

## HAZARD EVALUATION OF TEN ORGANOPHOSPHORUS INSECTICIDES AGAINST THE MIDGE, *CHIRONOMUS RIPARIUS* VIA QSAR

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Toxicities of ten organophosphorus (OP) insecticides were measured against midge larvae (*Chironomus riparius*) under varying temperature (11, 18, and 25°C) and pH (6, 7, and 8) conditions and with and without sediment. Toxicity usually increased with increasing temperature and was greater in the absence of sediment. No trend was found with varying pH. A series of unidimensional parameters and multidimensional models were used to describe the changes in toxicity. Log  $K_{ow}$  was able to explain about 40–60% of the variability in response data for aqueous exposures while molecular volume and aqueous solubility were less predictive. Likewise, the linear solvation energy relationship (LSER) model only explained 40–70% of the response variability, suggesting that factors other than solubility were most important for producing the observed response. Molecular connectivity was the most useful for describing the variability in the response. In the absence of sediment,  $^1\chi^v$  and  $^3\kappa$  were best able to describe the variation in response among all compounds at each pH (70–90%). In the presence of sediment, even molecular connectivity could not describe the variability until the partitioning potential to sediment was accounted for by assuming equilibrium partitioning. After correcting for partitioning, the same molecular connectivity terms as in the aqueous exposures described most of the variability, 61–87%, except for the 11°C data where correlations were not significant. Molecular connectivity was a better tool than LSER or the unidimensional variables to explain the steric fitness of OP insecticides which was crucial to the toxicity.

**Keywords:** Organophosphate insecticides; midges; molecular connectivity; QSAR

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## INTRODUCTION

Understanding the factors that govern the toxicity of compounds released to the environment is important for the evaluation of hazard. Such understanding can be achieved through the development and use of quantitative structure-activity relationships (QSARs) as a tool to predict the biological impact and environmental fate of chemicals. Such models rely upon the chemical's intrinsic topological and structural properties to describe toxicity and the potential environmental hazards of chemicals in varying environments [1, 2].

Traditionally, the *n*-octanol/water partition coefficient ( $K_{ow}$ ), which represents lipophilicity of chemicals, has been adopted as a general descriptor of the toxicity and environmental behavior of neutral membrane narcotics such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and various other halogenated hydrocarbons. These neutral hydrophobic compounds lack specific functional groups capable of interacting with particular biological target sites such as enzymes or protein receptors. Instead, these membrane narcotics passively partition across lipid membranes, and ultimately dissolve in lipids of the myelin sheath around axons to cause a nonspecific narcosis [3]. Therefore, the environmental fate and biological activity of membrane narcotics depend heavily on water/lipid partitioning so that  $\log K_{ow}$  values of hydrophobic, nonpolar narcotic chemicals can be used to predict their environmental fate [4–6] and biological impact [7–9].

Acetyl cholinesterase (AChE) inhibitors, such as organophosphorus (OP) compounds, are used primarily as insecticides in agriculture and urban pest control. Their skeletal complementarity with the active site of target enzyme, acetyl cholinesterase (AChE), mimics the gross molecular shape of the natural substrate, acetylcholine (ACh) [10]. Thus, OP compounds exercise their toxicity through the inhibition of a specific nervous system enzyme, AChE [11]. Even though some OP's are moderately lipophilic ( $\log K_{ow} > 3$ ), OP's are comparatively hydrophilic and chemically reactive. In addition, they are generally biodegradable, thus, they show only a slight tendency for bioaccumulation [12]. Moreover, AChE inhibitors can be activated and/or detoxified through oxidative processes, for example oxidative desulfuration of organic phosphorothionates and conversion of the thioether moiety to the respective sulfoxide and sulfone [13, 14]. In contrast to membrane narcotics, lipophilicity is not sufficient to explain the fate and toxicity of OP's in the environment [15, 16]. However, key characteristics of OP functional groups that bestow the insecticidal activity can be encoded in multi-

dimensional descriptors and used to predict the fate and toxicity of these insecticides [14, 15, 17].

Basak *et al.* [18] reported that two multidimensional molecular connectivity (MC) indices,  $^1\chi$  and  $^1\chi^v$ , showed high correlations with  $LC_{50}$  values for industrial pollutants in fathead minnows. Fisher *et al.* [15] demonstrated the utility of two multidimensional molecular models, linear solvation energy relationship (LSER) and MC, in regression analyses, as comprehensive indices of OP and carbamate toxicity in aquatic systems. Similarly, the  $LC_{50}$  of OP compounds was found to be best described by a series of parameters and indices that incorporate not only the character of the molecular shape but also the characteristic of the leaving group and the electronic character of the oxone form [17]. In present study, LSER and MC parameters were used in the regression analyses to evaluate their potential as chemical descriptors of OP toxicity under varying environmental conditions of different temperatures (11, 18, and 25°C), pH (6, 7, and 8) and in the presence and absence of sediment as a binding phase.

## MATERIALS AND METHODS

### Organisms

Fourth instar midge larvae, *Chironomus riparius*, were used as test organisms. The midges were raised in the laboratory [19]. The midge larvae were initially collected in 1974 from the Jackson Pike Water Treatment Plant in Columbus, OH and have been maintained in culture with periodic outbreeding to wild midges.

### Compounds

Reagent grade organophosphorus compounds ( $\geq 97\%$  purity) were used for all toxicity tests (Tab. I). All chemicals were purchased from Chem Service Inc., West Chester, PA. These chemicals demonstrate a wide range of water solubility and functional groups (Tab. I).

### Unidimensional Parameters for Pesticides Used in the Tests

$\log K_{ow}$  and water solubility values were generally obtained from the literature [16, 20–23]. However, no value for coumaphos was found and the  $\log K_{ow}$  was estimated using LSER regression with the current compounds

TABLE I Organophosphorus insecticides used in the toxicity tests

Pesticide (common name)	Chemical name	log $K_{ow}$	Mol. vol. ( $cm^3/mol$ ) <sup>f</sup>	Water sol. ( $mmol/L$ )	Mol. Wt.
Azinphos-Methyl	<i>O,O</i> -Dimethyl-S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl methyl] phosphorodithioate	2.7 <sup>a</sup>	209.25	0.095 <sup>a</sup>	317
Chlorpyrifos	<i>O,O</i> -diethyl <i>O</i> -(3,5,6-trichloro-2-pyridinyl) phosphorothioate	5.27 <sup>c</sup>	215.03	0.0009 <sup>a</sup>	351
Coumaphos	<i>O</i> -(3-chloro-4-methyl-2-oxo-2H-1-benzo-pyran-7-yl) <i>O,O</i> -diethyl phosphorothioate	5.16 <sup>b</sup>	249.9	0.0041 <sup>g</sup>	362
Diazinon	<i>O,O</i> -diethyl <i>O</i> -[6-methyl-2-(methyl-ethyl)-4-pyrimidinyl] phosphorothioate	3.81 <sup>c</sup>	230.3	0.131 <sup>a</sup>	304
Dicrotophos	( <i>E</i> )-3-(dimethyl amino)-1-methyl-3-oxo-1-propenyl dimethyl phosphate	0.0 <sup>c</sup>	174.77	miscible <sup>d</sup>	237
Disulfoton	<i>O,O</i> -diethyl <i>O</i> -[6-methyl-2-(methyl-ethyl)-4-pyrimidinyl] phosphorothioate	4.0 <sup>a</sup>	204.83	0.091 <sup>a</sup>	274
Fensulfothion	<i>O,O</i> -diethyl <i>O</i> -[4-(methyl sulfinyl) phenyl] phosphorothioate	2.23 <sup>d</sup>	218.73	5.0 <sup>g</sup>	308
Fenthion	<i>O,O</i> -dimethyl <i>O</i> -[3-methyl-4-(methyl thio) phenyl] phosphorothioate	4.1 <sup>a</sup>	198.77	0.18 <sup>a</sup>	278
Fonofos	<i>O</i> -ethyl <i>S</i> -phenyl ethyl phosphonodithioate	3.9 <sup>c</sup>	187.03	0.053 <sup>d</sup>	246
Terbufos	<i>S</i> -[[[(1,1-dimethyl ethyl)thio]methyl] <i>O,O</i> -diethyl-phosphorodithioate	4.48 <sup>c</sup>	218.92	0.016 <sup>d</sup>	288

<sup>a</sup> Suntio *et al.* [20];

<sup>b</sup> Calculated from LSER:  $\log K_{ow} = 0.41(1.3) + 5.11(1.1) V_d/100 - 5.9(2.3)\alpha - 2.40(0.71)\beta$   $r^2 = 0.83$ ,  $p < 0.001$ ,  $n = 14$ ;

<sup>c</sup> Hansch *et al.* [23];

<sup>d</sup> Montgomery [22];

<sup>e</sup> De Bruijn and Hermens [16];

<sup>f</sup> Data calculated according to McGowan [24];

<sup>g</sup> Worthing and Hance [21].

and additional compounds in Fisher *et al.* [15]. Characteristic molecular volumes were calculated following the method of McGowan [24] (Tab. I).

