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Relative Sensitivity of Three Freshwater Benthic Macroinvertebrates to Ten Contaminants

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Abstract. The objective of this study was to determine the suitability of *Hyalella azteca, Chironomus tentans* and *Lumbriculus variegatus* as representative species for the assessment of sediment toxicity. Ten chemicals were tested at the U.S. EPA Environmental Research Laboratory-Duluth, always using *H. azteca* and *C. tentans* and, occasionally, *L. variegatus*. The exposures were water-only, flow-through tests with measured chemical concentrations, which were conducted for 10 days in Lake Superior water. Chemicals tested included five metals (copper, lead, zinc, nickel, cadmium) and five pesticides (chlorpyrifos, dieldrin, *p,p'*-DDD,*p,p'*-DDE,*p,p'*-DDT). The amphipod was quite sensitive to the metals, while the midge often was exceptionally sensitive to the pesticides. No one of the three species was most (or least) sensitive to the toxicants. Toxicity of the contaminants to the three species was compared to the genus mean acute and chronic data found in U.S. EPA Water Quality Criteria (WQC) documents, as well as information from the AQUIRE database. The results of these comparisons indicated that the three species reasonably represent the range of sensitivities of other aquatic test species, and occasionally are among the most sensitive species when compared to others in the WQC database.

To promote a cohesive regulatory strategy for dealing with contaminated sediments, it is necessary to have a suite of well-characterized standard methods. Among the most frequently utilized species for assessing the toxicity of freshwater sediments are the amphipod *Hyalella azteca* and the midge *Chironomus tentans* (Nebeker *et al.* 1984; Borgmann *et al.* 1989; ASTM 1992; Ankley *et al.* 1993). Methods for bioaccumulation tests with freshwater sediments are not as advanced as for toxicity tests. One species that has shown good promise for estimating the bioaccumulation of sediment-associated contaminants is the oligochaete *Lumbriculus variegatus* (Ankley *et al.* 1992; Phipps et al. 1993). All three species recently were identified as those for which standard sediment test methods would be developed by the U.S. EPA (US EPA 1992). In support of this effort, and to derive and validate sediment quality criteria, the U.S. EPA research laboratory in Duluth, MN has performed a number of studies evaluating the relative sensitivity of the three species to sediment-associated contaminants. Some of this work has been done with environmental samples in which the character of the contaminants of concern was not fully defined, making it difficult to truly evaluate relative sensitivity (Ankley *et al.* 1991a; Schubauer-Berigan and Ankley 1991; West *et al.* 1993). Additionally, we have generated a significant amount of comparative toxicity data from single chemical, water-only tests with *H. azteca, C. tentans* and *L. variegatus*. Some of this information has been published in conjunction with various studies (Ankley *et al.* 1991b, 1994; Hoke *et al.* 1994a, 1994b; Schubauer-Berigan *et al.* 1993; West *et al.* 1993); however, much of the data have not been published.

The objectives of this analysis were two-fold. First, all published and unpublished single-chemical toxicity data were compiled from 10-day water-only tests conducted in our laboratory to evaluate relative differences in sensitivity among *H. azteca, C. tentans,* and *L. variegatus*. These tests were conducted with 10 potentially important sediment contaminants, including five metals (Cd, Ni, Cu, Zn, Pb) and five pesticides or pesticide metabolites - DDT, DDD, DDE, dieldrin, and chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)-phosphorothioate). The second objective was to compare toxicity information for the three test species to high-quality toxicity data generated for other freshwater species; sources for this information were chemical-specific water quality criteria documents (USEPA 1980a, 1980b, 1980c; 1984a, 1984b, 1984c; 1986a, 1986b) ACQUIRE information retrieval system (U.S. EPA 1984d).

