Modeling Oral Rat Chronic Toxicity

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The chronic toxicity is fundamental for toxicological risk assessment, but its correlation with the chemical structures has been studied only little. This is partly due to the complexity of such an experimental test that embraces a plethora of different biological effects and mechanisms of action, making (Q)SAR studies extremely challenging. In this paper we report a predictive *in silico* study of more than 400 compounds based on two-dimensional chemical descriptors and multivariate analysis. The root mean squared error of the predictive model is 0.73 (in a logarithmic scale) on a leave-one-out cross-validation and is close to the estimated variability of experimental values (0.64). The analysis of the model revealed that the chronic toxicity effects are driven by the bioavailability of the compound that constitutes a baseline effect plus excess toxicity possible described by a few chemical moieties. The results obtained give confidence that this model can be useful for establishing a level of safety concern in the absence of hard toxicological data.

INTRODUCTION

Food chemical risk assessment is the scientific process used to characterize the health significance of potentially harmful chemicals in food. Classically, it comprises four steps: i) hazard identification, ii) hazard characterization, iii) exposure assessment, and iv) risk characterization. In general, hazard identification relies on toxicological data obtained in experimental animals, mainly in rodents. Because ingestion of low doses of chemicals over long periods of time is a common scenario of food-mediated chemical exposures, chronic toxicity studies are considered essential for food chemical risk assessment. In general, chronic toxicity is addressed by feeding experimental animals with various doses of test materials over a long period of time up to a lifetime. The chronic studies are designed to obtain a dose−response covering overt toxic effects, mild effects (the Lowest Observed Adverse Effect, LOAEL), and no effects (the No Observed Effect Level, NOAEL).

It has been estimated that there are over 5 million man-made chemicals known, of which 70000 are in use today.[1] In addition, there are about 100000 naturally occurring chemicals of known structure. The application of continuously improving analytical methods has revealed that many of these chemicals can enter the food chain and result in human exposure. Since for the vast majority of these chemicals, toxicological information is absent or limited, the assessment of the health risks associated with them is therefore not possible. Nevertheless, the detection of these chemicals in food may cause alarm and sometimes trigger heavy management actions such as public recalls. The handling of such situations would significantly benefit from a very quick understanding of the potential health risks involved.

Solutions to this general issue are not straightforward. Obviously, from a resource perspective it is not feasible to generate a complete set of toxicological experimental data for any chemical which could potentially enter the food chain. In addition, experimental toxicology is not a practical tool to deal with a crisis requiring fast decision-making. Furthermore, even if sufficient facilities to perform toxicological testing within a relevant time frame were available, it still can be questioned whether testing all these substances would be a rational and practical approach. In this context, the challenge is to develop alternative strategies to identify chemicals of food safety concern in the absence of specific toxicological data. This will help in prioritizing the limited resources on issues of public health significance.

In this context, *in silico* predictive models have obvious advantages in terms of time, cost, and also animal protection. Although this type of approach is receiving increasing public, regulatory, and industrial attention,[2−4] only a few (Q)SAR models are available for chronic toxicity which is often considered as a far too heterogeneous and complex end point to be encoded in a single predictive model. Although this drawback has to be recognized, initial attempts have suggested the feasibility to develop models providing meaningful predictions of chronic toxicity.[5−10] In the present work, a new *in silico* model has been constructed based on a large data set of chronic toxicity values selected on strict criteria to ensure homogeneity. The performance of the model has been tested. The root-mean-square error of the model on a leave-one-out cross-validation was revealed to be close to the estimated variability of experimental values.

MATERIALS AND METHODS

Sources of Experimental Data. Munro et al.[5] published in 1996 a large database containing LOAEL values of over 550 chemical substances. Later, Venkatapathy et al.[7] collected some 600 chemicals, pooling together oral rat chronic values from the U.S. EPA′s OPP, the U.S. EPA′s IRIS, HEAST, and PTV databases. Additional sources of data were the U.S. EPA′s ECOTOX database[11] (about 230 chemicals)

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and other sparse different sources (about 300 compounds), e.g. JECFA, JMPR, NCI, and NIH. In total about 1750 values were retrieved.

