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Sublethal responses to endrin in sediment by *Styiodrilus heringianus* (Lumbriculidae) as measured by a ¹³⁷cesium marker layer technique

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Sediment reworking rates of *Styiodrilus heringianus* (Oligochaeta: Lumbriculidae) were measured in microcosms containing sediments dosed with the chlorinated pesticide, endrin. Reworking rates were measured at 10°C by monitoring a ¹³⁷cesium marker layer burial in contaminated and uncontaminated microcosms. Endrin concentrations ranged from 3.1 to 42 000 ng/g dry sediment. Alterations in reworking rates were observed at sediment concentrations five and one half orders of magnitude below the LC₅₀ (1 650 µg/g). For the lower concentrations, marker layer burial rate data suggested possible stimulatory effects in the first 300 to 600 h, followed by significant rate decreases relative to controls. For higher concentrations, rates were equal to or slower than control rates in the first 600 h, followed by dramatic decreases in the last 600 h. High final surficial sediment endrin concentrations at the end of experiments implied worm mediated upward transport. Worm mortalities were 9.3 to 28% for the two highest concentrations (42 000 and 11 500 ng/g) and 0 to 6.7% for all other concentrations including controls. Post experimental worm dry weights were inversely related to high concentrations. Bioaccumulation factors ranged from 34 to 67 on a g dry organism to g dry sediment basis.

Key words: Sediment toxicity; Oligochaete; Biogenic sediment mixing; Bioturbation

INTRODUCTION

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 macrobenthic organisms is largely unknown. Compound fates depend on complex combinations of sediment characteristics, xenobiotic chemical and physical properties, and the activity patterns of the benthos

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(Petr, 1977). Sediment mixing is directly related to the latter (Robbins, 1982), and of particular interest are the oligochaetes that rework sediments in a conveyor-belt fashion (Davis, 1974a; Rhoads, 1974; Robbins and Edgington, 1975; Krezoski, 1976; Robbins et al., 1977; Krezoski et al., 1978; Robbins et al., 1979; McCall and Fisher, 1980; Krezoski, 1981; Robbins, 1982; Robbins et al., 1984; Krezoski and Robbins, 1985; Robbins, 1986). Worms ingest subsurface sediments and convey them to the sediment-water interface, egesting the sediment particles as fecal pellets. The continuance of this feeding behavior (and other related modes such as funnel-feeding by marine polychaetes) affects stratigraphic records and physical-chemical properties of bottom sediments (Kikuchi and Kurihara, 1966; McCall and Tavesz, 1982). Radionuclide and pollen grain distributions from lake bottom cores and laboratory microcosm studies reflect a zone of uniform mixing of surface sediments to depths that correlate with oligochaete abundance and feeding patterns (Davis, 1974a; Robbins and Edgington, 1975; Edgington and Robbins, 1976; Fisher et al., 1980; Robbins et al., 1988). Similar observations of marine polychaetes have also been reported (Cadee, 1979; Baumfalk, 1979). Additionally, the effects of aquatic worms on sediment diagenesis and transport of materials across the sediment-water interface have been demonstrated (Davis, 1974b; Davis et al., 1975; Petr, 1977; Aller, 1978; Aller, 1980; Aller, 1982; Rippey and Jewsen, 1982; Fisher, 1982; Krezoski et al., 1984; Matisoff et al., 1985; Karickhoff and Morris, 1985).

Rates at which buried sediments are egested and subsequently reburied (the reworking rate) have been determined under laboratory conditions for *Stylocdrilus heringianus* (Krezoski, 1981; Robbins et al., 1984) and for mixed tubificid assemblages (predominantly *Limnodrilus hoffmeisteri*, McCall and Fisher, 1980; Fisher et al., 1980). Only very recently have contaminated sediment-oligochaete (tubificids) interactions been examined under laboratory conditions. Tubificids transported toxicants (hexachlorobenzene, pentachlorobenzene, and trifluralin) to the sediment surface in microcosms. The reworking activities resulting in the toxicant transport were assumed to be independent of the toxicant's presence (Karickhoff and Morris, 1985).

The quantification of burial rates is crucial to studies of both contaminated and uncontaminated sediments. Previously, reworking rates were inferred from either fecal pellet evolution or marker horizon (such as fluorescent glass beads, Karickhoff and Morris, 1985) studies. In both cases, only gross movements of surface sediments were measured and/or microcosms were destroyed or disrupted during measurement. However, use of the gamma scan method for monitoring laboratory microcosms is both non-destructive and gives high resolution (Robbins et al., 1979). Rate determinations are repeatable and dependent on the conditions imposed upon microcosms (such as oxygen fluctuations, Robbins et al., 1984).

Measurements of sediment reworking reflect the intimate interactions between these sediment-confined organisms and their surroundings. Potential alterations (either increases or decreases) in reworking rates over relatively long periods in con-

taminated sediments can therefore be compared to reworking rates established in uncontaminated sediments. Such a system is ideal for testing the influences of xenobiotics on conveyor-belt feeding oligochaetes.

