

Models for estimating the non-specific toxicity of organic compounds in short-term bioassays

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The solvation parameter model is used to construct equations for the estimation of the non-specific toxicity of neutral organic compounds to five organisms used for short-term toxicity testing. For the bacteria *Vibrio fischeri* (MicrotoxTM test) and *Pseudomonas putida*, the protozoan *Tetrahymena pyriformis* (Tetratox test), the green alga *Scenedesmus quadricauda* and the brine shrimp *Artemia salina*, the main factors resulting in increased non-specific toxicity are size (dominantly) and lone-pair electron interactions, with hydrogen-bond basicity the most important solute property reducing toxicity. Species differences in relative non-specific toxicity are largely related to differences in cohesion and hydrogen-bond acidity of the biomembranes. The models for non-specific toxicity are proposed as an alternative to the octanol–water distribution constant for the determination of baseline toxicity. Failure of the octanol–water distribution constant to model non-specific toxicity is quantitatively explained by its inability to adequately characterize the sorption properties of the biomembranes for compounds with varied properties.

Introduction

The pervasive use of synthetic and natural organic chemicals with different potentials for toxic or adverse effects on humans and other higher order animals has created the need for fast and affordable methods of toxicity assessment.^{1–3} These tests usually fall into two groups. Short-term tests, using bacteria or simple protozoa, for example, which are simple to perform, economical and yield results quickly.^{4,5} Long-term tests, involving fish or animals, that are orders of magnitude more expensive and time consuming, but may be desirable to provide a more cogent toxicity estimate for risk assessment.^{6,7} It is common practice to use short-term tests for initial evaluation and screening purposes and long-term tests for confirmatory purposes and when regulatory requirements dictate their use.

The molecular basis of toxicity is complex and poorly understood. For our present purposes, it is useful to divide organic compounds into two categories recognized as non-specific toxic (or narcosis) and specific (or reactive) toxic. Non-specific toxicity occurs by the general disruption of cell membrane functions. It results from the bioconcentration of organic compounds in cellular membranes where interference in essential biochemical processes occurs and becomes chronic above a critical minimum (toxic) concentration of the compound. Thus, non-specific toxicity should be related to the distribution properties of the biocompartment associated with the toxic action and is expected to be independent of other factors. Therefore, it should be possible to model non-specific toxicity as a general property of all compounds that can be modeled by a distribution constant. Another name for non-specific toxicity is baseline toxicity. Specific toxicity is measured by the excess toxicity from the calculated baseline value and results from specific chemical reactions at the toxic site. Some examples of specific toxicity include compounds acting as oxidative phosphorylation uncouplers, respiratory inhibitors, electrophiles, acetylcholinesterase inhibitors, etc. Specific toxicity cannot be deduced from chemical structure alone, which can only indicate the possibility that a compound could function in a certain manner. Whether it does or does not depends on the site of toxic action being structurally equipped

for the chemical reaction, a property that is possibly not universal across different organisms.

Wet octanol is often chosen as a chemical model for cell membrane sorption properties, and the octanol–water distribution constant as a molecular descriptor to estimate the non-specific toxicity of organic compounds.^{8,9} On this basis, compounds exhibiting non-specific toxicity can be further divided into class I (non-polar narcotics) and class II (polar narcotics) based on their separate and different correlations with the octanol–water distribution constant. It has been argued by others^{10,11} and ourselves¹² that the difference in mode of action for non-polar and polar narcotics is artificial and results from the choice of wet octanol as a model for membrane sorption. In other words, it reflects the failure of wet octanol to model adequately the polar interactions associated with the distribution of the class II polar narcotics into the biological membrane. These differences manifest themselves as family-dependent correlations between experimental toxicity and the octanol–water distribution constant, resulting in different slopes and intercepts for family groups (e.g., different homologous series varying only by size).

