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Short-Term Lethality and Sediment Avoidance Assays with Endrin-Contaminated Sediment and Two Oligochaetes from Lake Michigan

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Abstract. Mean 96-hr LC_{50} values and standard deviations for the oligochaetes *S. heringianus* and *L. hoffmeisteri* exposed to endrin-contaminated sediment were 2,588 ± 1,974 μg/g dry weight sediment for 4 assays and 2,725 ± 955 μg/g for 2 assays, respectively. Mixed species testing data suggested that the toxicity to *L. hoffmeisteri* was reduced in the presence of *S. heringianus*, yet further testing is required. Ninety-six hour EC_{50} burrowing avoidance values for both species (19 and 15.3 μg/g for *S. heringianus* and 59 μg/g for *L. hoffmeisteri*) were approximately 46 and 150 times lower than their respective mean 96-hr LC_{50} values. Both *S. heringianus* and *L. hoffmeisteri* initially burrowed in contaminated sediment and then returned to the surface in numbers somewhat proportional to the sediment concentration and the length of exposure. Future use of oligochaete behavioral responses to sublethal sediment contamination for pollutant impact on benthic communities is promising.

Toxic compounds with high partition coefficients that enter the Laurentian Great Lakes adsorb significantly to fine particles and settle to the bottom. The fate of these compounds and their interactions with the larger benthic organisms is largely unknown. Compound fates normally depend on complex combinations of sediment and xenobiotic chemical/physical properties and the activity patterns of the larger benthos (Petr 1977). Oligochaetes are of particular interest because they are frequently the dominant macrobenthic taxa, and because they rework (mix) sediments in a conveyor-belt fashion (Davis 1974; Krezoski *et al.* 1978; Robbins *et al.* 1979; McCall and Fisher 1980; Robbins 1982; Krezoski and Robbins 1985; Robbins 1986), which can result in profound alterations of sediment characteristics (Robbins 1982; Fisher 1982; McCall and Tevesz 1982). Despite the importance of the oligochaetes, very few studies have examined contaminated sediment-oligochaete interactions.

In previous freshwater oligochaete studies, contaminants were usually placed in solution, either without a sediment substrate or in the presence of uncontaminated sediment. The responses of several freshwater oligochaete species under these conditions have been determined for cadmium, mercury, pentachlorophenol, pulp mill effluent, and sewage sludge in water, and in water and uncontaminated sediment under a range of dissolved oxygen and temperatures (Chapman *et al.* 1982a, 1982b, 1982c). Death and changes in respiration were used as the measures of response. In most experiments, 96-hr LC_{50} values increased (toxicity was decreased) when uncontaminated sediments were added. Survival was also enhanced and a decrease in community respiration was observed when *Limnodrilus hoffmeisteri* (Tubificidae) and *Tubifex tubifex* (Tubificidae) were exposed together to the above pollutants without sediment (Chapman *et al.* 1982a). Additionally, the toxicities of twenty-three insecticides to *Branchiura sowerbyi* (Tubificidae) were generally reduced when uncontaminated sediment was added to test solutions (Naqvi

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1973), as were the toxicities of heavy metals and nitroaromatic compounds to *Lumbriculus variegatus* (Lumbriculidae) (Bailey and Lui 1980). Oligochaetes were exposed directly to contaminated sediments in only one study (Karickhoff and Morris 1985). The intent of their study, however, was to examine the role of tubificids in toxicant transport, and not to determine lethal and sublethal responses by the organisms.

By virtue of their persistence and relative toxicity, compounds with high partition coefficients that sorb to the fine grained, organic fraction of the sediments pose serious potential health hazards to man. Although benthic organisms are an integral part of the aquatic food web, lethal testing of oligochaetes in contaminated sediments has not been reported. Contaminated sediments should afford a more natural testing medium than water (Chapman *et al.* 1982b) and provide an optimal medium for exposures to hydrophobic toxicants. Use of contaminated sediments also allows potential sublethal responses to be examined and quantified, such as changes in burrowing behavior at exposure levels approaching levels that have been found in the environment.