In this research, we employ the gamma scan system to determine the existence and magnitude of these alterations in behavior using the chlorinated pesticide endrin as a representative sediment-bound toxicant and the lumbricolid *Stylocdrilus heringianus* as the test organism. We hypothesized that the sediment-bound toxicant should stimulate or depress relative to its concentration the normal reworking/feeding rates of the representative conveyor-belt oligochaete. Additionally, because oligochaete particle selective feeding results in a significant upward redistribution of fine-grained sediments (Robbins, 1986), and toxicants bound to sediments are often associated with the smaller, organic fractions (Karickhoff and Morris, 1985), we further hypothesized that oligochaete reworking should result in sediment-bound toxicant redistribution, with potentially high concentrations at or near the sediment-water interface.

MATERIALS AND METHODS

Two 1300-h experiments were conducted (experiments 1 and 2). Each tested four sediment endrin concentrations and a control in triplicate. The gamma scan system as described in detail by Robbins et al. (1979) was the primary investigative tool. The system consisted of a well-collimated NaI scintillation detector supported by a hydraulic elevator. The apparatus was used to vertically scan small microcosms contained in an aquarium modified as an environmental chamber. Before experimental organisms were added to the microcosms, a sub-millimeter layer of the gamma emitting radioisotope ^{137}Cs adsorbed on illite clay particles was added to the sediment surface of each microcosm. A single channel analyzer system was used to isolate counts from the 0.662 MeV energy peak of the ^{137}Cs . The top 1.5–3.5 cm of each microcosm was scanned with the NaI detector in mm increments (100 s counts/mm) every 2–5 days after the addition of oligochaetes. Depths of maximum gamma activity were calculated by fitting count data to a Gaussian distribution (Robbins et al., 1979; Krezoski and Robbins, 1985; Robbins, 1986). The hydraulic elevator supporting the lead shielded detector and collimator was mounted on steel casters and angle iron tracks for lateral mobility to permit the scanning of multiple microcosms lined up on the inside wall of the aquariums. Thus, microcosms remained undisturbed for the duration of experiments. When determining the initial location of the peak activity (prior to the addition of the worms), an external mark was made on the track so that the detector could be repeatedly returned to the same location for each cell. After the worms were added, the entire tank was enclosed in black plastic to maintain dark conditions. Microcosm cells (5.5 cm \times 3.5 cm \times 30 cm) were constructed of glass (sealed with silicone) to reduce compound adsorption and holding tanks were filled with aerated epilimnetic Lake Michigan water at 10°C.

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, molecular weight = 381, Weil et al., 1974) was chosen as a representative toxicant based on its low chemical solubility in water (0.26 mg/l at 25°C, Biggar and Riggs, 1974), acute toxicity in aqueous solution to other aquatic species (reviewed in Grant, 1976), its high partition coefficient ($\log K_{ow} = 5.6$, Neeley et al., 1974) and its availability in a commercially prepared radiolabelled form (Pathfinder Laboratories, St. Louis, MO).

Sediments and water were collected in Lake Michigan approximately 10 km offshore from St. Joseph, Michigan, in 42 m of water. For standardization between experiments, sediments were dried at 60°C, passed through a 0.25-mm sieve, and reconstituted with lake water as needed. When reconstituting, a few ml of fresh sediment were added to provide an active bacterial flora. For each experimental concentration, endrin/¹⁴C endrin was added to 450 g dry weight sediment in 2 l lake water via an acetone carrier (< 1 ml, also added to controls) and stirred for 24 h at room temperature. After settling 72 h, overlying water/acetone was aspirated off and fresh lake water added. The new mixture was stirred for approximately 10 min and then poured equally into each of three microcosms. The slurries were allowed to settle for 10 days at 10°C. A submillimeter layer of ¹³⁷Cs adsorbed onto Lake Michigan sediments was then added to the sediment surface of each microcosm. After settling 24 h, each cell was scanned with the detector (100 s counts at 1 mm intervals for these and all subsequent scannings) to determine the initial position of the peak gamma activity. Seventy five worms were then added to each cell and allowed to burrow for 24 h, after which cells were scanned. Scanning continued every 2–5 days thereafter. Identical set-up and scanning procedures were followed for controls (without endrin).

Worms were collected in March 1984, and April 1985, at the location of sediment collection. They were maintained at 10°C in lake sediments in the dark in 200-l aquariums for a minimum of one month prior to use. Aquarium sediments were gently sieved (0.5 mm) to concentrate worms (greater than 25 mm in length) prior to each experiment. A fiber optic light (to prevent unnecessary heating) and a dissecting microscope were used to identify and count worms within a 48 h period prior to the start of experiments. To minimize any potential size bias between microcosms, worms were placed in vials of 20 ml aerated lake water in lots of 15 as they were selected. When all needed were obtained, the contents of 5 randomly selected vials were added to each microcosm to correspond to a density of 50 000 worm m⁻².