In this paper, we use the solvation parameter model to construct models for estimating the non-specific toxicity of neutral organic compounds to the photobacterium *Vibrio fischeri*, the bacterium *Pseudomonas putida*, the protozoan *Tetrahymena pyriformis*, the green alga *Scenedesmus quadricauda* and the brine shrimp *Artemia salina*. *Vibrio fischeri* is used in the widely applied MicrotoxTM test^{4,6,13} and *Tetrahymena pyriformis* in the Tetratox test.^{14,15} This general approach provides an interpretation of species-dependent, non-specific toxicity in terms of the characteristic membrane sorption properties, and quantitatively accounts for the inadequacies of the octanol–water distribution constant as a molecular descriptor for polar narcotic compounds. In earlier studies, the solvation parameter model was used to characterize a large number of varied biopartitioning systems,¹⁶ including the blood–brain barrier,¹⁷ skin permeation,¹⁸ soil–water distribution,¹⁹ tadpole narcosis²⁰ and the non-specific aquatic toxicity of neutral organic compounds to several species of fish and the water flea.¹²

Methodology

The solvation parameter model is based on a cavity model of solvation. Transfer of a solute from the donor to the acceptor phase requires creation of a cavity in the acceptor phase for the solute, an energetically unfavorable process that is opposed by solvent–solvent interactions. For transfer to occur, these unfavorable interactions must be compensated by energetically favorable solute–solvent interactions. Simultaneously, the reverse process occurs in the donor phase. The change in free energy for this process can be parameterized assuming a linear free energy model set out below in a form suitable for modeling the aquatic toxicity of neutral organic compounds^{16,21–23}

$$\log \text{SP} = c + mV_X + rR_2 + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^0 \quad (1)$$

The model equation is made up of product terms representing solute properties (descriptors) and system properties characteristic of the biopartitioning mechanism. Each product term represents the contribution of a defined intermolecular interaction to the correlated solute property (SP), in this case the negative logarithm of the concentration (mM) of organic compound causing 50% diminution of the bioluminescence of *Vibrio fischeri* in a fixed time (5 min, pT₅; 15 min, pT₁₅; and 30 min, pT₃₀), the negative logarithm of the concentration (mM) of organic compound causing 50% inhibition of cell growth in a fixed time (IGC₅₀) for *Pseudomonas putida* (16 h), *Tetrahymena pyriformis* (40 h) and *Scenedesmus quadricauda* (24 h), or the negative logarithm of the concentration (mM) for 50% kill in a fixed time (–log LC₅₀) for *Artemia salina* (24 h).

The solute descriptors are McGowan's characteristic volume V_X, excess molar refraction R₂, the solute's dipolarity/polarizability π_2^H , the solute's effective hydrogen-bond acidity $\Sigma\alpha_2^H$ and the solute's effective hydrogen-bond basicity $\Sigma\beta_2^0$. Solute descriptors are known for more than 4000 compounds²⁴ with others available through parameter estimates and by computational approaches.^{21–23} In other cases, the solute descriptors are obtained by simple arithmetic calculation (V_X and R₂) or measured experimentally in chromatographic or liquid–liquid distribution systems using standard methods.^{21–26}

The system constants in eqn. (1) are defined by their complementary interactions with the solute descriptors. They represent the difference in capacity of the two components of the system (water and the biomembrane) to interact in a specific manner: with solute n- or π -electrons (*r* constant); to take part in dipole–dipole and dipole–induced dipole interactions (*s* constant); differences in their relative hydrogen-bond basicity (*a* constant) and hydrogen-bond acidity (*b* constant); and the relative ease of forming a cavity in the two phases together with differences in dispersion interactions (*m* constant). The system constants are obtained by multiple linear regression analysis of experimental toxicity values for solutes with known descriptors. The only requirements are that the number of solutes and their range of requirement values must be sufficient to establish the statistical validity of the model and the absence of significant cross-correlation among the selected descriptors.²⁷

The values of pT₅, pT₁₅ and pT₃₀ for *Vibrio fischeri*,¹³ IGC₅₀ for *Pseudomonas putida*,²⁸ *Tetrahymena pyriformis*¹⁴ and *Scenedesmus quadricauda*²⁸ and –log LC₅₀ for *Artemia salina*^{1,29,30} were taken from the sources indicated. The solute descriptors are from an in-house database with most values taken from the literature.^{12,19,20,23} For the convenience of the reader all toxicity data and solute descriptors used to construct the models are supplied as Electronic Supplementary Information.† Multiple linear regression analysis was performed on a Gateway E-4200 computer (North Sioux City, SD, USA) using the program SPSS/PC + v.8.0 (SPSS, Chicago, IL, USA). Cross-correlation analysis of the solute descriptors was per-

formed for all final models presented in the text and unless otherwise stated was < 0.80.

