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Hazard Assessment

QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS FOR PREDICTING THE TOXICITY OF PESTICIDES IN AQUATIC SYSTEMS WITH SEDIMENT

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Abstract – The toxicity of a series of organophosphorus (OP) and carbamate insecticides was measured against the midge *Chironomus riparius* in aquatic systems with and without sediment. Five molecular descriptors (molecular volume, Henry's law constant, *n*-octanol/water partition coefficient (K_{ow}), molecular connectivity, and linear solvation energy) were used in regression analysis as potential predictors of insecticidal activity. The regressions were conducted for each descriptor against toxicity values for the series of chemicals.

Molecular volume and Henry's law constant showed no relationship with toxicity. However, log K_{ow} was moderately successful in describing the effect of sediment on toxicity ($r^2 = 0.508$). Prediction of toxicity was substantially improved when a linear solvation energy (LSE) or molecular connectivity (MC) model was used in regressions. In multiple regressions conducted on carbamates and OPs separately, use of MC or LSE parameters explained up to 95.8% of the variability in toxicity. Based on the results of regression analyses, sorptive interactions between these insecticides and sediment apparently dominate the processes affecting the toxicity of these compounds when sediment is present. In the absence of sediment, the regressions suggest that the molecular structure of the insecticides is more important than solubility or partitioning for determining toxicity.

Keywords – QSAR AChE inhibitors Prediction Toxicity

INTRODUCTION

Traditional models for predicting the environmental fate and toxicity of xenobiotics have studied the relationship of hydrophobic chemicals to the *n*-octanol/water partition coefficient (K_{ow}). For hydrophobic chemicals, environmental fate in aquatic systems is dramatically influenced by the presence of sediment. The organic carbon (OC) content of sediment, in particular, is thought to be decisive in determining the fate of hydrophobic, nonpolar contaminants because sediment OC acts as a sorbent for such chemicals. Hydrophobic sorption of lipophilic, nonpolar compounds to sediment removes the contaminants from the water column and thus decreases the exposure that would take place if the contaminants were freely dissolved [1,2].

The toxicity of hydrophobic, nonpolar contaminants is also related to the hydrophobicity of the compound as expressed by log K_{ow} [3–5]. Hydrophobic compounds passively accumulate in aquatic organisms by passage through lipid membranes and exert a nonspecific narcosis by solubilization in lipids of the myelin sheath surrounding axons. Because all aspects of exposure and intoxication depend on water/lipid partitioning, it is not surprising that log K_{ow} of hydrophobic, nonpolar chemicals can be used to predict the fate [6,7] and toxicity [8,9] of these compounds. This relationship has facilitated hazard assessment by providing a means of predicting toxicity and, therefore, environmental hazard in an inexpensive, time-effective manner.

At present few efforts have been made to develop predictive models for estimating the fate or toxicity of organophosphorus (OP) and carbamate compounds in aquatic systems. Although some OP and carbamate insecticides are quite lipophilic (log $K_{ow} > 3$), most OPs and carbamates are relatively water soluble and chemically reactive. OP and carbamates possess one or more polar functional groups that may interact with sediment com-

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ponents through a variety of mechanisms that include, but are not limited to, hydrophobic binding [10]. Thus, OP and carbamate fate in aquatic settings may not be correlated solely with the lipophilicity of the chemicals but instead with parameters that characterize the functional groups that impart activity to the molecules [11,12].

The prediction of toxicity of OP and carbamate insecticides in aquatic environments is also likely to be more complex than describing lipid partitioning with log K_{ow}. In contrast to the nonspecific narcosis produced by hydrophobic, nonpolar compounds, cholinergic insecticides exert their toxicity through inhibition of a specific nervous system enzyme, acetylcholinesterase (AChE) [13]. AChE inhibitors are also subject to oxidative metabolism, which can potentially activate and/or detoxify the molecules [14], whereas hydrophobic, nonpolar chemicals are frequently not metabolized [8,9].

At a minimum, any model that attempts to predict the fate or toxicity of OPs and carbamates must assess both the hydrophobicity and the specific binding interactions contributed by polar functional groups. Further, the model also must account for metabolism and possible detoxification of OPs and carbamates in both environmental media and the test organism. Finally, the predictive model must be able to describe the fit of the insecticide molecule into a specific target site—the active site of AChE.

At first glance, the development of predictive models for estimating the fate and toxicity of OPs and carbamates appears to be a formidable task. Because it was unlikely that K_{ow} alone would be successful, two additional physical parameters, molecular volume (MV) and Henry's law constant (HLC), were examined in this study, through correlation analysis, for their ability to explain variation in the toxicity of a series of cholinesterase-inhibiting insecticides. Aquatic systems with and without sediment were used. Finally, we used two multidimensional molecular models, linear solvation energy (LSE) and molecular connectivity (MC), in correlation analyses, as comprehensive descriptors of chemical characteristics that affect the fate and toxicity of OP and carbamate insecticides in the midge *Chironomus riparius*.

MATERIALS AND METHODS

Organisms

The midge *C. riparius*, a sediment-dwelling organism, was used as the experimental animal in all toxicity tests because of its ready availability and importance in aquatic food chains. Midges were raised in the laboratory according to the methods of Estenik and Collins [15]. Fourth-instar larvae were used in all tests.

Compounds

A series of 12 OP and carbamate insecticides were chosen for use as toxicants. A list of the test chemicals, along with their trade names, purities, and sources, are given in Table 1. The compounds were chosen to represent a wide range of water solubilities and anticholinesterase activities.

Physical parameters

MV, HLC [16], and log K_{ow} [17] values for each chemical were obtained from the literature. MC indexes were calculated from the identities of the individual atoms and their bonding connections in each structural formula [18] using the MOLCON [18] computer program. Four MC levels were expressed as χ^0, χ^1, χ^2, and χ^3. χ values were calculated through quantification of adjacency values or the number of nonhydrogen atoms adjacent to each individual atom. χ^0 was an expression of molecular size; χ^1 represented the environment of two atoms connected by a bond and was calculated through quantification of adjacency values. χ^2 and χ^3 reflected higher level connections in the molecule (i.e., two- and three-bond connections, respectively). All χ parameters were corrected for valence (double bonds and ionization); these χ values were denoted as ${}^v\chi^0$ to ${}^v\chi^3$. MC indexes were used to quantify the relationships among individual atoms within a molecule, with the larger levels representing more complex interactions. At the higher levels of connection, these indexes can represent aspects of three-dimensional organization. Ultimately, it is the three-dimensional aspects of structure that determine fit of the toxin to the biological receptor and thus should be relevant to the prediction of toxicity for covalently reacting agents.

