase inhibition	17	21	5
Central nervous system seizure or stimulant	9	9	1
Neurodepressant	6	7	0
Respiratory blocker or inhibitor	4	20	17
Uncoupler of oxidative phosphorylation	12	22	2
MOA not determined	116	0	0

Classification with Pipeline Pilot machine learning

The learning model was applied to the training set to check the quality of the MOA prediction as a function of the MOA indicated in the EPAFHM dataset. In this dataset, 116 derivatives had no defined MOA. With this learning model, each compound was classified by function to a specific class based on the highest probability obtained. Table 7 shows the number of chemicals predicted in each MOA for EPAFHM and HPV.

The correlation between the real values and those predicted by the CERMN model for the 358 derivatives classified as having a narcosis MOA (see Table 7) was high ($r^2=0.77$ and $s=0.54$). If we compare this result to the Toxtree result, the CERMN model handled nearly four times as many derivatives for a similar correlation. The same studies were conducted with the test set (HPV). For this dataset, 60% of the chemicals were classified as having a baseline narcosis MOA. The correlation was close to the results obtained with Toxtree ($r^2=0.64$ and $s=0.75$), but this classification predicted 136 derivatives compared to only 57 with Toxtree. For the EPAFHM dataset, we can strongly improve the correlation by classifying the compounds according to the probability (P) of belonging to a class (e.g. with $P=0.9$, the correlation was 0.88 for r^2 for 127 chemicals instead of 358), but with HPV we observed that the correlation did not substantially improve using the same cutoff.

Conclusion

The definition of a method able to classify compounds as having a narcosis MOA with a high probability is necessary to improve the prediction of toxicity for the majority of organic derivatives. Bayesian approaches with fingerprints have made important advances toward this definition, but it will be necessary to couple such methods with a knowledge base that includes definitions of structural alerts (chemical features leading to high toxicity). With a correct classification of narcosis for the MOA, the ECB and CERMN models should give the most relevant statistical results along with an explanation of the origin of the toxic effects for these derivatives. Analysis of the outliers associated with general models (whatever the models) has shown clearly the origin of the discrepancies for the predictions. Very highly toxic compounds led to important errors for the ECB and CERMN predictions. TOPKAT included structural alerts, but in this case several derivatives were overestimated for their toxicities (the nitrile feature as a structural alert, for instance). Therefore, for each structural alert, its characteristic must be clearly defined. Toward this objective, data included in the DEREK expert system should be very useful. The next steps of this study will be to improve this classification as well as to analyze the possibility of improving the predictions for derivatives classified as "multiple narcosis" and "reactants" for the MOA. Indeed, as described herein, it is possible to build a good QSAR equation for reactants with the same MOA (Equation (5)). This work is now in progress.

Declaration of interest

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