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GROWTH AND REPRODUCTION OF THE EARTHWORM *EISENIA FETIDA* EXPOSED TO SUBLETHAL CONCENTRATIONS OF ORGANIC CHEMICALS

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Summary—The determination of a short-term LC_{50} toxicity value for a chemical is a useful indicator of the potential biological effect of the chemical if released into the environment. However, it is possible that adverse effects of the chemical may occur at sublethal concentrations far below LC_{50} values. Using the earthworm *Eisenia fetida* (Savigny) as an indicator organism for soil ecosystems, concentrations of 10 organic chemicals were evaluated for sublethal effects on earthworm growth and reproduction.

Following short-term exposure to sublethal concentrations of carbaryl and dieldrin, *E. fetida* was allowed to resume growth and reproduction to determine whether such effects were permanent.

The effect on earthworm populations as a representative soil organism could be a sentinel for the effects of more destructive soil processes.

INTRODUCTION

Man's activities have resulted in increasing concentrations of potentially-toxic materials being deliberately or unintentionally distributed in soil ecosystems. Soil can no longer be considered a waste depository without consideration of effects on soil ecosystems. Earthworms, one of the largest and most easily quantified components of soil biota, play a major role in maintaining soil fertility and structure (Edwards and Lofty, 1977). The effect on earthworms as a representative soil organism could be a sentinel for the indication of more destructive soil processes.

A number of laboratories have developed earthworm toxicity tests (Davis, 1971; Stenersen, 1979; Lofs-Holmin, 1980, 1982; Goats and Edwards, 1982; Karnak and Hamelink, 1982). Most of these tests have involved measuring the LC_{50} of various chemicals, mostly pesticides. Lofs-Holmin evaluated the sublethal effects of pesticides on earthworm growth (Lofs-Holmin, 1980) and reproduction (Lofs-Holmin, 1982).

The LC_{50} of a given chemical is often not a useful indicator of the potential ecological consequences of the chemical, because reproduction may be inhibited or halted at chemical concentrations far below a given LC_{50} (Neuhauser *et al.*, 1984). Other sublethal effects on earthworms include decreased nervous system functioning (Drewes *et al.*, 1987; Callahan *et al.*, 1985). To gain an insight into the sublethal effects of organic chemicals, the effects of priority pollutants (US EPA, 1979) on earthworm growth and reproduction were examined. To further evaluate the persistence of sublethal effects of two chemicals, carbaryl and dieldrin, earthworms exposed to each chemical were subsequently allowed to grow and reproduce without further exposure to the chemicals.

METHODS AND MATERIALS

Growth and reproduction

The test components included basic organic media (horse manure), sand, the test chemical and deionized water. Glass Petri dishes, 100 by 20 mm were used as experimental containers. Industrial quartz sand (dominant fine sand with >50% of particles sized 0.05–0.2 mm) were used to provide drainage. Dry sand (30 g) was placed in the bottom of the dish. The sand was brought to 20 ± 2% moisture by adding the proper amount of water to the dish. A mixture of the horse manure and the test substrate (20 g wet wt) was placed on top of the sand. The controls contained only horse manure, sand and deionized water. The manure contained a moisture content of 75 ± 5% after the test chemical was added. If the test chemical added to the manure was not water-soluble, an organic solvent, such as acetone, was added in the same quantity to the control manure. Sequential weighings were made to insure that all of the organic solvent was evaporated before the worms were added to the manure. None of the chosen test chemicals were volatile. When the moisture content was <75% after the test chemical was added, deionized water was added to the manure to bring the moisture content up to the prescribed level. Care was exercised to insure that the test chemical was evenly distributed and mixed into the manure.