LSE parameters were calculated by Jim Hickey (U.S. Fish and Wildlife Service, National Fisheries Center–Great Lakes, Ann Arbor, MI) from a compilation of rules for their calculation [19]. Four parameters were assessed in the LSE model: (a) a scaled intrinsic molecular volume (VI/100) that measures the free energy needed to separate solvent molecules to form a cavity for the solute; (b) π^*, the dipolarity of the solute molecule, which is a measure of the solute-solvent interaction; (c) α, which describes the ability of the solute molecule

Table 1. Insecticides used in toxicity tests

Common name (trade name)	Chemical name	Mol. wt. (g/mol)	Purity (%)	Source
Aldicarb (Temick®)	2-Methyl-2-(methylthio)propionaldehyde-*O*-(methylcarbamoyl) oxime	190	98	Chem Service (West Chester, PA)
Carbaryl (Sevin®)	1-Naphthyl-*N*-methylcarbamate	201	99	Pfaltz and Bauer (Stamford, CT)
Carbofuran (Furadan®)	2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate	221	99	Chem Service
Dichlorvos (Vapona®)	2,2-Dichlorvinyl dimethyl phosphate	221	94	Shell Chem. Co.
Ethion (Nialate®)	*O,O,O′,O′*-Tetraethyl,*S,S′*-methylene-bis-(phosphorodithioate)	384	95	Chem Service
Leptophos (Phosvel®)	*O*-(4-Bromo-2,5-dichlorophenyl)-*O*-methyl phenyl phosphorothioate	412	95	Velsicol (Chicago, IL)
Malathion (Cythion®)	Diethyl mercaptosuccinate, *s*-ester with *O,O*-dimethyl phosphorodithioate	330	98	Chem Service
Methyl parathion (Penncap-M®)	*O,O*-Dimethyl-*O*-(*p*-nitrophenyl) phosphorothioate	263	80	Chem Service
Mexacarbate (Zectran®)	4-Dimethylamino-3,5-xylyl methylcarbamate	222	94.2	Union Carbide (Jacksonville, FL)
Parathion (Niran®)	*O,O*-Diethyl-*O*-(*p*-nitrophenyl) phosphorothioate	291	98.7	Chem Service
Phosdrin (Mevinphos®)	Methyl-(*E*)-3-hydroxycrotonate dimethyl phosphate	224	95	Chem Service
Propoxur (Baygon®)	*O*-Isopropoxyphenyl methyl carbamate	209	96	Chem Service

to donate a hydrogen (acidity), and (d) β, which estimates basicity, or the ability of the molecule to accept a hydrogen. In sum, the LSE parameters estimate steric and electronic phenomena involved in the solvation process. Because LSE and MC have the ability to describe various parts of the processes involved in accumulation of the insecticides as well as the subsequent biological fate in the midge, it was hypothesized that MC and LSE would be more useful in describing changes in toxicity than the less complex unidimensional descriptors.

Toxicity tests

Two types of toxicity tests were performed. In the first case, the toxicant was dissolved in acetone, after which 1 ml of a graduated series of test concentrations of insecticide was added to 500 ml of soft standard reference water [20] in a 1-L beaker. Three replicates of each concentration were made. Controls received 1 ml of acetone. Twenty fourth-instar midge larvae were then added to each beaker, and the beakers were placed in a Forma Scientific environmental chamber (model 37844, Marietta, OH) on a photoperiod of 14:10 h light:dark. After 24 h of exposure, impairment of midges was scored as failure to execute three figure-eight motions when pinched with a pair of forceps. Inability to execute the requisite number of figure eights indicated that the animals were sufficiently stressed that they would die within 24 h [15], a criterion widely employed to assess chironomid health in toxicity tests [21,22]. This mode of administering the toxins was referred to as the water-only method.

The second method of delivering the insecticides, denoted as the spiked-sediment method, was conducted according to the method of Lydy et al. [7]. Briefly, 1-ml aliquots of a graduated series of concentrations for each chemical were pipetted onto 5 g of Olentangy River (Columbus, OH) sediment contained in a 5-ml conical centrifuge tube. The Olentangy sediment was dried and sieved before use. The sediment, characterized by REALab (Wooster, OH), contained 3% OC. The solvent front of each concentration of chemical was allowed to descend to the bottom of the centrifuge tube, after which 10 ml of soft standard reference water was added to each tube. The contents of the tube were then mixed with a metal spatula and centrifuged at high speed in a Fisher Scientific (Fair Lawn, NJ) model clinical centrifuge for 5 min. The resulting supernatant was decanted and saved. The sediment was then resuspended with fresh water and centrifuged

two more times. Controls contained sediment with the same amount of acetone and were treated in the same fashion as previously described, except that no insecticide was present. Work was performed at a constant temperature of 22°C under yellow light to minimize phototransformation of insecticides.

Spiked-sediment toxicity tests were initiated by removing the spiked sediment from the centrifuge tube and placing it in a 1-L beaker. Next, the combined supernatant (approximately 30 ml) was added to the sediment, along with 465 ml of fresh standard reference water. Twenty midges were added to each beaker, and the beakers were placed in an environmental chamber as previously described.

Analysis

Toxicity data were analyzed using Finney's probit analysis [23] and were expressed as 24-h EC50 values. EC50 values were considered to be significantly different when 95% confidence limits did not overlap. The unit of measurement for all test concentrations was micrograms of compound per kilogram of medium. For water-only systems, 0.5 L of water was assumed to weigh 0.5 kg. For all systems possessing sediment, the weight of the sediment was added to the weight of the water to obtain a value of 0.5 kg for the environmental media to which the midges were exposed. All concentrations were nominal.