To construct a consistent LOAEL data set and to ensure data homogeneity our analysis was restricted to chronic (defined as longer than 180 days), rat (*Rattus norvegicus*), and oral (gavage, diet, drinking water) studies. Furthermore, it was noted that the source databases were often overlapping; therefore, multiple cross-referenced entries were dropped. In conclusion, out of the initial 1750, a data set of 567 entries referring to 445 different chemicals was constructed.

The reproducibility of LOAEL values is strongly limited not only by variations in the protocol employed (e.g., dose-spacing) but also by purity of the tested chemical and inconsistencies in the interpretation of dose−response curves. Thus, a rather low interlaboratory reproducibility, and, in turn, a large background experimental noise/error due to the incorporation of experimental data from different laboratories, is expected in the final data set, though carefully treated. The experimental variability was estimated using compounds showing at least two independent chronic rat oral test studies.

Data were transformed and modeled as a logarithmic scale.

Physicochemical and Structural Descriptors. The canonical SMILES strings of these compounds were retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/) by means of their CAS registry number or chemical name(s). DRAGON Plus version 5.4 − 2006 (http://www.talete.mi.it/) was used for the calculation of molecular descriptors.[12,13] In total, around 350 two-dimensional descriptors were calculated for each structure, spanning several diverse categories: constitutional, functional group counts, atom-centered fragments, and molecular properties (LogP, hydrophilic factor, molecular refractivity, etc.).

Statistical Methods. The data set was cleared from 65 not significant, constant variables ($\sigma = 0$). Then, empty values were discarded descriptors-wise (9 descriptors).

The resulting pool of descriptors was further screened using a PLS-GA tool.[14] This method is very useful in selecting the best subset of variables by combining genetic algorithms (GA) and partial least-squares (PLS) modeling, validated through y-randomization test.[15] GA is a stochastic global search method that mimics natural biological evolution. GAs operate on a population of potential solution, applying the principle of survival of the fittest to produce approximations to a solution. In the present study a population size of 30 chromosomes with 1% mutation and 50% cross-over rates were used, and the maximum number of selected variables was limited to 30 per run. PLS can deal with strongly collinear input data and is the preferred tool to robustly handle many descriptor variables as compared to the number of compounds. This technique attempts to identify a few latent variables, or linear combination of descriptors, that best correlate with the observations. The maximum number of components in this case was limited to 15. In total the whole PLS-GA procedure was repeated 10 times, and only variables that were selected at least once were retained for the following analysis.

Finally, leave-one-out stepwise multiple linear regression (LOO-SMLR) was used to generate the predictive model. The LOO-SMLR systematically removes one data point at a time from the training set and, on the basis of the reduced data set, constructs a multilinear regression model that is subsequently used to predict the removed sample. The best MLR model resulting is retained as the most predictive.

This model was further validated by y-randomization. In a y-randomization test the output values, i.e. LOAEL values, of the compounds are shuffled randomly, and a model is fitted on the scrambled data. This procedure is repeated several times (in this case 100); if there remains a strong correlation between descriptors and randomized response variable, then the significance of a proposed QSAR model is regarded as being suspicious.

All statistical analyses were carried out on standard PCs using MATLAB 7.0.1, the Statistics Toolbox 5.0.1, and the PLS-genetic algorithm toolbox for MATLAB.

RESULTS

Experimental Variability. All the entries collected from the different sources were initially screened chemically wise dropping mixture and unavailable or unclear chemical structures. Experimental data were then carefully checked, and only values referring to studies on rats (*Rattus norvegicus*) orally exposed (gavage, diet, water) to the test material for more than 180 days were retained.

Most of these entries are referred to different chemicals, but 94 compounds showed at least two different valid experimental values. These compounds were used to estimate the interlaboratory reproducibility of chronic rat oral test studies (as defined above), by calculating the variability of the experimental value of each of these 94 chemical compounds. Assuming a normal distribution, 95% of the observations for a single chemical fall within twice the standard deviation of the mean value. The standard deviation of the distributions of experimental values of each compound averages 0.32, with a maximum up to 1.49 mg/kg$_{bw}$/day (in logarithmic units). Therefore, the interlaboratory reproducibility of LOAEL values can be estimated as twice the mean standard deviation, i.e. 0.64 mg/kg$_{bw}$/day (in logarithmic units).