Results and discussion

Kaiser and Palabrica¹³ have compiled a multi-author database of toxicity values to *Vibrio fischeri*, which represents one of the largest sources of toxicity data for any single organism. To use this database for the modeling of non-specific toxicity, we proceeded as follows. Firstly, all inorganic compounds and salts were eliminated from consideration. Next, all compounds we did not have experimental solute descriptors for were eliminated to avoid introducing further uncertainties due to the use of estimation methods. This left 232 varied organic compounds. From this list, we eliminated all compounds that might be significantly dissociated at the pH used for measurements, indicated by Kaiser and Palabrica¹³ at between pH 5 and pH 9 in individual studies. Next, we removed compounds characterized as exhibiting specific toxicity based on a lack of fit in quantitative structure–activity relationship (QSAR) studies for *Vibrio fischeri*^{6,31–39} and fish toxicity mechanisms.^{7,12} An initial model was formed and then further optimized by removing all compounds with experimental toxicity values that were different from the model estimated values by more than twice the standard error for the model estimate. Finally, each compound eliminated for any reason was added back (one at a time) to the initial models and retained if the experimental toxicity differed from the model estimated toxicity by less than twice the standard error in the estimate for the combined model. The final models for PT₅, PT₁₅ and PT₃₀ are summarized in Table 1.

We first consider the compounds excluded from the general models for non-specific toxicity to *Vibrio fischeri*, summarized in Table 2. For convenience, these compounds are grouped into four categories. Group 1 contains compounds normally considered to exhibit reactive toxicity and possess excess toxicity orders of magnitude greater than their estimated baseline toxicity. These compounds probably behave in a similar manner in *Vibrio fischeri* as in other organisms. Group 2 contains compounds with low water solubility. The latter are estimated from the solute descriptors using the model of Abraham and Le⁴⁰ when experimental values were unavailable. For these compounds, the experimental toxicity is indicated as a significantly higher concentration than the model predicted toxicity. This we believe is the result of using inhomogeneous solutions for the toxicity measurements or additional solvents to solubilize the test compounds.⁴¹ Group 3 contains compounds that could be partially ionized under the conditions used for the toxicity measurements. The model equation predicts the non-specific toxicity of the compounds in their neutral form. Extension of the solvation parameter model to partially ionized weak acids is possible, but this requires an exact knowledge of the solution pH, which is unavailable.^{42,43} In the absence of facilitated transport, the accumulation of partially ionized solutes in the biomembrane should be less than the model predicted value for the neutral compound. This is possibly the reason for the lower than predicted toxicity for the carboxylic acids in Table 2. For most of the phenols excluded from the models, excess toxicity is observed. It is possible that the phenolate ion is more toxic than the parent phenol, but given the large number of phenols with a similar structure that are included in the models (see Electronic Supplementary Information), this seems unlikely. Anilines are well behaved with respect to model predictions and provide no clues to the observed excess toxicity of 4-ethylaniline. The alkylamines and dialkylamines are not a good fit to the models, but are too few in number to draw any global conclusions. The group 4 compounds are not structurally related and contain some

† Available as Electronic Supplementary Information. See <http://www.rsc.org/suppdata/an/a9/a907235g>.

compounds such as aldehydes that might exhibit reactive toxicity. This group probably consists of poor experimental values and compounds with weak specific toxicity or metabolism that is not easily judged from structure alone. The database of Kaiser and Palabrica¹³ contains data from more than 50 sources obtained by over 20 research groups, and the likelihood of a few poor experimental values seems high. In addition, a few of the compounds may have poorly determined solute descriptors contributing to errors in the model predicted values.

The models for non-specific toxicity in *Vibrio fischeri* (Table 1) are chemically sensible, statistically sound and provide an estimate of non-specific toxicity to about ± 0.5 pT. If the uncertainty indicated for multiple determinations of a single compound in the database (some examples given in Table 3) is taken as a reasonable estimate of the uncertainty for all values in the database (most are single entries), then greater predictive accuracy for the models is not expected. The extreme toxicity values indicated by the range in Table 3 are an example of how erroneous values could exist for single value compounds in the database and why over-interpretation of those compounds removed from the models (Table 2) is unjustified. Where multiple entries exist in the database, the toxicity values indicated as preferred values by Kaiser and Palabrica¹³ were used without dissent. In addition, the models for non-specific toxicity in *Vibrio fischeri* agree favorably with a preliminary study of the non-specific toxicity of a group of varied compounds from a single laboratory (none of these results are included in the data analyzed in Table 1) [pT₁₅, $m = 3.43$ (± 0.28), $r = 0.86$ (± 0.17), $s = a = 0$, $b = -1.35$ (± 0.26) with $\rho = 0.952$, SE = 0.316, $F = 142$, $n = 48$].¹² The larger number of compounds in the Electronic Supplementary Information supports the hypothesis that the solvation parameter model can be used to estimate the non-specific or baseline toxicity of neutral organic compounds to *Vibrio fischeri*.

