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Comparative Toxicity of Chlordane, Chlorpyrifos, and Aldicarb to Four Aquatic Testing Organisms

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Abstract. Laboratory toxicity data contrasting responses of aquatic organisms to insecticides are important for focusing on sensitive species (steepest exposure-response slope) exposed to aqueous concentrations of these insecticides in field studies. These data also allow prediction of expected responses of aquatic species to a range of insecticide concentrations *in situ.* Aqueous 48-h toxicity tests were performed to contrast responses of *Daphnia magna* Straus, *Hyalella azteca* Saussure, *Chironomus tentans* Fabricius, and *Pimephales promelas* Rafinesque to acetylcholinesterase-inhibiting insecticides: chlorpyrifos, aldicarb, and chlordane. As expected, invertebrates tested (*H. azteca*, *C. tentans*, and *D. magna*) were ≥ 200 times more sensitive than the vertebrate *P. promelas* to chlorpyrifos exposures. *H. azteca* was approximately 3.5 times more sensitive to chlorpyrifos (453% mortality/µg/L) than *D. magna* (128% mortality/µg/L). For both aldicarb and chlordane, *C. tentans* was the most sensitive species tested (2.44 and 2.54% mortality/µg/L, respectively). Differences in chlordane potency for test species varied only by a factor of approximately 2–3 (0.88% mortality/µg/L for *H. azteca* to 2.54% mortality/µg/L for *C. tentans*). Although point estimates of population responses such as LC50s, NOECs, and LOECs are of some utility for predicting effects of pesticides in aquatic systems, exposure-response slopes are also useful for extrapolation of laboratory data to diverse field situations, especially where sediment sorption may regulate insecticide exposure or bioavailability.

Comparative toxicity data provide important information on variations in responses of aquatic species to insecticides and are useful for determining margins of safety for aquatic biota, either prospectively (before manufacture and use) or retrospectively (after manufacture and use) (Adams 1995; Graney *et al.* 1994). Laboratory toxicity data also provide insight into expected effects of accidental spills, cropland runoff, pesticide aerial drift, or other events potentially adversely affecting nontarget organisms. In this series of laboratory experiments, effects of chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methano-1H-indene), chlorpyrifos [phosphorothioc acid O, O-diethyl O-(3,5,6-trichloro-2-pyridinyl) ester], and aldicarb [2-methyl-2-(methylthio) propanal O-[(methylamino)carbonyl) oxime] were determined for four commonly tested aquatic organisms in 48-h exposures (Table 1).

Between the 1940s and 1970s, pesticide use increased almost 40-fold, with new products such as organochlorines (*e.g.* chlordane) becoming prominent (Nimmo 1985). In 1995, aldicarb and chlorpyrifos applications were approximately 600,000 kg and 3,000,000 kg active ingredient, respectively (Economic Research Service 1996). Organochlorines were designed to be persistent and manufactured inexpensively. Because of growing concerns for environmental and human health, organochlorine pesticides have been largely replaced with less persistent but relatively active organophosphorus and carbamate pesticides. Chlorpyrifos, an organophosphorus insecticide, is sold under the trade names Dursban™ and Lorsban™. The carbamate aldicarb is manufactured with the trade name Temik™ as an insecticide, as well as an acaricide and nematicide. Because of its reported human oral and dermal toxicity, aldicarb is sold to certified applicators only in granular form, rather than as emulsified concentrates or liquids (EXTOXNET 1993). Before discontinuance of its manufacturing, chlordane was sold with trade names such as Octaklor™ as an insecticide.

Insecticides associated with agriculture and domestic sites are used primarily in terrestrial systems; however, due to frequent proximity of croplands and homes to aquatic systems, concerns have arisen regarding the margin of safety for these materials in aquatic systems (Kersting and van Wijngaarden 1992). Because these insecticides are designed to evoke rapid responses in target populations and degrade rapidly, measurements of short-term effects (*e.g.*, 48-h exposures) offer important information for evaluation of potential risks to aquatic systems. Although insecticides vary in their persistence in aquatic systems, a primary effect that is almost immediately apparent is lethality to nontarget species. Differential responses of organisms representing diverse physiological capabilities and niches in aquatic systems can help focus field studies where nontarget effects due to off-site movement of insecticides are suspected. The objectives of this study were to compare, contrast, and model responses (*i.e.*, survival) of populations of *Daphnia magna*, *Hyalella azteca*, *Chironomus tentans*, and *Pimephales promelas* to short-term (48 h) aqueous laboratory