The objective of this research was to compare the lethal and behavioral responses, including an ancillary examination of mixed species effects in lethality tests, to a sediment bound toxicant by two freshwater oligochaetes: *Stylodrilus heringianus* (Lumbriculidae) and *Limnodrilus hoffmeisteri* (Tubificidae).

Materials and Methods

The chlorinated pesticide endrin (1,2,3,4,10,10, hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,endo-5,8-dimethanonaphthalene) was chosen because of its sorption characteristics (log k_{ow} = 5.6, Neely *et al.* 1974), its toxicity to other aquatic species (Grant 1976), and its availability in a commercially radiolabeled form (Pathfinder Laboratories, Inc, St. Louis, MO). Although concentrations in the sediments of the Great Lakes today are negligible (Frank *et al.* 1981a, 1981b), endrin represents one of hundreds of highly-sorbed, potentially toxic compounds.

Lake Michigan sediments were collected in October, 1983, with a Ponar grab approximately 10 km offshore from St. Joseph, Michigan, at a water depth of 42 m. To create a uniform sediment for experiments, sediments were dried at 60°C, passed through a 0.25 mm mesh sieve, and thoroughly mixed. Sediments were reconstituted as needed with lake water and a few ml of fresh sediment to provide an active bacterial flora.

Worms were collected at the sediment collection site in March or April of 1984, 1985, and 1986 and maintained in the dark at 10°C in 200 L aquariums for a minimum of 1 month prior to use. Aquarium sediments were gently sieved (0.5 mm mesh) to concentrate worms. A fiber-optic light (to prevent unnecessary heating) and a dissecting microscope were used to sort and identify worms.

For each experimental concentration, endrin: ^{14}C-endrin (1000:1) was added to reconstituted sediments via <1 ml acetone carrier (<1 ml acetone carrier also added to controls), and the mixture was stirred for 24 hr in 2 L Lake Michigan water. After settling 72 hr, overlying water, containing the acetone, was aspirated off and enough fresh aerated lake water was added back to the sediments (and restirred for approx. 10 min) to allow the resulting slurry to be poured equally into 50 ml Griffen beakers. The slurries were allowed to settle for 72 hr at 10°C. Each beaker received approximately 25 g dry weight sediment and 25 ml water. Five to fifteen worms/beaker were added at this time. All experimental concentrations and controls were run in triplicate except where noted.

Four 96-hr lethal and two 96-hr sediment avoidance assays were completed between October 1984 and August 1986. Two of the 96-hr lethal assays included both single and mixed species testing. Death was defined as absence of color and response to touch, and an unmistakable degree of body degeneration. Observations of burrowing behavior were made at 0.17, 0.5, 2, 8, 16, 24, 48, and 96 hr during one EC_{50} assay for time series analysis. A worm was considered unburrowed if greater than an estimated 75% of its body was exposed on the sediment surface.

In single species lethality tests (assays 1–4), 10 worms/beaker (30 total/concentration) were used. Five worms/species/beaker were used in one mixed species LC_{50} assay and 10 worms/species/beaker in a follow-up assay (run simultaneously with single species assays 3 and 4, respectively) to better define a potential mixed species response. Fifteen worms/beaker (45 worms total/concentration) were used in the first sediment avoidance EC_{50} assay, and 10 worms/beaker (20 worms total/concentration) were used in the second assay. Fewer worms/beaker were used in the second avoidance assay to facilitate counting. Worms for the second sediment avoidance assay were added individually, and spaced out over the sediment surface. In all other assays, worms were temporarily placed in 20 ml vials of aerated lake water until sufficient numbers were obtained to begin an experiment. Typically, the worms formed a 'ball' which was gently added to each beaker. At higher concentrations, even though some worms burrowed and then emerged from the sediments, worms on the surface remained loosely entangled making exact counts difficult. The protocol of 10 worms/beaker in the second EC_{50} assay eliminated this difficulty. Twenty worms (duplicate instead of triplicate replication) were used in the second assay.