#### Calculation of Multidimensional Molecular Descriptor for Pesticides Used in the Tests

Linear Solvation Energy indices were calculated from values for molecular fragments [25] (Tab. II). In this model, chemical characteristics are associated with molecular structure of chemicals through the energy needed to dissolve a solute in solvent molecules and the energy that is increased or decreased when electrostatic and hydrogen bonds between chemicals and medium are formed. Four variables were computed: (a) The endoergic energy term  $V_i/100$  is the intrinsic (van der Waals) molecular volume divided by a factor of one hundred to scale the parameter. This term denotes the free energy needed to isolate the solvent molecules to produce the cavity for solute; (b) The dipolar term  $\pi^*$  measures solute-solvent dipole interactions which can explain the molecule's ability to stabilize a surrounding charge or dipole through non-specific dielectric interactions; (c) The hydrogen bonding term  $\alpha$  is an estimation of acidity representing the ability of a solute to accept an electron and of a solvent to release an electron; (d) Another hydrogen bonding term,  $\beta$ , represents basicity or the ability of the solute molecule to release an electron and of a solvent to accept an electron.

Molecular Connectivity parameters were calculated using the MOLCON-X computer program [26]. Five orders of simple connectivity indices,  ${}^0\chi$ ,  ${}^1\chi$ ,  ${}^2\chi$ ,  ${}^3\chi$ ,  ${}^4\chi$ ,  ${}^3\chi_c$  and  ${}^4\chi_c$  [27], and three orders of simple shape parameters  ${}^1\kappa$ ,  ${}^2\kappa$ , and  ${}^3\kappa$  [28, 29] values were calculated. All  $\chi$  and  $\kappa$  values were recalculated with valence correlations and these were denoted  ${}^0\chi^v$ – ${}^4\chi^v$  and  ${}^3\chi_c^v$ ,  ${}^4\chi_c^v$  and  ${}^0\kappa_\alpha$ – ${}^3\kappa_\alpha$ . (Note: A zero order value can be calculated for the valence corrected  $\kappa$  values but not for the uncorrected  $\kappa$  by the MOLCON-X program [28, 29]). Molecular connectivity values were also calculated for the oxone form of the various molecules and designated with a subscript "ox", *e.g.*,  ${}^1\chi_{\text{ox}}^v$ . The  $\chi$  values encode information from the molecular size at the lowest level of connection to implicit information about the three dimension configuration at the higher levels of connection representing the complexity of the molecule. The  $\kappa$  values encode information about the shape of the molecule and encodes a value relative to the maximum number of bonds in the isomeric star graph and the minimum number in the isomeric linear graph [28, 29]. While all of the connectivity and LSER values were explored for utility in regression equations only those that provided the highest  $r^2$  values and that were consistent across essentially all conditions are reported (Tab. II).

TABLE II Molecular connectivity and linear solvation energy parameters for regression equations presented

Pesticide	Molecular connectivity parameters						Linear solvation energy parameters			
	$^0\chi^v$	$^1\chi^v$	$^2\chi^v$	$^4\chi^v$	$^4\chi_c^v$	$^3\kappa$	$^1\chi_{ox}^v$	$V_i/100$	$\alpha$	$\beta$
Azinphos-Methyl	12.87	9.51	9.98	4.00	0.34	3.56	8.41	1.434	0.0	2.11
Chlorpyrifos	13.63	8.71	7.03	3.59	0.11	4.57	7.61	1.473	0.0	1.62
Coumaphos	14.89	9.65	7.67	4.12	0.11	4.31	8.55	1.711	0.0	1.68
Diazinon	13.75	8.89	7.17	3.38	0.11	5.48	7.80	1.579	0.0	1.56
Dicrotophos	10.41	5.61	4.70	1.36	0.04	5.58	5.61	1.198	0.05	1.53
Disulfoton	12.37	10.18	10.14	6.64	0.34	7.04	9.08	1.404	0.0	1.30
Fensulfothion	13.15	9.64	7.61	3.83	0.11	5.27	8.54	1.498	0.0	1.68
Fenthion	12.25	8.38	7.03	2.70	0.11	3.77	7.28	1.362	0.0	1.34
Fonofos	11.00	9.0	10.30	5.66	0.58	3.96	7.91	1.282	0.0	1.21
Terbufos	13.45	10.22	12.26	8.20	0.95	7.88	9.12	1.500	0.0	1.30

### Toxicity Tests

Chironomids were exposed in triplicate both in presence and absence of sediment under yellow light to reduce the photolysis of pesticides [30]. The *water only* test was initiated by putting 500 ml of hard standard reference water (SRW) [31], adjusted to the appropriate temperature and pH, into 1 L beakers. Each beaker received 0.5 ml of a specific toxicant concentration dissolved in reagent grade acetone. Range finding tests were used to establish the test concentrations. Controls received 0.5 ml of reagent grade acetone only. Thereafter, twenty fourth-instar midges that were previously acclimated to the experimental temperature for at least 12 h were added to each beaker, and the beakers were held in an environmental chamber (Forma Scientific model 37844, Marietta, OH) on a photoperiod of 14/10 hour light/dark. After 24 h of exposure, impairment of the midges was evaluated. The criterion for effect was the failure of midges to do three figure-eight motions when pinched lightly with forceps. Failure to perform three figure-eight motions indicates that midges are affected enough to die within 24 hours [19]. This criterion has been widely applied to the evaluation of the chironomid health [15, 30, 32–34]. Toxicity tests were conducted at selected pHs (6, 7, and 8) and temperatures (11, 18, and 25°C) to represent several different exposure conditions.

The second method of exposure employed *spiked sediment*. Olentangy River (Columbus, OH) sediment (5 g) was placed into disposable culture tubes (16 × 125 mm) and dosed by pipetting 0.5 ml of a compound onto the sediment. The organic carbon content of this sediment (0.9%) had been previously determined by REALab, Wooster, OH. Dosed sediment was allowed to sit for one hour or until the solvent front reached the bottom of the

tubes. Hard SRW (10 ml) was added to each tube, the tubes were stirred vigorously with a metal spatula and then centrifuged for 5 min at high speed in a clinical centrifuge (Fisher Scientific, Fair Lawn, NJ). The resulting supernatants were removed and saved. The mixing and centrifugation were performed two more times for each tube. Sediment and combined supernatants (total 30 ml) were combined into a 1 L beaker along with 465 ml of fresh hard SRW. Twenty midges were added to each beaker and beakers were placed in an environmental chamber on a photoperiod of 14/10 hours. Controls were treated in the same manner, except that no pesticides were added. The affected midges were checked in the manner previously described. The pH of the SRW was checked at 1, 4, and 25 h after sediment was added. The changes at all pHs were minimal ( $< 0.1$  pH units).

#### Data Analysis

Toxicity data were analyzed using a Probit Analysis computer program to give  $EC_{50}$  values and 95% confidence intervals [35].  $EC_{50}$  values were considered statistically different if their 95% confidence intervals did not overlap. The unit of measure adopted for all experiments was  $\mu\text{M}$  of insecticide. In the sediment exposures, one milliliter (ml) of water was considered to weigh one gram (g). Thus, the weight (5 g) of *spiked sediment* and the water used to dose the sediment (30 ml) were added to 465 g (ml) water to give 0.5 kg of exposure medium in each test.

The  $EC_{50}$  values were regressed against each molecular index using Systat<sup>®</sup> 7.0 [36]. The  $EC_{50}$  values were regressed against each individual unidimensional variable ( $\text{Log } K_{ow}$ , molecular volume, and water solubility). Thereafter, the toxicity data were regressed against each individual MC and LSER parameter and forward stepwise regression analysis was used to obtain the "best" regression. Because of the small number of samples ( $n = 10$ ), only two parameter regressions were examined to determine the major factors describing the response data. Nine data sets, from the three pHs and three temperatures, were examined separately. Data sets were combined for each pH with temperature as an additional regression parameter. Ultimately, all data were combined when it was found that pH did not significantly influence the results. When the data sets were combined, additional parameters could be included in the regressions to help describe the variability in toxicity without having an over-determined model, a model with too many independent variables for the number of data pairs. At pH 7 and 25°C, the data from Fisher *et al.* [15] were added to the data from this study to examine a wider diversity of compounds. Regressions

were considered significant at  $p < 0.05$ . Slopes and intercepts were compared using student  $t$  test and were considered significant at  $p < 0.05$ . Regressions of  $1/EC_{50}$  and  $\log(1/EC_{50})$  were also explored.

