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Sublethal responses to endrin in sediment by *Limnodrilus hoffmeisteri* (Tubificidae), and in mixed-culture with *Stylodrilus heringianus* (Lumbriculidae)

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Sediment reworking by *Limnodrilus hoffmeisteri* (Tubificidae) alone, and with *Stylodrilus heringianus* (Lumbriculidae) were measured in sediments dosed with endrin by monitoring the burial of a ^{137}cesium marker layer. Endrin concentrations ranged from 16.1 to 81 400 ng/g dry sediment weight. Alterations in reworking rates were observed at sediment concentrations two to five orders of magnitude below LC_{50} values. In single species experiments with *L. hoffmeisteri* at low endrin concentrations, marker layer burial rate data did not suggest stimulation of reworking, as had previously been found for *S. heringianus*. At higher concentrations, reworking rates were equal to or slower than control rates early in experiments, followed by dramatic decreases thereafter. Reworking rates with mixed species (1:1 species ratio) suggested that the presence of *S. heringianus* enhanced the reworking response of *L. hoffmeisteri*.

Post-experimental worm dry weights were inversely related to high sediment concentrations for both species. Reductions in post-experimental *L. hoffmeisteri* mortalities and increases in *L. hoffmeisteri* dry weights in mixed species tests at high endrin concentrations implied that *L. hoffmeisteri* benefits from the presence of *S. heringianus*, although the reverse was not observed.

High final sediment endrin concentrations in the upper three cm implied worm mediated upward contaminant transport. Bioaccumulation factors for *S. heringianus* ranged from 9.7 to 43.8 and were consistently three to four times greater than bioaccumulation factors for *L. hoffmeisteri* (1.7 to 13.6).

Key words: Sediment toxicity; Oligochaete; Species interaction; gamma-Scan system; Biogenic sediment mixing; Bioturbation

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INTRODUCTION

Oligochaetes, sediment mixing and toxics

Oligochaetes are a major benthic component of freshwater systems (Brinkhurst, 1974) that ingest subsurface bottom sediments and convey them to the sediment-water interface as fecal pellets in a conveyor-belt fashion (Robbins, 1982; Robbins, 1986). Rates at which buried sediments are egested and subsequently reburied (the reworking rate) have been determined under a variety of laboratory conditions for the lumbriculid *Stylodrilus heringianus* (Krezoski, 1981; Robbins et al., 1984; White et al., 1987; Keilty et al., 1988a) and for mixed tubificid assemblages (predominantly *Limnodrilus hoffmeisteri*, McCall and Fisher, 1980; Fisher et al., 1980). Tubificids have been found to transport sediment-bound organics (hexachlorobenzene, pentachlorobenzene, and trifluralin) to the sediment surface in laboratory microcosms (Karickhoff and Morris, 1985). Similarly, endrin was concentrated in microcosm surface sediments by *S. heringianus*. The presence of the pesticide altered reworking activities and consequently final sediment endrin distributions (Keilty et al., 1988a).

Oligochaete species interactions

Selective feeding by tubificid oligochaetes and interspecific interactions affecting respiration rates have been demonstrated. Eight heterotrophic bacteria in sediment samples from Toronto Harbor were all ingested by three tubificid species and different bacterial species survived passage through the guts of different worm species. These differences in the use of nutritional resources were thought to directly relate to the ability of the three unspecialized sediment feeders to coexist in the same microhabitat (Brinkhurst and Chua, 1969; Wavre and Brinkhurst, 1971). Additionally, maintaining *T. tubifex, L. hoffmeisteri*, and *Peloscolex multisetosus* (now *Quistadrilus multisetosus*) in mixed species cultures, improved growth and respiration. Community respiration was reduced by one-third, and the presence of *Q. multisetosus* increased the weight gain of *T. tubifex* and *L. hoffmeisteri* by a factor of three over a six-month period (Brinkhurst et al., 1972). *T. tubifex* and *L. hoffmeisteri* also had increased growth and reduced respiration in mixed cultures (Chua and Brinkhurst, 1973; Brinkhurst and Austin, 1979); but direct relationships between selective feeding and respiration were not demonstrated.

Survival and respiration responses of *L. hoffmeisteri* and *T. tubifex* cultures exposed to Cd, Hg, and sodium pentachlorophenol in solution, and changes in temperature, pH, and dissolved oxygen concentrations were examined by Chapman et al. (1982a). Acute toxicities of Cd, Hg, and sodium pentachlorophenol were significantly decreased when *L. hoffmeisteri* and *T. tubifex* were in mixed cultures relative to single species cultures, while no reduction in toxicities was observed at extremes of temperature, pH, and oxygen. Changes in respiration rates and the ability to regulate respiration did not correspond to the enhanced tolerances (Chapman et

al., 1982a). A similar conclusion was reached in a later study with sublethal concentrations of the same compounds (Brinkhurst et al., 1983). Thus, mixed species tubificid test cultures appear to be more tolerant to toxicants, but the mechanism(s) remains unresolved (Chapman and Brinkhurst, 1984).

In addition to the need to understand the specific mechanism(s) of tubificid species interactions in the presence of a toxicant, important questions remain unanswered: (1) how does a sediment-bound toxicant affect toxicity and potential species interactions? (2) are interactions in oligochaetes limited to species within the family Tubificidae? and (3) could increased tolerances be expected with mixed species at sublethal concentrations over long-term (40–50 day) chronic exposures? This research sought to determine sublethal responses to endrin by *L. hoffmeisteri* alone, and in mixed culture with *S. heringianus*.

MATERIALS AND METHODS

Four experiments (referred to as experiments 1–4) ranging from 980 to 1312 h were conducted. Sediments were dosed with a mixture of ^{14}C and ^{12}C endrin in an aqueous slurry. The ^{14}C activity and the specific activity of the dosing mixtures was used to determine sediment concentrations (Keilty et al., 1988a). Measured sediment concentrations ranged from 5.5 to 81 400 ng/g, 17.6 to 15 000 ng/g, 16.1 to 44 200 ng/g, and 9 200 to 35 400 ng/g in experiments 1–4, respectively. Reworking rates were determined radiometrically by measuring the burial of a submillimeter layer of ^{137}Cs in microcosms with the gamma scan system (Robbins et al., 1979; Keilty et al., 1988a). Cells were scanned every 2–5 days, and depths of the peak activity were plotted vs. time. Specific methods for all measured variables including information on field collections are in Keilty et al. (1988a).

Experiments 1 and 2 used only *L. hoffmeisteri* (75 worms/microcosm, equivalent to a density of 50 000 m^{-2}; 3 microcosms/treatment and control). In addition to using both species (1:1) for all treatments (37 worms/species per microcosm; 3 microcosms/treatment and control), experiment 3 also used only *L. hoffmeisteri* at the highest concentration (75 worms/microcosm; 3 microcosms). Two relatively high exposure levels were used in experiment 4. In each, both species were exposed alone (75 worms/microcosm; 2 microcosms/treatment), and in mixed culture (37 worms/species per microcosm; 3 microcosms/treatment and control).