The toxicity data, expressed as EC50 values, were regressed against each molecular descriptor in regression analyses [24]. For MC and LSE analyses, the toxicity data were regressed against each individual parameter (e.g., χ^0, χ^1, χ^2, and χ^3) and all possible combinations thereof. In some cases, the data were sorted by chemical class, that is, carbamate vs. OP. These modifications are indicated in the tables. The EC50 values for LSE and MC analyses were described as molar concentrations.

RESULTS

Toxicity tests

The toxicity of the 12 insecticides varied significantly between treatments (water only or spiked sediment, Table 2). In addition, there were major differences in susceptibility of the midges to individual compounds, as noted in the water-only toxicity tests. For this treatment, methyl parathion was the most toxic insecticide (EC50 = 1.58 μg/kg), whereas ethion was least toxic (EC50 = 135.8 μg/kg).

When sediment was added to the test systems, the resulting EC50s declined significantly for all insecticides except carbofuran, mexacarbate, phosdrin, and propoxur (spiked sediment, Table 2). For the spiked-sediment treatments, methyl parathion proved to be most toxic, with an EC50 of 4.03 μg/kg, whereas leptophos had such low toxicity that it was not possible to estimate an EC50 in the presence of sediment. The EC50 is thus expressed as >500 μg/kg because that was the highest tested concentration.

The effect of sediment on the toxicity of these 12 insecticides was summarized by expressing the change in toxicity as a ratio of spiked-sediment EC50 to that of the water-only EC50 (ratio, Table 2). From the ratio, it is clear that the toxicity of leptophos was most affected by the addition of sediment (ratio ≥31.7), whereas sediment had no significant effect on the toxicities of carbofuran,

Table 2. Toxicity of cholinergic insecticides to *Chironomus ripairus*

Compound	24-h EC50 (μg/kg)		Sediment:water ratio
	Water only	Spiked sediment	
Methyl parathion	1.58 (1.2–2.7)	4.03 (3.6–4.8)	2.55 (1.73–3.76)
Parathion	6.9 (6.3–7.4)	72.0 (56.0–116.0)	10.40 (7.60–13.99)
Carbaryl	110.0 (99.0–122.0)	442.0 (146.0–1,464.0)	4.01 (1.18–13.6)
Aldicarb	9.9 (9.1–10.3)	26.7 (23.3–30.6)	2.70 (1.96–3.85)
Malathion	4.12 (3.8–4.6)	6.73 (4.9–7.8)	1.63 (1.10–2.49)
Leptophos	15.8 (12.1–17.6)	>500.0	31.7
Carbofuran	8.6 (7.1–9.6)	7.9 (6.8–8.7)	0.92 (0.67–1.26)
Ethion	135.8 (124.6–141.3)	393.9 (271.1–406.8)	2.90 (2.26–3.71)
Mexacarbate	23.4 (21.6–26.9)	25.5 (23.2–29.1)	1.08 (0.78–1.48)
Phosdrin	22.5 (18.6–24.2)	26.5 (22.1–28.3)	1.17 (0.75–1.80)
Dichlorvos	8.73 (6.2–10.4)	16.7 (12.1–18.9)	1.91 (0.98–3.70)
Propoxur	94.4 (82.6–98.4)	108.8 (96.1–115.2)	1.15 (0.91–1.45)

95% confidence limits are given in parentheses.

Table 3. Unidimensional molecular descriptors for insecticides used in toxicity tests

Compound	Mol. vol.[a] (cm^3/mol)	Henry's law constant[a] (Pa m^3/mol)	Log K_{ow}[b]
Aldicarb	224.3	0.00032	1.10
Carbaryl	218.7	0.0013	2.81
Carbofuran	240.8	0.00051	2.32
Dichlorvos	167.5	0.190	1.40
Ethion	350.2	0.032	5.07
Leptophos	317.8	0.250	6.31
Malathion	319.1	0.0023	2.36
Methyl parathion	207.5	0.021	3.32
Parathion	251.9	0.012	3.80
Phosdrin	ND	ND	1.11
Propoxur	244.5	0.130	1.50

ND = not determined.
[a]Data from Suntio et al. [16].
[b]Data from Hansch and Leo [17].

Table 5. Linear solvation energy (LSE) parameters for cholinergic pesticides[a]

Compound	VI/100	π^*	α	β
Aldicarb	1.202	1.14	0.30	1.50
Carbaryl	1.183	1.17	0.30	0.90
Carbofuran	1.303	1.45	0.30	1.15
Dichlorvos	0.861	0.85	0.10	0.77
Ethion	1.800	1.14	0.10	2.00
Leptophos	1.643	1.43	0	1.01
Malathion	1.421	1.65	0	1.75
Methyl parathion	1.214	1.30	0.16	1.15
Mexacarbate	1.379	1.16	0.30	1.22
Parathion	1.410	1.28	0.16	1.16
Phosdrin	1.016	1.30	0.22	1.25
Propoxur	1.245	1.35	0.30	1.10

[a]LSE parameters calculated by Jim Hickey using method of Hickey and Passino-Reader [19].

mexacarbate, phosdrin, and propoxur, for which the ratios ranged from 0.92 to 1.17 (Table 2).

Regression analyses with unidimensional descriptors (HLC, MV, and log K_{ow})

The physical and structural parameters used as independent variables in regression analyses are given in Tables 3 to 5. When the insecticides were added directly to water without sediment, there was no correlation between HLV, MV, or log K_{ow} and toxicity. In contrast, log K_{ow} was significantly correlated with toxicity when sediment was present, although log K_{ow} described only 50% of the variability in toxicity data ($r^2 = 0.504$, $d.f. = 11$, $P > F = 0.05$; EC50 = 77.3 log K_{ow} − 24.7). When the ratio of spiked-sediment EC50 to water-only EC50 was used in correlation analysis with log K_{ow}, r^2 was improved to 0.824 ($d.f. = 10$, $P > F = 0.03$) and the predictive model for the ratio was 5.383 log K_{ow} − 7.75. There was a weak correlation between the ratio and HLC where ratio = 63.4 HLC + 2.016 ($r^2 = 0.373$, $d.f. = 10$, $P > F = 0.05$). Multiple regressions were not performed on these parameters.