At the end of each experiment, two microcosms per treatment were sampled for worm data (percent mortality, dry weight, endrin concentration) and one for a vertical profile of sediment endrin concentration and porosity. Microcosms for vertical profiles were removed from the experimental aquariums and immediately frozen in a dry ice-alcohol bath. By limiting the level of the bath to 2–3 cm below the sediment surface of a microcosm, distortion effects due to expansion of sediments were

minimized. To remove the frozen sediment, the silicone seal was cut to remove the bottom of the microcosm. As the outer surface warmed, the entire plug was then pushed free. The plug was wrapped in aluminium foil and cut in centimeter fractions with a fine blade hacksaw. Three 'blank' (no worms, only [^{14}C]endrin) microcosms were mixed and treated in the same manner for standards. Porosity was determined by whole fraction wet weight-dry weight/wet weight $\times 100$. Sediments were dried at room temperature and stored in a freezer prior to endrin extractions. Ashfree dry weights were determined on sediments of 'blank' cells to examine the relationship between organic carbon and the distribution of the pesticide. Sediments were ashed in pre-baked and weighed ceramic crucibles at 550°C for 4 h.

Post experimental endrin: [^{14}C]endrin concentrations were determined by 10 min liquid scintillation counts using a Packard 460C counter. Disintegrations per min were calculated for all samples by correcting the measured activities for background and counting efficiency with a standard quench curve. Endrin was removed from the sediment by 8 h soxhlet extraction in 240 ml hexane and 60 ml acetone after Sharom et al. (1980). Extraction volumes were reduced with a Bulcher flash evaporator to approximately 1 ml before analysis. Liquid scintillation determinations were initially verified with gas chromatography (Varian Aerograph Series 1200, column temp. 210°C , detector temp. 230°C). GC samples were cleaned up with florisil columns (after Pesticide Analytical Manual, 1979). The radiopurity of the labeled endrin, determined using thin layer chromatography (after Patil et al., 1970), was $>98\%$. For comparative purposes, distributions of endrin were plotted as the percent of the total microcosm endrin content found at each cm interval in experimental and 'blank' microcosms.

TABLE I

Percent mortalities of *Stygodrilus heringianus* from replicate microcosms for experiments 1 and 2.

Concentration	Percent mortality		
	Replicate 1	Replicate 2	Mean
Experiment 1			
42 000 ng/g	24.0	28.0	26.0
2 300 ng/g	6.7	0.0	3.4
218 ng/g	5.3	2.7	4.0
20.6 ng/g	4.0	4.0	4.0
Control	0.0	^a	0.0
Experiment 2			
11 500 ng/g	26.6	9.3	18.0
541 ng/g	1.3	2.7	2.0
27 ng/g	0.0	1.3	0.7
3.1 ng/g	5.3	4.0	4.7
Control	2.7	6.7	4.7

^a Not assayed (replicate 2 used in determinations of vertical profile variables).

Whole worm endrin concentrations were obtained by using a Thomas No. 04212 tissue grinder to pulverize dried worms (stored in a dessicator) in acetone. The slurry was centrifuged at 10 000 rpm for 10 min. The supernatant was then assayed for ^{14}C activity as above.

Primary statistical analyses included covariance slope comparisons of treatment and control cell ^{137}Cs burial rates (position of the peak activity vs. time) for early and later portions of experiments. All test results were considered significant if $P < 0.05$. Analyses of residuals indicated that assumptions of 'normality' and 'equal variances' were not seriously violated (no transformations were used). All computa-

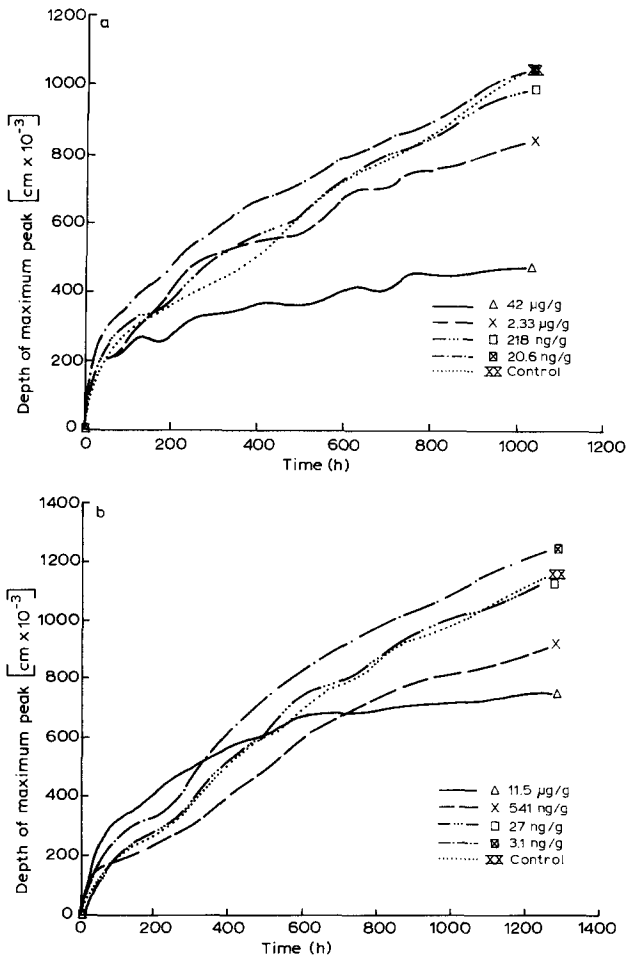


Fig. 1. a,b. Depths of mean ($n = 3$) ^{137}Cs peaks as buried by *Styldrilus heringianus* in experiments 1 and 2 for experimental sediment endrin concentrations and the control. To avoid cluttering the reworking responses, standard deviations were not plotted.

tions were made on the Michigan Terminal System using the University of Michigan's statistical package, MIDAS.