Primary statistical analyses included covariance slope comparisons of treatment and control cell ^{137}Cs burial rates (mean position of the peak activity vs. time) for early and later portions of experiments. Mean post experimental worm weights and bioaccumulation factors were compared with one way ANOVA ($P<0.05$ for all tests). Analyses of residuals indicated that assumptions of 'normality' and 'equal variances' were not seriously violated (no transformations were used). All computations were made on the Michigan Terminal System using the University of Michigan's statistical package, MIDAS.

TABLE I

Percent mortality of worms from replicate microcosms in experiments 1 and 2 (*L. hoffmeisteri* only).

Concentration	Percent mortality		
	Replicate 1	Replicate 2	Mean
Experiment 1			
81 400 ng/g	73.3	81.3	77.3
762 ng/g	6.7	13.3	10.0
5.5 ng/g	8.0	4.0	6.0
Control	4.0	6.7	5.4
Experiment 2			
15 000 ng/g	4.0	0.0	2.0
1 700 ng/g	10.7	6.7	8.7
17.6 ng/g	1.3	0.0	0.7
Control	0.0	4.0	2.0

RESULTS

Mortality

In experiments 1 and 2 using only *L. hoffmeisteri*, worm mortality ranged from 0.7 to 10%, excluding the 81 400 ng/g concentration in experiment 1, where mortality averaged 77.3% (Table I). In the first mixed species test (experiment 3), the mean mortality of *L. hoffmeisteri* alone was 44 percent at the 42 000 ng/g concentration, and only 11.7 in the presence of *S. heringianus* (Table II). Mean mortality of *S. heringianus* in the presence of *L. hoffmeisteri* was 42.4% (Table II). At the 6 300 ng/g sediment concentration mean mortalities of *L. hoffmeisteri* and *S. heringianus* were

TABLE II

Percent mortalities of worms from replicate microcosms in experiment 3 (L, S = single species microcosm, SL = mixed species microcosm, L = *L. hoffmeisteri*, S = *S. heringianus*).

Concentration	Percent mortality			
	Replicate 1	Replicate 2	Replicate 3	Mean
44 200 ng/g L	28.0	45.3	58.7	44.0
44 200 ng/g SL-L	16.2	5.4	13.5	11.7
44 200 ng/g SL-S	48.7	29.7	48.7	42.4
6 300 ng/g SL-L	8.1	8.1	2.7	6.3
6 300 ng/g SL-S	45.9	5.4	18.9	23.4
16.1 ng/g SL-L	16.2	16.2	[a]	16.2
16.1 ng/g SL-S	10.8	10.8	[a]	10.8
Control SL-L	8.1	16.2	[a]	12.2
Control SL-S	8.1	16.2	[a]	12.2

[a] One microcosm used for vertical endrin distribution.

TABLE III

Percent mortalities of worms from replicate microcosms in experiment 4 (L,S = single species microcosm, SL = mixed species microcosm, L = *L. hoffmeisteri*, S = *S. heringianus*).

Concentration	Percent mortality		
	Replicate 1	Replicate 2	Mean
35 400 ng/g L	21.3	20.0	20.7
35 400 ng/g S	12.0	20.0	16.0
35 400 ng/g SL-L	27.0	18.9	23.0
35 400 ng/g SL-S	16.2	32.4	24.3
9 200 ng/g L	0.0	0.0	0.0
9 200 ng/g S	0.0	8.0	4.0
9 200 ng/g SL-L	0.0	8.1	4.1
9 200 ng/g SL-S	21.6	0.0	10.8
Control SL-L	0.0	0.0	0.0
Control SL-S	13.5	8.1	10.8

6.3 and 23.4%, respectively. Mortalities at 16.1 ng/g and controls ranged from 10.8 to 16.2 percent (Table II). In the second mixed species test (experiment 4), mean mortalities at the high concentration of 35 400 ng/g for *L. hoffmeisteri* and *S. heringianus* alone were 20.7 and 16.0%, respectively (Table III). In mixed microcosms, mortalities were 23.0 and 24.3%, respectively. In both single and mixed species tests and the low concentration of 9 200 ng/g and controls, mortalities ranged from 0 to 10.8% (Table III).

Reworking rates

Microcosm sediments compacted to varying degrees in all experiments (0.3–1.1 cm). Compaction was greatest in cells with *L. hoffmeisteri* alone and relatively negligible in microcosms containing only *S. heringianus*. Compaction corrected mean depths of ^{137}Cs peaks were plotted for each experiment to interpret reworking responses (Figs. 1 and 2). To describe changes in reworking during the tests, a point where the lines visibly inflected was chosen to divide the data and calculate reworking rates (slopes/initial number of worms) for early and later portions of each experiment (Tables IV, V). Slopes were then compared using analyses of covariance (Tables VI–IX). All calculations were based on the mean of the responses. The variation between microcosms was low and approximately constant throughout experiments (similar to Keilty et al., 1988a). The mean coefficients of variation of the SD values for experiments 1–4 were 38.7 ± 22.8, 61.2 ± 7.7, 58.7 ± 18.7, and 56.8 ± 8.8, respectively.

At the high concentration of 81 400 ng/g in both early and later portions of experiment 1, reworking by *L. hoffmeisteri* was minimal (0.45 and 0.32×10^{-5} cm/h per worm, Table IV). Rates in the lower concentrations and the control were generally an order of magnitude faster (Table IV). Unexpectedly, the control reworking rate

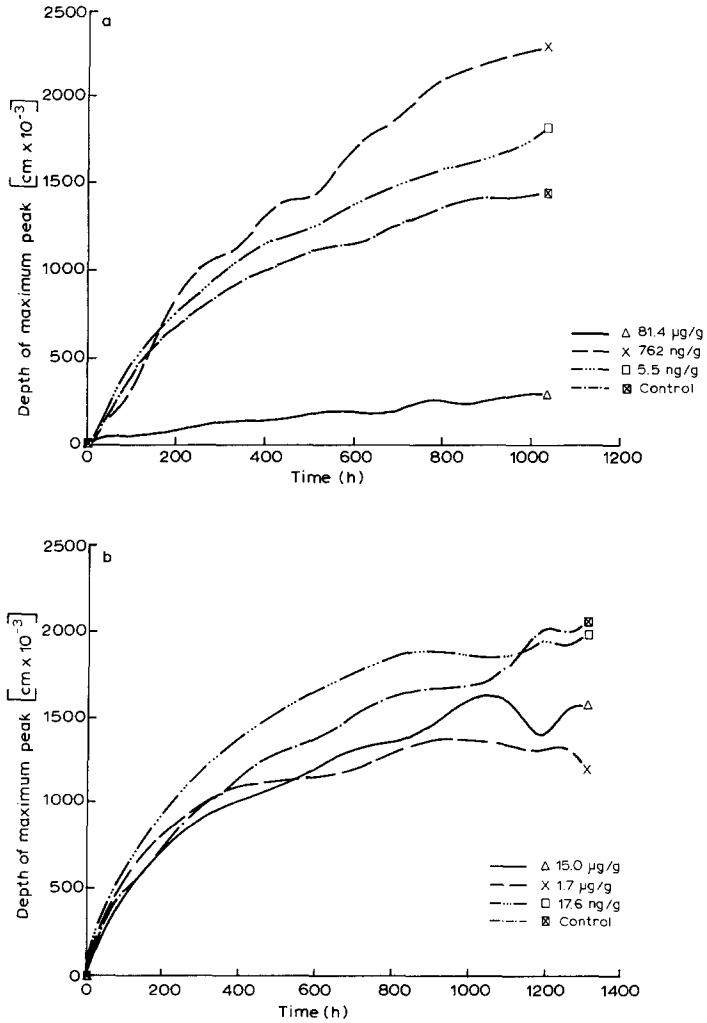

Fig. 1.a. Depths of mean ($n = 3$) ^{137}Cs peaks as buried by *L. hoffmeisteri* in experiment 1 for experimental sediment endrin concentrations and the control. b. Depths of mean ($n = 3$) ^{137}Cs peaks as buried by *L. hoffmeisteri* in experiment 2 for experimental sediment endrin concentrations and the control.

in the second half of the experiment was significantly slower than the rates of the intermediate and low concentrations of 762 ng/g and 5.5 ng/g (Table VI).