Regression analyses with multidimensional descriptors (MC and LSE)

When the more complex multidimensional molecular descriptors MC and LSE were used as descriptors, the ability to describe changes in toxicity generally improved compared to the unidimensional descriptors. When both OP and carbamate com-

Table 4. Molecular connectivity (MC) parameters for cholinergic pesticides[a]

Compound	χ^0	χ^1	χ^2	χ^3	χ^{3c}	$^v\chi^0$	$^v\chi^1$	$^v\chi^2$	$^v\chi^3$	$^v\chi^{3c}$
Aldicarb	9.7	5.4	5.1	3.4	1.7	8.6	4.7	4.3	2.5	1.7
Carbaryl	10.6	7.3	6.1	5.1	0.7	8.4	4.8	3.2	2.3	0.3
Carbofuran	12.1	7.3	6.5	3.9	1.7	9.8	5.2	4.2	2.2	1.0
Dichlorvos	8.0	4.6	3.6	2.8	0.9	7.2	4.1	3.2	2.0	0.3
Ethion	14.8	8.9	7.5	4.9	1.8	16.8	14.5	15.9	13.6	4.4
Leptophos	14.7	9.5	8.7	7.3	1.8	15.1	9.7	8.9	7.2	1.9
Malathion	14.9	8.9	7.4	5.5	1.6	13.9	9.8	9.7	7.8	2.5
Methyl parathion	12.2	7.5	6.7	5.3	1.6	10.4	6.7	5.8	4.2	1.0
Mexacarbate	12.3	7.5	6.9	4.9	1.4	10.4	5.1	3.9	7.3	0.7
Parathion	13.9	8.3	8.0	6.2	1.9	12.4	7.5	7.7	6.4	2.2
Phosdrin	10.3	6.1	4.8	3.4	0.7	11.3	8.1	5.7	3.6	0.5
Propoxur	11.3	7.1	6.2	4.2	1.0	9.1	4.8	3.3	1.6	0.4

[a]MC values calculated by method of Kier and Hall [18].

pounds were included together in multiple regressions with LSE parameters VI/100, π^*, and α as independent variables, an adjusted r^2 value of 0.36 was obtained for the water-only systems ($P > F = 0.09$, $d.f. = 11$) (Table 6). An adjusted r^2 value of 0.52 was obtained when VI/100, π^*, and β were used as independent variables against spiked-sediment toxicity values ($P > F = 0.05$, $d.f. = 11$). Predictive ability improved somewhat when the chemicals were separated according to chemical class. For carbamates in water-only systems, the best adjusted r^2 value using LSE parameters was 0.463 ($P > F = 0.125$, $d.f. = 4$); β was the only independent variable used in this regression (Table 6). However, the three LSE parameters, VI/100, π^*, and β, gave an adjusted r^2 value of 0.958 ($P > F = 0.131$, $d.f. = 4$) for carbamate toxicity in spiked-sediment systems. In contrast, the best r^2 value for OPs in water-only systems was achieved using π^* and β in a multiple regression ($r^2 = 0.517$, $P > F = 0.04$, $d.f. = 6$). Finally, the best r^2 value for OPs in spiked-sediment systems was achieved in a single regression in which VI/100 was employed as the independent variable ($r^2 = 0.517$, $P > F = 0.04$, $d.f. = 6$) (Table 6).

Overall, the best results were obtained when one or more MC parameters were used as descriptors of toxicity. For all compounds combined in water-only systems, the best relationship was determined when χ^0, χ^1, and χ^3 were employed in a multiple regression ($r^2 = 0.46$, $P > F = 0.04$, $d.f. = 11$) (Table 6). When sediment was present, χ^0, χ^1, and ${}^v\chi^{3c}$ described 85.0% of the variation in toxicity for all compounds combined ($P > F = 0.003$, $d.f. =$

Table 6. Summary of best regression analyses for linear solvation energy (LSE) and molecular connectivity (MC) descriptors

Descriptor type Compound class (treatment)[a]	Constant (± SE)	Independent variables[b] VI/100	π^*	α	β	N	P	Adjusted r^2
LSE								
OP, C (W)	2.68 (131.9)	−99.5 (72.5)	182.5 (37.4)	−291.6 (141.1)		12	<0.09	0.36
OP (W)	0.16 (0.15)		−0.34 (0.12)		0.27 (0.07)	7	<0.04	0.70
C (W)	1.25 (0.48)			−0.36 (0.41)		5	<0.13	0.46
OP, C (S)	−25.8 (62.4)	−96.6 (40.4)	109.7 (41.4)	−132.5 (70.0)	47.6 (27.3)	12	<0.05	0.52
OP (S)	−1.2 (0.59)	1.18 (0.43)				7	<0.04	0.52
C (S)	14.3 (1.67)	−4.97 (1.21)	−2.77 (0.72)		−3.36 (0.45)	5	<0.04	0.52

Descriptor type Compound class (treatment)[a]	Constant (± SE)	χ^0	χ^1	${}^v\chi^2$	χ^3	${}^v\chi^3$	χ^{3c}	${}^v\chi^0$	N	P	Adjusted r^2
MC											
OP, C (W)	0.25 (0.24)	0.28 (0.08)	0.53 (0.15)		−0.14 (0.07)				12	<0.05	0.46
OP (W)	0.28 (0.08)	−0.05 (0.07)		0.05 (0.005)					7	<0.002	0.92
C (W)	1.00 (0.09)						0.59 (0.07)		5	<0.003	0.95
OP, C (S)	2.16 (0.62)	−1.53 (0.21)	2.18 (0.27)			0.47 (0.12)			12	<0.003	0.85
OP (S)	0.54 (0.40)			−1.45 (0.41)		2.57 (0.62)	−2.55 (0.59)		7	<0.04	0.85
C (S)	3.75 (1.52)				1.04 (0.19)			−0.83 (0.16)	5	<0.04	0.92

[a]Organophosphorus compounds and carbamates are denoted OP and C, respectively. Treatment type is water only (W) or spiked sediment (S).
[b]Standard errors are given in parentheses.