All experiments were conducted in an environmental chamber maintained at 10°C in the dark. Darkness was maintained to eliminate light-stimulated burrowing responses (White unpublished data). Overlying water in each beaker was not replaced during experiments. In sediment avoidance experiments, counts of worms on the sediment surface were made under a dissecting microscope when necessary, and beakers immediately returned to the chamber.

Post experimental sediment endrin: ^{14}C-endrin concentrations were determined by liquid scintillation, using a Packard 460 C counter. Endrin was removed from the sediment by 8-hr Soxhlet extraction in 240 ml hexane and 60 ml acetone (Sharom *et al.* 1980). Extraction volumes were reduced with a Buchler flash evaporator to approximately 1 ml to concentrate samples. Liquid scintillation determinations were initially verified by gas chromatography (Varian Aerograph Series 1200, column temp.

Table 1. Measured sediment endrin concentrations in μg/g dry weight sediment for four 96-hr LC_{50} assays and two 96-hr sediment avoidance EC_{50} assays

96-hr LC_{50} assay	Sediment Concentrations
1	0, 72, 251, 298, 512, 829, 1092
2	0, 121, 279, 421, 982
3	0, 4.4, 91, 432, 1352
4	98, 414, 801, 1848
96-hr EC_{50} assay	Sediment Concentrations
1,2	0, 1.2, 10.7, 23.9, 52.4, 77.2, 104.1

210°C, detector temp. 230°C). Clean-up of samples for gas chromatographic analyses was with Florisil® and followed the procedure in the Pesticide Analytical Manual (1977). Prior to use, the radiopurity of the labeled endrin was determined by thin layer chromatography (Patil *et al.* 1970) and was found to be >98%.

EC_{50} and LC_{50} data with upper and lower 95% confidence limits were generated, using the Litchfield and Wilcoxon (1949) log probit nomographic method.

Table 2. Ninety-six hour LC_{50} values (μg/g endrin dry wt. sediment) with upper and lower 95% confidence limits for *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri* in single and mixed tests

		95% Confidence Limits	
Species	LC_{50} (μg/g)	Upper	Lower
S. heringianus			
Assay 1	1400	1442	1359
Assay 2	1050	1095	1007
Assay 3	2500	5416	1157
Assay 4	5400	10487	2781
L. hoffmeisteri			
Assay 3	2050	4045	1041
Assay 4	3400	5076	2277
Mixed Species			
Assay 3			
S. heringianus	2750	7838	965
L. hoffmeisteri	5600	13104	2393
Combined response	4000	10720	1493
Assay 4	[a]		

[a] no dose response

Results

Measured sediment concentrations in 96-hr LC_{50} assays 1–4 ranged from a low of 4.4 μg/g in assay 3 to a high of 1,848 μg/g in assay 4 (Table 1). The same sediments were used in both 96 hr EC_{50} assays and measured endrin concentrations ranged from 1.2 to 104 μg/g (Table 1).

No mortality occurred in any of the control beakers, and all control worms burrowed and remained in the sediment in all assays.

Ninety-six hour LC_{50} data with lower and upper 95% confidence limits for *S. heringianus* and *L. hoffmeisteri* were reasonably similar (Table 2). The mean LC_{50} and standard deviation for single species testing with *S. heringianus* was 2,588 ± 1,974 μg/g (n = 4). Most of the variance occurred as a result of the high (5,400 μg/g) value obtained in assay 4. The mean LC_{50} for *L. hoffmeisteri* was comparable (2,725 ± 955, n = 2). In mixed species testing (assay 3, Table 2), the LC_{50} for *S. heringianus* (2,750 μg/g) was not significantly different from the mean single species value (2,588 μg/g). A significant increase in tolerance (as measured by Litchfield-Wilcoxon slope comparison method, $p < 0.05$) was apparent for *L. hoffmeisteri* in the mixed species assay (5,600 μg/g vs 2,725 μg/g). However, in the mixed species aspect of assay 4, no dose response relationship was observed; thus, assay 4 could not be compared with assay 3.