In the first 342 h of experiment 2, reworking rates from all concentrations were similar (3.23 to 4.4×10^{-5} cm per h worm; Table IV) and compared well with those from the first experiment. Although these early rates were not significantly different (Table VII), during the final 960 h, the control working rate was significantly faster than all other rates. At the low concentration of 17.6 ng/g and the high concentra-

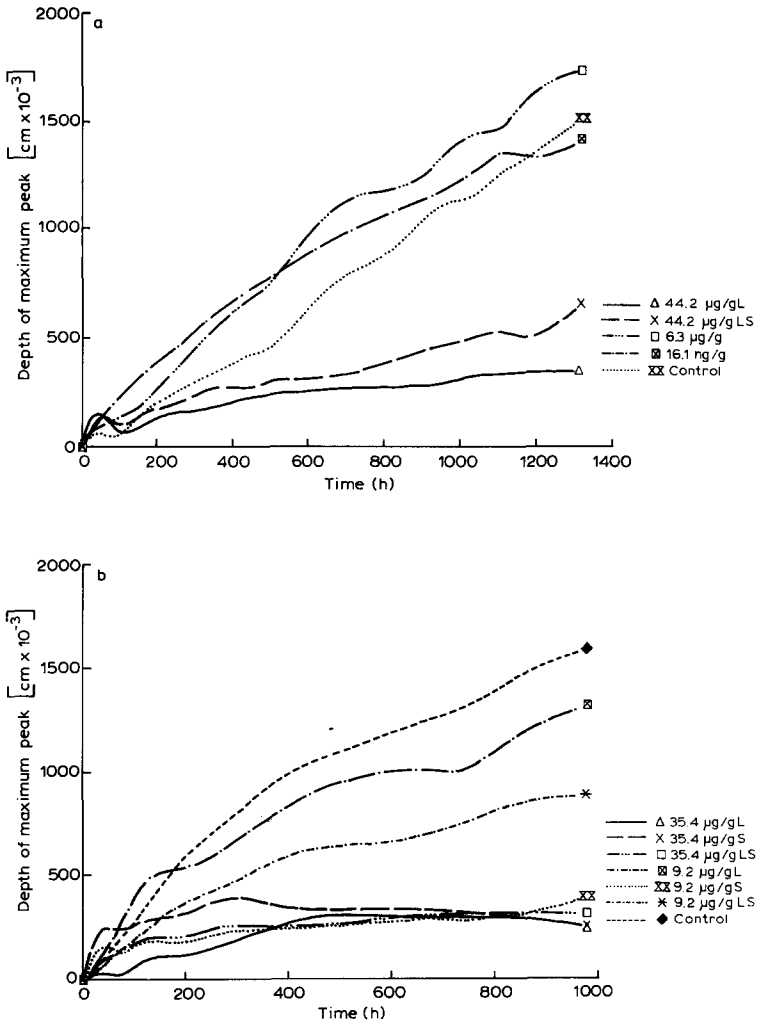

Fig. 2.a. Depths of mean ($n = 3$) ^{137}Cs peaks as buried by *L. hoffmeisteri* alone (L) and with *S. heringianus* (all others) in experiment 3. b. Depths of mean ($n = 3$) ^{137}Cs peaks as buried by *L. hoffmeisteri* alone (L), *S. heringianus* alone (S) and both species together (LS) in experiment 4.

tion of 15 000 ng/g reworking rates were faster than the reworking rates in the 1 700 ng/g microcosms, yet still significantly slower than the control rate (Table IV).

Reworking rates in the first mixed species test (experiment 3) ranged from 2.23×10^{-5} cm/h per worm at 16.1 ng/g to 0.21×10^{-5} cm/h per worm at 42 000 ng/g (Table V) and were similar to rates from the single species *L. hoffmeisteri* tests at comparable test concentrations. In the first 342 h, the reworking rate for *L. hoffmeisteri* alone was 0.35×10^{-5} cm/h per worm, and 0.68×10^{-5} cm/h per worm in

TABLE IV

Sediment reworking rates (cm/worm per h × 10^{-5}) calculated from ^{137}Cs burial rates for the first 336 h and the remainder of experiment 1, and the first 342 h and the remainder of experiment 2.

Experiment 1	24–336 h	336–1032 h
	Reworking rate (cm/worm per h × 10^{-5})	Reworking rate (cm/worm per h × 10^{-5})
81 400 ng/g	0.45 (0.03)[a]	0.32 (0.04)
762 ng/g	4.77 (0.44)	2.17 (0.18)
5.5 ng/g	3.99 (0.36)	1.35 (0.05)
Control	3.53 (0.36)	0.96 (0.08)
Experiment 2	**30–342 h**	**342–1302 h**
	Reworking rate (cm/work per h × 10^{-5})	Reworking rate (cm/worm per h × 10^{-5})
15 000 ng/g	3.33 (0.30)	0.71 (0.09)
1 700 ng/g	3.23 (0.38)	0.25 (0.11)
17.6 ng/g	4.41 (0.36)	0.68 (0.12)
Control	3.38 (0.19)	1.30 (0.04)

[a] Parentheses indicate SE.

TABLE V

Sediment reworking rates (cm/worm per h × 10^{-5}) calculated from ^{137}Cs burial rates for the first 352 h and the remainder of experiment 3, and the first 260 h and the remainder of experiment 4.