11). Separating the chemicals into structural classes further increased descriptive ability. In the case of carbamates, the use of χ^{3c} gave the best result in water-only systems ($r^2 = 0.947$, $P > F = 0.003$, $d.f. = 4$) (Table 6), whereas χ^3 and ${}^v\chi^0$ produced the highest adjusted r^2 value for carbamates in the spiked-sediment systems ($r^2 = 0.92$, $P > F = 0.04$, $d.f. = 4$). MC parameters were also useful in describing OP toxicity. Using χ^0 and ${}^v\chi^2$ as independent variables gave the best results in water-only systems ($r^2 = 0.923$, $P > F = 0.002$, $d.f. = 6$), whereas the combination of ${}^v\chi^2$, ${}^v\chi^3$, and ${}^v\chi^{3c}$ gave the highest adjusted r^2 value in systems with sediment ($r^2 = 0.845$, $P > F = 0.035$, $d.f. = 6$). Relevant statistical data that summarize the best regression for each type of analysis are given in Table 6.

DISCUSSION

The insecticides were tested against midges under two distinct environmental conditions. First, toxicity was assessed in water-only exposures in the absence of any sorbent other than the midges. Second, sediment was added as a potential sorbent that could reduce exposure from water and add exposure through ingestion.

In both cases, three main processes were involved in the ultimate expression of toxicity in the midge: (a) factors dictating the amount of accumulation of each insecticide; (b) biological factors determining the disposition of each chemical in the midge, and (c) factors dictating the fit of each chemical to the receptor site of AChE. These processes were composed of several substeps. For instance, accumulation of each insecticide is determined by the amount of insecticide sorbing to sediment (when present), the solubility of each insecticide in water, the rate of volatilization, the hydrolytic stability of each chemical, the susceptibility to microbial degradation, and the amount of insecticide attaching to and penetrating into the midge. Penetration into the midge could occur through epicuticular absorption or uptake from ingested sediment. Once inside the midge, various biological factors become relevant. These include the distribution of each chemical within the midge, the kind and amount of metabolism, and the rate of elimination. Finally, the fit of each chemical into the active site of AChE, the inherent capacity for inhibition of AChE, and possible involvement of noncholinergic target sites must be considered.

The physical and structural parameters used to describe changes in toxicity were of two types. Log K_{ow}, HLC, and MV were employed as unidimensional descriptors that were individually capable of describing parts of the accumulation process. Log K_{ow} was used to describe the relative lipophilicity of the OP and carbamate insecticides and to estimate hydrophobic interactions with sediment and lipophilic partitioning into the midges. HLC values, also known as air/water partition coefficients, were used to give an estimate of the ability of each chemical to leave the test system through evaporative processes [25]. HLC is relevant to this study because highly volatile insecticides could escape from water and thus not be available for absorption into the midge. Finally, MV was used to determine whether the molecular size of each insecticide could affect partitioning, membrane penetration, and accumulation of the insecticides in the midge.

In contrast, MC and LSE are multidimensional descriptors that may be capable of estimating various parts of the accumulation process, the subsequent biological fate of each chemical in the midge, and the fit of each molecule into the three-dimensional AChE receptor. Because AChE inhibition in midges is ultimately determined by all three processes, it is likely that multidimensional descriptors would be needed to relate the changes in toxicity of the OP and carbamate insecticides to structural properties.

Results with unidimensional descriptors

None of the unidimensional descriptors was sufficient to describe the variation in toxicity among the tested cholinergic pesticides. In the water-only systems, there were no physical impediments to absorption of the insecticides by the midge other than volatilization from water and absorption to glassware. HLC values for leptophos and propoxur were high (Table 3), suggesting that loss of insecticide through volatilization could decrease exposure of the midges to the insecticides. However, midges exposed to leptophos and propoxur showed cholinergic symptoms within 30 min of exposure. Thus, volatilization apparently did not dissipate sufficient chemical to prevent intoxication.

Several studies have demonstrated the utility of MV in explaining the toxicity of narcotic chemicals. For instance, McGowan and Mellors [26] found a strong correlation between molecular size and toxicity for many narcotic chemicals in fish. In that study, the tested chemicals had a size distribution similar to that of the 12 insecticides employed for this work and included a variety of functional groups. However, the correlations between pre-

dicted and observed toxicity for chemicals with polar functional groups, for example, ethanediol and 2,2′-dihydroxydiethylamine, were poor, which was attributed to the interaction of the polar functional groups with water [26]. Such interactions are likely for the cholinergic compounds used in this study [11,27] and may partially account for the failure of MV to describe the toxicity of these insecticides. Of potentially greater importance in explaining the lack of correlation between MV and midge toxicity is that all of the insecticides used in this study had a specific mode of action to which MV does not adequately relate, because MV does not describe the fit of the insecticides to the target site receptor or the distribution, metabolism, or elimination processes that occur with these insecticides. MV appears to have greater explanatory power for chemicals that exert toxicity by means of a nonreactive chemical narcosis, in which case toxicity is believed to be caused by the physical presence of a molecule in a biological membrane [28].

Log K_{ow} has been used to predict both fate [4,29] and toxicity [8,30] of a large number of neutral, lipophilic chemicals with a nonspecific, narcosis-type toxicity. Like MV, which is correlated with log K_{ow} for nonpolar compounds, log K_{ow} was insufficient to describe the toxicity of the studied insecticides, as might be expected as the insecticides were inhibitors of AChE and not primarily indifferent narcotics. Thus, toxicity would be related not only to lipid partitioning but also to a host of other factors, such as metabolism, distribution in the midge, and three-dimensional fit of each molecule into the receptor site. These phenomena are, at best, only partially described by log K_{ow} [26]. Even so, De Bruijn and Hermens [31] found a strong correlation between log K_{ow} and uptake (k_1) and elimination (k_2) rate constants for a series of 12 OP compounds in guppies. The lack of a consistent relationship between toxicity and log K_{ow} in our study suggests that the predictive ability of K_{ow} breaks down in accounting for internal processes in the midge. In addition, most of the insecticides studied by De Bruijn and Hermens [31] were not metabolized during the short-term uptake phase, and only a few were metabolized during the longer elimination phase. Those compounds metabolized during the measurement of k_2 departed significantly from the regression between log K_{ow} and elimination [31]. It is reasonable for the kinetic behavior of unmetabolized OPs to resemble that of neutral lipophilic contaminants in these restricted measurements.