Materials and Methods

Test Methods

Toxicity data were derived from water-only 10-d toxicity tests with *H. azteca, C. tentans* and *L. variegatus*. The tests were conducted in minidiluter systems described by Benoit *et al.* (1982). All tests were conducted in Lake Superior water, with the exception of the chlorpyri-

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fos exposure, which was performed using dechlorinated tap water from the city of Superior, WI. General test conditions for the various toxicants were similar; for some of the chemicals, these conditions are described in greater detail elsewhere (cadmium and nickel, Ankley *et al.* 1991b; copper, West *et al.* 1993; dieldrin, Hoke *et al.* 1994a; DDT, DDD, DDE, Hoke *et al.* 1994b; chlorpyrifos, Ankley *et al.* 1994b). Each exposure typically consisted of five experimental concentrations and a clean water control. Two-five replicate exposures were conducted in side-drilled and screened high-form 300 ml beakers contained in each test tank. Water solutions of toxicants for exposures were either dissolved and contained in 19 L glass stock bottles (metals), or generated from toxicant coated glass fiber packed into tubes (generator columns) through which dilution water was passed (pesticides). Water flow rates to the exposure beakers were 15-20 volume additions per day. Tests were conducted at 21 to 24°C with a 16L:8D photoperiod. Ten organisms from on-site cultures were typically used in each replicate beaker; however, on occasion, five *C. tentans*/replicate were used. *Hyalella azteca* were fed a yeast-Cerophyll®-trout chow mixture (US EPA 1989), and *C. tentans* were fed a Tetrafin® suspension daily (Ankley *et al.* 1993). *Lumbriculus variegatus* were not fed during the tests. *Hyalella azteca* were provided with a small piece of screen and *C. tentans* a monolayer of sand as substrates. Test concentrations of the metals were determined using atomic absorption spectroscopy, while pesticide concentrations were measured with gas chromatography/electron capture detection (Ankley *et al.* 1991a, 1994b; Hoke *et al.* 1994a, 1994b; West *et al.* 1993). LC_{50} values from measured contaminant concentrations were calculated using the trimmed Spearman-Karber technique (Hamilton *et al.* 1977), after adjustment for control mortality using Abbotts formula (Finney 1971).

Data Analysis

Water quality criteria documents (WQCD) were used as sources of comparative acute and chronic toxicity data for all chemicals except DDD and DDE. Comparative toxicity data for DDD and DDE were derived from the AQUIRE data base (USEPA 1984d), and genus mean acute and chronic values were calculated using data from the WQCD that includes, for freshwater organisms, 48-h EC_{50} and LC_{50} values for cladocerans and 96-h EC_{50} and LC_{50} values for other species. Chronic toxicity data includes full and partial life-cycle, and early life-stage tests. Tests with *H. azteca, C. tentans,* and *L. variegatus* were 10-days in duration, which is longer than WQCD tests, but typically shorter than WQCD chronic tests. Hence, they fall between the two WQCD categories, which is an important distinction for understanding relative sensitivity comparisons between our tests and the others described herein.

Results and Discussion

Comparison of 10-day LC_{50} values for *C. tentans, H. azteca,* and *L. variegatus* for the five metals and five pesticides indicates there was no one species consistently most sensitive to the 10 toxicants (Table 1). *Hyalella azteca* was most sensitive to the five metals and three of the five insecticides. However, in two instances, *C. tentans* was more sensitive than *H. azteca* to pesticides (chlorpyrifos, dieldrin), which are more specifically targeted to insects than DDT, DDD, and DDE. It appears that *H. azteca* is more sensitive to broad spectrum toxicants than the other two test species. *Lumbriculus variegatus* was never the most sensitive species tested; however, for at least two of the metals (copper and lead), the oligochaete was more sensitive than the relatively tolerant midges.

Table 1. Relative sensitivities of *H. azteca, C. tentans* and *L. variegatus* to 10 chemicals in 10-d, water-only exposures. The LC_{50} values are expressed in μg/L

Chemical	Species *H. azteca*	*C. tentans*	*L. variegatus*
Copper	31	54	35
Zinc	73	1125[a]	2984
Cadmium	2.8[b]	N/T[c]	158
Lead	<16[d]	N/T	740
Nickel	780	N/T	12160
p,p'-DDT	0.07	1.23	N/T
p,p'-DDD	0.19	0.18	N/T
p,p'-DDE	1.66	3.0	>3.27[e]
Dieldrin	7.6	1.1	N/T
Chlorpyrifos	0.086	0.07	N/T

[a] 50% mortality at highest dose
[b] 70% mortality at lowest dose tested
[c] Not tested
[d] 8-d test
[e] Highest dose tested

Figures 1 and 2 present graphical comparisons between our data and acute or chronic toxicity data for other freshwater test species. These relationships are discussed in detail below.