Experiment 3	20–352 h	352–1312 h
44 200 ng/g L[a]	0.35 (0.13)[c]	0.21 (0.02)
44 200 ng/g SL[b]	0.68 (0.10)	0.56 (0.04)
6 300 ng/g SL	2.01 (0.19)	1.59 (0.08)
16.1 ng/g SL	2.23 (0.07)	1.12 (0.06)
Control SL	1.29 (0.13)	1.68 (0.04)
Experiment 4	**24–260 h**	**260–980 h**
	Reworking rate (cm/worm per h × 10^{-5})	Reworking rate (cm/worm per h × 10^{-5})
35 400 ng/g L	0.86 (0.11)	−0.09 (0.07)
35 400 ng/g S	0.94 (0.06)	−0.21 (0.04)
35 400 ng/g SL	0.83 (0.11)	0.13 (0.03)
9 200 ng/g L	2.95 (0.60)	1.00 (0.13)
9 200 ng/g S	0.45 (0.06)	0.29 (0.06)
9 200 ng/g SL	2.07 (0.12)	0.73 (0.06)
Control SL	3.91 (0.15)	1.37 (0.06)

[a] L = *L. hoffmeisteri*;
[b] S = *S. heringianus*.
[c] Parentheses indicate SE.

the presence of *S. heringianus*. Although the increase in reworking rates was not significant, the increase from 0.21 to 0.56×10^{-5} cm/h per worm in the final 960 h was. Reworking rates from both the 6300 ng/g and 16.1 ng/g concentrations were significantly faster than the control rate and the high concentration rate in the first 352 h, while the control rate was significantly faster than all concentrations except the 6300 ng/g (no diff.) in the final 960 h (Table VIII).

In the second mixed species test (experiment 4), the early reworking rate for *L. hoffmeisteri* and *S. heringianus* in the high concentration of 35400 ng/g was 0.83×10^{-5} cm/h per worm, compared with 0.86×10^{-5} cm/h per worm for *L. hoff-*

TABLE VI

Statistical comparison of reworking rates for the first 336 h and the remainder of experiment 1 (alpha < 0.05).

81.4 μg/g	–			24–336 h
762 ng/g[a]	F[b]	–		
5.5 ng/g	F	n.d.	–	
Control	F	n.d.	n.d.	–
	81.4	762	5.5	Control
81.4 μg/g	–			336–1032 h
762 ng/g	F	–		
5.5 ng/g	F	S	–	
Control	F	S	S	–
	81.4	762	5.5	Control

[a] Example: 762 ng/g is significantly faster than 81.4 μg/g.
[b] Read from left, then down, F = faster, S = slower, n.d. = no difference.

TABLE VII

Statistical comparison of reworking rates for the first 342 h and the remainder of experiment 2 (alpha < 0.05).

15.0 μg/g	–			30–342 h
1.7 μg/g[a]	n.d.[b]	–		
17.6 ng/g	n.d.	n.d.	–	
Control	n.d.	n.d.	n.d.	–
	15.0	1.7	17.6	Control
15.0 μg/g	–			342–1302 h
1.7 μg/g	S	–		
17.6 ng/g	n.d.	F	–	
Control	F	F	F	–
	15.0	1.7	17.6	Control

[a] Example: 1.7 μg/g is not different from 15.0 μg/g.
[b] Read from left, then down. F = faster, S = slower, n.d. = no difference.

TABLE VIII

Statistical comparison of reworking rates for the first 352 h and the remainder of experiment 3 (alpha <0.05).

44.2 μg/g L					20–352 h
44.2 μg/g SL	n.d.	–			
6.3 μg/g SL[a]	F[b]	F	–		
16.1 ng/g SL	F	F	n.d.	–	
Control	F	F	S	S	–
	44.2 L	44.2 SL	6.3 SL	16.1 SL	Control
44.2 μg/g L	–				352–1 312 h
44.2 μg/g SL	F	–			
6.3 μg/g SL	F	F	–		
16.1 ng/g SL	F	F	S	–	
Control	F	F	n.d.	F	–
	44.2 L	44.2 SL	6.3 SL	16.1 SL	Control

[a] Example: 6.3 μg/g is significantly faster than 44.2 μg/g with *L. hoffmeisteri* only.
[b] Read from left, then down. F = faster, S = slower, n.d. = no difference.

meisteri and 0.94×10^{-5} cm/h per worm for *S. heringianus* alone (Table V). Reworking in single species microcosms stopped in the later half of the experiment, but continued in the mixed species microcosms at a slow rate of 0.13×10^{-5} cm/h per worm. At the lower concentration of 9 200 ng/g, the mixed species rate was 2.07×10^{-5} cm/h per worm, and 2.95×10^{-5} cm/h per worm for *L. hoffmeisteri* alone and 0.45×10^{-5} cm/h per worm for *S. heringianus* alone (Table V).

In both early and later portions of experiment 4, reworking rates in the 9 200 ng/g sediments were significantly faster than the rates in the 35 400 ng/g sediments (Table IX). The mixed species reworking rate in 9 200 ng/g was significantly faster than the single species rate of *S. heringianus*, but not significantly different from the single species rate of *L. hoffmeisteri*. The control rate in the first 260 h was significantly faster than all rates except the 9 200 ng/g *L. hoffmeisteri* single species rate (no diff.). In the final 720 h, the 35 400 ng/g mixed species rate was significantly faster than both single species rates. The 9 200 ng/g mixed and single species *L. hoffmeisteri* rates were not significantly different, however, both were significantly faster than the single species *S. heringianus* rate. The control rate was significantly faster than all other rates (Table IX).

Worm weights

Post experimental mean dry weights per worm of *L. hoffmeisteri* in single species experiment 1 ranged from 0.246 to 0.700 mg for worms exposed at 81 400 ng/g and 762 ng/g sediment endrin concentrations respectively (Table X). Worm weights from 762 ng/g and 5.5 ng/g exposures, and controls were significantly higher than worm weights from 81 400 ng/g exposures. Weights from 762 ng/g exposures were significantly higher than the 5.5 ng/g and control, while no weight difference was observed between the 5.5 ng/g and control.

TABLE IX

Statistical comparison of reworking rates for the first 260 h and the remainder of experiment 4 (alpha < 0.05).

35.4 µg/g L						24–260 h	
35.4 µg/g S	n.d.	–					
35.4 µg/g SL	n.d.	n.d.	–				
9.2 µg/g L[a]	F[b]	F	F	–			
9.2 µg/g S	F	F	F	S	–		
9.2 µg/g SL	F	F	F	n.d.	F	–	
Control SL	F	F	F	n.d.	F	F	–
	35.4 L	35.4 S	35.4 SL	9.2 L	9.2 S	9.2 SL	Control
35.4 µg/g L	–						260–980 h
35.4 µg/g S	n.d.	–					
35.4 µg/g SL	F	F	–				
9.2 µg/g L	F	F	F	–			
9.2 µg/g S	F	F	F	S	–		
9.2 µg/g SL	F	F	F	n.d.	F	–	
Control	F	F	F	F	F	F	–
	35.4 L	35.4 S	35.4 SL	9.2 L	9.2 S	9.2 SL	Control

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

[b] Example: 9.2 µg/g is significantly faster than 35.4 µg/g with *L. hoffmeisteri* only.

TABLE X

Mean worm dry weights (mg $\times 10^{-3}$, m = mean, s = SD, $n = 3$) from replicate microcosms in experiments 1 and 2 (*L. hoffmeisteri* only).

Sediment conc.	Worm dry weight (mg $\times 10^{-3}$)					
	Replicate 1		Replicate 2		Mean	
	m	s	m	s	m	s
Experiment 1						
81 400 ng/g	246	101	[a]		246	101
762 ng/g	647	80	727	128	700	100
5.5 ng/g	94	91	420	17	506	109
Control	561	108	431	83	496	111
Experiment 2						
15 000 ng/g	539	60	628	134	584	105
1 700 ng/g	489	30	503	48	496	37
17.6 ng/g	682	88	710	122	696	96
Control	484	48	665	45	575	108

[a] Combined into replicate 1.