In our case, however, the biological measurement used in the regressions was mortality, presumably due to cholinergic poisoning. It represents the cumulative sum of all of the events leading to accumulation (including passage through the membrane) and all of the biochemical events that subsequently occur in the midge. Because most of the latter require specific types of interaction with three-dimensional receptors, nonspecific descriptors such as log K_{ow} were insufficient to describe the suite of processes leading to toxicity. The situation was further complicated by metabolism. As previously noted, metabolism obscured the relationship between k_2 and log K_{ow} among the OPs examined by De Bruijn and Hermens [31]. Our toxicity data were scored after 24 h of exposure, at which point all of the insecticides had undergone some metabolism in the midge [7,32] and possibly transformation in the water and sediment [7]. An additional difference between the current study and that of De Bruijn and Hermens [31] is that they worked with a homologous series of *O,O*-dimethyl-*O*-phenylphosphorothioates. Our group of test chemicals included not only OP chemicals of various types but also carbamates of different structural classes. It has been shown elsewhere that the descriptive value of many unidimensional QSAR parameters is reduced when chemicals of different classes are analyzed simultaneously [8].

The absence of correlation between toxicity of the insecticides in water-only systems and log K_{ow} implies that transport processes other than lipophilic partitioning are contributing to toxicity in the midge. When sediment was present, log K_{ow} was effective in describing 50% of the variation in toxicity among the 12 tested chemicals (Table 6). Thus, hydrophobic binding of the insecticides to sediment appears to be a process that can alter toxicity of most of the insecticides by reducing exposure of the midges to the chemicals. This influence is even more noticeable when the relative toxicities in the presence and absence of sediment are compared. In this case, log K_{ow} described over 80% of the variation in the ratio of the two EC50 values, indicating that hydrophobic binding of the insecticides to sediment is a primary difference between the two systems. However, the failure to fully describe changes in toxicity in the sediment systems indicates that additional sorption reactions, which are specific in nature (e.g., hydrogen bonding) and are not described by log K_{ow}, could be occurring. For instance, parathion can sorb to sediment through a variety of binding mechanisms in addition to hy-

drophobic interactions [32,33]. The specific sorption reactions that parathion undergoes vary with physical conditions and are not predictable from log K_{ow} [12,32].

Results with multidimensional descriptors (LSE and MC)

LSE attempts to describe biological phenomena such as toxicity in terms of solubilization of the toxicant molecule in different media. In our case, toxicity ultimately depended on the transfer of dissolved insecticide in water into a second solvent phase, represented by accumulation into the midge and multiple solutions and transfers during distribution within the midge. The solubilization process in a given medium is thought to consist of three distinct steps: (a) a cavity must be formed in the solvent into which the solute (toxicant) can enter; (b) individual toxicant molecules must be separated from bulk solute and be deposited into the cavity; and (c) interactions between the solvent and the toxicant can then occur, for example, hydrogen bonding, which alter solubility [34]. The four LSE parameters, VI/100, π^*, α, and β, describe these processes according to the steric and electronic properties of the toxicant molecule [34]. LSE has been useful in predicting the toxicity of chemicals with diverse functional groups in organisms ranging from bacteria [28] to fish [35].

In the absence of sediment, the ability of LSE to describe changes in toxicity was limited. LSE described only 36% of the variation in toxicity when OP and carbamate insecticides were analyzed together. The adjusted r^2 values improved to 0.70 and 0.46 for OPs and carbamates, respectively, when the compounds were separated by chemical class; for both the OPs and the carbamates analyzed separately, β emerged as a dominant factor in the regressions (Table 6). The prominence of β is interesting, although a variety of implications of this finding are possible. For instance, high β favors solubility in water [35], which should decrease the penetration of the insecticides into the midge and lower toxicity. Our data do not suggest that penetration into the midge was impeded. Alternatively, changes in β among the insecticides could reflect susceptibility to alkaline hydrolysis in water or nucleophilic attack leading to phosphorylation or carbamylation in the midge. Attempts failed to correlate toxicity with literature-derived hydrolysis rate constants for the 12 studied insecticides. Thus, the LSE β parameter may not be describing solubility or stability in water but the relationship to enzymatic reactions, of various sorts, in the midge, wherein degradative and activating metabolism may be acting in opposition. The potential for these competing reactions to occur prevents the tying of β to any particular chemical or biological process without further research.

When sediment was present, LSE r^2 values did not change significantly when compared to the water-only results, except when carbamates were analyzed alone (Table 6). In this case, an adjusted r^2 value of 0.96 was achieved using VI/100, α, and β as independent variables. In the sediment systems, β still emerged as a significant independent variable when carbamates were analyzed alone and when OPs and carbamates were analyzed together. Thus, the ability of each insecticide to act as a hydrogen acceptor appears to be critical to one or more processes involved in toxicity. VI/100, describing the energy necessary to separate insecticide molecules from bulk solution, was prominent in all three regressions involving sediment. The energetics of the solvation process are likely changed by sediment sorption, thus explaining the consistent importance of VI/100 in the presence of sediment. Unlike log K_{ow}, which encodes information primarily about hydrophobicity of the chemical, VI/100 may have the power to describe not only lipophilic interactions, but specific binding mechanisms peculiar to particular functional groups as well.

Like LSE, MC parameters have been used successfully to describe and predict the toxicity of narcotic chemicals with a range of functional groups to a large number of species [36,37]. MC indexes have potential advantages over strictly physical parameters such as HLC, log K_{ow}, and MV, because MC indexes include additional information about molecular size, skeletal branching, unsaturation, heteroatom content, and tertiary structure [37]. Thus, although MC has shown utility primarily in the prediction of toxicity of narcotic chemicals, it encodes enough information to make it potentially useful in the description of toxicity of metabolizable chemicals with specific modes of action.