From the system constants in Table 1, the factors that contribute to increased non-specific toxicity to *Vibrio fischeri* are the solute size (most important) and lone-pair electron interactions (m and r system constants are positive). Compounds that are strong hydrogen-bond bases are less toxic than other compounds of a similar size (b system constant is negative). The dipolarity/polarizability and hydrogen-bond acidity of a compound are unimportant (s and a system constants are 0). The biomembrane is not particularly cohesive, being somewhat similar in this respect to wet octanol, but is quite polar, being as dipolar/polarizable as water with similar hydrogen-bond basicity. It has significantly weaker hydrogen-bond acidity compared to water. Cronin and Schultz³⁶ state that equilibrium is achieved within 5 min in the Microtox test, while

Backhaus *et al.*⁴⁴ indicate that, for compounds with non-specific toxicity, similar results were obtained for 30 min and 24 h assays, with significant differences only observed for compounds with reactive toxicity mechanisms. The data in Table 1 suggest that small but significant differences exist in the non-specific toxicity determined in the 5 min and 30 min assays. With increasing time, the m system constant becomes smaller and the b system constant larger (more positive) with a good fit to a linear or logarithmic function of time (three time values only). Small compounds of high hydrogen-bond basicity will demonstrate higher toxicity in the 30 min assay compared to the 5 min assay. For compounds acting by a non-specific toxicity mechanism, it seems likely that an equilibrium state is not reached in less than 30 min. Additional data at times greater than 30 min would be required to demonstrate that equilibrium had been reached.

A smaller database for the inhibition of cell growth in the bacterium *Pseudomonas putida*²⁸ was analyzed in the same manner as described for *Vibrio fischeri*. We had solute descriptors for 81 varied compounds and, after removal of six compounds, obtained the model given in Table 1. The model is suitable for estimating non-specific toxicity to *Pseudomonas putida* to about ± 0.3 IGC₅₀. The compounds removed were butanal (excess toxicity 0.83), vinyl acetate (excess toxicity 2.00), styrene (excess toxicity 0.97) and morpholine (excess toxicity 0.97), suspected of acting by a reactive toxicity mechanism; benzoic acid (underestimate of toxicity -1.05), suspected of being partially ionized; and 2-nitrophenol (excess toxicity 1.32), as a statistical outlier. The reported toxicity of 0.9 mg L⁻¹ for 2-nitrophenol may be a typographical error. A value of 9 mg L⁻¹ would fit the model and be of a similar magnitude to the values reported for 3-nitrophenol and 4-nitrophenol. Of interest is that hydroquinone and allylamine, considered reactive toxicants in *Vibrio fischeri*, are fitted by the general model for non-specific toxicity in *Pseudomonas putida*, perhaps indicating species differences in reactive toxicity. The general trends in non-specific toxicity in *Pseudomonas putida* are similar to those observed in *Vibrio fischeri*, with the bio-compartment of *Pseudomonas putida* being slightly more cohesive and less hydrogen-bond acidic than that of *Vibrio fischeri*. Quantitative differences in relative toxicity between the two species are expected for compounds possessing a range of hydrogen-bond basicity.

Schultz¹⁴ has provided a large database for the inhibition of cell growth (IGC₅₀) in the protozoan *Tetrahymena pyriformis* Tetratox test, for which we had solute descriptors for 118 compounds. These compounds represent three classes of toxic action as indicated by Schultz.¹⁴ After removing 19 compounds,

Table 1 Models for the non-specific toxicity of organic compounds to different species

