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Interspecies Modeling of Narcotics Toxicity to Aquatic Animals

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The evaluation of the environmental risk of existing chemicals, especially the so-called high production volume chemicals, requires an extensive array of information, including data for physicochemical properties as well as acute and chronic toxic endpoints. The large number of experimental data gaps for toxicity creates the need for further development of estimation and extrapolation methodologies. Although the present status of the estimation techniques can produce reliable predictions of some toxicological data, there are still endpoints where the results are much less robust and no useful estimates can be expected. The same observation holds true for the evaluation of the distribution of sensitivities among different organisms. Usually, on the basis of limited experimental data, we must judge which species occupies the most vulnerable part of the ecosystem (Van Leeuwen et al. 1992). This makes the prediction problem more complicated because extrapolation are required not only for new chemicals but also for different endpoints.

As noted by Bradbury and Lipnick (1990), the vast majority of organic contaminants exhibit a reversible physiological phenomenon, which lack any covalent interactions between toxicant and organism. These chemicals are the so-called narcotics (van Wetzel and Opperhuizen 1995). The motivation for this study was the numerous publications on interspecies correlation of toxicological data showing that, at least for non-covalent acting chemicals, there is remarkable similarity in relative toxicity. For example, the juxtapose of hydrophobic-dependent neutral narcotic toxicity models for fish survivability, ciliate growth impairment, and bacterial bioluminescence inhibition revealed that the agreement is quite harmonious between endpoints (Schultz et al. 1990). More recent investigations comparing fish and ciliate toxicity (see Bearden and Schultz 1998) revealed good agreement for narcotics, weak acid respiratory uncouplers, and soft electrophiles. On the other hand, differences in toxic potency were demonstrated for proelectrophiles and esters (Bearden and Schultz 1998). Recently, Kaiser (1998) presented an updated review of the studies devoted to comparisons of a prokaryotic to selected eukaryotic organisms. He demonstrated that the bacterial bioluminescence assay had a very high degree of commonality with mean lethality to fishes and crustaceans. Results from intravenous exposure with mammals, such as rats and mice, also showed general correlation (Kaiser 1998).

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The traditional application of quantitative structure-activity relationships (QSARs) in risk assessment of pollutants is two-fold (Van Leeuwen et al. 1992). First, to predict the toxic potency of a variety of untested chemicals on the basis of a limited data set, and second, to help in understanding of the mechanism of action of toxicants. The scope of the present work was to extend the role of QSARs for quantitative extrapolation of narcotic toxicological effects from one organism to others by developing an interspecies (i.e., interendpoint) model. To achieve this model, toxicity data for ciliate, water flea, and fish were simultaneously regressed against conventional descriptors of hydrophobicity and electrophilicity. External validation of the interspecies model was performed using data for three other organisms: mosquito larva, pond snail, and tadpole.

METHODS AND MATERIALS

Chemicals belonging to the neutral or non-polar narcosis group according to Veith et al. (1983), Verhaar et al. (1992), and Russom et al. (1997) were selected for evaluation in this study. Toxicity data for other non-covalent acting substances including esters (with exception to fish), pyridines, and nitrobenzenes with alkyl and halogen substituents were also included in the domain of the modeled chemicals. Karabunarliev et al. (2000) presented a complete description of the toxicant selection rules.

The training set of narcotic chemicals was selected from literature for three species: a ciliate, water flea, and fish. Schultz (1997) reported the 40- or 48-hr 50% inhibitory growth concentration (IGC_{50}) measured in a static bioassay with the ciliate (*Tetrahymena pyriformis*). Zhao et al. (1998) reported the 24-hr 50% immobilization concentration for the water flea (*Daphnia magna*), while the median lethal concentrations of fathead minnow (*Pimephales promelas*) for 96-hr (LC_{50}) measured in flow-through experiments were taken from Nendza and Russom (1991).

External model validation was performed on the basis of toxicity data for three other organisms. The toxicity data of primary alkanols against the mosquito (*Culex tarsalis*) larva were reported as mortality after 24-hr exposure in static experiments (Hammond and Kubo 1999). The static 96-hr lethality data (LC_{50}) for the pond snail (*Lymnaea stagnalis*) were taken from several authors (Leeuwangh et al. 1975; Bluzat and Seuge 1979; Slooff et al. 1983; Urrestarazu-Ramos 1998). The static, time-independent minimum narcosis concentration data ($NC_{minimum}$) for the tadpole (*Rana temporaria*), originally reported by Overton (1901), were secured from Lipnick (1989). These data sets, while not exhaustive, were selected to represent different animal classes and different trophic levels.