RESULTS

Measured sediment concentrations in the first experiment ranged from 20 to 42 000 ng/g and 3.1 to 11 500 ng/g in the second experiment. In both experiments, mortality was low, although at the two highest concentrations, mortality was significantly higher than other experimental concentrations and controls (Table I). Replicate 2 of the control cells in experiment 1 was eliminated from final analyses due to an apparent mid-experiment catastrophe. At the end of the experiment, the sediment in the microcosm was uncharacteristically black with no life.

Mean depths of ^{137}Cs peaks were plotted for each experiment to interpret the reworking response (Fig. 1a,b). To describe changes in reworking during the tests, a point where the lines visibly inflected was chosen to divide the data and reworking rates (slope/75 worms) were calculated for both the early and later portions (Table II). Slopes were then compared using analyses of covariance (Tables III and IV). All calculations were based on the mean of the responses. Variation among microcosms was low and somewhat constant throughout both experiments. The mean coefficient of variation ($n = 5$) for SD in experiment 1 was 39.0 ± 15.8 , and 47.4 ± 13.3 in experiment 2.

TABLE II

Sediment reworking rates (cm/worm per h $\times 10^{-5}$) calculated from ^{137}Cs burial rates for the first 290 hours and the remainder of experiment 1 and for the first 600 h and the remainder of experiment 2.

Sediment conc.	24-290 h	290-1 032 h
	Reworking rate (cm worm $^{-1}$ h $^{-1}$ $\times 10^{-5}$)	Reworking rate (cm worm $^{-1}$ h $^{-1}$ $\times 10^{-5}$)
42 000 ng/g	0.80 (0.11) ^a	0.25 (0.03)
2 300 ng/g	1.83 (0.09)	0.62 (0.04)
218 ng/g	1.52 (0.15)	0.90 (0.03)
20.6 ng/g	1.73 (0.11)	0.76 (0.02)
Control	1.03 (0.09)	1.12 (0.04)
Sediment conc.	24-600 h	600-1 272 h
	Reworking rate (cm/worm per h $\times 10^{-5}$)	Reworking rate (cm/worm per h $\times 10^{-5}$)
11 500 ng/g	1.14 (0.13)	0.14 (0.01)
541 ng/g	1.07 (0.06)	0.53 (0.03)
27 ng/g	1.42 (0.06)	0.80 (0.03)
3.1 ng/g	1.64 (0.07)	0.79 (0.02)
Control	1.39 (0.04)	0.86 (0.04)

^a Parentheses indicate SE.

TABLE III

Statistical comparison of reworking rates for the first 290 hours and the remainder of experiment 1 ($\alpha < 0.05$).

42.0 $\mu\text{g/g}$	—				24–290 h
2.3 $\mu\text{g/g}$	n.d. ^a	—			
218 ^b ng/g	F	n.d.	—		
20.6 ng/g	F	n.d.	n.d.	—	
Control	n.d.	S	S	S	—
	42.0	2.3	218	20.6	Control
42.0 $\mu\text{g/g}$	—				290–1 032 h
2.3 $\mu\text{g/g}$	F	—			
218 ng/g	F	F	—		
20.6 ng/g	F	F	n.d.	—	
Control	F	F	F	F	—
	42.0	2.3	218	20.6	Control

^a Read from left, then down, F = faster, S = slower, n.d. = no difference.

^b Example: 218 ng/g is significantly faster than 42 $\mu\text{g/g}$.

During the first 290 h of experiment 1, mean reworking rates from 20.6 ng/g, 218 ng/g, and 2 300 ng/g microcosms were significantly faster ($P < 0.05$) than those from the highest exposure concentration of 42 000 ng/g. The mean control cell rate was not significantly different from the 42 000 ng/g rate (Table III). In the later half of the experiment, all treatment reworking rates slowed relative to the control rate, while the later half control rate was not significantly different from the early control

TABLE IV

Statistical comparison of reworking rates for the first 600 hours and the remainder of experiment 2 ($\alpha < 0.05$).

11.5 $\mu\text{g/g}$	—				24–600 h
541 ng/g	n.d. ^a	—			
27.0 ng/g ^b	F	F	—		
3.1 ng/g	F	F	F	—	
Control	F	F	n.d.	S	—
	11.5	541	27.0	3.1	Control
11.5 $\mu\text{g/g}$	—				600–1 272 h
541 ng/g	F	—			
27.0 ng/g ^b	F	F	—		
3.1 ng/g	F	F	n.d.	—	
Control	F	F	n.d.	F	—
	11.5	541	27.0	3.1	Control

^a Read from left, then down. F = faster, S = slower, n.d. = no difference.

^b Example: 27.0 ng/g is significantly faster than 11.5 $\mu\text{g/g}$.