Species		System constants ^a						Statistics ^b			
		m	r	s	a	b	c	ρ	SE	F	n
<i>Vibrio fischeri</i>	pT ₅	3.73 (0.17)	1.08 (0.10)	0	0	-1.79 (0.16)	-3.05 (0.16)	0.923	0.493	374	198
	pT ₁₅	3.57 (0.19)	1.01 (0.12)	0	0	-1.51 (0.18)	-2.92 (0.18)	0.918	0.468	289	166
	pT ₃₀	3.43 (0.18)	1.10 (0.11)	0	0	-1.29 (0.16)	-2.98 (0.17)	0.916	0.479	325	192
<i>Pseudomonas putida</i>	IGC ₅₀	3.00 (0.17)	1.08 (0.11)	0	0	-2.01 (0.16)	-2.33 (0.16)	0.961	0.283	285	75
<i>Tetrahymena pyriformis</i>	IGC ₅₀	2.88 (0.08)	0.64 (0.06)	0	0	-2.51 (0.14)	-2.48 (0.11)	0.978	0.220	690	98
<i>Scenedesmus quadricauda</i>	IGC ₅₀	3.25 (0.25)	1.86 (0.20)	0	0	-1.05 (0.40)	-3.36 (0.21)	0.949	0.413	139	50
<i>Artemia salina</i>	LC ₅₀	3.41 (0.14)	0.13 (0.09)	0	0	-4.46 (0.12)	-2.02 (0.11)	0.988	0.233	564	44

^a Numbers in parentheses are the standard errors. ^b ρ is the multiple linear correlation coefficient, SE is the standard error in the model estimate, F is the Fischer F statistic and n is the number of compounds.

we obtained the model summarized in Table 1, which is suitable for estimating non-specific toxicity to about ± 0.22 IGC₅₀. Of the compounds removed, eight are homologous alkylamines and four are alkanals representing all the compounds of that type from the database used for modeling. Compounds identified as exhibiting excess toxicity by the solvation parameter model (Table 4) are in broad agreement with the conclusions of Schultz and co-workers using QSAR models for family groups.^{45–50} These authors used the octanol–water

Table 2 Compounds whose experimental toxicities to *Vibrio fischeri* are not adequately predicted by the solvation parameter models in Table 1

Compound	Difference in experimental and model predicted toxicity		
	pT ₅	pT ₁₅	pT ₃₀
(1) Compounds capable of reactive toxicity			
Hydroquinone	3.14	3.34	3.21
Formanilide	3.43	3.21	2.95
Formaldehyde	3.00	2.79	2.78
Morpholine	1.73	1.54	1.34
1,4-Dinitrobenzene	1.27	1.80	1.85
Allylamine	1.93	1.89	
Acrolein	3.55		
(2) Compounds of limited water solubility			
Acridine	−2.41	−2.11	−2.39
Carbazole	−2.27	−1.93	−2.02
2-Aminobiphenyl	−1.42	−1.44	−1.54
4-Aminobiphenyl	−1.33	−1.32	−1.50
Diphenylmethane	−1.18	−1.23	−1.31
Octane	−2.45		−2.06
1-Chlorohexane	−1.23		
1-Chlorooctane	−2.33		−2.10
Bornyl acetate	−2.69	−2.22	−2.14
Diethyl phthalate	−2.00	−2.28	−2.23
1,2,4,5-Tetrachlorobenzene	−1.39	−1.03	
Phenyl ether			−1.37
(3) Compounds containing an ionizable group			
Phenylacetic acid	−1.12	−1.21	−1.32
Salicylic acid	−1.03		
4-Iodobenzoic acid	−1.88	−2.69	−1.71
4-Ethylphenol	2.30	2.33	2.32
4-Bromophenol	1.32		1.36
4-Hydroxybenzonitrile	1.33	1.26	1.31
4-Chloro-3-methylphenol	1.32	1.35	1.33
3,4-Dimethylphenol	1.26	1.31	1.35
4-Aminophenol	2.05	2.09	1.24
4-Ethylaniline	1.71	1.71	1.65
Methylamine	2.38		
n-Butylamine	−1.34		−1.53
Cyclohexylamine	−1.44		
Dimethylamine		2.21	
Diethylamine		1.39	
(4) Miscellaneous compounds			
Methanol	−1.18	−1.70	−5.06
Ethanol			−1.11
Propan-2-ol	−1.08		
Ethylene glycol	1.09		
N-Phenylacetamide	−1.17	−1.19	−1.26
Dimethyl sulfoxide	−1.15	−1.53	−1.72
Acetaldehyde	1.32		
Butanal	1.82		
tert-Butyl methyl ether	1.64	1.39	1.46
Thiazole	1.64	1.53	1.39
2-Aminothiazole	−1.00		−1.07
Thiophenol		1.13	1.17
1,1,1,2-Tetrachloroethane	1.22		1.28
Pentachloroethane	1.19		1.29
Hexachloroethane			1.55
Cyclohexanone	1.18		
Benzophenone			−1.74
4-Nitrobenzamide			1.22
Benzenesulfonamide	−0.88	−1.07	−1.22