QSAR Model. Table 1and Figure 1 summarize results of the QSAR model that is in the form

$$\log_{10}(\text{LOAEL}) = b \cdot B + c \qquad (1)$$

where b is the vector of coefficients, B is the matrix of descriptors, and c is the intercept, and the result is given in mg/kg$_{bw}$/day, after transformation into natural scale.

In total 19 descriptors are involved, including general molecular descriptors and more atom/fragment based ones. The model's statistical parameters for the 567 entries indicate good stability: the squared correlation coefficient (along with the F and p values for the regression) and root mean squared error are $R^2 = 0.54$ ($F = 31.426$, p-val $= 0$) and rmse $= 0.700$. The stability of the model was also confirmed by the leave-one-out cross-validation coefficient, $R^2_{CV} = 0.50$, and the root mean squared error, $\text{rmse}_{CV} = 0.727$, and by a y-randomization test (Figure 1b). For the assessment of the goodness-of-fit of chronic toxicity predictive models, the percentages of compounds within a given predictive error are considered to give a better picture[7−10] than standard regression statistics.

The percentage of LOAELs predicted accurately to be within an error of 0.64 (σ_e), 1, 2, and 3 of experimental values were 64, 85, 99, and 100%, respectively. The majority

Table 1. Best MLR Predictive Model[a]

b	se	pval	*B*	descriptor
0.05150	0.01338	≪0.05	RBN	number of rotatable bonds
−0.16676	0.02385	≪0.05	nN	number of nitrogen atoms
0.68498	0.24292	<0.05	nArCOOH	number of carboxylic acids (aromatic)
0.46493	0.11054	≪0.05	nOHp	number of primary alcohols
0.17824	0.03574	≪0.05	O-058	=O
0.13760	0.03923	≪0.05	Hy	hydrophilic factor
0.07871	0.02553	≪0.05	MLOGP	Moriguchi octanol−water partition coeff
−0.02219	0.00516	≪0.05	$MLOGP^2$	squared Moriguchi octanol−water partition coeff
−0.91947	0.37352	<0.05	nHM	number of heavy metals
−0.11135	0.02181	≪0.05	nX	number of halogen atoms
−0.50694	0.12192	≪0.05	nCXr=	number of X on ring C(sp^2)
0.52406	0.24934	<0.05	nCconjX	number of X on exoconjugated C
−1.36840	0.11688	≪0.05	P-117	Y3-*P* = Y (phosphate)
−0.32953	0.13622	<0.05	nRSR	number of sulfides
−1.78510	0.51186	≪0.05	nSO	number of sulfoxides
−0.26345	0.08066	<0.05	S-107	R2S/RS-SR
−0.13636	0.06585	<0.05	nArOR	number of ethers (aromatic)
−0.02561	0.01006	<0.05	H-052	H attached to C^0(sp^3) with 1Y attached to next C
−0.16168	0.07008	<0.05	H-054	H attached to C^0(sp^3) with 3Y attached to next C
−0.09491	0.03510	<0.05	nCt	number of total tertiary C (sp^3)
1.80710	0.06690			intercept

[a] *b* is the coefficient of the corresponding descriptor; se is the standard error of *b*; pval is the p-value for testing whether *b* is 0. R represents any group linked through carbon; X represents halogen atom; Y represents any electronegative atom (O, N, S, P, Se, halogens); Ar represents aromatic groups.

of the compounds in the data set is thus predicted within the experimental error (as estimated before), and only 15% has an error greater than an order of magnitude. The plot of the residuals of the model (Figure 1c) shows a rather symmetric distribution, meaning that the model was equally successful in modeling the compounds exhibiting high and low toxicity.