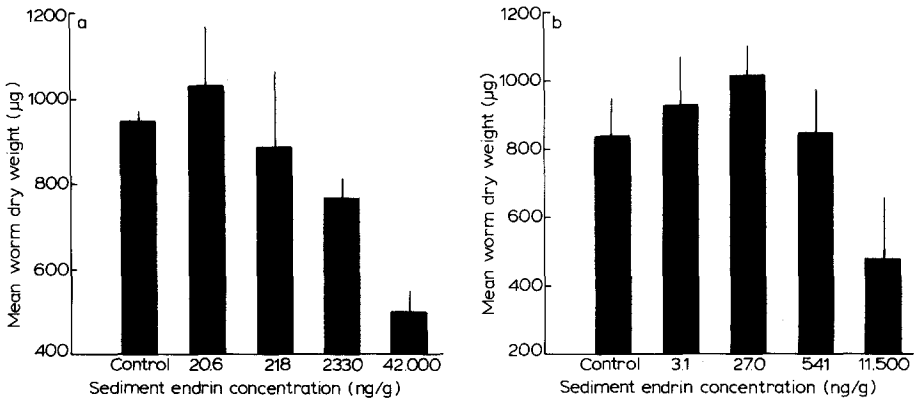


Fig. 2. a,b. Post experimental mean worm dry weights and standard deviations ($n=6/\text{concentration}$) as a function of sediment endrin concentration for experiments 1 and 2.

rate. During the first 600 h of experiment 2, the mean 27 ng/g, 3.1 ng/g, and control rates were faster than the high concentration of 11 500 ng/g and the intermediate concentration of 541 ng/g (Table IV). Of the two lower concentrations, only the 3.1 ng/g rate was significantly faster than the control rate in the first half of the experiment. Unlike the 42 000 ng/g microcosm in experiment 1, reworking rates in the high concentration of 11 500 ng/g and the intermediate concentration of 541 ng/g in experiment 2 were significantly slower than the control reworking rate during the first half of the experiment.

At the end of studies, worm dry weights after exposure to high endrin concentrations were reduced relative to control weights. Worms exposed to lower concentrations, however, tended to weigh more than control worms (Fig. 2a,b).

Vertical profiles of porosity in the treatment cells did not differ significantly from control profiles, however, porosity profiles between microcosms with worms and without worms were slightly different.

Vertical endrin distributions in microcosms with reworking activity reflected an apparent upward deposition of the compound relative to 'blanks' (endrin contaminated sediment microcosms without worms; Fig. 3a-d). Organic carbon content (as ashfree dry weight) closely followed the distribution of the compound in the 'blank' microcosms (Fig. 4), consistent with the results of Karickhoff and Morris (1985).

The bioaccumulation of endrin by *S. heringianus* was found to range from 34 to 67 times in the five highest experimental sediment concentrations on a dry weight basis. Bioaccumulation factors from the lower concentrations in both experiments were not determined because of detectability problems. There did not appear to be a relationship between sediment concentration and bioaccumulation.

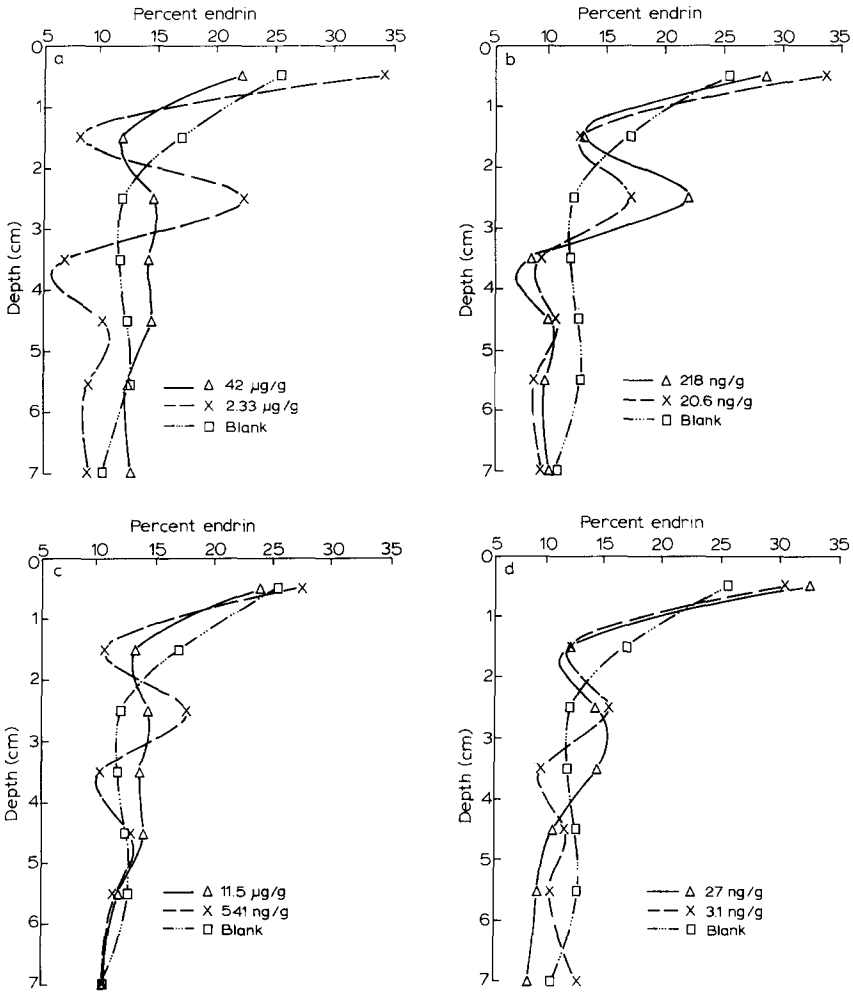


Fig. 3. a-d. Vertical endrin distribution as the percent of the total compound at each centimeter depth interval for microcosm sediments in experiments 1 and 2. Blank distribution reflects endrin position without oligochaete sediment mixing.