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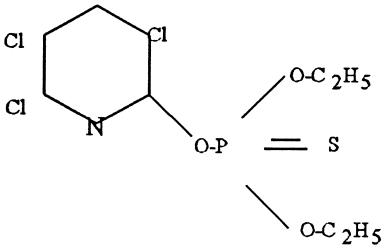
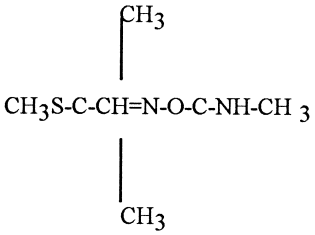
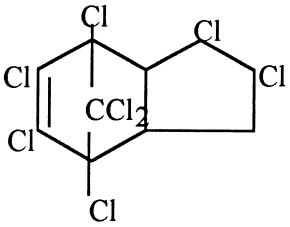
Table 1. Physical properties and fate characteristics of chlorpyrifos, aldicarb, and chlordane

	Chlorpyrifos	Aldicarb	Chlordane
			
Molecular weight (g/mol)	350.62	190.25	406
Water solubility (mg/L)[a,b]	2	6000	0.15
K_{ow}[a,b]	66,000	1.36	3.00×10^5
K_{oc}[a]	6070	20–80	1.4×10^5
Specific gravity (g/cm^3)[a]	1.398	1.195	1.59–1.63
Vapor pressure (mm Hg)[a]	1.87×10^{-5}	3.0×10^{-5}	1.0×10^{-5}
Melting point (°C)[a]	41.5–44	99–100	104–107
Water persistence, $T_{1/2}$ (days)[a,c]	0.5–4	5–10	<10
Soil persistence, $T_{1/2}$ (days)[a]	60–120	30–45	1460

[a] EXTOXNET, 1993
[b] US EPA, 1980
[c] Verschueren, 1983

exposures of chlordane, chlorpyrifos, and aldicarb. These insecticides were studied as commercial preparations used in agricultural and domestic practices.

Materials and Methods

Test Organism Culture Procedures

All test organisms were cultured in the University of Mississippi Department of Biology culturing facility. *D. magna* and *P. promelas* (fathead minnow) culturing procedures followed the methods of Peltier and Weber (1985). Culturing procedures for *H. azteca* followed the methods of de March (1981). *C. tentans* culture methods followed those of Townsend *et al.* (1981).

Experimental Design

All static aqueous toxicity tests (48 h) were conducted in incubators at 20 ± 1°C with a 16-h light/8-h dark photoperiod, and were initiated by adding 10 *H. azteca* (2–3 weeks), 10 *D. magna* (<24 h), six *C. tentans* (10–13 days), and 10 *P. promelas* (<24 h) to each of three replicate 250-ml glass beakers per concentration. Two 1.4-cm diameter maple leaf discs were placed in each *H. azteca* test beaker for substrate. Glass beads (150–212 µm, Sigma Chemical Co., St. Louis, MO) were used as substrate in *C. tentans* tests to allow for tube building and reduce stress (Suedel *et al.* 1996). *C. tentans* was fed one drop of Cerophyll™ per beaker at test initiation to decrease predation. *D. magna* and *P. promelas* were not fed during the 48-h exposure. Following 48-h exposures, organisms were gently prodded with a dissecting probe and survival was determined by observation of organism responses. Water temperature, pH, conductivity, dissolved oxygen, alkalinity, and hardness were measured according to APHA (1992).

Dilution Water

Test dilution water was spring water collected at the University of Mississippi Biological Field Station (UMBFS) (Deaver and Rodgers 1996; Gillespie *et al.* 1996). Water was filtered using MFS 0.45-µm polymembrane filters. Hardness and alkalinity of the filtered water were adjusted with $NaHCO_3$ and $CaCl_2$ (Fisher Scientific, Pittsburgh, PA) to values between 60–80 mg/L as $CaCO_3$.

Insecticide Stock Solutions

Insecticide stock solutions for testing were prepared by dissolving Lorsban™ (44.9% active ingredient chlorpyrifos), Temik™ (15% active ingredient aldicarb), and chlordane (44% active ingredient chlordane) in one liter of Milli-Q™ water. Lorsban™ and chlordane stock solutions were prepared from aqueous insecticides, while granular Temik™ was used to prepare the aldicarb stock solution. After stock solutions were mixed, dilution water and stock solutions were added to each of three replicate test beakers (200 ml total volume) to obtain nominal exposure concentrations. Ranges in nominal aqueous exposure concentrations of chlorpyrifos, aldicarb, and chlordane were 0.1–1000 µg/L, 20–50,000 µg/L, and 1–100 µg/L, respectively.