DISCUSSION

In this paper we report a predictive *in silico* study of more than 400 compounds based on two-dimensional chemical descriptors and multivariate analysis. Its general prediction performance was thoroughly evaluated. On a leave-one-out cross-validation the root mean squared error of the model was 0.73 (in a logarithmic scale). To be interpreted, this error was compared to the estimated variability of experimental values. Indeed *in silico* predictive models cannot perform better than the data used for training. The variability of experimental data was estimated to be 0.64, a value similar to the error of the model. The percentages of compounds within a given predictive error were also applied to assess the performance of the model. The majority of the compounds in the data set were predicted within the experimental error, and only 15% had an error greater than 1 order of magnitude. Altogether, these data indicate that the performance of the present model is likely to be sufficient to provide meaningful and useful information. Since the plot of the residuals of the model presented a rather symmetric distribution, this conclusion applies to compounds exhibiting high and low toxicity.

Often, the assessment of model reliability places greater emphasis on the accuracy of the predictions with respect to many different chemicals than on the reproducibility of *in silico* models within and between laboratories. The fact that the present linear model uses only two-dimensional descriptors ensures its reproducibility, transfer, and reliability.

In general the analysis of the nature of the key descriptors of QSAR models provides some insight about their plausibility and understanding. In the present model, eight (RBN, nN, nArCOOH, nOHp, O-058, Hy, MLOGP, $MLOGP^2$) out of the 20 features selected were strongly correlated with bioavailability. These descriptors constitute the general baseline model.[16,17] More than 83% of the LOAELs were actually underestimated by this baseline model, which accounted for roughly 50% of the variability of the whole QSAR model. Thus, this baseline model reflects the minimum exert toxicity, that is intuitively dependent on the availability of the compound at the target organ.

The finding of a close relationship between specific descriptors, bioavailability and toxicity, is in agreement with previous data correlating structure, bioavailability and bioactivity. For example, according to Lipinski's Rule[18] (which determines if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans), a good intestinal absorption or permeation is more likely when the orally administered compound has not more than 5 hydrogen bond donors (OH and NH groups), not more than 10 hydrogen bond acceptors (notably N and O), a molecular weight lower than 500 Da, and a partition coefficient log *P* less than 5 (low lipophilicity). These rules have spawned many extensions, and it has been shown in several publications[19−22] that reduced molecular flexibility (as measured by the number of rotatable bonds) and total hydrogen bond counts (sum of H-bond donors and acceptors) or low polar surface area (PSA) may be important predictors of significant oral bioavailability, independent of molecular weight. Lipophilicity (via the octanol/water partition) shows generally a good correlation with the membrane permeation rate. It is therefore not surprising that as observed in the present analysis, an increase in RBN (number of rotatable bonds), hydrogen bond donors and acceptors (nN, nArCOOH, nOHp, O-058), and in the hydrophilicity (Hy)