## RESULTS

### Toxicity Tests

The toxicity of the 10 OP compounds varied significantly (Tabs. III and IV). In the *water only* treatment, chlorpyrifos was most toxic to *Chironomus riparius* at all 3 pH's, while midges were least susceptible to dicrotophos at

TABLE III Twenty-four hour  $EC_{50}$  values (nmol/L) of OP insecticides at three different temperatures (11, 18, and 25°C) and three different pHs (6, 7, and 8) in *water only* treatment to *Chironomus riparius*. Ninetyfive percent confidence intervals are given in parentheses

Compound (common name)	Temp. (°C)	24-hour $EC_{50}$ (nmol/L)		
		pH 6	pH 7	pH 8
Azinphos-Methyl	11	8.6(7.1–10.3)	19.5(16.3–22.4)	10.8(9.2–12.4)
	18	4.0(3.5–4.5)	4.6(4.1–4.4)	4.6(4.1–5.3)
	25	1.9(1.6–2.2)	3.9(3.5–4.4)	3.1(2.8–3.4)
Chlorpyrifos	11	1.9(1.8–2.1)	0.83(0.74–1.0)	1.5(1.3–1.6)
	18	0.49(0.40–0.57)	0.31(0.29–0.34)	0.74(0.66–0.83)
	25	0.28(0.23–0.31)	0.29(0.26–0.31)	0.37(0.34–0.43)
Coumaphos	11	99.8(73.5–146.8)	54.1(43.5–66.3)	103.0(82.3–130.4)
	18	67.1(56.9–77.1)	44.8(36.5–55.5)	31.8(26.1–38.6)
	25	17.1(13.5–21.8)	50.6(42.5–59.3)	23.2(19.1–28.0)
Diazinon	11	182.5(132.1–248.4)	213.5(166.5–269.3)	211.8(156.9–286.5)
	18	43.1(33.9–54.9)	80.3(60.4–109.9)	56.9(44.7–72.7)
	25	70.4(52.6–92.0)	38.2(32.4–52.8)	57.2(44.5–73.0)
Dicrotophos	11	688.2(433.2–1290)	865.8(705.5–1054)	474.7(353.6–662.4)
	18	645.8(509.5–839.9)	381.6(316.4–464.4)	580.2(447.7–765.0)
	25	325.0(275.8–375.9)	178.1(137.2–229.3)	388.9(327.2–472.2)
Disulfoton	11	177.0(114.0–271.2)	126.3(88.6–180.4)	82.1(55.0–122.4)
	18	103.5(75.0–141.2)	22.4(16.2–31.2)	63.5(46.9–83.2)
	25	28.5(21.1–39.5)	22.8(17.6–29.3)	23.6(16.4–33.4)
Fensulfothion	11	30.2(17.8–49.7)	13.2(9.6–17.7)	13.2(8.1–21.5)
	18	12.0(8.0–17.2)	5.5(4.0–7.5)	2.1(1.3–3.0)
	25	3.1(2.2–4.4)	4.3(2.9–6.1)	8.7(6.0–13.2)
Fenthion	11	20.2(17.2–23.4)	21.0(17.2–27.4)	10.1(8.2–12.2)
	18	9.7(8.1–11.6)	7.2(6.3–8.3)	9.5(8.5–10.8)
	25	5.3(4.5–6.2)	4.4(3.9–4.9)	4.4(3.8–5.0)
Fonofos	11	69.9(55.0–92.0)	122.5(111.9–132.8)	134.1(107–185)
	18	47.6(38.2–59.3)	41.9(38.4–46.0)	56.1(43.2–72.5)
	25	41.0(35.0–47.3)	49.9(44.4–56.0)	21.9(18.4–26.0)
Terbufos	11	185.2(134.9–272.2)	204.5(175.9–251.9)	248.9(200.7–337.6)
	18	113.3(91.6–140.9)	103.2(89.5–117.7)	60.9(50.0–72.3)
	25	58.0(45.5–71.7)	106.3(94.5–127.2)	23.9(19.5–28.3)

TABLE IV Twenty-four hour  $EC_{50}$  values of OP insecticides at three different temperatures (11, 18, and 25°C), and three different pHs (6, 7, and 8) in *spiked sediment* treatment to *Chironomus riparius* are shown in nmol/L. Ninety-five percent confidence intervals are given in parentheses

Compound (common name)	Temp. (°C)	24-hour $EC_{50}$ (nmol/L)		
		pH 6	pH 7	pH 8
Azinphos-Methyl	11	21.5 (16.0–28.2)	127.2 (109.5–143.2)	14.2 (10.9–18.1)
	18	11.7 (10.0–13.5)	22.3 (19.4–25.2)	8.5 (6.4–10.9)
	25	6.7 (5.4–7.9)	10.6 (8.7–12.2)	7.9 (6.7–9.0)
Chlorpyrifos	11	12.8 (11.1–14.6)	32.7 (29.5–36.8)	13.0 (11.1–15.2)
	18	2.1 (1.6–2.8)	10.2 (9.3–11.2)	3.4 (2.7–4.2)
	25	3.0 (2.5–3.7)	5.3 (4.5–6.2)	3.8 (3.0–4.7)
Coumaphos	11	561.3 (432.0–698.1)	646.5 (495.4–805.0)	486.9 (392.8–589.8)
	18	209.1 (166.2–258.0)	116.2 (80.5–164.6)	156.6 (110.4–192.8)
	25	142.1 (95.2–179.3)	156.2 (114.5–192.2)	38.5 (25.3–56.1)
Diazinon	11	810.0 (665.6–956.3)	263.5 (191.5–346.5)	455.8 (363.3–556.7)
	18	174.6 (121.1–238.9)	168.5 (118.7–227.1)	241.2 (178.1–308.6)
	25	177.9 (130.9–221.1)	194.0 (157.1–232.2)	188.5 (146.3–225.6)
Dicrotophos	11	412.4 (297.6–559.1)	476.8 (387.1–584.4)	444.6 (349.1–557.8)
	18	346.0 (291.8–405.8)	467.1 (375.8–587.4)	340.1 (279.8–407.3)
	25	298.8 (219.5–378.4)	264.3 (180.6–306.2)	278.1 (204.9–352.7)
Disulfoton	11	108.7 (61.9–188.4)	299.1 (237.7–366.1)	110.9 (81.3–142.3)
	18	115.4 (38.9–178.6)	72.6 (43.0–114.9)	47.0 (28.5–69.4)
	25	22.3 (12.5–36.5)	159.1 (91.2–208.5)	159.8 (114.9–204.1)
Fensulfothion	11	55.1 (35.5–75.3)	84.2 (63.1–105.6)	97.2 (60.6–141.4)
	18	40.5 (27.9–53.4)	40.5 (29.8–51.7)	10.9 (7.8–14.7)
	25	9.1 (6.3–12.6)	10.4 (7.5–14.0)	9.4 (6.7–13.00)
Fenthion	11	29.5 (23.1–38.1)	48.3 (39.7–59.4)	28.8 (23.0–37.2)
	18	18.4 (15.3–21.8)	16.2 (12.3–20.6)	21.4 (18.7–24.5)
	25	14.9 (12.2–17.2)	7.9 (6.0–9.6)	8.5 (6.7–10.3)
Fonofos	11	672.0 (580.0–767.4)	548.7 (464.2–640.9)	447.6 (345.0–616.9)
	18	326.6 (281.1–377.2)	374.1 (309.2–451.4)	205.1 (162.6–266.1)
	25	215.2 (182.5–245.6)	343.4 (298.7–393.3)	175.9 (144.1–208.1)
Terbufos	11	233.4 (192.3–284.2)	396.1 (305.3–542.8)	212.3 (171.1–262.5)
	18	143.1 (112.5–190.0)	135.5 (108.1–171.7)	121.6 (95.6–157.3)
	25	167.2 (139.5–197.2)	97.5 (78.4–117.2)	57.3 (47.3–69.5)

all pH's tested. Although changing pH appeared to significantly affect toxicity in some cases, there was no consistent relationship between pH and toxicity. In the *spiked sediment* treatment (Tab. IV), chlorpyrifos again was most toxic, and dicrotophos was generally the least toxic insecticide. However, at 11°C, chlorpyrifos was still most toxic, while diazinon was least toxic at pH 6 and dicrotophos at pH 7 and pH 8 (Tab. IV).

Temperature exerted the major change in toxicity in *water only* and *spiked sediment* systems. While toxicity generally increased with increasing temperature, some exceptions were observed. For instance, in *water only* treatments, coumaphos at pH 7 and dicrotophos at pH 8 were not significantly affected by the temperature. In the *spiked sediment* system, dicrotophos at

pH 6 and 8 and diazinon at pH 7 did not respond significantly to the changing temperature. For other chemicals like disulfoton and fonofos, there were no significant increases in toxicity between adjacent temperatures *i.e.*, 11 and 18°C or 18 and 25°C. However, statistically significant differences in toxicity were always found between 11 and 25°C (Tabs. III and IV). Except for the few exceptions previously mentioned, increases in toxicity ranged from 2 to 10 fold in the *water only* treatment and ranged from 2 to 13 fold in the *spiked sediment* treatment across the range of temperature.