Ninety-six hour EC_{50} assays for *S. heringianus* yielded values of 19 and 15.3 μg/g, compared with a value of 59 μg/g for *L. hoffmeisteri* (Table 3). *L. hoffmeisteri* was not used in assay 1, because at the time it was not in the protocol.

EC_{50} data generated over different time frames demonstrated that both species initially burrowed into highly contaminated sediment and then returned to the surface. At 0.5 hr, the EC_{50} for *S. heringianus* was 1,000 μg/g and 300 μg/g for *L. hoffmeisteri* (Table 4). After 2 hr, EC_{50} values dropped to 180 μg/g and 255 μg/g, respectively. Each species reached an approximate equilibrium in response (8 hr for *S. heringianus* and 32 to 48 hr for *L. hoffmeisteri*) well before the 96 hr termination (Figures 1 and 2).

Discussion

Lethal Responses

Endrin is extremely toxic to aquatic organisms, producing 96-hr LC_{50} values of 1 μg/L or less for bluegills, trout, salmon, fathead minnows (Grant 1976) and flagfish (Hermanutz *et al.* 1985). It is slightly less toxic to freshwater Crustacea with reported LC_{50} values of 1.3 to 3 μg/L for two species of *Gammarus* and 320 μg/L for mature crayfish (Sanders 1972). Although the literature is sparse, the oligochaetes are more resistant to endrin (Naqvi 1973) as well as to DDT, Sevin, malathion, and methoxychlor (Bailey and Lui 1980). However,

Table 3. Ninety-six hour burrowing avoidance EC_{50} values (μg/g endrin dry weight sediment) with upper and lower 95% confidence limits for *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri*

Species	EC_{50} (μg/g)	95% Confidence Limits	
		Upper	Lower
S. heringianus			
Assay 1	19.0	22.4	16.1
Assay 2	15.3	21.1	11.1
L. hoffmeisteri			
Assay 1	[a]		
Assay 2	59	38	92

[a] not used in assay 1

Table 4. Burrowing avoidance EC_{50} values (μg/g endrin dry weight sediment) with upper and lower 95% confidence limits for *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri* at selected time intervals

	Species					
	S. heringianus	95% C.L.		*L. hoffmeisteri*	95% C.L.	
Time (hr)	EC_{50}	Upper	Lower	EC_{50}	Upper	Lower
0.17	95	210	43	300	628	143
0.5	1000	4410	227	300	628	143
2	180	349	93	255	504	129
8	17.5	30.7	9.9	118	172	81
16	16.0	23.1	11.0	[a]		
24	15.3	21.1	11.1	90	129	63
32	[a]			66	101	43
48	15.3	21.1	11.1	59	92	38
96	15.3	21.1	11.1	59	92	38

[a] no observation made

all previous acute oligochaete testing has involved aqueous test solutions.

In pesticide studies involving oligochaetes (Whitten and Goodnight 1966, Naqvi 1973), toxicities of test solutions were significantly reduced when sediment was added (Naqvi 1973). The ability of sediments to modify toxicity was also demonstrated with chlorinated pesticides and fish (Ferguson *et al.* 1965), kepone and chironomid larvae (Adams *et al.* 1985), and a hexachlorobiphenyl isomer and amphipods (Lynch and Johnson 1982). The modifying effect(s) of the sediment in these studies was attributed to a reduction in the bioavailability of the toxicants due to sorption, particularly with the most hydrophobic compounds. Addition of sediment also resulted in higher survival rates of twelve oligochaetes exposed to pentachlorophenol, black liquor, mercury, cadmium, and sewage (Chapman *et al.* 1982b). The ability to better cope