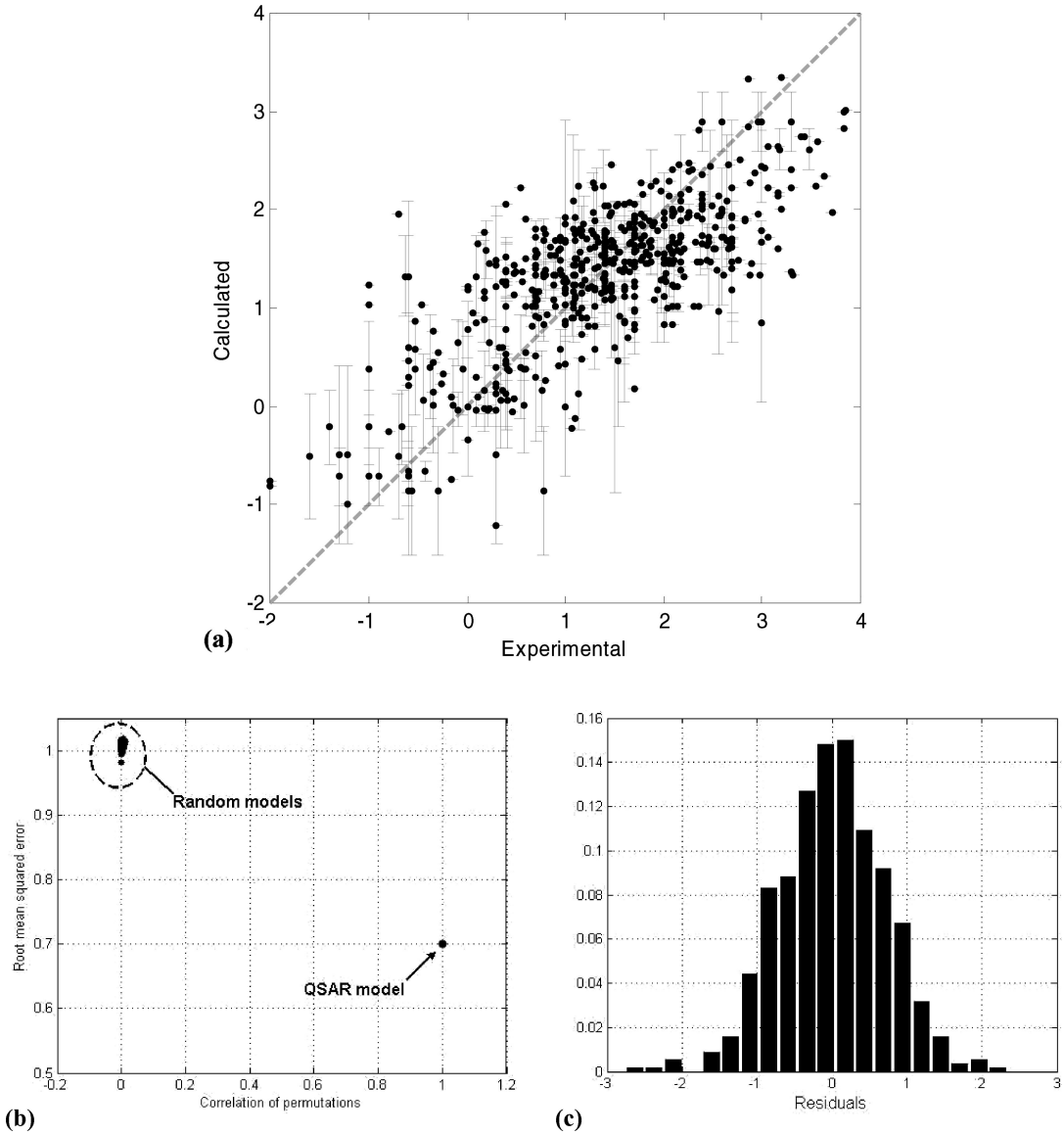

Figure 1. Statistical plots of the final QSAR model: (a) plot of the calculated versus experimental values in logarithmic scale of the chronic toxicity of compounds [Length of the whiskers are equal to 2 σ_e of experimental values of the corresonding compound.]; (b) y-randomization test; and (c) distribution plot of the prediction error/residuals.

correspond to a decrease in the chronic toxicity, though not linearly. The relationship between mucous membrane permeability, which serves as the body′s primary barrier to absorption of chemicals, and lipohilicity (MLogP) can be seen from the literature.[22−26]

LOAEL values reflect a plethora of different toxic effects and even more diverse mechanisms of action spanning cardiovascular, gastrointestinal, renal, hepatic, neurological, reproduction effects, and much more. As such, simple linear models based on a few structural descriptors will hardly capture the whole complexity of these end points. Nevertheless, some of the selected descriptors encode specific substructures (e.g., halocarbons, sulfides, sulfoxides, phosphates, heavy metals, etc.), as shown in Table 2. Interestingly, most of these substructures are well documented to determine chemical properties related to toxicity and metabolism.

For example the general nHM (number of heavy metals) and P-117 descriptors detect respectively organometal compounds and organophosphorus. Organometals[27,28] and organophosphorus compounds[29] are widely recognized to raise

Table 2. Example Moieties Responsible for Extra Toxicity[a]

nSO	nCXr=	nCconjX
nRSR	S-107	P-117

[a] X: halogen; Y = O or S.

toxicological concern. Halogen rich compounds are among the most toxic compounds in the data set, and their toxicity is underestimated by the general baseline as applied alone. The presence of halogen substituents is a well-known general alert of toxicity. In addition halogen-substituted carbon atoms

are not readily metabolized, and a halogen substituent may block metabolism at that carbon atom or at adjacent atoms. The absence of metabolism, combined with increased lipid solubility of the molecule, can give rise to accumulation.[30]