Toxicity decreased significantly in *spiked sediment* treatment compared to the *water only* treatment. A ratio of the *spiked sediment* EC<sub>50</sub> to the *water only* EC<sub>50</sub> (S:W ratio) was calculated to summarize the influence of sediment on toxicity (Tab. V). From the ratio, it was found that chlorpyrifos toxicity was

TABLE V The ratio of toxicity of *spiked sediment* treatment to *water only* treatment. Ninety-five percent confidence intervals are given in parentheses

Compound (common name)	Temp. (°C)	pH 6 (Sediment:water ratio)	pH 7 (Sediment:water ratio)	pH 8 (Sediment:water ratio)
Azinphos-Methyl	11	2.51 (1.56–3.94)	6.52 (4.87–8.79)	1.31 (0.87–1.96)
	18	2.92 (2.19–3.82)	4.86 (4.42–6.15)	1.82 (1.20–2.63)
	25	3.45 (2.44–4.80)	2.70 (1.98–3.45)	2.59 (1.95–3.19)
Chlorpyrifos	11	6.71 (5.35–8.25)	39.55 (30.44–49.73)	8.80 (6.85–11.31)
	18	4.35 (2.80–7.00)	32.63 (27.08–39.40)	4.61 (3.31–6.43)
	25	10.61 (7.81–16.13)	18.60 (14.27–24.00)	10.23 (7.13–13.75)
Coumaphos	11	5.62 (2.94–9.49)	11.96 (7.47–18.50)	4.72 (3.01–7.16)
	18	3.11 (2.16–4.51)	2.59 (1.45–4.50)	4.92 (2.86–7.38)
	25	8.30 (4.38–13.32)	3.08 (1.93–4.53)	1.65 (0.90–2.94)
Diazinon	11	4.30 (2.68–7.24)	1.23 (0.711–2.08)	2.15 (1.27–3.55)
	18	4.06 (2.20–7.07)	2.09 (1.08–3.76)	4.23 (2.45–6.90)
	25	2.53 (1.42–4.20)	5.07 (2.97–7.17)	3.29 (2.00–5.07)
Dicrotophos	11	0.59 (0.23–1.29)	0.55 (0.37–0.83)	0.93 (0.84–1.57)
	18	0.53 (0.35–0.79)	1.22 (0.81–1.86)	0.58 (0.37–0.91)
	25	0.91 (0.58–1.37)	1.38 (0.78–2.23)	0.71 (0.43–1.08)
Disulfoton	11	0.59 (1.65–4.37)	2.36 (1.32–4.13)	1.35 (0.66–2.58)
	18	1.12 (0.28–2.38)	3.24 (1.38–7.09)	0.74 (0.34–1.48)
	25	0.78 (0.31–1.73)	6.97 (3.11–11.83)	6.76 (3.44–12.42)
Fensulfothion	11	1.81 (0.71–4.23)	6.35 (3.56–10.94)	7.35 (2.82–17.55)
	18	3.47 (1.62–6.68)	7.38 (3.96–13.04)	5.23 (2.62–11.04)
	25	2.90 (1.43–5.72)	2.44 (1.22–4.89)	1.08 (0.50–2.16)
Fenthion	11	1.45 (0.98–2.21)	2.29 (1.45–3.46)	2.86 (1.87–4.55)
	18	1.90 (1.31–2.69)	2.25 (1.47–3.28)	2.24 (1.73–2.90)
	25	2.79 (1.97–3.82)	1.81 (1.24–2.47)	1.91 (1.33–2.70)
Fonofos	11	9.61 (6.30–13.94)	4.47 (3.49–5.72)	3.33 (2.59–5.51)
	18	6.84 (4.72–9.88)	8.94 (6.72–11.75)	3.65 (2.24–6.13)
	25	5.24 (3.85–7.01)	6.88 (5.33–8.86)	8.04 (5.53–11.32)
Terbufos	11	1.26 (0.40–2.12)	1.93 (1.21–3.08)	0.85 (0.51–1.30)
	18	1.26 (0.79–2.07)	1.31 (0.92–1.92)	1.99 (1.32–3.14)
	25	2.88 (1.94–4.33)	0.91 (0.62–1.24)	2.35 (1.66–3.56)

affected most by the presence of sediment (S:W = 39 at pH 7, 11°C). However, the toxicity of dicrotophos, terbufos and disulfoton were not affected by the addition of sediment with ratios close to or less than 1.0 (Tab. V).

### Quantitative Structure Activity Relationships

This study investigates molecules from three classes of organophosphorus compounds: organophosphates, organophosphorothioates, and organophosphorodithioates. Thus, some of the molecules required biotransformation for activation while others in the group did not. The number of molecules in the study was limited, so the number of parameters allowed in any regression was also limited to prevent over parameterization of the data. A wide range of regressions were examined, but those that explained the majority of the data are presented here.

#### Regression Analyses with Unidimensional Descriptors (log $K_{ow}$ , MV and Water Solubility)

Log  $K_{ow}$  was not available for coumaphos from the literature. From a regression of log  $K_{ow}$  that included data from Fisher *et al.* [15] and LSER parameters (Tab. VI) (Fig. 1), an estimate for the log  $K_{ow}$  of coumaphos (5.16) was determined for use in investigating the variation of  $EC_{50}$ .

There were statistically significant linear correlations ( $p < 0.05$ ) between  $EC_{50}$  and log  $K_{ow}$  but not for molecular volume (MV) or for water solubility in the *water only* exposures. Under two conditions, pH 7, 25°C and pH 8, 11°C, no significant correlations were found for the *water only* exposures with log  $K_{ow}$ . In the *sediment* exposures, no significant correlations were observed. The correlations in *water only* systems were weak,  $r^2$  ranged from 0.53–0.64, and dicrotophos had strong leverage in each

TABLE VI Molecular connectivity and linear solvation energy parameters for insecticides from Fisher *et al.* [18]

Pesticide	log $K_{ow}$	Molecular connectivity parameters						Linear solvation energy parameters		
		$^0\chi^v$	$^1\chi^v$	$^2\chi^v$	$^4\chi^v$	$^4\chi^v_c$	$^3\kappa$	$V_{il}/100$	$\alpha$	$\beta$
Methyl Parathion	3.32	10.36	6.72	5.80	1.84	0.112	4.08	1.214	0.16	1.15
Parathion	3.80	11.78	7.89	6.06	3.25	0.112	5.27	1.41	0.16	1.16
Malathion	2.36	13.94	9.76	9.78	4.95	0.335	7.37	1.42	0.24	1.75
Leptophos	6.31	15.16	9.70	8.97	4.34	0.137	3.81	1.63	0.0	1.01
Ethion	5.07	16.75	14.48	15.93	13.71	0.671	8.0	1.80	0.0	2.0
Phosdrin	1.11	9.37	5.11	3.99	1.28	0.037	5.48	1.02	0.17	1.25
Dichlorvos	1.4	8.32	4.67	4.17	1.03	0.037	4.44	0.86	0.17	0.77

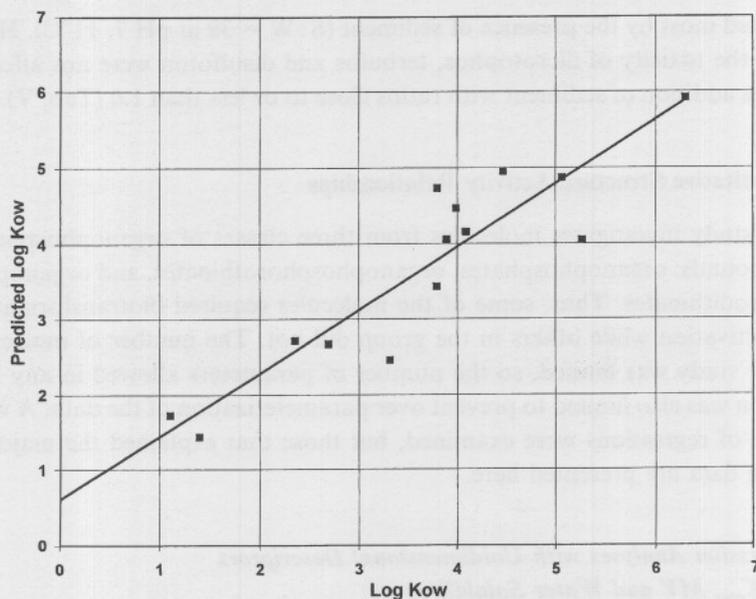


FIGURE 1 Comparison of predicted  $\log K_{ow}$  with literature values from the equation  $\log K_{ow} = 0.41(1.3) + 5.11(1.1) V_i/100 - 5.9(2.3)\alpha - 2.40(0.71)\beta$ ;  $r^2 = 0.83$ ,  $p < 0.001$ ,  $n = 14$ . Dicrotophos and fensulfthion were outliers in the data and not included in the regression. Numbers in parentheses are standard errors from the regression.

of the regressions because of its very low toxicity. Thus, the amount of the toxicity explained by factors that drive bioaccumulation is in the range of 50% of the overall variability.