Exposure Verification

Ohmicron RaPID Assay™ was utilized to confirm insecticide concentrations in aqueous exposure chambers by immunoassay (Kaufman and Clower 1995). Analytical ranges were 1–20 µg/L for chlordane, 0.22–3.0 µg/L for chlorpyrifos, and 1–100 µg/L for aldicarb. If concentrations in amended spring water exceeded analytical ranges, dilutions were performed prior to repeated analysis. Samples were analyzed at 450 nm with an Ohmicron RPA-1™ RaPID Photometer Analyzer.

Statistical Analyses

Insecticide concentrations and organism survival data were used to calculate median lethal concentrations (LC50s) using probit procedure (Stephan 1977) and Trimmed Spearman Karber analyses. Lowest-observed effects concentrations (LOECs) for each organism's response to chlordane, chlorpyrifos, and aldicarb were determined by statistically significant differences relative to controls ($p \leq 0.05$). One-way analysis of variance (ANOVA) was performed with Dunnetts multiple range test to test for significance compared with controls ($p \leq 0.05$) (Zar 1974). If the assumptions of a parametric ANOVA were not met, ANOVA on ranks with Dunn's multiple range test was performed. Insecticide concentrations and organism survival data were also used for determining organism- and insecticide-specific exposure-response slopes. Relative potency of these insecticides was determined for each test species by regression analysis. Exposure-response slopes illustrated the response (mortality) elicited per unit concentration in excess of the lower threshold for response.

Results and Discussion

Exposure Verification

Average recoveries of chlorpyrifos and aldicarb were 110.5 and 78.8%, respectively (Table 2). However, average recovery of chlordane concentrations was 66.4% relative to nominal concentrations. Previous studies (Hall *et al.* 1986) reported relatively rapid (<48 h) sorption of chlordane to surfaces of test vessels. The immunoassay responds to aqueous chlordane and should be indicative of aqueous exposure. Reported 48-h LC50 values were not corrected for recovery.

Table 2. Pesticide concentrations ($\bar{x}$) in exposure chambers during experiments (n = 2)

Pesticide	Nominal Concentrations (µg/L)	Mean Measured Concentrations (µg/L)	Standard Deviation	Average Recovery (%)
Control	0	0	0	0
Chlorpyrifos	0.10	0.10	0.00	110.5
	1.00	1.23	0.01	
	80	100.4	0.80	
	250	285.0	1.25	
	1000	1031.7	2.88	
Aldicarb	20.0	20.27	0.19	78.8
	500	219.6	3.40	
	1000	824.27	22.90	
	9000	7439.40	91.80	
	50000	41970.00	130.00	
Chlordane	1.00	0.93	0.03	66.4
	10.0	5.54	0.06	
	20.0	11.02	0.08	
	50.0	30.75	0.45	
	100	67.37	1.05	

Test Organism Responses to Aqueous Insecticide Exposures

Hyalella azteca. For chlorpyrifos, *H. azteca* was the most sensitive aquatic animal tested (Figure 1). The mean 48-h LC50 for *H. azteca* and chlorpyrifos was 0.1 µg/L in this experiment. *H. azteca* was orders of magnitude less sensitive to aldicarb and chlordane exposures (Figures 2 and 3) (Table 3), with mean 48-h LC50s of 3990 and 61.1 µg/L, respectively. Phipps *et al.* (1995) reported a 10-day LC50 for *H. azteca* and chlorpyrifos of 0.086 µg/L. In a thorough review of insecticide data, Moulton *et al.* (1996) reported an absence of published toxicity values for aldicarb and aquatic invertebrates. Verschueren (1983) reported a 96-h LC50 value of 97 µg/L for *H. azteca* and chlordane. Chlordane 96-h LC50 values of 26 and 40 µg/L were reported for the amphipods *Gammarus lacustris* and *G. fasciatus*, respectively (Sanders and Cope 1966; Sanders 1969).