Furthermore, atom-centered fragments, such as S-107 and P-117, or functional groups, such as RSR and SO, contribute significantly to the molar refractivity (MR) of the compound. MR is among the oldest and most successful descriptors for QSAR studies and has been successfully used to model several kinds of toxic actions, and, like many other descriptors, when experimental values are not available, it can be approximated from group-additive constants. MR is strongly correlated with molecular polarizability and ligand binding and is regarded as a measure of nonlipophilic interactions.[31,32]

It has to be noted that the previous 17 descriptors explain more than 90% of the model and that the remaining descriptors are of much less evident explanation. Though they are all statistically relevant (p-value <0.05), they are neither clearly related to bioavailability of the compound nor encode specific moieties that might be involved in a putative mechanism of actions. Their presence in the predictive model is thus probably useful in correcting and lowering the predicting error of some specific cases. The number of aromatic ethers (nArOR) corrects the toxicity prediction of a rather limited number of medium-low toxic compounds, while H-052 and H-054 affect many more compounds in the data set. These descriptors, in practice, count hydrogen atoms attached to strongly electronegative groups and therefore might encode potential hydrogen bond donors and as such being indirectly related to bioavailabilty. nCt is defined as the number of total tertiary C (sp^3) but in this data set identifies a special class of chemicals with a high number of electronegative atoms (Cl, O). In particular, this descriptor corrects the prediction of a group of chlorinated polycondensed rings that are highly toxic (e.g., chlordane, aldrin, heptox).

In the field of computational toxicology, the issue of the applicability domain of the developed models is a critical question. The study of the chemical space of the data set mined and the outliers allowed us to draw some conclusions on the applicability domain of the model. The following considerations derive from the descriptors involved in the baseline model that is considered the backbone of the QSAR model. Furthermore the baseline model is based on features describing the whole molecule, rather than the presence/absence of fragments that describe a chemical space too sparse for being informative. The diversity of the chemical in the data set both in terms of Hotelling's T-square[33,34] and probability density estimation[35,36] methods is not directly correlated with a predictive power. For example, the most diverse chemical in the data set, β-cyclodextrin (7585-39-9), a cyclic oligosaccharide with low toxicity that dramatically differs because of its high number of primary alcohols and elevated hydophilicity, is fairly well predicted (error $= -0.1946$). On the other hand, the worst predicted compound (error $= -2.6278$) is zeranol (55331-29-8), a nonsteroidal estrogenic growth stimulant that does not appear particularly far from the chemical space of the data set. Residuals do not clearly correlate to any descriptors in the data set, nor are they chemically class dependent. This suggests that the predictive ability of the model is not constrained by specific characteristics of the chemical compounds but is general enough to cover a wide range of different chemicals, e.g. pesticides, drugs, natural products, etc.

The main limitations of this model have then to be searched in the quality and the scarceness of experimental data rather than in the chemical space covered. Predictions with an average error much better than 0.64 (in logarithmic scale) cannot be expected because of the high intrinsic experimental variability. The question remained, however, as to whether a better performance of in silico models could be expected, using e.g. multidimensional nonlinear (Q)SAR methods and/or 3D-descriptors. The answer is most likely positive but with a dramatic impact on the interpretability and transferability of the model itself. Another limitation of this approach is clearly the incapability of including consecutive metabolic activation steps or taking into account three-dimensional chemistry, e.g. isomers and active conformations.

CONCLUSIONS

A QSAR model has been developed for chronic toxicity. From the analysis of the descriptors it appears that chronic toxicity is determined by a basal effect depending on bioavailability plus excess toxicity due to specific moieties i.e.:

$$\text{ChronicTox} = f(\text{bioavailability}) + \text{ExcessTox} + \varepsilon \quad (2)$$

This two-dimensional QSAR model for chronic toxicity could predict LOAEL values with an error as low as 0.70. Since this error approaches the postulated experimental error (0.64) due to the variation in the protocol used, the ability of the model to reasonably predict chronic toxicity is confirmed. Considering the size of the data set, the chemical diversity herein, and the complexity of the end point involved, these results give confidence that this model is reliable and relevant and may be used together with exposure estimates to establish a level of safety concern of chemicals in food for which hard toxicological data are missing.