#### *Regression Analyses with Linear Solvation Energy Relationships (LSER)*

LSER was not significantly better than the unidimensional parameters at describing the toxicity in the *water only* exposures, although significant regressions were obtained at each pH and temperature. The regressions had  $r^2$  values ranging from 0.41–0.70 and  $V_i/100$  was the only significant term. As with the unidimensional parameters, the dicrotophos has strong leverage in the regression. Also, as with the unidimensional parameters, no significant correlations were found for the *sediment* exposures. These models demonstrate that molecular size was a very important contributor to the toxicity. It is curious that  $V_i/100$  was so much more successful than characteristic molecular volume in describing the observed response since the two parameters presumably encode information for the same characteristic

and are directly proportional to each other. No explanation can be offered for the difference. In addition, dipolarity and hydrogen bonding characteristics did not contribute to the observed correlations.

### *Regression Analyses with Molecular Connectivity (MC) Indices*

When MC parameters were regressed against  $EC_{50}$  values, the ability to describe changes in toxicity was significantly increased and correlation coefficients ( $r^2$ ) ranged from 0.69 to 0.90 (Tab. VII). In *water only* exposures, the  $EC_{50}$  was best described by  $^1\chi^v$  and  $^3\kappa$  and the regressions were all leveraged by the presence of dicotophos as it was by far the least toxic compound. The two indices explain two important features in the toxicity process.  $^1\chi^v$  most likely accounts for the molecular size and accumulation processes while the shape index,  $^3\kappa$ , likely describes the fit of the molecule to the receptor. The thermal influence could be incorporated into the regression and highly significant regressions were found that described the variability for all temperatures at a given pH (Tab. VIII). These regressions were not significantly different from each other suggesting that the variability in the water-only exposures across pH for such short exposures was not significant. Thus, the organisms were responding under all conditions of pH in essentially the same manner (Fig. 2). Even though these are relatively strong relationships, the ability to predict the more toxic compounds is lower than the ability to predict the less toxic compounds because of the stronger leverage in the regressions by the less toxic insecticides.

The relationships between  $1/EC_{50}$  and  $\log 1/EC_{50}$  were investigated because of the strong leveraging by dicotophos and the desire to better

TABLE VII Regressions of  $EC_{50}$  ( $\mu\text{mol/l}$ ) for organophosphate insecticides with molecular connectivity indices for each pH and temperature

pH 6	
11 C	$EC_{50} = 0.91(0.22) - 0.129(0.023) ^1\chi^v + 0.077(0.02) ^3\kappa; r^2 = 0.84, n = 10, p = 0.002$
18 C	$EC_{50} = 0.96(0.22) - 0.128(0.023) ^1\chi^v + 0.058(0.02) ^3\kappa; r^2 = 0.83, n = 10, p = 0.002$
25 C	$EC_{50} = 0.51(0.10) - 0.067(0.01) ^1\chi^v + 0.028(0.01) ^3\kappa; r^2 = 0.86, n = 10, p = 0.001$
pH 7	
11 C	$EC_{50} = 1.28(0.23) - 0.18(0.025) ^1\chi^v + 0.092(0.024) ^3\kappa; r^2 = 0.89, n = 10, p < 0.001$
18 C	$EC_{50} = 0.57(0.11) - 0.079(0.012) ^1\chi^v + 0.039(0.01) ^3\kappa; r^2 = 0.87, n = 10, p < 0.001$
25 C	$EC_{50} = 0.20(0.09) - 0.031(0.009) ^1\chi^v + 0.024(0.009) ^3\kappa; r^2 = 0.69, n = 10, p = 0.02$
pH 8	
11 C	$EC_{50} = 0.57(0.20) - 0.086(0.02) ^1\chi^v + 0.064(0.02) ^3\kappa; r^2 = 0.75, n = 10, p = 0.007$
18 C	$EC_{50} = 0.94(0.17) - 0.12(0.02) ^1\chi^v + 0.050(0.02) ^3\kappa; r^2 = 0.88, n = 10, p < 0.001$
25 C	$EC_{50} = 0.65(0.10) - 0.085(0.011) ^1\chi^v + 0.031(0.01) ^3\kappa; r^2 = 0.90, n = 10, p < 0.001$

TABLE VIII Regression of  $EC_{50}$  ( $\mu\text{mol/l}$ ) and  $\log(1/EC_{50})$  for each pH with molecular connectivity parameters

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$EC_{50}$	
pH 6	$EC_{50} = 1.04(0.12) - 0.0065(0.0027) T^a - 0.118(0.013) {}^1\chi^v + 0.044(0.01)^3\kappa; r^2 = 0.78, n = 30,$ $p < 0.001$
pH 7	$EC_{50} = 0.83(0.16) - 0.0084(0.0035) T^a - 0.091(0.016) {}^1\chi^v + 0.047(0.015)^3\kappa; r^2 = 0.63, n = 30,$ $p < 0.001$
pH 8	$EC_{50} = 0.81(0.11) - 0.005(0.002) T^a - 0.094(0.01) {}^1\chi^v + 0.044(0.01)^3\kappa; r^2 = 0.78, n = 30,$ $p < 0.001$
$\log(1/EC_{50})^b$	
pH 6	$\log(1/EC_{50}) = -1.72(0.68) + 0.047(0.01) T^a - 0.33(0.08) {}^0\chi^v + 0.94(0.12) {}^1\chi^v - 0.47(0.06) {}^4\chi^v,$ $r^2 = 0.81, p < 0.001$
pH 7	$\log(1/EC_{50}) = -1.94(0.73) + 0.038(0.01) T^a - 0.19(0.07) {}^0\chi^v + 0.75(0.11) {}^1\chi^v - 0.40(0.06) {}^4\chi^v,$ $r^2 = 0.84, p < 0.001$
pH 8	$\log(1/EC_{50}) = -1.18(0.79) + 0.038(0.01) T^a - 0.16(0.09) {}^0\chi^v + 0.61(0.11) {}^1\chi^v - 0.33(0.07) {}^4\chi^v,$ $r^2 = 0.68, p < 0.001$

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<sup>a</sup>  $T$  equals temperature in degrees celcius.

<sup>b</sup> Chlorpyrifos omitted from the data set as an outlier.

predict toxicity of the more toxic insecticides. Regressions with  $1/EC_{50}$  were not particularly successful. Using  $\log(1/EC_{50})$  improved the predictability across all the data such that significant regressions were obtained so long as chlorpyrifos was omitted from the data set (Tab. VIII). Thus, chlorpyrifos remains an outlier and is not well predicted whether the regressions are based on  $EC_{50}$  or  $\log 1/EC_{50}$ . The regressions have  $r^2$  values ranging from 0.68 to 0.84. Again, there is no evidence that there is any real difference across pH among the responses to the various pesticides. Thus, the data could be described by a single regression (Fig. 3).

#### *QSAR for Sediment Exposures with Molecular Connectivity Parameters*

When the sediment was added to the systems, the ability of MC parameters to explain the changes in toxicity with varying environmental conditions was not successful. In this case, the toxicity of the insecticides is governed by: (1) partitioning to sediment that reduces the bioavailability; (2) the inherent toxicity of the compound; and (3) factors governing the accumulation in

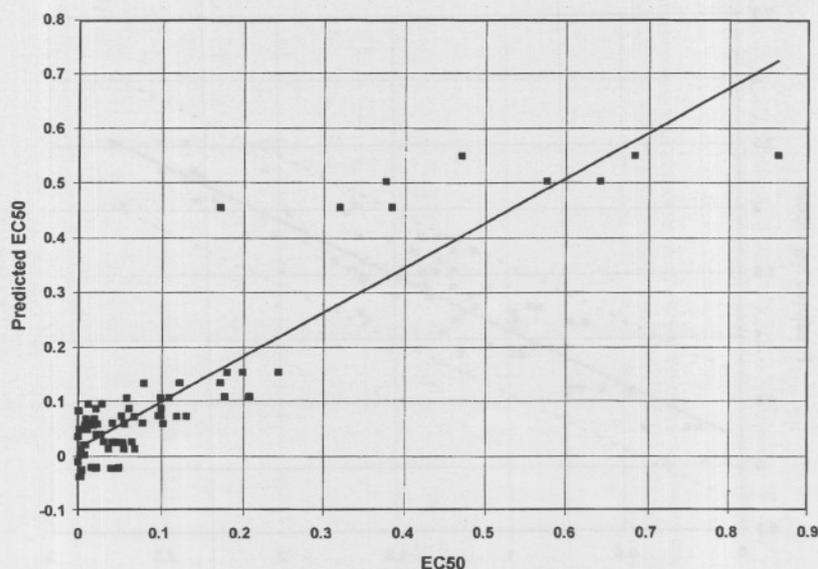


FIGURE 2 Predicted  $EC_{50}$  ( $\mu\text{mol/l}$ ) compared to measured values for all pH and temperatures using the equation:  $EC_{50} = 0.52(0.09) - 0.0067(0.0013)T - 0.393(0.007)^1\chi^r + 0.023(0.007)^3\kappa + 0.388(0.07)^1\chi_{ox}^r$ ,  $r^2 = 0.81$ ,  $p < 0.001$ ,  $n = 80$ . Numbers in parentheses are standard errors from the regression.

aqueous exposures. The combination of these factors resulted in large  $EC_{50}$  values under two conditions: (1) Compounds with high water solubility and low partitioning exhibited low toxicity because the compounds were not readily accumulated, *e.g.*, dicotophos; and (2) Compounds with high partitioning had reduced bioavailability because of sorption to the sediment, *e.g.*, chlorpyrifos. Thus, it was not surprising that regressions based on either univariate or multivariate parameters were not successful. The experimental design also complicated the predictive ability because of the very short exposures so that exposure from ingestion of sediment would have been minimal. Finally, pH-mediated hydrolysis would not be expressed in the short time period of the exposures.