distribution constant as a descriptor for membrane transport and molecular orbital quantum chemical descriptors to account for the reactive potential of alkylamines,⁴⁵ alkanals,^{46,47} nitrobenzenes,^{48,49} and phenols and anilines.⁵⁰ Bearden and Schultz⁵⁰ indicate that chemical transformation during incubation with *Tetrahymena pyriformis* contributes to the observed toxicity of 4-nitroaniline and 4-nitrophenol. We did not find evidence of excess toxicity for esters in *Tetrahymena pyriformis*⁴⁶ using the solvation parameter model to estimate baseline toxicity. The toxicity of benzophenone is overestimated by the model (−0.58) possibly due to conversion to a less toxic compound, or is an experimental artifact. The general model for *Tetrahymena pyriformis* is statistically sound and confirms our previous observation¹² that a single model for class I and II narcotic compounds is possible using the solvation parameter model.

A small database is available for the inhibition of cell growth in the green alga *Scenedesmus quadricauda*.²⁸ This was analyzed in the usual way and provided the model indicated in Table 1. The model is chemically sensible and capable of estimating non-specific toxicity to about ± 0.4 IGC₅₀. We started from 71 solutes with known solute descriptors, but 19 solutes were identified as exhibiting reactive toxicity (Table 5) and two solutes (butan-2-ol and benzoic acid) were removed as statistical outliers. Eleven of these compounds are esters possessing weak reactive toxicity in fish,^{7,12} but not in *Vibrio fischeri* and *Tetrahymena pyriformis* (this study). Also, all four alkylamines (but not anilines) demonstrated specific toxicity. The other compounds removed were butanal, hydroquinone, morpholine and allyl chloride: common reactive toxicants in many test species. The factors which contribute to non-specific toxicity in *Scenedesmus quadricauda* are similar to those for *Vibrio fischeri*, *Pseudomonas putida* and *Tetrahymena pyr-*

Table 3 Selected toxicity values for *Vibrio fischeri* from the database of Kaiser and Palabrica¹³ to illustrate experimental uncertainty

Compound	Test	Statistics			
		Mean	s	n	Range
Ethanol	pT ₅	−2.96	0.10	5	−2.83 to −3.08
Acetone	pT ₅	−2.48	0.10	5	−2.33 to −2.58
	pT ₁₅	−2.49	0.14	5	−2.34 to −2.70
Butan-1-ol	pT ₅	−1.80	0.55	5	−1.44 to −2.77
Benzene	pT ₅	0.34	0.88	6	1.59 to −0.44
Pentachlorophenol	pT ₅	2.62	0.45	6	2.31 to 3.52
	PT ₁₅	2.45	0.20	5	2.17 to 2.64
Phenol	pT ₅	0.53	0.11	13	0.35 to 0.72
2,4-Dichlorophenol	pT ₅	1.74	0.27	5	1.51 to 2.17
3,5-Dichlorophenol	pT ₅	1.58	0.15	6	1.32 to 1.77

Table 4 Compounds exhibiting excess toxicity to *Tetrahymena pyriformis*

Compound	Excess toxicity (IGC ₅₀)	Compound	Excess toxicity (IGC ₅₀)
(1) Alkylamines		(2) Alkanals	
n-Propylamine	1.34	2-Propenal	3.36
n-Butylamine	1.08	n-Butyraldehyde	1.14
sec-Butylamine	1.06	n-Valeraldehyde	0.99
n-Pentylamine	0.77	n-Hexanal	0.54
n-Hexylamine	0.63		
n-Heptylamine	0.65	(3) Miscellaneous	
n-Octylamine	0.66	Aniline	0.66
Benzylamine	0.76	4-Nitroaniline	0.93
		2-Nitrophenol	0.70
		4-Nitrophenol	1.15
		1,4-Dinitrobenzene	1.02
		Catechol	1.52

iformis. The biocompartment in *Scendesmus quadricauda* is more hydrogen-bond acidic than for the other organisms, and strong hydrogen-bond bases are expected to be relatively more toxic to *Scendesmus quadricauda*.