In this study, all endpoint concentrations were expressed in mol/L. The selected bioassays refer to different sets of chemicals, but in all cases the hydrophobicity, measured as the 1-octanol/water partition coefficient (log K_{ow}), were less than 5.0.

The computer software, Optimized Approach based on Structural Indices Set (OASIS), (Mekenyan et al. 1990, 1994) was used in this investigation to assist in quantification of molecular descriptors. This software incorporates automatic 3-D model builder from molecular connectivity, integrated molecular force-field optimizer, and interface to standard programs CLOGP (ClogP version 3.4 1988) and MOPAC (Stewart 1995), for computation of hydrophobicity measured as log K_{ow} and electrophilicity measured as the energy of the lowest unoccupied molecular orbital (E_{LUMO}). These variables have been found to be relevant descriptors of the domain of modeled non-congeneric narcotics (Karabunarliev et al. 2000) in the following generic model:

$$\text{Log (1/endpoint)} = b_0 + b_1 (\log K_{ow}) + b_2 (E_{LUMO}) \qquad \text{Eq. [1].}$$

Model adequacy was quantified with the coefficient of determination (r^2), squared correlation coefficient of predictions according "leave one out" procedure (Q^2), residual variance (σ), and Fisher-value for explained versus unexplained variance (F). The 95% confidence intervals (*t*-test) of the model parameters are also presented.

RESULTS AND DISCUSSION

The model derived in this study was based on toxicity data from several species and can be considered a further generalization of the response-surface theory as described by Schultz and Mekenyan (2000). The basis of this modeling approach is Overton's finding that there is "no sharp delineation, but rather a continuum of effects between the neutral and basic classes of organic chemicals" (cf. Lipnick 1986). This continuum can be explained by McFarland's probability hypothesis (McFarland 1970). Here, the organism response to the presence of a toxicant in the environment was considered to be a consequence of the combination influences of two different processes: uptake of the chemical into the biophase, and interaction with the site of action. While uptake is traditionally modeled by log K_{ow}, descriptors assessing the electrophilic character of molecules can delineate the interaction of the non-covalent acting chemicals. Such descriptors include the E_{LUMO}, electronegativity, average or maximum superdelocalizability, and maximum charge at non-hydrogen atom.

Regression analysis of the narcosis toxicity data for ciliate, fish, and water flea revealed the following interspecies QSAR:

$$\begin{aligned}\text{Log (1/endpoints)} = {} & b^{\,Organism}_{\,Endpoint} + (0.77 \pm 0.01) (\log K_{ow}) \\ & - (0.07 \pm 0.01) (E_{LUMO}) \qquad \text{Eq. [2]} \\ & n = 202,\ r^2 = 0.96,\ Q^2 = 0.95,\ F = 1154,\end{aligned}$$

where endpoints, number of single species data (n), and the values of the intercept parameters $b_{Endpoint}^{Organism}$ are presented in Table 1.

The standard deviations of the toxicity data are presented in Table 1. For the training sets, the accuracy of the fit for the interspecies model was nearly identical to that of the corresponding regressions for the separate endpoints (see last two columns in Table 1).

To more carefully explore the validity of the developed interspecies narcosis model, an additional validation test was applied using data for toxicity to the mosquito larva, the pond snail, and the tadpole. The standard deviations of the toxicity data modeled for a single species as delineated in Eq. [1], was compared to the fit of the data to the interspecies model, Eq. [2] (Table 1). However, the interspecies fit was done without re-estimation of the slope terms associated with partitioning and electronegativity (i.e., the slope terms estimated from protozoa, daphnids, and fish data were used to estimate toxic potency to insect larvae, mollusks, and early amphibians). It is evident by how close the standard deviations values are that the precision of the predicted potency was not affected by their extrapolation by the interspecies model, Eq. [2]. Furthermore, the regression of observed toxicity versus toxicity predicted based on Eq. [2] for all six endpoints reveals an intercept almost zero, a slope of about one, and an r^2 = 0.97.

The derived interspecies model represents non-specific and non-covalent toxicity to different aquatic animals with high accuracy. It was demonstrated that the statistical quality of this model is comparable with those of QSARs fitted on separate endpoints. We emphasize that the excellent statistical characteristics of these approximations were found without involving additional explanatory variables or corresponding parameters.