Since the exposures were so short, it was expected that the aqueous concentration would dominate the overall exposure of the midges. If the aqueous concentrations under the sediment regime could be determined, then some level of predictability might be found. To accomplish this, the aqueous concentration was calculated from equilibrium partitioning [37]. This assumed that the sediment and water were in equilibrium. When these calculations were performed, there were significant correlations between the calculated aqueous concentrations at the  $EC_{50}$  in the presence of sediment

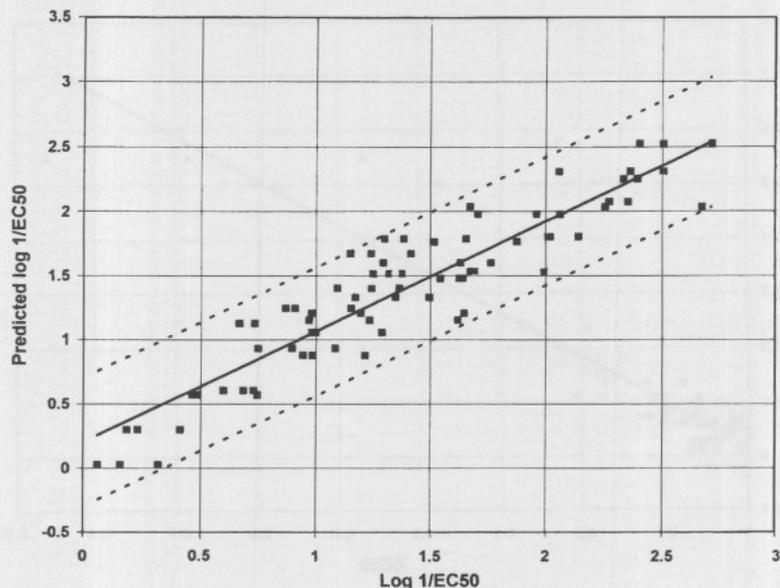


FIGURE 3 Prediction of  $\log(1/EC_{50})$  ( $\mu\text{mol/l}$ ) compared with measured values using the equation  $\log(1/EC_{50}) = -2.26(0.31) + 0.039(0.006)T - 0.30(0.04)^0\chi^y + 1.04(0.06)^1\chi^y - 0.72(0.05)^4\chi^y + 1.75(0.26)^4\chi^z$ ,  $r^2 = 0.86$ ,  $p < 0.001$ ,  $n = 81$ . Chlorpyrifos is an outlier in the data set. Numbers in parentheses are standard errors from the regression. Dotted lines represent  $\pm 0.5$  log units from the regression line.

and the water-only  $EC_{50}$  measured in the absence of sediment. The slope of the regression lines ranged from 0.58–0.61 and  $r^2$  values from 0.48–0.73 for each pH across all temperatures. A slope of less than one suggests that some of the exposure was coming directly from sediment and not through the water. However, the aqueous exposure route was dominant. Regressions with MC parameters were successful except for pH 6, pH 7, and pH 8, at 11°C (Tab. IX). For pH 7, fonofos was found to be a consistent outlier in the data set. While the regressions employed the same MC parameters as the water only exposures, the regressions were not as robust because of the contribution from direct exposure to the sediment in some cases, the likelihood that the systems were not actually at equilibrium, and the possibility that the  $\log K_{ow}$  values were not accurate, *e.g.*, the predicted values for coumaphos and the outliers in the LSER regression with  $\log K_{ow}$  given earlier. However, this did demonstrate that for the short-term exposures the aqueous concentration was dominant in the toxicity and that the toxicity was influenced by the ability of the compound to partition to sediment.

TABLE IX Regressions of EC<sub>50</sub> (μmol/l) for predicted water concentration from sediment with molecular connectivity indices

## pH 6

11C NS,  $p > 0.1$ 

18C EC<sub>50</sub> = 0.57(0.18) - 0.065(0.02) <sup>1</sup>χ<sup>v</sup> + 0.019(0.018) <sup>3</sup>κ;  $r^2 = 0.61$ ,  $n = 10$ ,  $p = 0.04$

25C EC<sub>50</sub> = 0.51(0.13) - 0.061(0.015) <sup>1</sup>χ<sup>v</sup> + 0.020(0.014) <sup>3</sup>κ;  $r^2 = 0.72$ ,  $n = 10$ ,  $p = 0.01$

pH 7 (Compound 9, fonofos was a consistent outlier at this pH and has been dropped from the data set)

11C NS,  $p > 0.1$ 

18C EC<sub>50</sub> = 0.78(0.17) - 0.099(0.02) <sup>1</sup>χ<sup>v</sup> + 0.037(0.015) <sup>3</sup>κ;  $r^2 = 0.87$ ,  $n = 9$ ,  $p < 0.01$

25C EC<sub>50</sub> = 0.32(0.11) - 0.046(0.01) <sup>1</sup>χ<sup>v</sup> + 0.030(0.012) <sup>3</sup>κ;  $r^2 = 0.74$ ,  $n = 9$ ,  $p = 0.017$

## pH 8

11C NS,  $p > 0.1$ 

18C EC<sub>50</sub> = 0.60(0.15) - 0.071(0.016) <sup>1</sup>χ<sup>v</sup> + 0.021(0.015) <sup>3</sup>κ;  $r^2 = 0.75$ ,  $n = 10$ ,  $p < 0.01$

25C EC<sub>50</sub> = 0.41(0.12) - 0.055(0.013) <sup>1</sup>χ<sup>v</sup> + 0.030(0.012) <sup>3</sup>κ;  $r^2 = 0.75$ ,  $n = 10$ ,  $p < 0.01$

**Combination of Studies**

This study followed the study design of Fisher *et al.* [15] making it possible to incorporate the data from that study with this one to determine whether additional insight can be gained. That study was performed at 22°C, pH 7 and used soft standard reference water. It was assumed that the thermal response of the small difference in temperature (3°C) between the two studies would not have affected the estimate of EC<sub>50</sub> and that the differences in water hardness would cause no measurable effect. Thus, two studies could be combined. If this was done, then the total number of compounds would expand to 17. Best regressions were again investigated. The EC<sub>50</sub> was best described by a combination of <sup>1</sup>χ<sup>v</sup>, <sup>2</sup>χ<sup>v</sup> and <sup>4</sup>χ<sup>v</sup>,  $r^2 = 0.81$  (Fig. 4). The predictability of the relationship was generally within about a factor of three compared to the measured EC<sub>50</sub> values and best for the non-toxic compounds, as was observed previously for the smaller data set. There were two compounds, azinphos methyl and fenthion, where the prediction fell below zero and chlorpyrifos toxicity was under-predicted by a factor of 120 while leptophos toxicity was over-predicted by about 130 between measured and predicted EC<sub>50</sub>. If instead of EC<sub>50</sub>, regressions are performed with log (1/EC<sub>50</sub>), then predictions using <sup>0</sup>χ<sup>v</sup>, <sup>1</sup>χ<sup>v</sup> and <sup>4</sup>χ<sup>v</sup> as parameters fall within 0.5 log units ( $r^2 = 0.86$ ) except for chlorpyrifos which is an outlier in this data set as with the smaller data set (Fig. 5). Because the temperature difference between the two studies might have influenced the data, the data for this regression were corrected using the thermal coefficient for pH 7. The regression was repeated and no appreciable change was found in the regression. Thus, the assumption that the two studies could be combined directly was supported.

