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Acute Toxicity of Several Pesticides, Organic Compounds, and a Wastewater Effluent to the Freshwater Mussel, *Anodonta imbecilis, Ceriodaphnia dubia,* and *Pimephales promelas*

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Under the Endangered Species Act (PL 100-707), the federal government is mandated to protect listed species. This protection may go as far as the establishment of label restrictions for pesticide use in areas with sensitive endangered species. However, it is impossible to set valid standards or limits without appropriate data. With the current designation of over 40 species of freshwater (unionid) mussels as endangered or threatened (USFWS 1992) and over 70 species under consideration for listing (USFWS 1991), it is important to assess the impact of pesticides, herbicides and other organic pollutants on this group in particular. It is also pertinent to compare the sensitivities of commonly tested zooplankton species (e.g., *Daphnia magna* and *Ceriodaphnia dubia*) with those of mussels since the use of the former as surrogates for freshwater mussels in toxicity tests would simplify data collection.

The goals of this research were to: (1) determine the acute toxicity of several pesticides, organic compounds and an organic effluent to juvenile *Anodonta imbecilis* mussels, (2) compare their sensitivities with common test organisms such as *D. magna, C. dubia* and *Pimephales promelas*, the fathead minnow and (3) compare the toxicity of toxaphene and chlordane to *A. imbecilis* in the presence and absence of sediment.

MATERIALS AND METHODS

A. imbecilis glochidia were cultured *in vitro* using methods described previously (Keller and Zam 1990) were put in soft reconstituted freshwater (40-50 mg/L as $CaCO_3$) (Peltier and Weber 1985) and used for tests usually within 2 days after their transformation. *C. dubia* were cultured in house. Larval *P. promelas* (fathead minnow) were obtained from EPA-Newtown, Ohio, via overnight mail and used within 24 h.

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Toxicity tests with pesticides and pure compounds were conducted for 48-h in an environmental chamber at a temperature of 22° ± 1° C with a 16L:8D photoperiod. Five test concentrations were used for each toxicant, plus a control which was soft reconstituted freshwater (Peltier and Weber 1985). Two replicates, each containing 10 juvenile mussels, were prepared per concentration in 15 X 60 mm glass Petri dishes with lids. Subchronic toxicity tests (7-d) developed by the USEPA (Horning and Weber 1985) were used to evaluate the toxicity of the wastewater effluent to *C. dubia* and *P. promelas*. Juvenile mussels used in the wastewater test were treated as described above for 48-h tests, but were exposed to the effluent for 7-d. All tests were static except for the wastewater effluent which was a static renewal test. *C. dubia* and *P. promelas* were fed as described in Horning and Weber (1985) during the wastewater test. Otherwise, test organisms were not fed prior to or during tests.

Hydrothol-191, an endothall derivative (Pennwalt Corp., Philadelphia, PA.), was dissolved directly in soft reconstituted freshwater (40-50 mg/L as $CaCO_3$) to make a stock of 530 mg/L. Na · PCP has low solubility in water. Therefore, it was dissolved in 0.01 N NaOH and pH was adjusted to 7.0. Stocks of the remaining compounds--Sodium Dodecyl Sulfate (SDS), methanol, acetone and EDTA--were prepared by dissolving the reagents directly in soft reconstituted freshwater. Reagents were obtained from Fisher Scientific Co., Orlando. Dilutions used in definitive tests were made as appropriate based on range-finding tests. Test solutions were made by 60% dilution of the stocks with soft reconstituted freshwater. All toxicant concentrations given are nominal except for those of toxaphene and chlordane.

Little was known about the toxicity of sediment-sorbed pesticides to mussels, or whether such infaunal molluscs are differentially susceptible to sediment-bound or aqueous concentrations. Toxaphene and chlordane, two chlorinated organic pesticides were chosen to test. Two sets of chambers were prepared for each of these insecticides. One series contained sediment, the other did not. Thirty ml of soft reconstituted freshwater were put into each 50-ml glass vial with or without sediment (5 g dried, 3% organic content). An appropriate volume of stock pesticide in acetone was injected directly into each vial using a micro-syringe, then the vial was capped with a teflon-lined top and mixed over night on a wrist-action shaker. The contents of the vials were then emptied into 50-ml beakers. Both sets of test chambers (with and without sediments) were left for 24 h prior to addition of the mussels, to allow sediments to settle and contents to equilibrate. Ten juvenile *Anodonta imbecilis* and five *Ceriodaphnia dubia* neonates were added to each of two chambers (replicates) at each test concentration both with and without sediment. Toxaphene concentrations ranged from 1.829

mg/L to 0 mg/L. Chlordane was used at concentrations of 0.905 mg/L to 0 mg/L.