TABLE XI

Mean worm dry weights (mg × 10^{-3}, m = mean, s = SD, $n = 3$) from replicate microcosms in experiment 3.

Sediment conc.			Worm dry weight (mg × 10^{-3})							
			Replicate 1		Replicate 2		Replicate 3		Mean	
			m	s	m	s	m	s	m	s
44 200	ng/g	L[a]	232	11	320	47	275	62	276	55
44 200	ng/g	SL-L	376	61	376	16	329	101	360	64
44 200	ng/g	SL-S	210	24	269	46	286	32	255	46
6 300	ng/g	SL-L	468	148	521	103	598	20	529	107
6 300	ng/g	SL-S	325	130	310	123	297	15	311	50
16.1	ng/g	SL-L	511	45	581	110	[b]		546	84
16.1	ng/g	SL-S	328	35	375	43	[b]		352	43
Control		SL-L	559	59	759	188	[b]		659	166
Control		SL-S	370	10	491	42	[b]		430	72

[a] L,S = single species microcosm, SL = mixed species microcosm, L = *L. hoffmeisteri*, S = *S. heringianus*.
[b] Frozen for other analyses.

In single species experiment 2 with *L. hoffmeisteri*, mean weights ranged from 0.496 to 0.696 mg/worm for exposures at 1 700 ng/g and 17.6 ng/g sediment endrin concentrations, respectively (Table X). The mean weight per worm from 17.6 ng/g exposure was significantly higher than the worm weights from all other exposures. No differences were observed between 15 000 ng/g and 1 700 ng/g and controls.

In the first mixed species test (experiment 3), weights of both species from the 42 000 ng/g sediment exposure ranged from 0.255 to 0.360 mg/worm (Table XI) and were significantly lower than weights from all other sediment exposure concentrations. *L. hoffmeisteri* weights from mixed microcosms were significantly higher than *L. hoffmeisteri* weights in single species microcosms (0.360 mg/worm vs. 0.276 mg/worm). *S. heringianus* was not tested in a single species format. In sediment concentrations of 6 300 ng/g, 16.1 ng/g and controls, *L. hoffmeisteri* weights were significantly higher than *S. heringianus* weights. *L. hoffmeisteri* weights from control sediments were significantly higher than *L. hoffmeisteri* weights from 16.1 ng/g and 6 300 ng/g sediment exposures, while *S. heringianus* weights from control sediments were significantly higher than *S. heringianus* weights from the 6 300 ng/g sediment exposure, but not the 16.1 ng/g sediment exposure.

In the second mixed species test (experiment 4), weights from single species 35 400 ng/g sediment exposure were 0.377 and 0.388 mg/worm for *L. hoffmeisteri* and *S. heringianus* respectively (Table XII). Weights from mixed species 35 400 ng/g sediments were 0.540 and 0.340 mg/worm, respectively. The increase of *L. hoffmeisteri* body weight in the mixed microcosms was significant. Weights from single species 9 200 ng/g sediments were 0.544 and 0.438 mg/worm for *L. hoffmeisteri* and *S. heringianus*, respectively. Weights from mixed species 9 200 ng/g sediments were

TABLE XII

Mean worm dry weights (mg × 10^{-2}, m = mean, s = SD, $n = 3$) from replicate microcosms in experiment 4.

Sediment conc.		Worm dry weight (mg × 10^{-3})					
		Replicate 1		Replicate 2		Mean	
		m	s	m	s	m	s
35 400 ng/g	L	44	9	31	7	37.7	10.4
35 400 ng/g	S	38	1	40	1	38.8	1.8
35 400 ng/g	SL-L	63	15	45	7	54.0	14.6
35 400 ng/g	SL-S	33	1	35	9	34.0	5.8
9 200 ng/g	L	42	7	67	12	54.4	16.0
9 200 ng/g	S	43	2	45	2	43.8	2.1
9 200 ng/g	SL-L	68	9	83	14	75.6	13.4
9 200 ng/g	SL-S	44	6	51	11	47.8	8.4
Control	SL-L	67	3	77	8	72.0	7.6
Control	SL-S	58	9	64	11	61.0	9.4

[a] L,S = single species microcosm, SL = mixed species microcosm, L = *L. hoffmeisteri*, S = *S. heringianus*.

0.756 and 0.478 mg/worm, respectively. Again, the increase in *L. hoffmeisteri* weight was significant. Weights of *S. heringianus* from mixed species microcosms were not significantly different from *S. heringianus* single species weights from 35 400 ng/g and 9 200 ng/g sediments.

Single species worm weight comparisons between exposures at 35 400 and 9 200 ng/g sediment yielded significantly higher *L. hoffmeisteri* weights from 9 200 ng/g sediments, but no difference of *S. heringianus* single species weights between 35 400 and 9 200 ng/g sediments. Both mixed species 9 200 ng/g *L. hoffmeisteri* and *S. heringianus* weights were significantly higher than mixed species 35 400 ng/g *L. hoffmeisteri* and *S. heringianus* weights.

Mixed species control *L. hoffmeisteri* weights were significantly higher than mixed species *L. hoffmeisteri* weights from 35 400 ng/g sediments, but not 9 200 ng/g sediments. Mixed species *S. heringianus* control weights were significantly higher than mixed species *S. heringianus* weights from both sediment endrin concentrations.

Bioaccumulation

Bioaccumulation factors (endrin concentration in whole worm tissue/sediment endrin concentration) for *L. hoffmeisteri* in single species experiments 1 and 2 ranged from 1.7 to 9.1 (Table XIII), with a mean value of 4.19 ± 2.54 ($n = 4$).

In the first mixed species test (experiment 3), bioaccumulation factors for *L. hoffmeisteri* ranged from 3.06 to 11.52 (Table XIV), with a mean value of 6.40 ± 3.16 ($n = 9$). Bioaccumulation factors for *S. heringianus* ranged from 9.77 to 43.83 (Table XIV), with a mean value of 20.42 ± 12.37 ($n = 8$). Highest bioaccumulation factors

for both species were observed at the intermediate concentration of 6300 ng/g. Single species bioaccumulation factors for *L. hoffmeisteri* were not significantly different from mixed species *L. hoffmeisteri* bioaccumulation factors at 42000 ng/g. Bioaccumulation factors at all sediment endrin levels for *S. heringianus* were usually 3–4 times higher than bioaccumulation factors for *L. hoffmeisteri*.

In the second mixed species test (experiment 4), bioaccumulation factors for single species *L. hoffmeisteri* microcosms ranged from 4.46 to 31.31 (Table XV), with a mean of 9.9 ± 3.87 ($n = 4$). Mixed species *L. hoffmeisteri* bioaccumulation factors ranged from 5.24 to 13.55, with a mean of 8.27 ± 3.74 ($n = 4$). Bioaccumulation factors for single species *S. heringianus* ranged from 17.62 to 29.03 (Table XV), with a mean of 22.52 ± 5.03 ($n = 4$). Mixed species *S. heringianus* bioaccumulation factors

TABLE XIII

Bioconcentration factors for *Limnodrilus hoffmeisteri* in experiments 1 and 2.