Two young worms, <10 mg and <1 week old, were added to each of five Petri dishes for each chemical concentration tested. The Petri dishes were kept at a temperature of 25°C for the duration of the test. This temperature was found to give maximum growth and reproduction for *Eisenia fetida* (Savigny) (Kaplan *et al.*, 1980). Under these circumstances

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Table 1. The mean final weights of *E. fetida* (g worm^{-1}) and total cocoon production worm^{-1} 8 weeks^{-1} for the concentration (mg kg^{-1}) of organic chemicals indicated ($N = 10$)

	Chemical concentration (mg kg^{-1})								*F* value
Carbaryl	0	25	50	100	150	200	250	500	
Weight	0.47^{a1}	0.35^{b}	0.31^{b}	0.17^{cd}	0.10^{d}	0.20^{c}	0.14^{cd}	Death	$F^{6}_{23} = 16.72^{*2}$
Cocoons	7.7^{a}	3.1^{b}	1.2^{b}	0^{b}	0^{b}	0^{b}	0^{b}		$F^{6}_{23} = 28.09^{*}$
Chloroacetamide	0	250	300	350	400	450	500	1000	
Weight	0.41^{a}	0.44^{a}	0.42^{a}	0.37^{a}	0.47^{a}	0.46^{a}	0.44^{a}	Death	$F^{6}_{27} = 1.08$
Cocoons	7.4^{a}	5.7^{a}	5.5^{a}	5.8^{a}	8.8^{a}	8.8^{a}	7.4^{a}		$F^{6}_{27} = 2.45$
1,2 *Dichloropropane*	0	34,800	46,300	57,800	69,300	80,800	92,300		
Weight	0.51^{a}	0.50^{a}	0.52^{a}	0.47^{a}	0.57^{a}	0.53^{a}	Death		$F^{5}_{17} = 2.02$
Cocoons	7.1^{a}	6.4^{a}	8.3^{a}	5.3^{a}	7.0^{a}	6.3^{a}			$F^{5}_{17} = 2.75$
Dieldrin	0	25	50	100	150	200	250	500	
Weight	0.45^{a}	0.48^{a}	0.41^{a}	0.42^{a}	0.32^{b}	0.24^{b}	0.14^{b}	Death	$F^{6}_{24} = 14.15^{*}$
Cocoons	8.4^{a}	4.6^{b}	3.5^{b}	0^{c}	0^{c}	0^{c}	0^{c}		$F^{6}_{24} = 14.85^{*}$
Dimethylphthalate	0	5900	11,800	23,600	47,200	70,800	94,400		
Weight	0.41^{a}	0.39^{a}	0.39^{a}	0.38^{a}	0.36^{a}	0.35^{a}	Death		$F^{5}_{24} = 0.49$
Cocoons	7.7^{a}	9.3^{a}	8.2^{a}	7.2^{a}	7.3^{a}	2.9^{b}			$F^{5}_{24} = 7.34^{*}$
N-Nitrosodiphenylamine	0	1400	1600	1800	2000	2200	2400		
Weight	0.52^{a}	0.57^{a}	0..39^{a}	0.46^{a}	0.44^{a}	0.39^{a}	Death		$F^{5}_{10} = 2.58$
Cocoons	8.7^{a}	5.0^{b}	1.2^{b}	2.4^{b}	3.7^{b}	1.2^{b}			$F^{5}_{10} = 4.43^{*}$
Fluorene	0	250	500	750	1000	1500			
Weight	0.57^{a}	0.49^{a}	0.51^{a}	0.47^{a}	0.43^{a}	Death			$F^{4}_{19} = 2.14$
Cocoons	7.1^{a}	6.2^{a}	6.3^{a}	3.6^{b}	4.3^{a}				$F^{4}_{19} = 4.56^{*}$
4-*Nitrophenol*	0	600	900	1200	1500	1800	2000		
Weight	0.49^{a}	0.47^{a}	0.50^{a}	0.43^{a}	0.46^{a}	0.42^{a}	Death		$F^{5}_{16} = 1.01$
Cocoons	7.4^{a}	4.5^{ab}	3.4^{bc}	1.9^{c}	2.8^{c}	0.3^{d}			$F^{5}_{16} = 3.13^{*}$
Phenol	0	2400	2900	3400	3900	4900	5900	6900	
Weight	0.50^{a}	0.47^{a}	0.48^{a}	0.53^{a}	0.53^{a}	0.54^{a}	0.56^{a}	Death	$F^{6}_{24} = 1.01$
Cocoons	8.4^{a}	10.3^{a}	9.6^{a}	8.7^{a}	6.9^{a}	6.2^{a}	9.5^{a}		$F^{6}_{24} = 1.41$
2,4,6 *Trichlorophenol*	0	100	200	300	400				
Weight	0.43^{a}	0.50^{a}	0.46^{a}	0.42^{a}	Death				$F^{3}_{13} = 2.84$
Cocoons	6.9^{a}	5.0^{ab}	3.4^{b}	4.0^{b}					$F^{3}_{13} = 3.93^{*}$

[1]Means with a common letter in the same row are not significantly different, $P < 0.05$. $N = 10$ for all points.
[2]*Indicates significant *F* value at degrees of freedom indicated, $P < 0.05$.