Daphnia magna. The microcrustacean *D. magna* was also relatively sensitive to chlorpyrifos exposure with a mean 48-h LC50 of 0.6 µg/L. Tomlin (1994) reported a 48-h LC50 for *D. magna* and chlorpyrifos of 1.7 µg/L. Kersting and Wijngaarden (1992) measured 24-h and 48-h LC50s for *D. magna* and chlorpyrifos of 3.7 and 1 µg/L, respectively. Recent studies by Foe and Sheipline (1993) reported 96-h LC50s values for *D. magna* and chlorpyrifos between 0.08 and 0.13 µg/L.

The other insecticides, aldicarb and chlordane, were an order of magnitude less toxic to *D. magna* with mean 48-h LC50s of 583 and 98.4 µg/L, respectively. Hall *et al.* (1986) measured a 48-h LC50 for *D. magna* and technical chlordane of 270 µg/L, while US EPA (1980) reports a *D. magna* and technical chlordane (undisclosed duration, probably 48 h) LC50 value of 35 µg/L.

Chironomus tentans. As anticipated, the midge larva *C. tentans* was also relatively sensitive to chlorpyrifos exposure with a mean 48-h LC50 of 0.3 µg/L. Karnak and Collins (1974) measured a 24 h LC50 of 6.4 µg/L for *C. tentans* and chlorpyrifos, and Ankley *et al.* (1994) reported a 10-day LC50 of 70 ng/L for *C. tentans* and chlorpyrifos. In this experiment, *C. tentans* was the most sensitive species tested for aldicarb and chlordane exposures with mean 48-h LC50s of 20 and 5.8 µg/L, respectively.

Pimephales promelas. P. promelas was the least sensitive species tested for chlorpyrifos and aldicarb, with mean 48-h LC50s of 162.7 and 8860 µg/L, respectively. Johnson and Finley (1980) reported 96-h LC50 values of 2.4 and 7.1 µg/L for chlorpyrifos and *Lepomis machrochirus* (bluegill) and *Oncorhynchus mykiss* (rainbow trout), respectively. An aldicarb 96-h LC50 of 1370 µg/L for *P. promelas* was reported by Pickering and Gilliam (1982). Johnson and Finley (1980) measured aldicarb 96-h LC50s for bluegill and rainbow trout of 50 and 560 µg/L, respectively.

In this experiment, *P. promelas* was relatively sensitive to chlordane with a 48-h mean LC50 of 21.4 µg/L. Verschueren (1983) reported a chlordane 96-h LC50 of 36.9 µg/L for *P. promelas,* while Johnson and Finley (1980) reported a 96-h LC50 of 115 µg/L for the same species.

Relative Potency of Chlorpyrifos, Chlordane, and Aldicarb Based on Responses of Test Organisms

Organism response slopes were calculated using the linearized portion of the exposure-response curves (between 20% and 80% mortality). Lower threshold responses occurred at the lowest test concentration of insecticide where survival was significantly different from the control.