Cadmium (Figure 1a)

The WQCD has 13 species chronic and 44 genus mean acute values. The range of toxicity values for cadmium was 8,325 μg/L in an acute test with goldfish. (*Carassius auratus*), to a chronic value of 0.14 μg/L for *Daphnia magna.* Two chronic and one acute values are lower than the approximate 10-d LC_{50} of 2.8 μg/L for *H. azteca. Chironomus tentans* was not tested with cadmium, while *L. variegatus* was ranked 29th of 59. The brown trout, *Salmo trutta,* was most sensitive in short-term tests with a 96-h acute value of 1.6 μg/L. Moreover, salmonids occupy four of the first eight ranks and appear to be particularly sensitive to this metal. There is a value for *H. azteca* in the WQCD database that occupies the 30th rank of 59. However, that test was conducted for only 96-h in a water harder than Lake Superior water.

Nickel (Figure 1b)

The nickel toxicity data ranged from a genus mean chronic value of 86 μg/L for *D. magna* to an acute value of 43,550 μg/L for the banded killifish, *Fundulus diaphanus.* There were 18 genus mean acute values and four species mean chronic values. Our 10-d LC_{50} values for *H. azteca* and *L. variegatus* were 780 μg/L and 12,160 μg/L, respectively. *Chironomus tentans* was not tested with this metal. *Hyalella azteca* was ranked fifth of 24, exceeded in sensitivity only by the four chronic values in the WQCD database. *Lumbriculus variegatus* was ranked 14th, with an LC_{50} value similar to that for the marine polychaete worm *Nais sp.*