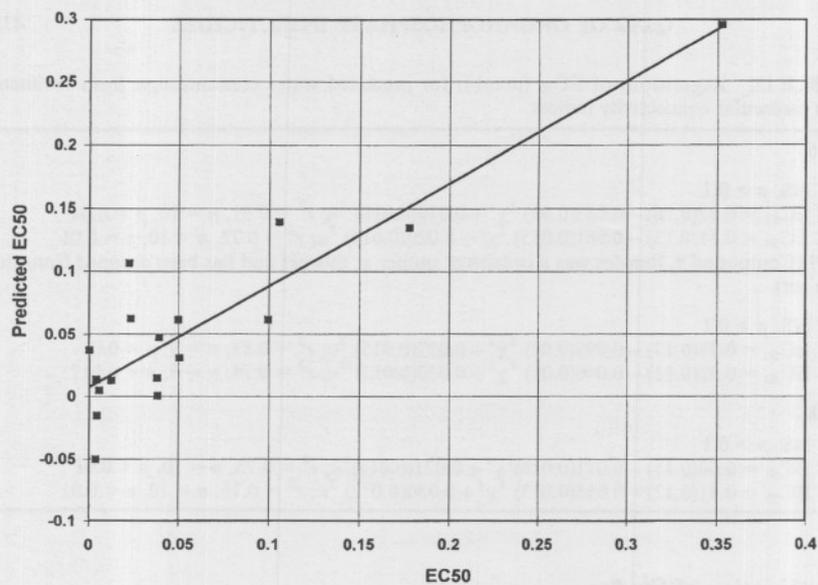


FIGURE 4 Prediction of  $EC_{50}$  ( $\mu\text{mol/l}$ ) for pH 7 using the  $25^{\circ}\text{C}$  data from this work and the  $22^{\circ}\text{C}$  data from Fisher *et al.* [15] using the equation:  $EC_{50} = 0.22(0.06) - 0.022(0.01) {}^1\chi^v - 0.033(0.013) {}^2\chi^v + 0.067(0.011) {}^4\chi^v$ ,  $r^2 = 0.81$ ,  $n = 17$ ,  $p < 0.001$ . Numbers in parentheses are standard errors from the regression.

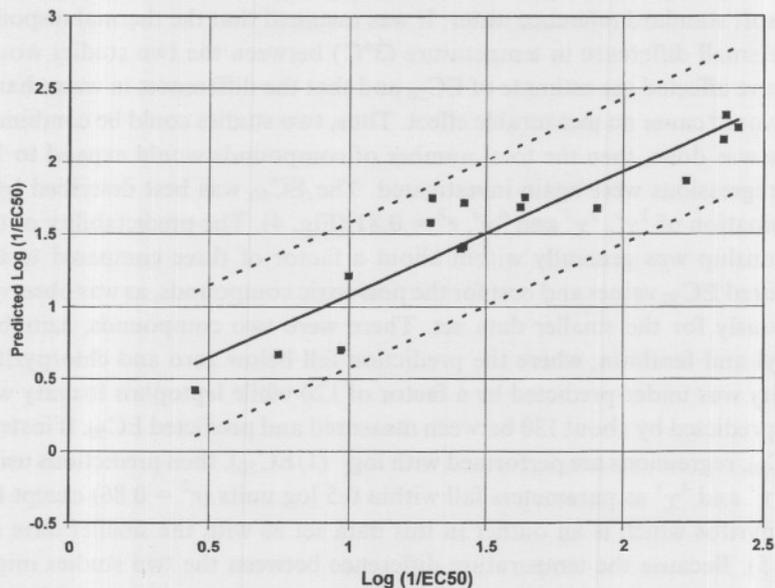


FIGURE 5 Predictions of  $\log(1/EC_{50})$  ( $\mu\text{mol/l}$ ) compared with measured values for pH 7 using  $25^{\circ}\text{C}$  data from this work and the  $22^{\circ}\text{C}$  data from Fisher *et al.* [15] using the equation  $\log(1/EC_{50}) = 1.09(0.43) - 0.35(0.08) {}^0\chi^v + 0.78(0.12) {}^1\chi^v - 0.45(0.05) {}^4\chi^v$ ,  $r^2 = 0.86$ ,  $n = 16$ . Chlorpyrifos was an outlier in the data and was not included in the regression. Numbers in parentheses are standard errors from the regression. Dotted lines represent  $\pm 0.5$  log units from the regression line.

## DISCUSSION

The toxicity of 10 OP insecticides was tested against the midge larvae under varying environmental conditions of pH (6, 7, and 8), temperature (11, 18, and 25°C) and in the presence and absence of sediment, to examine the effects of changing environmental conditions on toxicity. Changing temperature had a conspicuous and generally consistent effect on toxicity. In both *water only* and *spiked sediment* systems, insecticides became more toxic to the midge larvae as temperature increased. Lydy *et al.* [30] also found that parathion toxicity increased as much as 100 times, when temperature increased from 10°C to 30°C. Increased toxicity of OP insecticides with increasing temperature may be due to increased bioaccumulation or increased activation of insecticides as the temperature is elevated. Increased accumulation kinetics have been observed for the midge with increasing temperature [38] which supports the notion that lower water concentrations could lead to equal or greater accumulation as temperature increased. Further, the metabolic conversion of parathion to paraoxon in the midge increased as temperature increased from 10°C to 30°C. This enhanced activation supports the finding of increased toxicity with increasing temperature. The combination of these two mechanisms is adequate to account for the role of temperature in the increased toxicity of the insecticides.

In contrast, toxicity of OP's was hardly influenced by pH. In fact, once the thermal response is accounted for there was no statistical difference in the response across the range of pH. The absence of a pH effect agreed well with the work of Mayer and Ellersieck [39]. It was expected that changing the pH would affect the stability of the various insecticides differentially and perhaps affect the physiology of the midge larvae. However, the short duration of the test probably precluded the influence of pH on the stability of the compound, as hydrolysis half-lives range from 1.5 d at pH 8 to multiple days for those compounds where half life was available in the literature [40] and they are more stable at lower pH values. Change in pH could also have influenced the physiology of the midge. However, this narrow range is well within the pH of surface waters and apparently was not a sufficient range to alter insecticide accumulation or to stress the midge to lower its resistance to the toxins.

### Influence of $\log K_{ow}$

$\log K_{ow}$  is a powerful tool for explaining the environmental fate and biological activity of many xenobiotics. For instance,  $\log K_{ow}$  has been used to predict the bioconcentration factors of lipophilic organic chemicals

including organochlorine pesticides [4], cytotoxicity of lipophilic chemicals to goldfish cells [41], and toxicity of surfactants to fish [42]. However, the use of  $\log K_{ow}$  as a predictive tool seems to be restricted to the highly lipophilic neutral compounds that accumulate through passive partitioning and have a nonspecific mode of action. OP insecticides, however, have relatively high water solubility in addition to specific mode of action. Thus,  $\log K_{ow}$  was expected to be less useful in describing toxicity of OPs [43]. Moreover, OP's go through many types of biochemical transformations including hydrolysis, oxidation, reduction and conjugation reactions [44–46]. Thus, a low correlation of toxicity with unidimensional descriptors like  $\log K_{ow}$ , MV and water solubility in regression analysis was expected. However,  $\log K_{ow}$  did explain about 50% of the variability in the data. This suggests that the accumulation process as modeled by  $\log K_{ow}$  was important in conferring toxicity.  $\log K_{ow}$  was correlated to the uptake and elimination rate constants for twelve OP insecticides [16] suggesting that  $\log K_{ow}$  appropriately models accumulation processes even for these fairly water soluble and reactive compounds.

#### Results with Unidimensional Descriptors

Each unidimensional descriptor used individually failed to describe the variation in toxicity of these cholinergic insecticides. Hodson *et al.* [8] showed that  $LC_{50}$  values were highly correlated with molecular size as encoded in molecular weight ( $r = -0.90$ ) and in Parachor (molecule volume) ( $r = -0.89$ ). McGowan and Mellors [48] reported that there was strong correlation between MV and fish toxicity of non-reactive hydrophobic chemicals like phenols, toluene and naphthalene. However, the correlation between MV and the toxicity for compounds with polar functional groups, such as ethanediol and diethanolamine, was poor because MV could not explain the interaction of these compounds with water. The same interaction may partly account for the lack of correlation between MV and OP toxicity. More importantly, all chemicals used have the specific mode of action which MV alone cannot adequately describe. MV, like  $\log K_{ow}$ , seems to have greater explanatory powers for chemicals with a mode of action of non-reactive narcosis, where toxicity is presumably caused primarily by the presence of a chemical molecule in the biological membrane.

As water solubility increases, the residence time in organisms decreases since the absorption of chemicals into the lipophilic portion of an organism decreases and the excretion increases [48]. As a result, there is less of a

tendency for the chemicals to build up to toxic concentrations in organism tissue. Dhanaraj *et al.* [49] reported that uptake and bioconcentration of aldrin and phorate by a protozoan (*Tetrahymena pyriformis*) were inversely dependent on their water solubility. However, Geen *et al.* [50] showed that predictions of bioconcentration of an OP insecticide, acephate, based on water solubility did not match with experimental data. In this work, water solubility also failed to explain the toxicity changes with variety of OP chemicals at different environmental conditions.

### Results with Multidimensional Descriptors

The toxicity of organophosphate insecticides ultimately results from interference with acetylcholine neurochemistry such that the toxin is inserted into the acetylcholine receptor. This requires a series of steps from the exposure through distribution to the final association with the receptor. Thus, for aquatic organisms exposed in aqueous media the factors that govern bioaccumulation, internal distribution, biotransformation and the shape and fit of the molecule into the receptor all exert influence on the value measured as the  $EC_{50}$ . To be predictive, quantitative structure activity models need to incorporate the factors that dominate the toxicokinetic process to best explain the observed toxic response for a range of compounds. The best models incorporated two or more parameters to describe the toxicity.