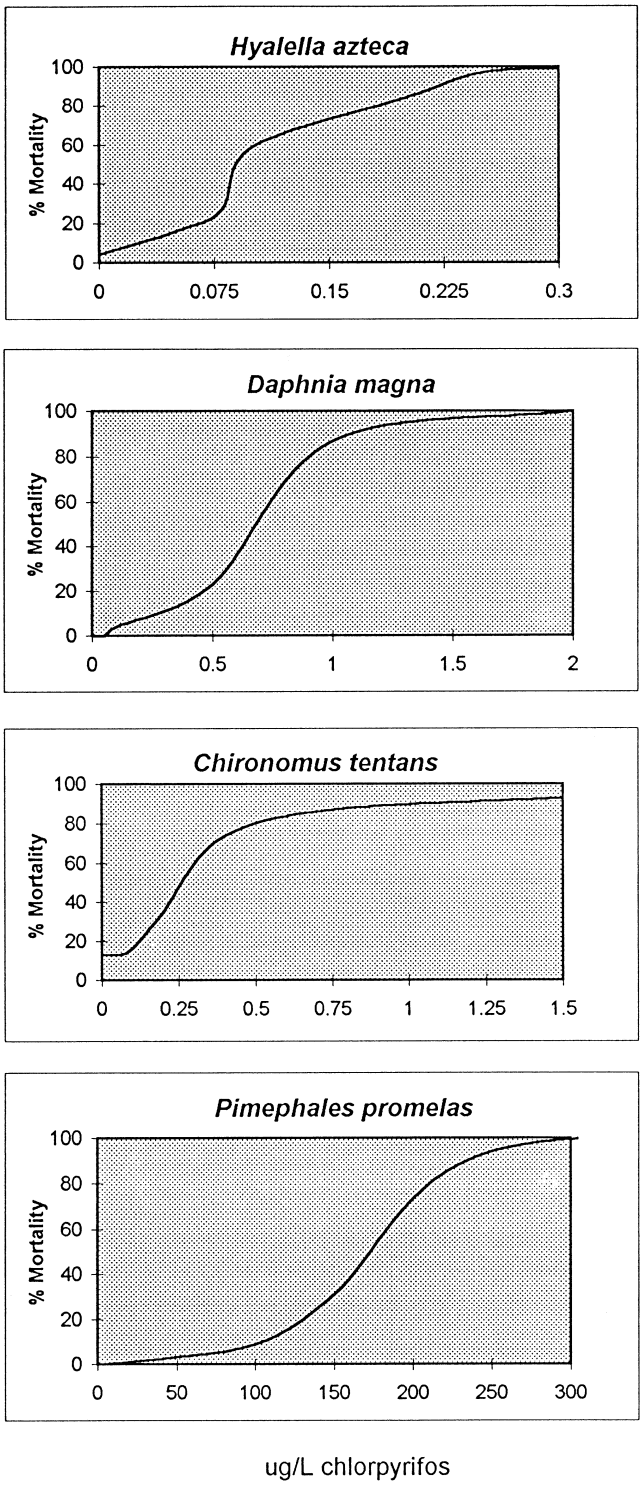


Fig. 1. Chlorpyrifos 48-h exposure-response curves for *H. azteca, D. magna, C. tentans,* and *P. promelas* in order of sensitivity

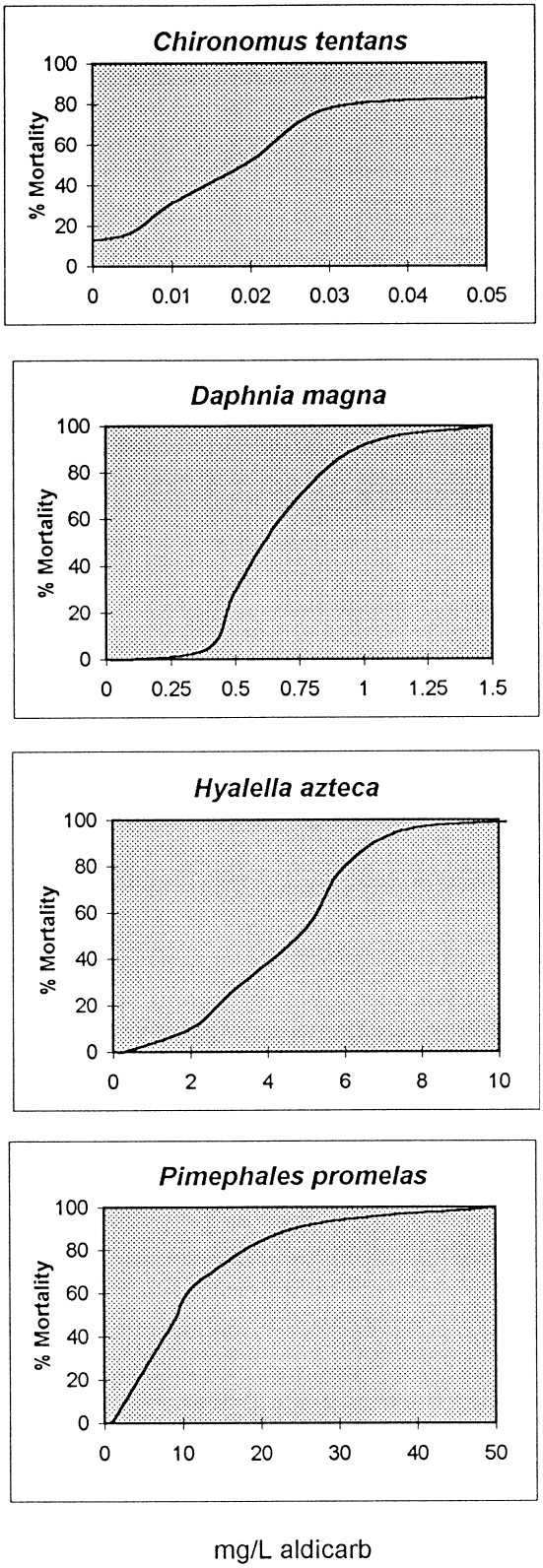


Fig. 2. Aldicarb 48-h exposure-response curves for *C. tentans, D. magna, H. azteca,* and *P. promelas* in order of sensitivity