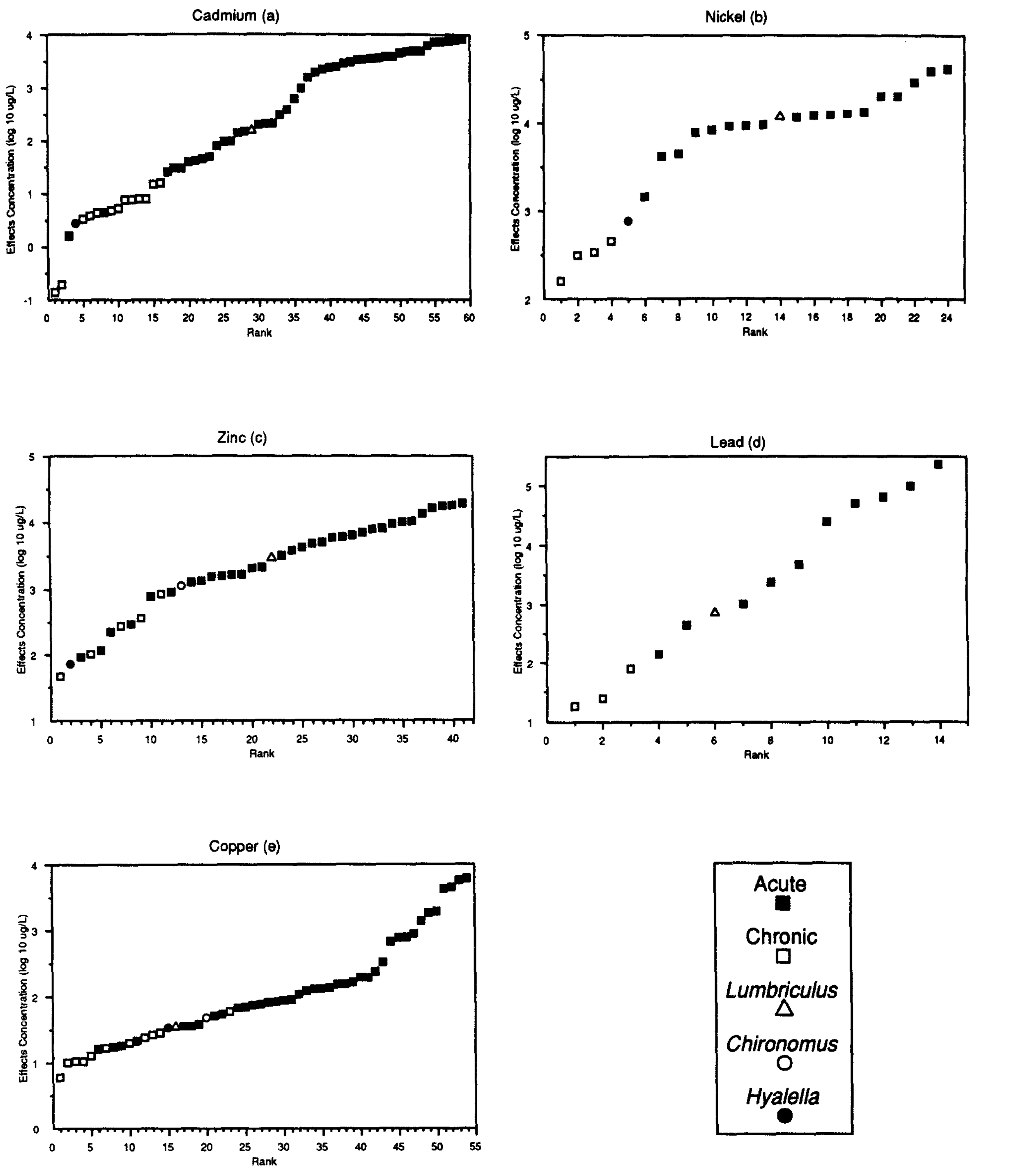

Fig. 1. Sensitivity of *Hyallela azteca, Chironomus tentans* and *Lumbriculus variegatus* to cadmium, nickel, zinc, lead and copper compared to species-specific toxicity data from EPA Water Quality Criteria Documents and/or AQUIRE

Zinc (Figure 1c)

The zinc WQCD has six species mean chronic values and 33 genus mean acute values. Our 10-d LC_{50} value of 73 μg/L for *H. azteca* was exceeded in sensitivity only by the flagfish, *Jordinella floridae,* with a chronic value of 47 μg/L. *Chironomus tentans* and *L. variegatus* 10-d LC_{50} values were 1,125 μg/L and 2,984 μg/L, respectively, and were higher than all six chronic values from the WQCD. However, *C. tentans* was more sensitive than 27 acute values and *L. variegatus* was more sensitive than 19 other genus mean acute values. A value of 9,712 μg/L for *L. variegatus* was found in the WQCD compared to a 10-d