The value of MC in the toxicological analysis of cholinergic chemicals is evident in Table 6. MC parameters yielded r^2 values ≥ 0.85 in every case, except when OPs and carbamates were considered together in water-only systems, for which the adjusted $r^2 = 0.46$. However, when sediment was present, r^2 improved to 0.85 when χ^0, χ^1, and χ^{3c} were used as independent variables. It is clear from the consistent appearance of χ^3 and χ^{3c} that the three-dimensional structure of the molecules is im-

portant in determining the toxicity of both OPs and carbamates in water-only and spiked-sediment systems. Because LSE parameters and log k_{ow}, which characterize various aspects of solubility and sorption, described about 50% of the variation in toxicity data in the presence of sediment, the improvement in adjusted r^2 to >0.90 with MC indexes suggests that MC indexes may be describing higher level interactions where three-dimensional fit of the molecules into specific receptors is required. This interpretation is especially compelling in water-only systems, where sorption to sediment is not relevant and toxicity could not be explained by solubility-driven parameters such as log K_{ow} or LSE. It is likely that the very high adjusted r^2 values for MC indexes indicate that they are defining interactions of the insecticides with biological target sites that govern the processes of accumulation, distribution, biotransformation, and inhibition of AChE.

Although MC indexes were useful in describing changes in observed toxicity, various problems are evident. First, there exists the possibility of chance correlation of MC indexes with toxicity. Hall and Kier [37] estimated that the probability of generating random correlations for r^2 values > 0.49 was 0.011%. However, the probability of chance correlation ultimately depends on the ratio of observations to independent variables for which a ratio >10 has been prescribed [36]. The number of chemicals used in this study was too small to meet that criterion in every case. Thus, the results of this MC analysis must be considered preliminary. A second difficulty is that it is not possible to attribute particular biological or physical effects to specific molecular characteristics or functional groups. Additional work will be required to discern whether the consistent appearance of χ^3 and χ^{3c} in the MC analyses is describing a particular biological process or whether it represents an estimation of all reactions combined. Finally, as with most other QSAR approaches, selecting the appropriate groups of MC indexes to describe a process must be done by regression analysis. Changes in the group of molecules being described may necessitate the use of different indexes to assess variability in biological effects. For example, in the water-only exposures, changes in carbamate toxicity were best described by χ^{3c}, whereas OPs were best described by a combination of χ^1 and χ^2. When these two groups were combined, χ^0, χ^1, and χ^3 were required to yield the best description of the effect of chemical structure on toxicity. Thus, the descriptor or combination of descriptors that yields the best regression cannot be predetermined.

In general, these data indicate that sorption is a primary determinant of insecticidal activity in systems with sediment, even for chemicals that are rather water soluble. Thus, log k_{ow} was significantly correlated with toxicity in systems with sediment. However, once sorption is accounted for, the ability of each chemical to be dissolved in water and absorbed into the midge, then to negotiate various biochemical processes en route to the target site, becomes important. Because the latter involves a suite of potentially competing processes, the complexity of determining toxicity renders unidimensional descriptors such as HLC and MV ineffective. Our hypothesis that multidimensional descriptors would better describe the several processes leading to toxicity was upheld. LSE and MC indexes provided better descriptions of variation in toxicity among the insecticides than the analyzed unidimensional parameters. However, we are still not able to identify which chemical or biological processes are being described by the more effective multidimensional descriptors. Nor are we able to attribute changes in toxicity to any specific functional group. Our data suggest that it will become possible to expand predictive capabilities from nonpolar chemicals of high hydrophobicity to polar chemicals with diverse functional groups. Using MC and LSE models may provide this ability when more is known about which processes are being described. Alternatively, research with MC indexes or LSE parameters may not itself provide this information, but may help to identify those chemical and biological processes that should be assessed with less complex descriptors.

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REFERENCES

1. **Knezovich, J.P.** and **F.L. Harrison.** 1988. The bioavailability of sediment-sorbed chlorobenzenes to larvae of the midge, *Chironomus decorus. Ecotoxicol. Environ. Saf.* **15**:226–241.