The test organisms in this study range widely in physiology and niches occupied in aquatic systems. Since four species were used, the relative potency of the three insecticides based on these species takes on additional meaning. The test compounds are designed to be insecticides. Thus, one could expect that an insect (*C. tentans* in this case) or perhaps a microcrustacean would be the most sensitive of the species tested. For both aldicarb and chlordane, *C. tentans* was the most sensitive species tested in terms of potency (Table 4). *H. azteca* was approximately 3.5 times more sensitive to chlorpyrifos than *C. tentans.* Also as expected, the invertebrates tested (*H. azteca, C.*

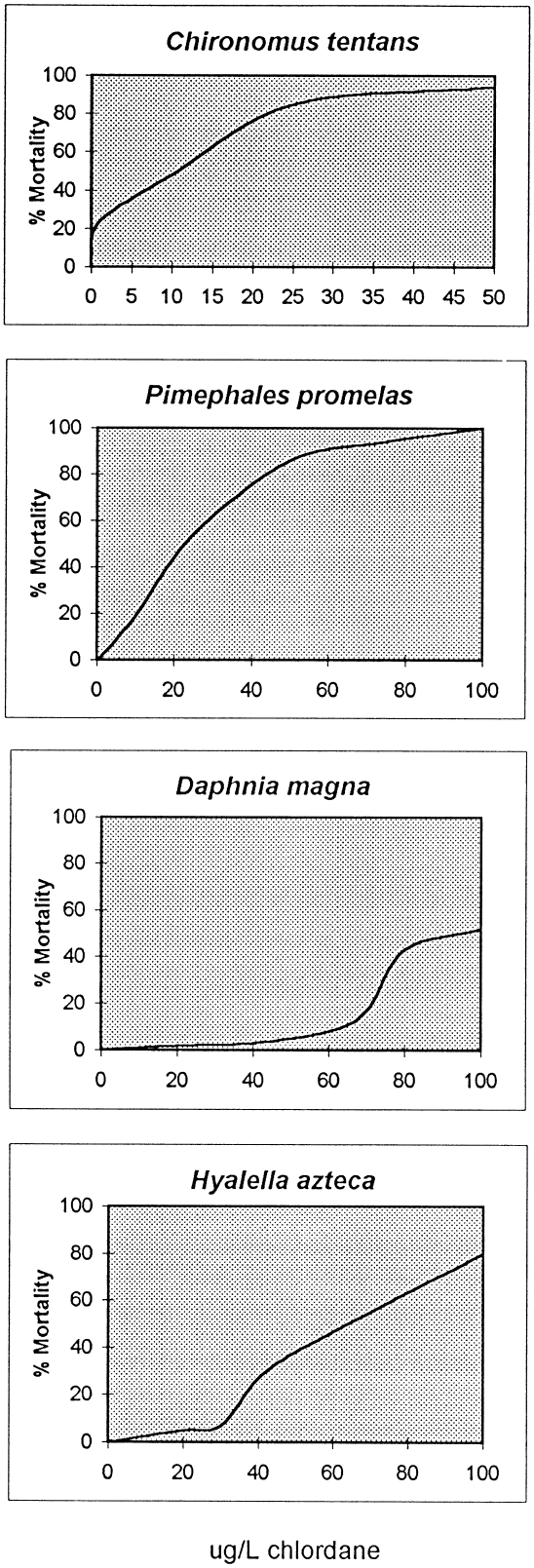


Fig. 3. Chlordane 48-h exposure-response curves for *C. tentans, P. promelas, D. magna,* and *H. azteca* in order of sensitivity

tentans, and *D. magna*) were ≥ 200 times more sensitive in terms of potency than the vertebrate *P. promelas. C. tentans* was about two orders of magnitude more sensitive to aldicarb than the other tested species. Both *H. azteca* and *P. promelas* responded similarly to increasing aqueous concentrations of aldicarb after the lower threshold concentrations for response were reached at 5,000 and 4,500 µg/L, respectively. The differences in potency responses of the test species to chlordane varied only by a factor of approximately 2–3. *P. promelas* was more sensitive (in terms of potency responses) to chlordane than to either chlorpyrifos or aldicarb. For the 48-h exposure duration, aqueous solutions of chlordane approaching solubility limits (0.1 mg/L) were required to elicit 80–100% mortality in *H. azteca, C. tentans,* and *P. promelas;* however, only approximately 50% mortality in *D. magna* occurred in chlordane solutions approaching solubility limits with 48-h exposures.