Sediment conc.	Bioconcentration factor		
	Replicate 1	Replicate 2	Mean
Experiment 1			
81400 ng/g	5.12	13.11	9.12
762 ng/g	1.65	1.74	1.70
5.5 ng/g	3.88	2.54	3.21
Experiment 2			
15000 ng/g	3.99	3.73	3.86
1700 ng/g	5.63	2.19	3.91
17.6 ng/g	3.70	3.02	3.36

TABLE XIV

Bioconcentration factors for *Limnodrilus hoffmeisteri* and *Stylodrilus heringianus* from single (*L. hoffmeisteri* only) and mixed species microcosms in experiment 3.

Sediment conc.	Bioconcentration factor				
	Rep. 1	Rep. 2	Rep. 3	Mean	SD
44200 ng/g L[a]	3.06	7.41	5.69	5.39	2.19
44200 ng/g SL-L	5.01	3.11	5.09	4.40	1.12
44200 ng/g SL-S	2.39	17.10	10.79	13.43	3.28
6300 ng/g SL-L	11.52	5.30	11.44	9.42	3.57
6300 ng/g SL-S	32.42	43.83	25.21	33.82	9.39
16.1 ng/g SL-L	n.d.[b]	n.d.	n.d.	n.d.	n.d.
16.1 ng/g SL-S	9.77	11.82	[c]	10.80	[c]

[a] Species composition within microcosms for each concentration delineated by L, S, and LS (L = *L. hoffmeisteri*, S = *S. heringianus*, and LS = both species 1:1).

[b] n.d., not detectable.

[c] One microcosm used for vertical endrin distribution.

TABLE XV

Bioconcentration factors for *Limnodrilus hoffmeisteri* and *Stylodrilus heringianus* from single and mixed species microcosms in experiment 4.

Sediment conc.		Bioconcentration factors		
		Replicate 1	Replicate 2	Mean
35 400 ng/g	L[a]	4.46	10.01	7.24
35 400 ng/g	S	19.68	17.62	18.65
35 400 ng/g	SL-L	5.24	8.24	6.74
35 400 ng/g	SL-S	20.38	24.97	22.68
9 200 ng/g	L	11.85	13.31	12.58
9 200 ng/g	S	23.73	29.03	26.38
9 200 ng/g	SL-L	13.55	6.04	9.80
9 200 ng/g	SL-S	41.88	43.27	42.58

[a] Species composition within microcosms for each concentration dlineated by L, S and LS (L = *L. hoffmeisteri*, S = *S. heringianus*, and LS = both species 1:1).

ranged from 2.38 to 43.27, with a mean of 32.63 ± 11.65 ($n = 4$). The single species bioaccumulation factor for *L. hoffmeisteri* was not significantly different from the mixed species bioaccumulation factors at both the 35.4 and 9 200 ng/g sediment endrin concentrations. The single species bioaccumulation factor for *S. heringianus* was not significantly different from the mixed species bioaccumulation factor at 35 400 ng/g, however, it was at the 9 200 ng/g sediment endrin concentration. Once again, bioaccumulation by *S. heringianus* was significantly greater than bioaccumulation by *L. hoffmeisteri*.

Vertical distribution of endrin in microcosms

Post experimental vertical endrin distributions were characterized by increases of the compound in the upper 2–3 cm of experimental microcosms relative to 'blanks' (endrin contaminated sediment microcosms without worms (Fig. 3a–d)). Where little or no reworking occurred, endrin redistribution was minimal. Organic carbon content followed the vertical distribution of endrin in the 'blank' microcosms (Keilty et al., 1988a) and in experimental cells (Fig. 4).

DISCUSSION

Mortality

Endrin is very toxic to aquatic organisms (reviewed in Grant, 1976; Keilty et al., 1988a,b). Although the literature 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). In a recent study, 96 h LC_{50} values for *S. heringianus* and *L. hoffmeisteri* with endrin contaminated sediment yielded mean values of $2\,588 \pm 1\,974$ and $2\,725 \pm 995$

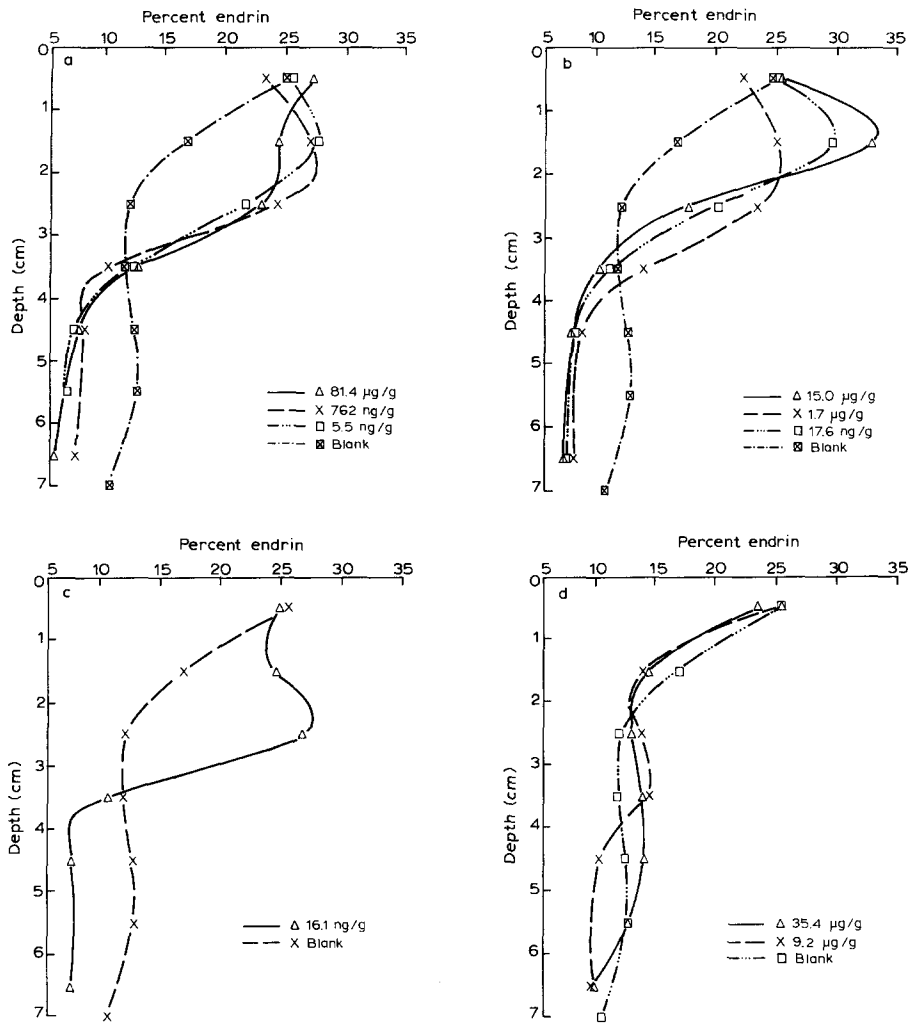

Fig. 3.a–d. Post experimental vertical endrin distribution as the percent of the compound at each centimeter depth interval for experimental and blank microcosms in experiments 1–4, respectively. Blank distribution reflects endrin position without oligochaete sediment mixing. Note: Only one distribution is reflected from exp. 3 because all higher concentration microcosms were sacrificed for mortality data.