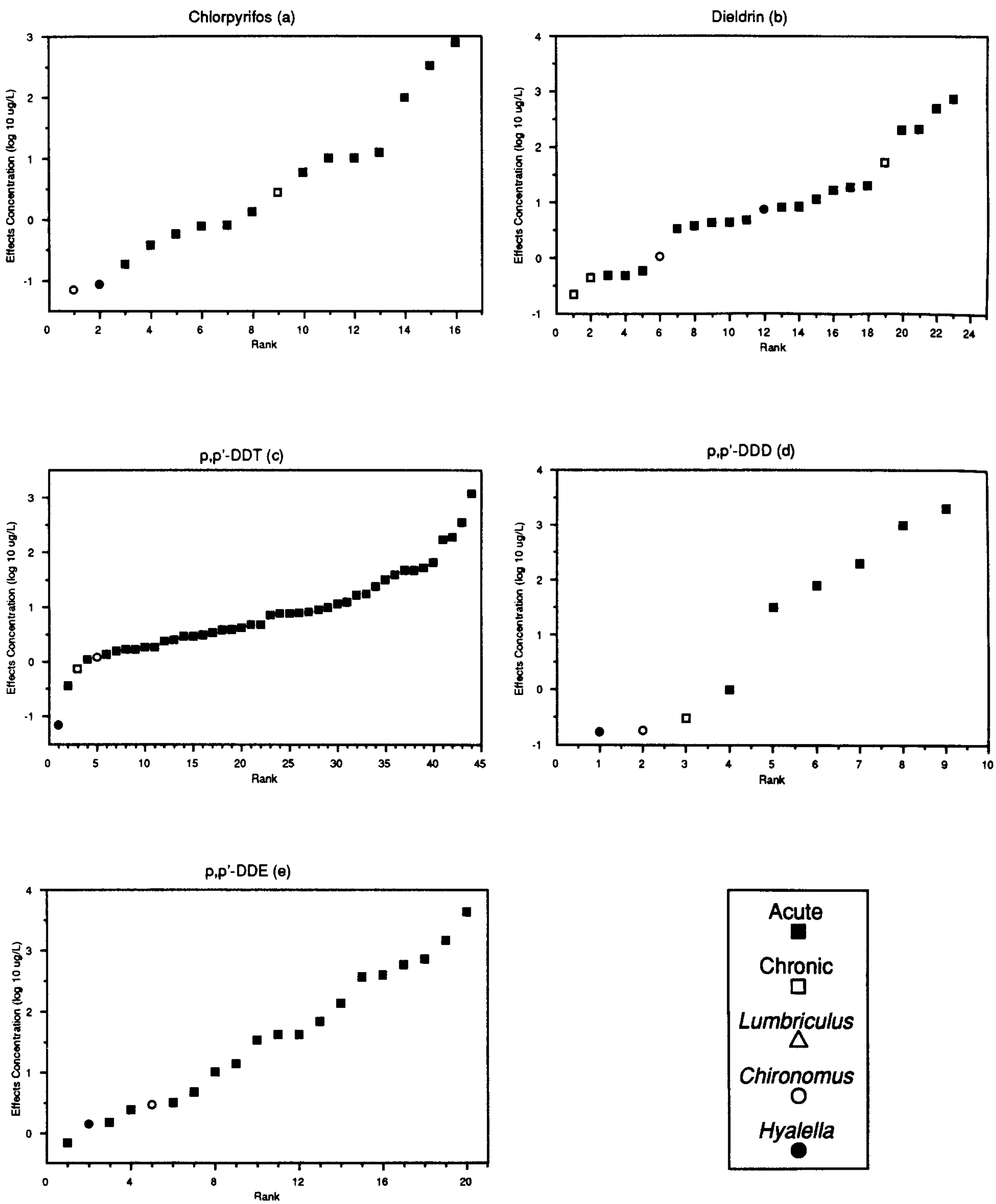

Fig. 2. Sensitivity of *Hyallela azteca, Chironomus tentans* and *Lumbriculus variegatus* to chlorpyrifos, dieldrin, *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE compared to species-specific toxicity data from EPA Water Quality Criteria Documents and/or AQUIRE

LC_{50} of 2,984 μg/L from our study, but again, the former value was from a 96-h test in water harder than Lake Superior water.

Lead (Figure 1d)

The WQCD database for lead is small. It has three genus mean chronic and ten genus mean acute values. The WQCD data ranged from 18.9 μg/L for a rainbow trout *(Oncorhynchus mykiss)* chronic test to an acute value of 235,900 μg/L for the midge *Tanytarsus dissimilus*. *Lumbriculus variegatus* had a 10-d LC_{50} of 794 μg/L in our laboratory. A preliminary 8-d test with *H. azteca* produced an LC_{50} value of less than 16 μg/L, compared to a value for another amphipod, *Gammarus pseudolimnaeus*, of 143 μg/L. *Chironomus tentans* was not tested with this metal.

Copper (Figure 1e)

The database for copper is fairly extensive, with 40 acute and 11 chronic values from the WQCD. The WQCD acute database ranged from 16.7 μg/L for the northern squawfish *Ptychochelus oregonensis* to 10,240 μg/L for the stonefly *Acroneuria lycorias*. The chronic database ranged from 6.1 μg/L for the amphipod *G. pseudolimnaeus* to 60.4 μg/L for northern pike *Esox lucious*. *Hyalella azteca* was the most sensitive species, with a 10 day LC_{50} of 31 μg/L followed closely by *L. variegatus,* with a value of 35 μg/L and ranks of 15 and 16, respectively. *Chironomus tentans* was the least sensitive species with a 10-d LC_{50} of 54 μg/L and a rank of 20.