Water samples from chambers used in chlordane and toxaphene tests were analyzed by gas chromatography to determine aqueous concentrations. Samples were extracted with three 10-ml aliquots of methylene chloride and later transferred to iso-octane. The extracts were blown down to 1 ml with nitrogen and stored in crimpseal vials until analyzed on a Varian 3700 gas chromatograph. A 30-m DB-5 column with a 0.53-mm diameter and a 1-um coating was used with an initial temperature of 150° C ramped to 250° C at 5° C per minute. The injector temperature was 22° C and the detector was set at 300° C. The recovery rate for both pesticides was 28%. Values were corrected for recovery.

An industrial effluent sample was obtained from the Buckman Street Wastewater Treatment Facility, Jacksonville, FL for use in assessing the relative sensitivity of juvenile *A. imbecilis* mussels versus those of standard effluent test organisms. The 7-d effluent toxicity tests were performed using moderately hard (80-100 mg/L as $CaCO_3$) reconstituted freshwater (Horning and Weber 1985) as diluent and control water.

Forty *A. imbecilis* juveniles were exposed to each effluent concentration, 20 in each of two replicate 15 mL chambers. Ten *Ceriodaphnia dubia* neonates and 20 *Pimephales promelas* larvae were exposed to each test concentration, the former in individual 30 ml plastic containers to permit monitoring of reproduction, the latter in two groups (replicates) of 10 at each dilution in 1 L pyrex beakers. Toxicity was assessed based on protocols established in the *C. dubia* survival and reproduction test, and the fathead minnow survival and growth test (Horning and Weber 1985). Survival of test organisms was recorded daily until termination at 7-d. Zooplankton reproduction and larval fathead minnow growth were used as indicators of sublethal affects (Horning and Weber 1985). Water was changed daily in all test chambers.

Survival data from each test were analyzed by a computerized Probit Analysis (Peltier and Weber 1985). Results of these analyses (LC50s) were then used to determine differences in toxicity among the chemicals with ANOVA and Duncan's multiple range test. All statistical analyses except LC50s were performed using SAS procedures (GLM with Duncan's) (SAS 1986).

RESULTS AND DISCUSSION

Of the eight organic compounds tested for toxicity to *A. imbecilis*, PCP was the most toxic, while methanol was the least toxic (Table 1). Forty-eight hour LC50s for *A. imbecilis* exposed to acetone and methanol

were 37.02 and 36.3 mg/L, respectively (Table 1). Forty-eight hour LC50 values for *D. magna* are 0.0039 mg/L acetone (Macek and McAllister 1970) and 11 mg/L methanol (Poirier et al. 1986). The sensitivity of *S. gairdneri* (rainbow trout) to methanol was 29.40 mg/L, intermediate between the values for the two invertebrates (Mayer and Ellersieck 1986).

The 48-h LC50s for *A. imbecilis* exposed to SDS, often used as a reference toxicant in tests with zooplankton, was 19.04 mg/L (Table 1). There are no established benchmark values for SDS toxicity to mussels. However, mussels were about as sensitive to the surfactant as *D. magna* (Table 1) based on published values (Lewis and Weber 1985). A. imbecilis was relatively insensitive to the

Table 1. Acute toxicities of several organic compounds to juvenile *Anodonta imbecilis*, *Daphnia magna* and *Lepomis macrochirus*. LC50s values for *A. imbecilis* with the same superscript numbers were not significantly different from each other ($p<0.05$).

	48-h LC50 (mg/L)		96-h LC50 (mg/L)
Chemical	*A. imbecilis*	*D.magna*	*L. macrochirus*
Methanol	37.02 (4.7)[1]	11.0[eg]	29.40[fg]
Acetone	33.83 (11.31)[12]	0.003[d]	--
SDS	19.04 (4.19)[3]	10.3[c]	--
Hydrothol	4.85 (2.29)[4]	0.360[i]	0.940[j]
EDTA	1.35 (0.35)[4]	--	--
PCP	0.61 (0.26)[4]	0.33[c]	0.24[d]
Chlordane[a]	0.88	0.029[dk]	0.092[d]
Toxaphene[a]	0.74	0.010[d]	0.007[d]