µg/g dry weight sediment respectively (Keilty et al., 1988b). When sorbed to sediment, endrin's toxicity to oligochaetes appears to be reduced for both short and long-term exposures (Keilty et al., 1988a,b). Mortality from relatively long-term low and intermediate experimental endrin loads in this study (Tables I–III) was not significantly different from control worm mortality, and was similar to previously reported mortality for *S. heringianus*. Because the mortality of *L. hoffmeisteri* was

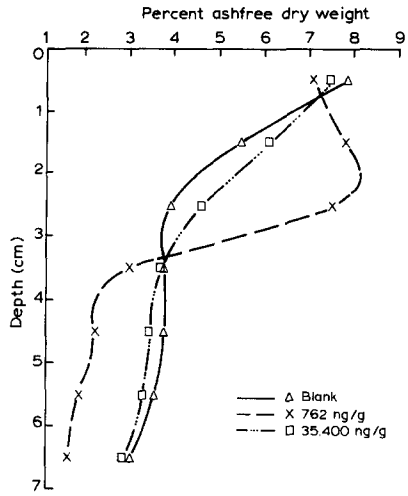

Fig. 4. Percent ashfree dry weight distributions in blank and experimental microcosms.

four-fold lower (44 to 11.7%, Table I) in the presence of *S. heringianus* at a high sediment endrin concentration, it is possible that *S. heringianus* may reduce the toxicity of endrin to *L. hoffmeisteri*. However, mortality alone may not be the most desirable measure of potential mixed species interactions influencing toxic effects because significant mortality only occurs at very high concentrations. Inclusion of sublethal responses, such as alterations in sediment reworking, as measured with a gamma scan system, and worm body weight changes, provide a more sensitive measure.

Compaction effects

Unlike the reworking of *S. heringianus*, the reworking of *L. hoffmeisteri* resulted in the marked compaction (up to 1.1 cm) and irregular sculpturing of microcosm surficial sediments. Compaction may be the result of the greater reworking rate exhibited by *L. hoffmeisteri*. The measured rates for each species were within the range of previously reported reworking rates (reviewed in Fisher et al., 1980; Krezoski et al., 1984). Single species control rates for *L. hoffmeisteri* (corrected from compaction) ranged from 9.5×10^{-6} to 3.5×10^{-5} cm/h per worm (this study), while control rates for *S. heringianus* ranged from 0.85 to 1.3×10^{-5} cm/h per worm (Keilty et al., 1988a). If *L. hoffmeisteri* reworking data were not corrected for compaction, rates would be approximately three times those of *S. heringianus*. This suggests that *L. hoffmeisteri* exerts a greater influence on the physical integrity of the sediments than *S. heringianus*. Irregularly shaped surface deposits resulting from the feeding of *L. hoffmeisteri* apparently do not, however, influence the integrity of the ^{137}Cs layer, for no unusually broad ^{137}Cs distributions were observed over the duration of

experiments. A broadening of the tracer profile is expected when the layer eventually reaches the depth of the zone of maximum oligochaete feeding (Robbins et al., 1979).

Sediment reworking responses

By using the gamma scan system, longterm chronic effects of endrin at concentrations as great as five orders of magnitude below the 96-h LC_{50} values were observed. Reworking responses to endrin contaminated sediment by *L. hoffmeisteri* alone (Fig. 1a,b) follow the same general pattern observed for *S. heringianus*, typified by marked reductions in reworking at high endrin loads, relative to control rates and low concentrations. Reductions are most prominent in the later portions of experiments. The stimulation of *S. heringianus* rates early in previous experiments was not observed for *L. hoffmeisteri*. Perhaps the latter species has an overall greater resistance to endrin. Such resistance is further suggested for *L. hoffmeisteri* by threefold higher burrowing avoidance EC_{50} values and 3–4 times lower bioaccumulation factors than *S. heringianus* (Keilty et al., 1988b).

Characteristic reductions in reworking by both species at high concentrations indicate decreased feeding rates. Reductions in worm weights reinforce this. Because the 'high' concentrations are still two orders of magnitude lower than previously determined 96 hour LC_{50} for each species, the deleterious effects of long-term, chronic exposures to sediment associated toxicants are clearly demonstrated.

As initially suggested by the mortality data, reworking data from mixed species microcosms indicated that a beneficial interaction occurred for *L. hoffmeisteri*. Without altering the relative densities of each species within microcosms to determine the relative contribution to the 'mixed' reworking rate, it is difficult to elaborate on the nature and extent of this beneficial effect; however, increases in *L. hoffmeisteri* body weights in the presence of *S. heringianus* at high endrin concentrations further substantiated this gain. Similar interactions, where one species clearly benefits by the presence of another (only among tubificids), have been demonstrated with tubificid feeding, respiration, and short-term response patterns to lethal physical and chemical factors (Brinkhurst and Chua, 1969; Brinkhurst et al., 1972; Chua and Brinkhurst, 1973; Chapman et al., 1982a,b,c; Brinkhurst et al., 1983).

Reworking rate response trends from mixed species tests (Fig. 2a,b) were consistent with single species trends and provide insight into species interactions. At the lower concentrations early in experiment 3, reworking rates significantly exceeded control rates, most likely reflecting the expected stimulation of *S. heringianus* (Keilty et al., 1988a). At the high concentrations in experiments 3 and 4, early individual reworking rates were not significantly different from the mixed species reworking rates, but the mixed species rates later in the experiments were significantly faster than the individual rates. This suggests that the underlying mechanism(s) of interspecific interaction manifested in enhanced reworking was slow to develop. An insufficient length of exposure (both time and the mode; i.e. bound to sediment), as

well as insufficient time for the potential redistribution of bacterial resources through selective feeding and differential feeding depth distributions, all may contribute to the length of time required to identify a mixed species response to a sediment bound toxicant.

The redistribution and partitioning of resources may influence the depth at which each species feeds. Previous studies implied that the feeding zone of tubificids extended deeper into the sediments than the feeding zone of *S. heringianus* (Fisher et al., 1980; Krezoski and Robbins, 1985). With their normal tendency of feeding deeper in the sediments, *S. heringianus* in mixed species microcosms may encourage the vertical extension of *L. hoffmeisteri*'s feeding zone to greater depths within the microcosms and subsequent exposure to lower endrin loads (based on endrin distributions in 'blank' microcosms). Or, the presence of *S. heringianus* may not extend *L. hoffmeisteri*'s feeding zone, but may only result in *L. hoffmeisteri* spending less time in the uppermost sediments. In either case, the overall exposure of *L. hoffmeisteri* to endrin may be reduced (relative to *S. heringianus*), resulting in increased mixed species reworking rates relative to single species rates at high endrin concentrations. Consistent, slightly higher *L. hoffmeisteri* bioaccumulation factors for single species versus mixed species *L. hoffmeisteri* bioaccumulation factors in experiments three and four (Tables XIV and XV) again suggested that *L. hoffmeisteri* might have had limited exposure to endrin in the mixed species test. Consistently higher bioaccumulation factors of *S. heringianus* suggested that this species had a higher feeding zone and consequently spent more time in and ingested more sediment with the highest endrin concentrations. Additionally, post experimental endrin distributions suggested that *L. hoffmeisteri*'s feeding zone extended deeper than *S. heringianus*'s feeding zone. *S. heringianus* tended to increase the concentration of endrin in the top cm of microcosms (Keilty et al., 1988b), while *L. hoffmeisteri* tended to increase endrin concentrations 2–3 cm below the surface (Fig. 3a–c).