A small database is available for the lethal toxicity of organic compounds to the brine shrimp *Artemia salina*.²⁹ There are an insufficient number of compounds with hydrogen-bond acid properties in this database to evaluate the biomembrane hydrogen-bond basicity. To circumvent this problem, additional toxicity data from Calleja *et al.*³⁰ for methanol, ethanol, propan-2-ol, ethylene glycol, phenol, pentachlorophenol, 1,1,1-trichloroethane, paracetamol, nicotine, theophylline, propranolol, phenobarbital, amphetamine, atropine, aspirin and caffeine, and from Toussaint *et al.*¹ for n-octanol, were added. A few additional values from Sanchez-Fortun *et al.*^{51–53} were tested, but in nearly all cases were systematically larger than both the model predicted values and the experimental values in common with Abernathy and Mackay²⁹ and Calleja *et al.*³⁰ Sanchez-Fortun *et al.*⁵¹ ascribe the poor agreement in the results to differences in bioassay techniques and purity of the solvents. Because of the systematic difference in the data sources, the Sanchez-Fortun *et al.* toxicity data for *Artemia salina* were not used in these studies. We had solute descriptors for 50 compounds in the combined data sets. Using the process described above, six compounds were removed to give the model summarized in Table 1. The model is statistically sound and allows the estimation of non-specific toxicity in *Artemia salina* to about $\pm 0.23 \log \text{LC}_{50}$. Four of the compounds removed show excess toxicity (acetylsalicylic acid 1.65; caffeine 1.91; theophylline 1.97; and paracetamol 0.91), which is not unexpected from assumptions about their general mechanism of toxic action.⁷ Nicotine (−2.63) and 1,1,1-trichloroethane (−1.71) are less toxic than predicted by the model. Nicotine is unstable and rapidly metabolized in many biological systems so that its poor fit can be accounted for, but there is no obvious reason for the lack of fit for 1,1,1-trichloroethane. This was removed from the model on statistical grounds alone. Significant cross-correlation exists between the R_2 and π_2^H solute descriptors ($r = 0.858$), but is not a problem. Using π_2^H and R_2 together results in π_2^H being eliminated as statistically insignificant, and using π_2^H alone, π_2^H is rejected from the new model as statistically insignificant. For the best fit model in Table 1, R_2 is only weakly significant and does not make a large contribution to the model estimate of non-specific toxicity. In the absence of additional data for a more varied group of compounds, the model should provide a reasonable estimate of the non-specific toxicity to *Artemia salina*. The model suggests that the biocompartment is moderately cohesive with very weak hydrogen-bond acidity. Compounds with significant hydrogen-bond basicity should exhibit lower non-specific toxicity in *Artemia salina* than for the other organisms discussed in this paper.

Table 5 Compounds exhibiting excess toxicity in the green alga *Scendesmus quadricauda*

Compound	Excess toxicity (IGC ₅₀)	Compound	Excess toxicity (IGC ₅₀)
Butanal	1.24	Ethyl acetate	1.98
Hydroquinone	1.50	Propyl acetate	1.37
Morpholine	2.49	Isopropyl acetate	0.66
Allyl chloride	1.90	Butyl acetate	1.10
Ethylamine	3.26	Isobutyl acetate	0.58
n-Butylamine	3.22	Amyl acetate	0.12
Allylamine	2.82	Vinyl acetate	0.48
Cyclohexylamine	2.58	Phenyl acetate	0.87
		Ethyl butyrate	0.76
		Methyl propionate	2.07
		Ethyl propionate	1.65

Conclusions

There are gross similarities as well as differences in the molecular basis of non-specific toxicity for the five organisms studied in this work. The system constants summarized in Table 1 provide the key to interpreting the non-specific toxicity data. The model constant, c term, indicates species differences as indicators of non-specific toxicity. The c term varies by over an order of magnitude, with *Artemia salina* the most responsive to sample concentration and *Scendesmus quadricauda* the least. Species differences in reactive toxicity were also noted among the compounds analyzed. The biomembranes are moderately cohesive and show similar dipolarity/polarizability and hydrogen-bond basicity to water ($s = a = 0$) for all organisms. Consequently, the solute dipolarity/polarizability and hydrogen-bond acidity do not contribute to the non-specific toxicity. Only the solute size and hydrogen-bond basicity are really important, together with a small contribution from lone-pair electron interactions. The range of membrane hydrogen-bond acidity for the different organisms is quite large, and the greatest difference in relative toxicity for these organisms will be observed for compounds with significant hydrogen-bond basicity. In general terms, hydrogen-bond bases are most toxic to *Scendesmus quadricauda* and least toxic to *Artemia salina*. Solute size differences affect the relative toxicity through differences in cohesion of the biomembranes (m constant). Increasing solute size has most influence on the relative toxicity for *Vibrio fischeri* and *Artemia salina* and least for *Tetrahymena pyriformis*.