DISCUSSION

Endrin is one of the most toxic pesticides to fish (Grant, 1976). Ninety-six h LC₅₀ values of 1 µg/l or less were found for bluegills, trout, salmon, fathead minnows (Grant, 1976) and flagfish (Hermanutz et al., 1985). Endrin is slightly less toxic to freshwater Crustacea with reported LC₅₀ values of 1.3 to 3.0 µg/l for two species of *Gammarus* and 320 µg/l for mature crayfish (Sanders, 1972). Although the literature

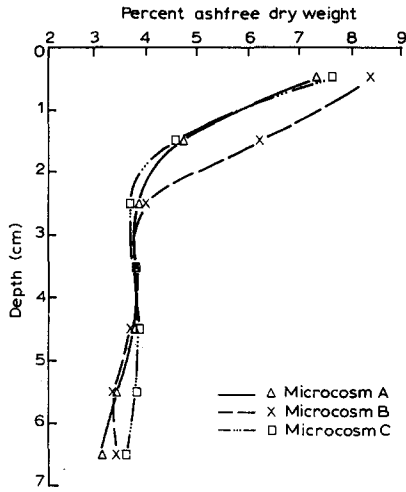


Fig. 4. Percent ashfree dry weights of sediments at cm depth intervals in 'blank' microcosms.

is sparse relative to other invertebrates, the oligochaetes appear to be relatively resistant to endrin (Naqvi, 1973) and other pesticides (DDT, chlordane, sevin, malathion, and methoxychlor (Bailey and Lui, 1980)). This resistance is typified in a recent study where 96 h LC_{50} values for *S. heringianus* with endrin averaged $2588 \pm 1974 \mu\text{g/g}$ dry weight sediment (Keilty et al., 1988).

Studies of oligochaete-sediment-toxicity interactions are rare (Karickhoff and Morris, 1985). The tolerance of the tubificid *Branchiura sowerbyi* was determined for twenty three insecticides, including four chlorinated hydrocarbon pesticides (aldrin, dieldrin, DDT, and endrin) (Naqvi, 1973). No mortality was observed at the high concentration of 4 mg/l at 21°C with the chlorinated compounds, consistent with the high tolerance of other tubificids such as *Tubifex* sp. and *Limnodrilus* sp. to DDT (Whitten and Goodnight, 1966). With other pesticides where mortality occurred, significant increases in tolerances were found by adding sediment to the test solutions (Naqvi, 1973). The addition of sediment presumably decreased the bioavailability of the toxicants (Ferguson et al., 1965; Adams et al., 1985; Lynch and Johnson, 1982; for fish, chironomids, and amphipods respectively). A similar response was observed for *Tubifex tubifex* and *Limnodrilus hoffmeisteri* exposed to sodium pentachlorophenol (Chapman and Brinkhurst, 1984).

Aside from a reduction in toxicity/bioavailability, the presence of sediment affords a more natural testing environment, particularly when investigating subtle behavioral changes in response to toxicant exposures by infaunal organisms. Unfortunately, very little information is available on the behavioral responses of freshwater oligochaetes to pesticide-contaminated sediment. However, sufficient sediment and/or water concentrations of chlorinated and organophosphate

pesticides significantly inhibited both the rate and magnitude of marine polychaete burrowing/feeding behavior (Rubinstein, 1979; McLeese et al., 1982; Mohlenberg and Kiorboe, 1983). In these studies worm responses and sediment movements were quantified by either simple observations or time series photographs of sediment surfaces. Although these techniques were suitable for the given experimental designs, neither determine subsurface activities.

Fluorescent glass beads are limited as a tracer of particle movement because they can only be detected close to the microcosm walls, and environmental chambers must be opened to collect data. Opening the test systems is cumbersome and requires 'safelight conditions' to prevent evoking a negative phototropic response from the worms. Use of a gamma scan system avoids these difficulties and affords long term, undisturbed, and highly accurate assessments of sediment burial/oligochaete reworking rates.

By using the gamma scan system, the longterm chronic effects of endrin contaminated sediment on *S. heringianus* reworking rates were measured at concentrations up to five and one half orders of magnitude lower than the LC_{50} . Although the LC_{50} measurement requires a lower investment of time and resources, it does not permit extrapolation to responses induced by long-term, low level exposures. Measures of reworking patterns require a greater effort, yet more closely mimic a 'real world' exposure, and therefore a 'real world' response.