Table 3. 48 h LC50 values ($\bar{x} \pm$ SD) for the *H. azteca, D. magna, C. tentans,* and *P. promelas* (n = 3)

Organism	Chlorpyrifos (µg/L)	Aldicarb (µg/L)	Chlordane (µg/L)
H. azteca	0.1 (± 0.04)	3990 (± 791)	61.1 (± 4.89)
D. magna	0.6 (± 0.04)	583 (± 40)	98.4 (± 6.68)
C. tentans	0.3 (± 0.07)	20 (± 3.71)	5.8 (± 1.27)
P. promelas	162.7 (± 13.7)	8860 (± 393)	21.4 (± 0.89)

Since potencies of these insecticides were compared and contrasted based upon slopes of exposure-response relationships after initial thresholds (where slope > 0) (Table 4), it is important to consider not only slopes, but also lower thresholds or intercepts as well as upper thresholds or saturation of the response (mortality in this case) (Figures 1–3). In the case of chlorpyrifos (Figure 1), lower thresholds for response followed a similar pattern as the relative potency relationships (slopes) with *H. azteca, C. tentans, D. magna,* and *P. promelas* lower thresholds (LOECs) of 0.1, 0.38, 0.5, and 150 µg/L, respectively. Upper thresholds of response to chlorpyrifos exposures also followed a similar pattern with 100% mortality observed for *H. azteca, C. tentans, D. magna,* and *P. promelas* at 0.3, > 1.5, 2.0, and 300 µg/L, respectively.

Lower threshold responses of organisms to aldicarb exposures followed a similar pattern to the potency relationships. Lower thresholds for *C. tentans, D. magna, P. promelas,* and *H. azteca* were 0.02, 0.5, 4.5, and 5.0 µg/L, respectively. However, upper thresholds varied somewhat as indicated by the slopes of the potency relationships. Upper thresholds of response (100% mortality) were observed for *D. magna, P. promelas,* and *H. azteca* at 1.5, 50, and 15 µg/L, respectively. For *C. tentans,* approximately 83% mortality (maximum mortality observed for this particular experiment) was reached at 0.05 µg/L.

Organisms exposed to chlordane followed a similar pattern of lower threshold responses to that of the potency relationships. Lower thresholds for *C. tentans, P. promelas, H. azteca,* and *D. magna* were 1.0, 25, 40, and 70 µg/L, respectively. Upper thresholds varied somewhat because of approaching solubility limits for chlordane. Only *P. promelas,* at 100 µg/L, reached the upper threshold of 100% mortality. Responses of *D. magna* and *H. azteca* were limited by chlordane solubility to 52% and 80% mortality, respectively at 100 µg/L for the 48-h exposures.

While point estimates of population responses such as LC50s, NOECs, and LOECs are of some utility for predicting effects of pesticides in aquatic systems, exposure-response slopes are also useful for extrapolation of laboratory data to diverse field situations, especially where sediment sorption may regulate exposure or bioavailability. These slopes serve as

Table 4. Potency of chlorpyrifos, aldicarb, and chlordane with their respective exposure–response slopes (% mortality/µg/L)

Organism Sensitivity	Chlorpyrifos	Aldicarb	Chlordane
	H. azteca (453)	*C. tentans* (2.44)	*C. tentans* (2.54)
More sensitive	*D. magna* (128)	*D. magna* (0.13)	*P. promelas* (1.68)
Less sensitive	*C. tentans* (109)	*H. azteca* (0.02)	*D. magna* (1.17)
	P. promelas (0.63)	*P. promelas* (0.003)	*H. azteca* (0.88)

diagnostic models for effects of insecticides on both target and nontarget species. This knowledge will also allow researchers to focus on those target and nontarget species that are sensitive to specific insecticides and the exposures encountered, rather than expending efforts on more resilient species or even on hypersensitive species that may respond before field investigations can be mobilized. In subsequent field studies, we can also focus on species responses that are diagnostic of the insecticide exposure, because these laboratory data can provide guidance for field sampling as well as other strategies such as caged organisms for evaluating the insecticide's fate and effects.

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