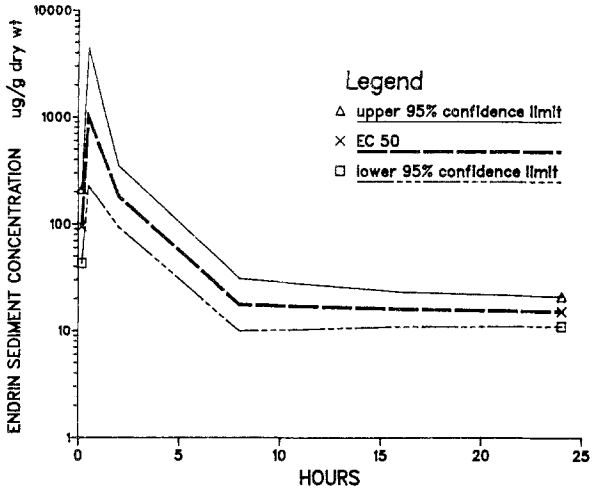

Fig. 1. Sediment avoidance EC_{50} data with upper and lower 95% confidence limits for *Stylodrilus heringianus*. Forty-eight and ninety-six hour values are equal to the twenty-four hour value and are not plotted.

with stress under the more natural conditions afforded by the sediment as well as a reduction in toxicant availability were considered responsible for many one-order of magnitude increases in observed LC_{50} values. The less stressful conditions associated with the use of sediments was illustrated by worms exhibiting heightened tolerances to higher water temperatures in the presence of sediment (Chapman *et al.* 1982b).

The present exposures using sediment bound endrin resulted in very high 96-hr LC_{50} values for both oligochaete species (Table 2) when compared to the LC_{50} values for other aquatic species tested in aqueous endrin solutions. In all likelihood, the high sorptive properties and concurrent reduction in availability of the toxicant are primarily responsible for the low toxicity, and the presence of the sediment acting as a more natural medium secondary. Regardless, the data suggest that oligochaetes may be able to withstand substantial amounts of highly toxic, sorbed pollutants.

Species interactions among tubificids increase the tolerance to some pollutants in aqueous test solutions (Chapman *et al.* 1982a). We desired to see if a similar relationship existed between a lumbriculid and a tubificid exposed to contaminated sediment. Although it appeared from the mixed species testing in assay 3 that the toxicity to *L. hoffmeisteri* was reduced (Table 2), when repeated using twice the number of individuals (10 worms/species/beaker) in assay 4, no dose response was observed. Although tolerance changes due to species interactions are suggested, further work is clearly necessary.

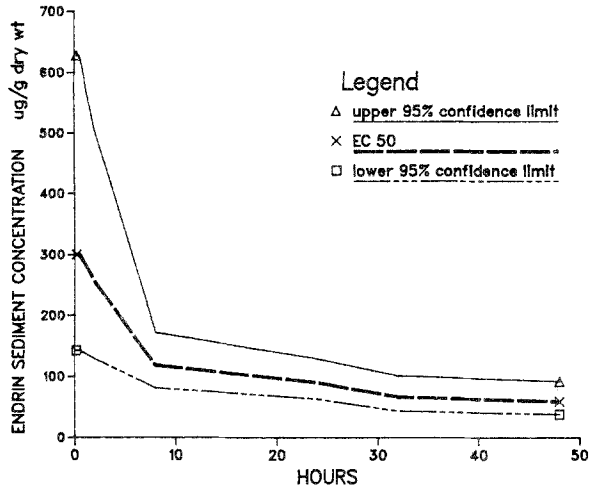

Fig. 2. Sediment avoidance EC_{50} data with upper and lower 95% confidence limits for *Limnodrilus hoffmeisteri*. The ninety-six hour value equals the forty-eight hour value and is not plotted.

Sublethal Responses

Sublethal impairment of an animal's development or its capacity to live and compete in its environment reduces the chances of survival for both the individual, and in extreme cases, the entire species (Anderson and d'Apollonia 1982).