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Supporting Information Available: Constructed data set of molecular structures (encoded as canonical SMILES strings) with LOAEL values. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

(1) Kroes, R.; Galli, C.; Munro, I.; Schilter, B.; Tran, L.-A.; Walker, R.; Würtzen, G. Threshold of toxicological concern for chemical substances present in the diet: A practical tool for assessing the need for toxicity testing. *Food Chem. Toxicol.* **2000**, *38*, 255–312.

(2) Cronin, M. T. D.; Walker, J. D.; Jaworska, J. S.; Comber, M. H. I.; Watts, C. D.; Worth, A. P. *Use of QSARs in International Decision-Making Frameworks to Predict Ecologic Effects and Environmental Fate of Chemical Substances Environmental Health Perspectives*, 2003; Vol. 111.

(3) Walker, J. D.; Carlsen, L.; Hulzebos, E.; Simon-Hettich, B. Global government applications of analogues, SARs and QSARs to predict aquatic toxicity, chemical or physical properties, environmental fate parameters and health effects of organic chemicals. *SAR QSAR Environ. Res.* **2002**, *13*, 607–616.

(4) *Quantitative Structure-Activity Relationships (QSAR) for Pesticide Regulatory Purposes*, 1st ed.; Elsevier Science: May 7, 2007; 532 pp.
(5) Munro, I. C.; Ford, R. A.; Kennepohl, E.; Sprenger, J. G. Correlation of Structural Class with No-Observed-Effect Levels: A Proposal for Establishing a Threshold of Concern. *Food Chem. Toxicol.* **1996**, *34*, 829–867.
(6) Mumtaz, M. M.; Knauf, L. A.; Reisman, D. J.; Peirano, W. B.; DeRosa, C. T.; Gombar, V. K.; Enslein, K.; Carter, J. R.; Blake, B. W.; Huque, K. I.; Ramanujam, V. M. S. Assessment of effect levels of chemicals from quantitative structure-activity relationship (QSAR) models. I. Chronic lowest-observed-adverse-effect level (LOAEL). *Toxicol. Lett.* **1995**, *79*, 131–143.
(7) Venkatapathy, R.; Moudgal, C. J.; Bruce, R. M. Assessment of the Otal Rat Chronic Lowest Observed Adverse Effect Level Model in TOPKAT, a QSAR Software Package for Toxicity Prediction. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1623–1629.
(8) Final Report to the U.S. Environmental Protection Agency; Cincinnati, OH, in fulfillment of Contract No. 68-C4-0006; HDi Inc.: Rochester, NY, 1995.
(9) Ensle, K. In *Advances in Molecular Toxicology*; Reiss, C., Labbe, G., Parvez H., Eds.; VSP: Utrecht, The Netherlands, 1998; pp 141−164.
(10) Tilaoui, L.; Schilter, B.; Tran, L.-A.; Mazzatorta, P.; Grigorov, M. Intergrated Computational Methods for Prediction of Lo west Observable Adverse Effect Level of Food-Borne Molecules. *QSAR Comb. Sci.* **2007**, *26*, 102–108.
(11) U.S. Environmental Protection Agency. *ECOTOX User Guide: ECOTOXicology Database System. Version 4.0*; 2006. www.epa.gov/ecotox/(accessed Dec 12, 2006).
(12) Todeschini, R.; Consonni, V. *Handbook of Molecular Descriptors*; Mannhold, R., Kubinyi, H., Timmerman, H., Eds.; VCH: 2000.
(13) *DRAGON for Windows (Software for Molecular Descriptor Calculations)*, *version 5.4−2006*; Talete srl.
(14) Leardi, R.; Lupiáñez, A. Genetic algorithms applied to feature selection in PLS regression: how and when to use them. *Chemom. Intell. Lab. Syst.* **1998**, *41*, 195–207.
(15) Van de Voet, H. Comparing the predictive accuracy of models using a simple randomization test. *Chemom. Intell. Lab. Syst.* **1994**, *25*, 313–323.