2. **Landrum, P.F.** and **J.A. Robbins.** 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In R. Baudo, J.P. Giesy and H. Muntau, eds., *Sediments: Chemistry and Toxicity of In-Place Pollutants.* Lewis, Ann Arbor, MI, pp. 237–263.
3. **Karickhoff, S.W., D.S. Brown** and **T.A. Scott.** 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Res.* **13**:421–428.
4. **Adams, W.J., R.A. Kimerle** and **R.G. Mosher.** 1985. Aquatic safety assessments of chemicals sorbed to sediments. In R.D. Cardwell, R. Purdy and R.C. Bahner, eds., *Aquatic Toxicology and Hazard Assessment: Seventh Symposium.* STP 854. American Society for Testing and Materials, Philadelphia, PA, pp. 429–453.
5. **Landrum, P.F.** and **W.R. Faust.** 1993. Effect of variation in sediment composition on the uptake rate coefficient for selected PCB and PAH congeners by the amphipod, *Diporeia* spp. In M.A. Mayes and M.G. Barron, eds., *Aquatic Toxicology and Hazard Assessment: Fourteenth Volume.* STP 1124. American Society for Testing and Materials, Philadelphia, PA (in press).
6. **Veith, G.D., D.L. Defoe** and **B.V. Bergestedt.** 1978. Measuring and estimation of the bioconcentration factor of chemicals in fish. *J. Fish. Res. Board Can.* **36**:1040–1048.
7. **Lydy, M.J., K.A. Bruner, D.M. Fry** and **S.W. Fisher.** 1990. Effects of sediment and route of exposure on the toxicity and accumulation of neutral lipophilic and moderately water soluble metabolizable compounds in the midge, *Chironomus riparius.* In W.G. Landis and W.H. van der Schalie, eds., *Aquatic Toxicology and Risk Assessment: Thirteenth Volume.* STP 1096. American Society for Testing and Materials, Philadelphia, PA, pp. 140–164.
8. **Eriksson, L., J. Jonsson, S. Hellberg, F. Lindgren, M. Sjostrom** and **S. Wold.** 1991. A strategy for ranking environmentally occurring chemicals. V. The development of two genotoxicity QSARs for halogenated aliphatics. *Environ. Toxicol. Chem.* **10**:585–596.
9. **Van Leeuwen, C.J., P.T.J. Van Der Zandt, T. Aldenberg, H.J.M. Verhaar** and **J.L.M. Hermens.** 1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I. Narcotic industrial pollutants. *Environ. Toxicol. Chem.* **11**: 267–282.
10. **Hill, E.F.** and **W.J. Fleming.** 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Environ. Toxicol. Chem.* **1**:27–38.
11. **Sharom, M.S., J.R.W. Miles, C.R. Harris** and **F.L. McEwen.** 1980. Persistence of 12 insecticides in water. *Water Res.* **14**:1089–1093.
12. **Briggs, G.G.** 1969. Molecular structure of herbicides and their sorption by soils. *Nature* **223**:1288.
13. **Aldridge, W.N.** and **E. Reiner.** 1972. *Enzyme Inhibitors as Substrates.* Interactions of Esterases with Esters of Organophosphorus and Carbamic Acids. North-Holland, Amsterdam, The Netherlands.
14. **Miyamoto, J.** and **H. Ohkawa.** 1979. Oxidative processes in pesticide transformation. In H. Geissbuehler, ed., *Advances in Pesticide Science*, Part 3. Pergamon, Oxford, UK, pp. 508–515.
15. **Estenik, J.F.** and **W.J. Collins.** 1978. In vivo and in vitro studies of mixed function oxidase in an aquatic insect, *Chironomus riparius.* In M.A.Q. Khan, J.J. Lech and J.J. Menn, eds., *Pesticide and Xenobiotic Metabolism in Aquatic Organisms.* ACS Symposium Series 99. American Chemical Society, Washington, DC, pp. 349–370.
16. **Suntio, L.R., W.Y. Shiu, D. Mackay, J.N. Seiber** and **D. Glotfelty.** 1988. Critical review of Henry's law constants for pesticides. *Rev. Environ. Toxicol. Chem.* **103**:1–59.
17. **Hansch, C.** and **A. Leo.** 1979. *Substituent Constants for Correlation Analysis in Chemistry and Biology.* John Wiley & Sons, New York, NY.
18. **Kier, L.B.** and **L.H. Hall.** 1986. *Molecular Connectivity in Structure-Activity Analysis.* John Wiley & Sons, New York, NY.
19. **Hickey, J.P.** and **D.R. Passino-Reader.** 1991. Linear solvation energy relationships: "Rules of thumb" for estimation of variable values. *Environ. Sci. Technol.* **25**:1753–1760.
20. **U.S. Environmental Protection Agency.** 1975. Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. EPA 6601/3-75-009. Washington, DC.
21. **Detra, R.L.** 1982. Organophosphate toxicity of larval *Chironomus riparius* Meigen (Diptera: Chironomidae). Ph.D. thesis. Ohio State University, Columbus, OH.
22. **Fisher, S.W.** 1985. Effects of pH on the toxicity and uptake of ^{14}C-lindane in the midge, *Chironomus riparius. Ecotoxicol. Environ. Saf.* **10**:202–208.
23. **Finney, D.J.** 1971. *Probit Analysis.* Cambridge University, London, UK.
24. **SAS Institute.** 1982. *SAS® User's Guide. Version 5 Edition.* Cary, NC.
25. **Kenega, E.E.** and **C.A.I. Goring.** 1980. Relationship between water solubility, soil sorption, octanol-water partitioning and the concentration of chemicals in biota. In T.G. Eaton, P.R. Parrish and A.C. Hendricks, eds., *Aquatic Toxicology: Third Volume.* STP 707. American Society for Testing and Materials, Philadelphia, PA, pp. 78–115.
26. **McGowan, J.C.** and **A. Mellors.** 1986. Molecular volumes and the toxicities of chemicals to fish. *Bull. Environ. Contam. Toxicol.* **36**:881–887.
27. **Kuhr, R.J.** and **H.W. Dorough.** 1976. *Carbamate Insecticides: Chemistry, Biochemistry and Toxicology.* CRC, Cleveland, OH.
28. **Kamlet, M.J., R.M. Doherty, G.D. Veith, R.W. Taft** and **M.H. Abraham.** 1986. Solubility properties in polymers and biological media. 7. An analysis of toxicant properties that influence inhibition of bioluminescence in *Photobacterium phosphoreum* (the Microtox test). *Environ. Sci. Technol.* **20**:690–695.
29. **Rogers, C.L.** 1980. A study of penetration and knockdown in the housefly with five organophosphate insecticides of significantly different partition coefficients. M.S. thesis. Ohio State University, Columbus, OH.
30. **Hodson, P.V., D.G. Dixon** and **K.L.E. Kaiser.** 1988. Estimating the acute toxicity of waterborne chemicals in trout from measurements of median lethal dose and the octanol water partition coefficient. *Environ. Toxicol. Chem.* **7**:443–454.
31. **de Bruijn, J.** and **J. Hermens.** 1991. Uptake and elim-

ination kinetics of organophosphorus pesticides in the guppy (*Poecilia reticulata*): Correlation with the octanol/water partition coefficient. *Environ. Toxicol. Chem.* **10**:791–805.

32. **Metcalf, R.L.** 1971. Structure-activity relationships for insecticidal carbamates. *Bull. WHO* **44**:43–78.
33. **Saltzman, S.** and **S. Yariv.** 1976. Infrared and X-ray study of parathion-montmorillonite sorption complexes. *Soil Sci. Am. J.* **40**:34–38.
34. **Kamlet, M.J., R.M. Doherty, J.M. Aboud, M.H. Abraham** and **R.W. Taft.** 1986. Solubility: A new look. *Chemtech* **202**:566–576.
35. **Kamlet, M.J., R.M. Doherty, M.H. Abraham, G.D. Veith** and **D.J. Abraham.** 1987. Solubility properties in polymers and biological media. 8. An analysis of the factors that influence toxicities of organic nonelectrolytes to the golden orfe fish (*Leuciscus idus melanotus*). *Environ. Sci. Technol.* **21**:149–155.
36. **Protic, M.** and **A. Sabljic.** 1989. Quantitative structure-activity relationships of acute toxicity of commercial chemicals on fathead minnows: Effect of molecular size. *Aquat. Toxicol.* **14**:47–64.
37. **Hall, L.H.** and **L.B. Kier.** 1984. Molecular connectivity of phenols and their toxicity in fish. *Bull. Environ. Contam. Toxicol.* **32**:354–362.