The interspecies model's most specific feature is the nature of its parameters. These parameters can be divided into two groups. One group reflects the similarity of the toxicological effects of the chemicals on the array of target organisms or endpoints. They account for the global sensitivity of toxicity to the molecular descriptors and thus to changes in a toxicants' ability to penetrate into the biophase and to interact with the site of action. Because these parameters are common to the explanation of toxic potency in all the assays, the target sites and changes of toxicant intracellular concentration should be very similar for the reported manifestations in the studied aquatic animals. At present, we assumed that biological membranes are the sites of action of non-covalent acting toxicants. Specifically, such narcotics penetrate the lipid-bilayer region of membranes and alter lipid properties and fatty acid composition (Beavan et al. 1982; Sikkema et al. 1995; Bearden et al. 1999).

Table 1. Single organism and interspecies models.

				Statistical Comparisons	
Organism	Endpoint	N	Intercept	σ, Eq. [1]	σ, Eq. [2]
Training data					
T. pyriformis	IGC_{50}^{48-h}	125	$b_{IGC50}^{T.\ pyriformis} = 1.12 \pm 0.06$	0.189	0.201
P. promelas	LC_{50}^{96-h}	43	$b_{LC50}^{P.\ promelas} = 1.49 \pm 0.04$	0.237	0.290
D. magna	IC_{50}^{24-h}	34	$b_{IC50}^{D.\ magna} = 1.86 \pm 0.06$	0.283	0.309
Validation data					
C. tarsalis[a]	LC_{50}^{24-h}	13	$b_{LC50}^{C.\ tarsalis} = 0.93 \pm 0.15$	0.239	0.239
L. stagnalis[b]	LC_{50}^{96-h}	12	$b_{LC50}^{L.\ stagnalis} = 1.57 \pm 0.26$	0.282	0.389
R. temporaria[c]	$NC_{minimum}$	9	$b_{NC_{minimum}}^{R.\ temporaria} = 1.02 \pm 0.04$	0.038	0.051

[a]Hammond and Kubo (1999).
[b]Leeuwangh et al. (1975); Bluzat and Seuge (1979); Slooff et al. (1983); Urrestarazu-Ramos (1998).
[c]Overton (1901); Lipnick (1989).

Separate factors measure the toxicological distinction between the different bioassays. For the modeled non-specific and non-covalent ecotoxicity to aquatic species, only the intercepts belong to this group, thus, revealing that there is a constant bias between different endpoints. The difference between magnitudes of $b_{Endpoint}^{Organism}$ is due to a variation in the testing protocols and differences in biological complexities of the target organisms. Our model parameters afford a sophisticated means to make a direct comparison between the assays and endpoints.

QSAR models obtained by the traditional modeling approach are based on a single bioassay, and it should not be expected that they would be applicable to other organisms. Thus, these models tend to be limited in their applicability for estimating the hazardous effect of the environmental chemicals to ecosystems. In contrast, the formulated multiple-response QSAR enables the fit of different endpoints at the same time within a single model. Handling many responses at the same time is favorable because parts of the model parameters are bioassay independent. As was previously mentioned in this study, the biological and experimental specificity is reflected by one parameter only — the intercept. This makes the toxicity extrapolation to other organisms and endpoints quite easy. The

interspecies model extension to a non-studied bioassay of interest can be based on limited and appropriately designed measurements. Because non-specific and non-covalent narcotics are a predominant part of all industrial chemicals (Bradbury and Lipnick 1990), especially those of high production volume, the derived model can be extremely valuable in chemical risk assessment.

In summary, an ecotoxicity data set for narcotic chemicals was selected from literature for aquatic species belonging to four phyla: Protozoa, Mollusca, Arthropoda, and Vertebrata. The toxicity data for *T. pyriformis*, *D. magna*, and *P. promelas* were regressed against hydrophobicity and electrophilicity to form a single high quality model, Eq. [2]. This QSAR was applied to model the narcosis toxicity to three other organisms: *C. tarsalis*, *L. stagnalis*, and *R. temporaria* without re-estimation of the slope parameters associated with partitioning and electronegativity of the test substances. The accuracy of the predicted toxic potency was not affected by extrapolation and was comparable with that obtained from the separate fits of the data for individual bioassays. The derived interspecies model for non-covalent acting chemicals is easily interpretable and affords a straightforward method for the extrapolation of toxic potency of non-covalent acting chemicals from one organism to another.

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