Reworking rates for the controls were consistent with other oligochaete reworking determinations (reviewed in Fisher et al., 1980 and Krezoski et al., 1984). The exposure to endrin resulted in both the elevation and reduction of reworking rates, dependent on the length of the exposure and the sediment concentration. While changes in reworking for *S. heringianus* also result from variations in the ambient temperature and dissolved oxygen levels (Khlar, 1981; Robbins et al., 1984; White et al., 1987), this is the first demonstration that oligochaete reworking/feeding behavior changes due to a sediment bound toxicant.

Reworking data suggest that the presence of the toxicant at low concentrations stimulates reworking behavior for a considerable length of time, after which reworking decreases. An increased metabolic rate due to the chemical stress may be responsible for the heightened reworking response. Elevated respiration rates of both lumbricid and tubificid worms occur as a response to aqueous pollutants, such as pulp mill effluent (Whitley, 1968; Whitley and Sakora, 1970; Chapman et al., 1982a, 1982b, 1982c; Brinkhurst et al., 1983). The later half of the exposure shows decreases in reworking rates that are likely due to the accumulated toxic effect. At the high concentration of 42 000 ng/g, there was no stimulation in the first half of the experiment, while the reduction in the later portion was most dramatic (Fig. 1a). The decrease in reworking/feeding results in significant reductions in post experimental worm weights from the 42 000 ng/g microcosms. Such responses illustrate the deleterious effects of long-term exposures at concentrations over one and one half orders of magnitude less than the LC_{50} value.

Reworking rates and general trends from experiments 1 and 2 were comparable. However, in the second experiment, only the 3.1 ng/g cells exhibited significantly faster reworking than the control early in the experiment. At the intermediate and high concentrations, the reworking rates were slower than the controls early on, suggesting a more toxic response than from the higher concentration of 42 000 ng/g in experiment 1. Differences in sensitivity of the worms between the experiments may be attributed to the use of worms from different collections. For ideal replication and comparison, both experiments should have been run simultaneously, with worms from one Lake Michigan sampling. Equipment and space constraints made this impossible. Although the reworking rate from 27 ng/g was not significantly different from the control rate in experiment 2, the control reworking rate did exceed all other treatment rates in the later portion of the experiment, and the high concentration (11.5 $\mu\text{g/g}$) once again showed the most dramatic reduction in reworking.

Changes in reworking rates somewhat paralleled post experimental worm dry weights and sediment endrin loads. In the first experiment, weights were much lower in worms exposed to the high concentrations relative to controls, whereas worms exposed to low concentrations were similar to, and greater than controls (Fig. 2a). The observed changes in body weights probably resulted from the altered feeding patterns manifested in the altered reworking patterns. A similar, yet less distinct trend was observed in experiment 2 (Fig. 2b). Again, worms exposed to the high concentration of 11 500 ng/g had a diminished reworking rate and a greatly reduced worm weight, while those exposed to low concentrations had similar and elevated weights relative to the control weights. Changes in body weights did not correspond to microcosm sediment concentrations as well as they did in experiment 1. Data at the high concentrations from both experiments do, however, clearly indicate the value of post contaminant exposure body weight measurements. Although they are 'end-point' measures, they reflect the integrated physiological response to long term contaminant exposure and provide support for the reworking data.

Post experimental vertical endrin profiles suggest upward worm mediated movement of the compound (Fig. 3a-d). A similar response was observed using tubificid worms and hexachlorobenzene, pentachlorobenzene, and trifluralin (Karickhoff and Morris, 1985). Transport of endrin by *S. heringianus* appears to depend on the concentration in the sediment. At the highest concentration in experiment 1 (42 000 ng/g), the endrin distribution was very similar to the 'blank' distribution (Fig. 3a) presumably because the worms did little feeding/reworking. At a concentration of 2 300 ng/g (Fig. 3a) and lower (Fig. 3b), significantly higher reworking activities occurred along with significant upward endrin transport. In each case, the upwardly transported compound was preceded by a marked decrease in the centimeter fraction beneath it. In the 2-3 cm interval another small peak was usually present, consistent with the current oligochaete feeding model which predicts characteristic waves (Robbins, 1986). Similar responses were observed in experiment 2 (Fig. 3c,d). In the 11 500 ng/g microcosm, the endrin distribution was very similar to the

'blank', consistent with the 42 000 ng/g cell in experiment 1. The vertical endrin distribution in the 541 ng/g cell more closely resembles the distributions at lower concentrations (Fig. 3d) with slightly less compound in the surface fraction. Thus, upward transport of a sediment bound toxicant corresponds to significant oligochaete sediment reworking. The worms tend to selectively ingest compounds with high partition coefficients that are associated with the finer, organic fractions (McCall and Tevesz, 1982). This selectivity is the result of the size limitation of the worm's prostomium and of the higher food quality due to the rich bacterial flora found on the organic component (Brinkhurst and Chua, 1969; Wavre and Brinkhurst, 1971; Brinkhurst et al., 1972; Chua and Brinkhurst, 1973; Brinkhurst and Austin, 1979). The organic carbon vertical profiles (as ashfree dry weight, Fig. 4) of the 'blanks' closely followed the endrin distributions in the 'blanks' corroborating this notion. A relationship between organic carbon and highly sorbed toxicants was also observed by Karickhoff and Morris (1985).