(16) Lipnick, R. L. Base-line toxicity predicted by quatitative structure-activity relationships as a probe for molecular mechanism of toxicity. In *Probing Bioactive Mechanisms*; Magee, P. S., Henry, D. R., Block, J. H., Eds.; American Chemical Society: Washington, DC, 1989; Vol. 413, pp 366−389.
(17) Öberg, T. A QSAR for Baseline Toxicity: Validation, Domain of Application, and Prediction. *Chem. Res. Toxicol.* **2004**, *17*, 1630–1637.
(18) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **1997**, *23*, 3–25.
(19) Martin, Y. C. A bioavailability score. *J. Med. Chem.* **2005**, *48*, 3164–3170.
(20) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **2002**, *45*, 2615–2623.
(21) Yoshida, F.; Topliss, J. G. QSAR model for drug human oral bioavailability. *J. Med. Chem.* **2000**, *43*, 2575–2585.
(22) Lu, J. J.; Crimin, K.; Goodwin, J. T.; Crivori, P.; Orrenius, C.; Xing, L.; Tandler, P. J.; Vidmar, T. J.; Amore, B. M.; Wilson, A. G. E.; Stouten, P. F. W.; Burton, P. S. Influence of molecular flexibility and polar surface area metrics on oral bioavailability in rat. *J. Med. Chem.* **2004**, *47*, 6104–6107.
(23) Kitagawa, S.; Li, H.; Sato, S. Skin permeation of parabens in excised guinea pig dorsal skin, its modification by penetration enhancers and their relationship with n-octanol/water partition coefficients. *Chem. Pharm. Bull* **1997**, *45*, 1354–57.
(24) Potts, R. O.; Guy, R. H. Predicting skin permeability. *Pharm. Res.* **1992**, *9*, 663–69.
(25) Siegel, I. A. Effect of chemical structure on nonelectrolyte penetration of oral mucosa. *J. Invest. Dermatol.* **1981**, *76*, 137–40.
(26) Siegel, I. A. Permeability of the rat oral mucosa to organic solutes measured in vivo. *Arch. Oral. Biol.* **1984**, *29*, 13–16.
(27) Dopp, E.; Hartmann, L. M.; Florea, A. M.; Rettenmeier, A. W.; Hirner, A. V. *Environmental distribution, analysis, and toxicity of organometal(loid) compounds. Crit. Rev. Toxicol.* **2004**, *34*, 301–333.
(28) Florea, A.-M.; Büsselberg, D. Occurrence, use and potential toxic effects of metals and metal compounds. *BioMetals* **2006**, *19*, 419–427.
(29) Costa, L. G. Current issues in organophosphate toxicology. *Clin. Chim. Acta* **2006**, *366*, 1–13.
(30) Kroes, R.; Renwick, A. G.; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma, A.; Schilter, B.; Schlatter, J.; van Schothorst, F. G.; Vos, J.; Würtzen, G. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem. Toxicol.* **2004**, *42*, 65–83.
(31) Hansch, C.; Steinmetz, W. E.; Leo, A. J.; Mekapati, S. B.; Kurup, A.; Hoekman, D. On the Role of Polarizability in Chemical-Biological Interactions. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 120–125.
(32) Verma, R. P.; Hansch, C. A. comparison between two polarizability parameters in chemical-biological interactions. *Bioorg. Med. Chem.* **2005**, *13*, 2355–2372.
(33) Hotelling, H. The generalization of Student′s ratio. *Ann. Math. Statist.* **1931**, *2*, 360–378.
(34) Eriksson, L.; Jaworska, J.; Worth, A.; Cronin, M. T. D.; McDowell, R. M.; Gramatica, P. Methods for reliability and uncertainty assessment and for applicability evaluations of classification and regression-based QSARs. *Environ. Health Perspect.* **2003**, *111*, 1351–1375.
(35) Silverman, B. W. *Density Estimation for Statistics and Data Analysis*; CRC Press: Boca Raton, FL, U.S.A., 1986; 176 pp.
(36) Jaworska, J.; Nikolova-Jeliazkova, N.; Aldenberg, T. QSAR Applicability Domain Estimation by Projection of the Training Set in Descriptor Space: A Review. *ATLA* **2005**, *33*, 445–459.

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