LSER was selected as a potential model because it explains the biological activities in transfer of chemicals from one medium to another, namely, from an aqueous solution to more lipophilic compartments as in an organism such as the midge. The events that lead to toxicity—dissolution of the insecticide in water, absorption by the midge, and the multiple dissolutions as the insecticide is transferred from tissue to blood to the nervous system—represent a complex series of solvent-solute interactions [51]. Thus, it is possible that LSER could explain OP toxicity in the midge. While three-dimensional complementarity of OP's to the target site is very important to toxicity, the solubilization process responsible for the transport of the toxicants to the target site is also important. Further, LSER has effectively correlated chemical parameters to the toxicity of a series of polar chemicals such as amines, carboxylic acids, aldehydes, carboxylic acid esters, alkanes, alkyl benzenes, and chlorobenzenes, to bioluminescence inhibition of bacteria *Photobacterium phosphoreum* [48] and of organic non-electrolytes to the golden orfe fish, *Leuciscus idus melanotus* [52].

In water-only exposures, the correlation coefficients using LSER variables were comparable to the use of  $\log K_{ow}$  and the only parameter affecting the regression was the steric factor  $V_i/100$ . Thus, the LSER model only explained the portion of the variability that was occurring due to the partitioning behavior which, like  $\log K_{ow}$ , accounted for about 50% of the variability.

MC parameters have been successfully employed to predict the biological effect of highly lipophilic compounds to fish [53, 54]. In the *water only* system, high correlation coefficient values (Tab. VII) were obtained in most cases. The importance of both  ${}^1\chi^v$  and  ${}^3\kappa$  demonstrate the importance of both the uptake process and the shape required to fit the receptor.

The fit of this model compared to other models describing other OP insecticide toxicity was generally similar. Molecular connectivity has been used in combination with other parameters to describe the toxicity of OP insecticides to *Daphnia magna* [17]. For 22 compounds, the best relationship required six parameters and a constant, and predicted the  $\log(1/EC_{50})$  within 0.67 log units (a factor of about 5). Similar parameters, specifically  $\log K_{ow}$ ,  $(\log K_{ow})^2$  and  ${}^3\kappa$ , used with the combined data from this work and Fisher *et al.* [15], yielded a correlation with an  $r^2 = 0.53$  after omitting the outlier chlorpyrifos. This correlation gave predicted values with an error of 0.45 log units (a factor of about 3). This was certainly comparable to the work done with *Daphnia* presented above. Improved  $r^2$  values may have been achievable with more parameters but that would have led to an over-determined model and not provided any better representation of the data.

Our final regression for the combined data set used  ${}^0\chi^v$ ,  ${}^1\chi^v$  and  ${}^4\chi^v$  and gave a substantially better fit ( $r^2 = 0.86$ ) and error for the estimates was 0.25 log units (Fig. 5). The shift in parameters from  ${}^3\kappa$  to a combination of  ${}^0\chi^v$  and  ${}^4\chi^v$  splits the components of the  ${}^3\kappa$  value into those of molecular size represented by  ${}^0\chi^v$  and those of conformation in  ${}^4\chi^v$ . These supply improved indicators of the factors that influence the toxicity of the OPs for the midge.

A similar attempt to predict toxicity of organophosphorothioates compounds to fish had good success with  $\log K_{ow}$  in combination with various measures of the electronic state of the molecule, including molecular orbital descriptions and reactivity descriptions [14]. The observed regression coefficients for this single compound class were high with  $r^2$  values in the 0.82–0.9 range. These were comparable to the regression coefficients found using molecular connectivity with the  $EC_{50}$  and  $\log(1/EC_{50})$  in this study except that this work included three classes of OP compounds.

The models employed for describing the toxicity do not explicitly account for the rate or extent of biotransformation. Some of the molecules require

biotransformation to the oxone form for toxicity to be elicited while others in the set do not. Further, biotransformation differences may well detoxify some compounds faster than others. Thus, there will likely be differences in the rate and extent of presentation of the oxone form to the receptor. Inclusion of an MC parameter for the oxone form appeared to contribute somewhat to the relationship between the  $EC_{50}$  and the MC parameters that described the toxic response. In this case,  ${}^1\chi^v$  and  ${}^1\chi_{ox}^v$  along with the  ${}^3\kappa$  were the terms that described the regression (Fig. 2). The coefficients associated with  ${}^1\chi^v$  and  ${}^1\chi_{ox}^v$  were of about the same magnitude but of different sign. Thus, the two terms describe the influence of the difference between the sulfur containing form and the oxone. This may account to some extent for the need for conversion to the oxone but it is not necessarily explicit. Further, exploration of the role of biotransformation in the variation observed in the predictions of the toxic response may account for some of the residual variation that remained from the QSAR models.

The most disturbing finding was the inability to predict the chlorpyrifos either within the data generated in this study or when combined with the data from Fisher *et al.* [15]. In all cases, this compound exhibited considerably more toxicity than would be expected based on its characteristics. It is not possible to simply say that the toxicity was mismeasured since the results were consistent for nine tests in water only and nine tests in the presence of sediment. It is clear that other types of parameters than those used in this study will be required to explain the effect. The use of molecular orbital calculations may lead to an improved understanding for this compound in comparison to the others. Use of a more highly shape-descriptive parameter (if and when developed) may also prove more useful.

The pH did not apparently affect the toxicity. All the data were combined to produce a single regression that incorporated four MC parameters since the number of independent observations was now 81, omitting the outlier chlorpyrifos (Fig. 3). This equation provides a good description of toxicity and the error for predicted values falls within  $\pm 0.5$  log units for all estimates of the  $\log 1/EC_{50}$ . This equation was used to predict the toxicity in the form of  $\log 1/EC_{50}$  for the data from Fisher *et al.* [15] and the resultant slope of the predicted *versus* measured values was 1.14 and the  $r^2$  was 0.73 but the intercept was  $-0.66$ . Thus, this equation under-predicts the  $EC_{50}$  by about a factor of five between the two studies. Common comparisons between laboratories are in the range of a factor of 2–3 when all the conditions are the same. While the work of Fisher *et al.* [15] was performed in the same laboratory and the temperature effects were accounted for in the regression

equation, the studies were performed by different individuals, with different water types, and the tests were displaced by more than 5 years. During this time the midge culture crashed and had to be regenerated by obtaining organisms from another facility. It is likely that genetic differences in the two midge populations contributed to the differential susceptibility. Thus, this is not an unreasonable prediction between the two studies.

### Spiked Sediment

The presence of sediment clearly reduces the exposure of the midge to the OP insecticides in most cases in these short-term bioassays. Because the bioassays are only 24-h duration, ingestion is likely not an important factor in the accumulation of dose. Thus, until the partitioning between sediment and water was accounted for through the use of equilibrium partitioning, no regressions were possible regardless of the model. This was different than had been previously found for a mixture of carbamate and organophosphorus insecticides [15]. However, in the previous study the regression for a combination of OP and carbamate insecticides was not very strong, explaining only about 40% of the variability even though it was significant. After accounting for the partitioning, the regressions for the data in this work (Tab. IX) were generally similar but somewhat lower than that found for the water only case with the exception of the 11°C temperature at all pHs. The inability to find significant regressions for the 11°C data suggests that the systems at this temperature were further from equilibrium and perhaps the organisms were less active, which affected their exposure in some unknown manner in the presence of sediment. The lower correlation coefficients and particularly the finding that fonofos was an outlier at pH 7, further suggests that the systems were not truly at equilibrium. Thus, the assumption of equilibrium was adequate to explain the driving force for the observed toxicity, *e.g.*, accumulation of the dose from water. However, short-term assays cannot completely assess the role of sediment in the toxicity of the compounds in the environment where longer-term exposures are expected.

Attempts to examine the data across experiments for sediment exposures, this work and Fisher *et al.* [15], as was done in the water only exposures were not successful. This is likely due in large part to differences in the sediment used in the experimental work. The sediments came from two different locations and had different characteristics, *e.g.*, 0.9 and 3% organic carbon respectively. Differences in composition on the bioavailability of contaminants cannot always be accounted for by normalization to organic carbon

[55]. Thus, calculations of the equilibrium water concentration were likely not comparable between different sediments. Since the concentration in the water dominates the response, failure to accurately account for the water concentration would explain failure to match the two experiments.

### Environmental Implications

Measures of the toxicity of organophosphate insecticides and the ability to predict their effects for organisms are useful for the development of environmental risk assessment. This work advances our understanding of both the toxic response and the factors controlling this response in aqueous exposures. While the predictions are generally as good as others found in the literature, there is significant room for improvement. This is particularly the case for the failure to adequately predict the toxicity of chlorpyrifos in any of the models. The incorporation of molecular orbital information, similar to the work of Schüürmann [14], or other more shape descriptive factors may well improve the ability to predict all the compounds. It is clear from this work that features governing the accumulation and fit into the acetylcholine receptor must both be included to obtain reasonable regression relationships with effects.

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