Porosity can be influenced by oligochaete sediment mixing, particularly in sandy sediments, where surface water content increases as a result of tubificid feeding (Tevesz et al., 1980). Worms selectively remove the subsurface finer grained particles and transport them to the surface (McCall and Tevesz, 1982). However, in very fine grained sediments, tubificid oligochaetes did not influence the surface water content of Lake Erie muds apparently due to compaction (McCall and Fisher, 1980). Porosity profiles from microcosms with worms in our experiments differed slightly from the vertical porosity profile of the 'blank'. Microcosms without worms generally had a slightly higher porosity in the 0-1 and 1-2 cm fractions than both experimental and control microcosms with worms. The 'blank' also continued to decrease in porosity with depth after 2 cm, while the others did not. These differences were probably a result of further compaction of the sediments by the conveyor-belt feeding worms. It is possible that a 10 day settling of microcosm sediments prior to the addition of worms may be too short. There were, however, no measurable differences between the control cell and the experimental cells in both experiments, suggesting that the toxicity of the compound was not manifested in this measurement. Additionally, porosity did not relate to the vertical distribution of endrin.

Bioaccumulation of endrin by *S. heringianus* was significant, ranging from 34-67 times the concentration of the sediment. Concentration factors by the tubificids, *Limnodrilus hoffmeisteri* and *Tubifex tubifex*, of pp'DDE, Mirex, and PCB in spiked samples from Lake Ontario were approximately five times lower than our observed values (Oliver, 1987). Considering the use of different species, sediments, compounds, experimental designs, etc., the determinations are reasonably similar and suggest that aquatic oligochaetes may sequester substantial quantities of persistent pollutants.

Based on this study and previous research with endrin (Keilty et al., 1988), expected oligochaete responses at various concentrations juxtaposed with the types of

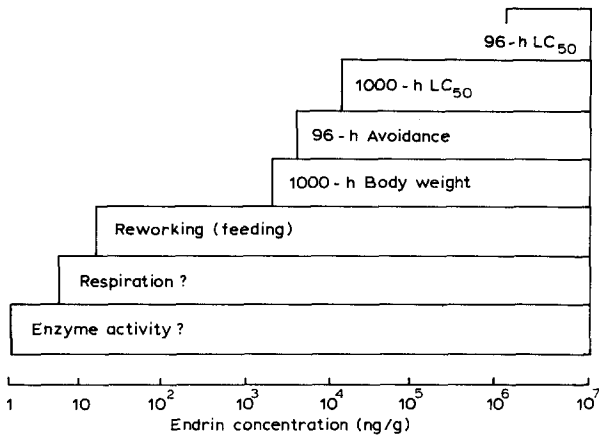


Fig. 5. Summary of measured and hypothesized responses by *Stylodrilus heringianus* to endrin-laden sediments in various assays. Values are estimations placed along a presumed continuum of response.

assays employed can be summarized to provide perspective between the assay types and the sensitivity of the responses (Fig. 5). A 96-h LC₅₀ requires the highest sediment concentration. An approximate 1000-h LC₅₀, a 96-h sediment avoidance EC₅₀ (Keilty et al., 1988), and 50% body weight losses after approximately 1000-h exposures, are all about equally sensitive at 1–2 orders of magnitude lower sediment concentration compared to the 96-h LC₅₀. The significant chronic reduction in reworking rate occurs at about 5 orders of magnitude below the 96-h LC₅₀ and at levels that have been observed in Great Lakes sediments. Because the reworking rate is an integrated physiological response, even more sensitive endpoints such as changes in respiration or enzyme activity may be found. However, the reworking rate is the most sensitive oligochaete bioassay for sediment associated toxicants to date and because it is an integrated physiological response, all aspects of bioavailability, organism compensatory mechanisms and organism function are incorporated in the response.

CONCLUSION

Reworking rates of *S. heringianus* measured with a gamma scan system were significantly altered by the presence of a sediment bound toxicant in concentrations up to 5.5 orders of magnitude lower than the 96-h LC₅₀ value. At low concentrations, reworking data suggested possible stimulatory effects during the first 300–600 h, after which there were significant reductions relative to control rates. At high concentrations, rates were equal to or lower than control rates in the first 300–600 h, followed by a definite reduction relative to control rates.

Post experimental worm dry weights tended to be inversely related to high endrin sediment concentrations, presumably reflecting decreased feeding and/or increased stress associated with the higher sediment endrin loads.

Relatively high post experimental surficial endrin concentrations demonstrated worm mediated upward toxicant transport in microcosms with actively feeding worms. Transport did not occur in microcosms where reworking was significantly retarded.

Bioconcentration of endrin by *S. heringianus* was significant, ranging from 34 to 67 times the sediment concentrations.

The importance of sublethal (and even lethal) testing of freshwater oligochaetes in sediments has been neglected. Sublethal impairment of an animal's development or its capacity to live and compete in its environment can greatly reduce the chances of survival for both the individual, and in extreme cases, the entire species (Anderson and d'Apollonia, 1982). Because oligochaetes are one of the most important components of the infaunal macrobenthos of lacustrine systems, the need exists to examine the effects of low level chronic toxicant exposure to these organisms and their role in the fate and persistence of xenobiotics.

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