We can use the data in Table 1 to answer three further questions. Is the octanol–water distribution constant a suitable model for the non-specific toxicity in the above organisms and, if not, can we provide a molecular basis for its failure? What is the origin of family-dependent relationships for the non-specific toxicity of different homologous series and their octanol–water distribution constants? Can we predict the non-specific toxicity in higher organisms from the results obtained in short-term bioassays? For the correlation of two free energy linear relationships, it is not necessary that they have identical system constants, but only that the ratios of their system constants are (nearly) identical. The system constant ratios for non-specific toxicity of the organisms in Table 1, the tadpole,²⁰ water flea (*Daphnia magna*),¹² fathead minnow (*Pimephales promelas*),¹² guppy (*Poecilia reticulata*),¹² and golden orfe (*Leuciscus idus melanotus*)¹² and the octanol–water distribution constant²¹ are summarized in Table 6. Wet octanol is not as dipolar/polarizable and its hydrogen-bond basicity is different from that of the organisms studied here. The most useful correlations are expected with *Tetrahymena pyriformis* for compounds with either a narrow range or small π_2^H descriptors. For members of a homologous series, the solute descriptors are generally constant except for V_X and R_2 . Variation in R_2 is small, so

Table 6 System constant ratios for non-specific toxicity models and the octanol–water distribution constant

Model	System constant ratios			
	r/m	s/m	a/m	b/m
<i>Vibrio fischeri</i> (pT ₅)	0.290	0	0	−0.480
<i>Pseudomonas putida</i>	0.360	0	0	−0.670
<i>Tetrahymena pyriformis</i>	0.220	0	0	−0.872
<i>Scendesmus quadricauda</i>	0.572	0	0	−0.323
<i>Artemia salina</i>	0.040	0	0	−1.308
Fathead minnow	0.071	0	0.118	−1.08
Guppy	0.180	0	0.108	−0.946
Golden orfe	0.500	0	0.364	−0.775
Water flea	0.100	0	0.202	−1.102
Tadpole	0.243	−0.219	0	−0.746
Octanol–water	0.146	−0.273	0.008	−0.901

effectively V_X is the dominant variable. Since the contributions from s/m and a/m are either zero or constant, correlations with the octanol–water distribution constant are reduced to a single parameter equation with b/m as the significant variable. Mathematically, equations that reduce to a single common variable should always correlate. For compounds that do not belong to a homologous series, the solute descriptors will not be constant, except by coincidence, and therefore any correlation would be fortuitous and unlikely to hold for a group of compounds with varied properties. There are no good matches of the system constant ratios for the organisms studied here and the higher order species in Table 6. Except for the tadpole, there is a poor match for hydrogen-bond acid compounds, which are expected to exhibit different non-specific toxicity in the two groups of organisms. *Tetrahymena pyriformis* is the best match in terms of hydrogen-bond acidity, and is a good match in terms of dipolarity/polarizability, and would be expected to be the best indicator species for the fish and water flea. Since individual compounds have a wide range of descriptor properties, the quality of any fit for the non-specific toxicity of one species regressed against another would be a function of the variation of descriptor properties as well as experimental error. Mathematically, there is no fundamental reason to expect good correlations except in fortuitous cases and specific examples of similarity in descriptor properties. Therefore, any models built in this way will not be global and are subject to unacceptable precision. The solvation parameter model offers a better alternative for predicting non-specific toxicity in different organisms. Once a model has been formulated, as in Table 1, the non-specific toxicity of any compound with known solute descriptors can be calculated for each organism. Solute descriptors are available for over 4000 compounds and rapid experimental and computational techniques are under development that would allow new values to be determined with no greater difficulty than determining the octanol–water distribution constant.^{21,22,25–27,40}

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