Chlorpyrifos (Figure 2a)

Cholrpyrifos is a acetylcholinesterase inhibitor, and the WQCD database suggests that the chemical is a well-targeted insecticide since, with the exception of the stonefly *Pteronarcys californica,* all fish and crayfish are less sensitive than other invertebrates such as insects and amphipods. With respective LC_{50} values of 0.086 and 0.07 μg/L, *H. azteca* and *C. tentans* were more sensitive than any freshwater species tested for the WQCD. The data ranged from 0.07 μg/L (rank 1) for the *C. tentans* test to 806 μg/L for an acute exposure with channel catfish *Ictaluris punctatus*. The *H. azteca* 10-d LC_{50} of 0.086 μg/L (rank 2) was four-times lower than that of another amphipod *G. fasciatus,* with a value of 0.32 μg/L. There was one chronic value for the fathead minnow, *Pimephales promelas,* of 2.76 μg/L. *Lumbriculus variegatus* was not tested with this chemical.

Dieldrin (Figure 2b)

There are 18 genus mean acute values and three chronic values in the dieldrin WQCD. The acute toxicity data for this organochlorine insecticide range from 0.5 μg/L for two stonefly species to 740 μg/L for the crayfish *Orconectes nais*. Two chronic values for rainbow trout, *O. mykiss,* and the guppy *Poecilia reticulata* were the lowest in the dieldrin database at 0.22 and 0.45 μg/L, respectively. *Chironomus tentans,* which ranked sixth, was the most sensitive species with a 10-d LC_{50} of 1.1 μg/L, while *H. azteca* (10-d LC_{50}, 7.6 μg/L) was ranked 12th. *Lumbriculus variegatus* was not tested with dieldrin in the study, however, the WQCD had an acute value for *L. variegatus* of 21.8 μg/L at a rank of 18.

p,p'-DDT (Figure 2c)

The WQCD for *p,p'*-DDT has 41 acute values ranging from 0.36 μg/L for *D. pulex* to 1,230 μg/L for a planarian, *Polycellis felina*. The database suggests that even though it was designed as an insecticide, DDT is not solely targeted at insects, as it is also quite toxic to fish and other aquatic invertebrates. *Hyalella azteca* was the most sensitive of our test species with a 10-d LC_{50} of 0.07 μg/L; this value was lower than any other in the WQCD. The 10-d LC_{50} for *C. tentans* was 1.23 μg/L, which occupied the 5th rank. *Lumbriculus variegatus* was not tested with DDT.

p,p'-DDD (Figure 2d)

Toxicity data for *p,p'*-DDD are scarce. There was only one chronic and six acute values from the AQUIRE database. The acute values ranged from 1 μg/L for *Bosmina longirostris* to 2,360 μg/L for a flatworm. *Nitocra spinipes* had a chronic value of 0.3 μg/L. The *C. tentans* and *H. azteca* 10-d LC_{50} values were lower than any others found at 0.18 and 0.17 μg/L, respectively. *Lumbriculus variegatus* was not tested with *p,p'*-DDD.

p,p'-DDE (Figure 2e)

There were 18 acceptable acute values in the AQUIRE database, ranging from 0.68 μg/L for *Gammarus sp.* to 4,400 μg/L for the fathead minnow, *P. promelas*. The 10-d LC_{50} values for *H. azteca* and *C. tentans* were 1.66 and 3.0 μg/L and ranked third and sixth, respectively. The 10-d LC_{50} for *L. variegatus* was greater than 3.3 μg/L.

Summary and Conclusions

An examination of the toxicity data for *H. azteca, C. tentans* and *L. variegatus* revealed a number of trends and relationships. Foremost, there was no single most sensitive species for the chemicals tested. *Hyalella azteca* was consistently most sensitive to the metals, however, *C. tentans* was the most sensitive to two of the pesticides. Similarly, although *L. variegatus* was never the most sensitive species, neither was it consistently the least sensitive. These observations highlight the importance of testing multiple species in sediment assessments, particularly in situations where unknown contaminants are present. In addition, our analysis indicates that the three benthic species are comparable in sensitivity to other organisms tested with the 10 chemicals used in these studies. In fact, although the 10-d exposures used for *H. azteca, C. tentans* and *L. variegatus* are considered short-term tests (US EPA 1992), in several instances the results of the assays indicated a sensitivity comparable to, or exceeding that of, early life stage, partial life cycle or life cycle tests defined as chronic for WQC development. It should be noted that this sensitivity is a function not only of the test species, but the test regime, i.e., many of the tests used for WQC development were static rather than flow-through, and many used nominal rather than measured chemical concentrations to calculate effects. Both of these practices would result in apparent effects concentrations being higher than "true" effects concentrations.

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