Reworking data from the 9200 ng/g microcosms in experiment 4 suggested that *L. hoffmeisteri* might have been responsible for the low, but significant, mixed species reworking pattern. Again, a depper feeding zone by *L. hoffmeisteri* may have reduced its endrin exposure. At this concentration, and the much lower concentrations of experiment 3, reworking rates did not, however, indicate that the presence of *S. heringianus* enhances the reworking of *L. hoffmeisteri*. Apparently this occurs, or at least can only be measured, at high concentrations where significant toxicity occurs.

In the latter half of experiments, control rates were in almost all cases faster than reworking rates from experimental microcosms. The result is consistent with previous *S. heringianus* data (Keilty et al., 1988a) and indicated that the toxic effect accumulates over an extended period at both low and high exposure levels.

Worm weights

Post experimental worm dry weights have been shown to parallel changes in

reworking rates and sediment endrin loads for *S. heringianus* (Keilty et al., 1988b). Similar trends were evident in both single and mixed species post experimental *L. hoffmeisteri* weights, reflecting the altered feeding patterns manifested in the altered reworking patterns. In experiment 3, the mixed species *L. hoffmeisteri* weight at the highest concentration of 42 000 ng/g was significantly higher than the single species *L. hoffmeisteri* weight. *L. hoffmeisteri* weights from mixed species microcosms at both 35 400 and 9 200 ng/g concentrations in experiment 4 also were significantly higher than *L. hoffmeisteri* single species weights. These data imply that *L. hoffmeisteri* benefit from the presence of *S. heringianus* at high sediment endrin concentrations, and demonstrate the utility of post-contaminant exposure body weight measurements to complement the reworking rate measure by reflecting an integrated physiological response.

Bioaccumulation factors

Bioaccumulation of endrin by *L. hoffmeisteri* and *S. heringianus* was considerably lower than reported bioconcentration factors from water for other aquatic species (see Keilty et al., 1988a).

Bioaccumulation factors for *S. heringianus* were generally 3–4 times greater than bioaccumulation factors for *L. hoffmeisteri*. Differences in total lipid content may relate to this, but recent lipid analyses of *S. heringianus* and tubificids (reported as predominantly *L. hoffmeisteri*) indicated that both average 10 to 20% lipid material (Gardner et al., 1985a,b). As discussed previously, a higher feeding zone for *S. heringianus* was likely responsible for its higher bioaccumulation factor, although differences in bioaccumulation factors between the two species were slightly enhanced by using the mean sediment concentration for calculations. Observed values for *L. hoffmeisteri* were consistent with recently reported values for *L. hoffmeisteri* and pp'DDE, Mirex, and PCB (Oliver, 1987).

Distribution of endrin in microcosms

Post experimental vertical endrin profiles suggested upward worm mediated movement of the compound (Fig. 3a–c), similar to responses observed using tubificid worms and hexachlorobenzene, pentachlorobenzene, and trifluralin (Karickhoff and Morris, 1985), and *S. heringianus* and endrin (Keilty et al., 1988a). In the latter study, marked increases of endrin in the top 1 cm relative to 'blank' microcosms (settled sediment with endrin and no worms) were observed. These peaks were characteristically preceded by marked decreases in the cm fraction below them, implying that subsurface defecation was minimal for *S. heringianus*, consistent with the results of Krezoski and Robbins (1985). Reworking activities of *L. hoffmeisteri* also resulted in upward endrin transport, however, the resultant distributions were characterized by increases in the top 2–3 cm and were again preceded by decreases with slopes steeper than decreases over the same depths in the 'blanks' (Fig. 3a–c). The relatively wider and deeper peak of endrin probably

reflected a deeper feeding zone for *L. hoffmeisteri* and potentially significant subsurface defecation for this species. Where reworking was greatly diminished in the mixed species microcosms, redistribution of the compound was minimal (Fig. 3d). Surprisingly however, at the very high concentration of 81 400 ng/g in experiment 1 using only *L. hoffmeisteri*, redistribution occurred, although not as extensively as in the lower concentrations. This may relate to a previous observation of *L. hoffmeisteri*, where many worms initially burrowed even in highly contaminated sediments and then returned to the surface (Keilty et al., 1988b).

The upward worm mediated transport of the compound is not surprising, because most compounds with high partition coefficients are associated with the finer, organic fractions. This was observed for both 'blank' and experimental sediments (Fig. 4). A 1:1 relationship between fine organic carbon and highly sorbed toxicants was also observed by Karickhoff and Morris (1985). Worms tended to selectively ingest this component (McCall and Tevesz, 1982) because of the size limitation of their prostomium and for 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). Data imply that oligochaetes may be responsible for retaining hydrophobic compounds in the biologically active surface sediments of aquatic systems.

CONCLUSION

Single species reworking rates of *L. hoffmeisteri* measured with a gamma scan system were significantly altered by the presence of a sediment bound toxicant. At low concentrations, reworking data did not indicate stimulation effects during the first 350 hours as had been observed for *S. heringianus*. At high concentrations, reworking rates were equal to or lower than control rates in the first 350 hours, followed by reductions relative to control rates thereafter.

Mixed species reworking data suggested that the presence of *S. heringianus* enhanced the reworking rate of *L. hoffmeisteri* exposed to high sediment endrin concentrations (44 200 and 35 400 ng/g). Mixed species rates from low sediment endrin concentrations (6 300 ng/g and 16.1 ng/g) were stimulated early in experiments, presumably due to a stimulated response by *S. heringianus*.

Post experimental worm dry weights for both species were somewhat inversely related to high endrin sediment concentrations, most likely reflecting decreased feeding and/or increased stress associated with the higher sediment endrin loads. Additionally, weights from mixed species tests indicated that the presence of *S. heringianus* reduced the toxicity of endrin to *L. hoffmeisteri* at high concentrations.

Bioaccumulation of endrin by *L. hoffmeisteri* and *S. heringianus* were significant, ranging from 1.7 to 13.55 for all *L. hoffmeisteri* tests and 9.77 to 43.83 for all *S. heringianus* tests.

Relatively high post experimental surficial endrin concentrations reflected worm

mediated upward toxicant transport. *L. hoffmeisteri* consistently increased endrin in the top 2–3 cm of microcosms where significant reworking occurred.

Since oligochaetes are often the most important component of the infaunal macrobenthos of lacustrine systems, the effects of low level chronic toxicant exposure to these organisms and their role in the fate and transport of xenobiotics is important in assessing the impact of in-place sediment associated pollutants.

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