While conducting the lethal assays, it was noted that, at concentrations one to two orders of magnitude below the LC_{50}, *S. heringianus* did not burrow. Since this observation had not been previously quantified for freshwater oligochaetes in contaminated sediments, EC_{50} assays were performed, using burrowing as a response. Because sublethal testing often involves exposure far closer to levels sometimes encountered in the environment, low level behavioral response assays may be a more realistic assessment of pollutant impact. The problem with this type of testing is the identification and subsequent quantification of the sublethal response. For oligochaetes, burrowing is an excellent response variable. Additionally, observations of burrowing behavior allow collection of nondestructive time series data, and the ease of conducting an experiment is equal to that of a lethal assay, making its potential promising for assessing pollutant impact on benthic communities.

Although very little is known about freshwater oligochaete behavioral responses to pesticide or other toxicant contaminated sediment, sufficient sediment and/or water concentrations of toxic compounds have been shown to influence burrowing behavior of three species of marine polychaetes. *Nereis virens* emerged from sediment contaminated with endosulfan, chlordane, dieldrin, DDT, and endrin (McLeese *et al.* 1982). Sediments contaminated with parathion, methyl parathion, and malathion caused impaired and almost complete cessation of burrowing by *Nereis versicolor* (Mohlenberg and Kiorboe 1983). Significant inhibition of both the rate and magnitude of lugworm feeding activity was observed for *Arenicola marina* in kepone contaminated sea water (Rubinstein 1979).

In the present study, mean 96-hr EC_{50} values for *L. hoffmeisteri* and *S. heringianus* are approximately 46 and 150 times lower than their respective mean 96-hr LC_{50} values (Tables 2 and 3). This level of response suggests that sorbed pollutants have the potential to alter benthic communities without directly or immediately killing individuals. Along with other factors, changes in species composition in some instances may be the result of inefficiencies in life-sustaining functions induced in less tolerant species by longterm, low level exposures to dozens, perhaps hundreds, of toxicants residing in lake bottom sediments. Relative to *S. heringianus*, the higher EC_{50} value observed for *L. hoffmeisteri* may relate to this, as *L. hoffmeisteri* is a eutrophic to mesotrophic species. Even at endrin concentrations of 1,800 μg/g in lethal assays, one to three (of ten) worms consistently would burrow, whereas in concentrations of 50–75 μg/g burrowing behavior ceased altogether for the characteristically more oligotrophic *S. heringianus*. This is in contrast to findings of Chapman *et al.* (1982b) where in LC_{50} assays oligotrophic species were often most tolerant to specific chemical pollutants (eutrophic species were more tolerant to sewage sludge and anoxia). One would not anticipate Chapman's result with oligotrophic species based on oligochaete field distribution patterns. In our study, LC_{50} values for *S. heringianus* and *L. hoffmeisteri* were not significantly different, also a result not anticipated in light of field distributions. The observed behavioral responses were, however, consistent with the respective trophic status of each species, implying such tests may be more sensitive indicators of environmental impact of chemical pollutants.

The sensitivity of the behavioral response is further demonstrated in the time series analyses of burrowing behavior *S. heringianus* emerged and remained on the surface after only 8 hr (Figure 1), while *L. hoffmeisteri* returned to the surface after 32 to 48 hr (Figure 2). The faster response of *S. heringianus* (4 times faster than *L. hoffmeisteri*) in returning to the sediment surface may again reflect the anticipated sensitivity associated with the oligotrophic species. The underlying mechanism(s) for this is unknown. The respective abilities to reg-

ulate respiration may be a factor as *L. hoffmeisteri* is considered a regulator and *S. heringianus* a partial regulator; however, oligochaete respiratory responses to sublethal stress are variable, and changes in LC_{50} values do not correlate with changes in respiration (reviewed in Chapman and Brinkhurst 1984). In the present study, both *S. heringianus* and *L. hoffmeisteri* would burrow into contaminated sediment and then return to the surface in numbers somewhat proportional to the sediment concentration and the length of exposure. Future use of oligochaete behavioral responses to sublethal sediment contamination for assessment of pollutant impact on benthic communities is promising.

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