[a]96-h LC50. [c]Lewis and Weber (1985). [d]Macek and McAllister (1970). [e]*Ceriodaphnia dubia*. [f]*Salmo gairdneri*. [g]Poirier *et al.* (1986). [i]Pennwalt Corp. (1980). [j]Johnson and Finley (1980). [k]*Daphnia pulex*.

herbicides and insecticides that were tested (Table 1). The 48-h LC50 for the aquatic herbicide Hydrothol-191 to mussels was 4.85 mg/L. In comparison, the 48-h LC50 for *Ceriodaphnia dubia* is 0.190 mg/L and the 96-h value for fathead minnow larvae is 0.468 mg/L (Keller *et al.* 1988a, 1988b).

Table 2. Comparative toxicity of an effluent from the Buckman Street Wastewater Treatment Facility, Jacksonville, Florida to *A. imbecilis*, *C. dubia* and *Pimephales promelas*.

	% Wastewater	
Organism	96-h LC50	7-d LC50
A. imbecilis	35.35	16.24
C. dubia	7.08	4.97
P. promelas	N/C*	N/C

*N/C=not calculable; all fathead minnows died in 24-h even in only 6% effluent.

Literature values for Hydrothol toxicity to other aquatic organisms are much lower than those of the mussels (Pennwalt Corp. 1980; Johnson and Finley 1980). *A. imbecilis* was also less sensitive to toxaphene and chlordane than other test organisms (Table 1). Acute toxicity for most aquatic organisms range from 0.002-0.040 mg/L for toxaphene and from 0.003-0.112 mg/L for chlordane (Johnson and Finley 1980). After four days' exposure, half of the mussels were killed by 0.740 ± 0.007 mg/L toxaphene and 0.880 ± 0.005 mg/L chlordane in chambers without sediment. However, neither toxaphene (up to 1.83 mg/L) nor chlordane (up to 0.90 mg/L) was toxic to *A. imbecilis* at 48 h in chambers with sediment.

PCP was acutely toxic to juvenile *A. imbecilis* mussels (Table 1). It was the only pesticide of the three tested to which mussels were as sensitive as other species. The 48-h LC50 was 0.610 mg/L for mussels, 0.330 mg/L for *D. magna* (Lewis and Weber 1985) and 0.240 mg/L for bluegill (Macek and McAllister 1970). PCP is known to be toxic to virtually all biota, including molluscs (Ware 1978) and has been used as a molluscicide for years. The 48-h LC50s for two snail species, *Lymnaea stagnalis* and *Gillia altilis*, were found to be 0.240 mg/L and 0.810 mg/L PCP (USEPA 1986).

The industrial effluent was less toxic to mussels than to either *C. dubia* or *P. promelas* (Table 2). Juvenile mussels were 4-5 times less sensitive than were the zooplankton, and even less sensitive than that compared to fathead minnows which died at the lowest effluent concentration (6%) in 24-h. Ninety-six hour LC50s for *A. imbecilis* and *C. dubia* were 35.35% and 7.08%, respectively, while at 7-d the LC50s had decreased

to 16.24% for the mussels and 4.97% for *C. dubia*. Reproduction levels in the controls were too low to determine subchronic effects on *C. dubia*. While chemical analyses of the effluent used in the toxicity test were not performed, previous analyses identified several organic compounds including diazinon (Koopman *et al.* 1989).

In contrast to the results of toxicity tests with metal pollutants (Keller and Zam 1991), *A. imbecilis* was found to be generally less sensitive to organic pollutants than were standard toxicity organisms such as *D. magna*, *Ceriodaphnia dubia*, the fathead minnow, and bluegill sunfish. The reasons for the apparent tolerance of these mussels to pesticides, herbicides and effluents, all having different chemical structures, characteristics and mechanisms is unknown. There may be protective physiological adaptations or short-term behavioral responses that allow mussels to survive longer.

D. magna is the invertebrate commonly used to derive water quality criteria in the United States. Since *A. imbecilis* was found to be no more sensitive to the tested pollutants after a 48-h exposure than was *D. magna*, mussels *may* be adequately protected by current water quality standards. However, it is impossible to determine from these data what the effects of chronic exposures to pesticides or other organic compounds might be. *Further testing is necessary* to determine whether long-term exposure of mussels to pesticides or other organic compounds is responsible for the loss of mussels from rivers and streams where they were once plentiful.

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