Table 2. The lowest concentration of test chemicals that showed a significant difference in growth or reproduction from the control *E. fetida*

Chemical	Lowest concentration of chemical (mg kg^{-1}) to show significant difference from control for: Growth	Reproduction
Carbaryl	25	25
Dieldrin	150	25
2,4,6 Trichlorophenol	Death at 400	200
Chloroacetamide	Death at 1000	NE[1]
Fluorene	Death at 1500	750
4-Nitrophenol	Death at 2000	900
N-Nitrosodiphenylamine	Death at 2400	1400
Phenol	Death at 6900	NE
1,2 Dichloropropane	Death at 92,300	NE
Dimethylphthalate	Death at 94,400	70,800

NE[1]—no effect observed at the highest non-lethal concentration.

growth and reproduction were not limited by the amount of manure (Neuhauser *et al.*, 1980). The complete exposure was for 8 weeks although worms were also evaluated for growth and reproduction at 4 and 6 weeks. At these times, cocoons were removed and the worms were re-fed new manure, to which the appropriate concentration of test substance was added. The range of concentrations tested for each chemical included one concentration that had no sublethal effect, at least four concentrations that had a sublethal effect and one concentration that had a lethal effect. Selection of these concentrations helped to optimize the possibility of separating threshold concentrations for impairment of growth and reproduction.

The final earthworm weight and mean cocoon production per worm per 8 weeks for each chemical concentration were tested for significant differences using one-way analysis of variance. When a significant F value was obtained, Duncan's new multiple range test (Steel and Torrie, 1960) was used to determine differences.

Recovery

To evaluate the possible residual effects of persistent chemicals, the worms exposed to carbaryl and dieldrin were tested for an additional 8 weeks in manure without added chemicals. The worms were evaluated for growth and reproduction at every 2 weeks. The old manure and cocoons were removed and the worms were re-fed fresh untreated manure at each observation opportunity. The final earthworm weights and mean cocoon production per worm for weeks 8–16 were tested for significant differences using one-way analysis of variance. When a significant F value was obtained, Duncan's new multiple range test (Steel and Torrie, 1960) was used to determine differences.

RESULTS AND DISCUSSION

Growth and reproduction

The final weights and total cocoon production achieved by *E. fetida* exposed to the various concentrations of test chemicals show that only carbaryl and dieldrin caused statistically-significant reductions in both growth and cocoon production at sublethal chemical concentrations, whereas five other chemicals had a significant effect on cocoon production alone (Table 1).

The highest sublethal concentrations of the other chemicals tested showed no significant weight reductions when compared to controls, although all tests were designed to evaluate both sublethal as well as lethal effects during the exposure period. Dimethylphthalate, *N*-nitrosodiphenylamine, fluorene, 4-nitrophenol and 2,4,6 trichlorophenol significantly reduced reproduction at concentrations that did not kill the worms. Chloroacetamide, 1,2 dichloropropane, and phenol caused no reduction in cocoon production or growth even at the highest concentrations tested.

Carbaryl and dieldrin have been established as neurotoxins to earthworms (Drews and Vining, 1984). These two chemicals affected earthworm growth and reproduction at the lowest concentrations of the ten chemicals tested (Table 2). Venter and Reinecke (1988) demonstrated that exposing *E. fetida* to dieldrin concentrations of 30 mg kg^{-1} decreased cocoon production when compared to controls and dieldrin concentrations of 50 mg kg^{-1} decreased the rate of clitellate formation.

Recovery

To evaluate the possibility of recovery from sublethal effects after exposure to carbaryl and dieldrin, the horse-manure test-chemical substrate was replaced with horse manure only and earthworm weight and cocoon production were monitored for an additional 8 weeks. Earthworm growth in the presence and absence of carbaryl (Fig. 1) and dieldrin (Fig. 2) show that the worms exposed to sublethal concentrations of each chemical were able to resume normal growth rates when the chemical was removed from their environment.

Growth rates for both carbaryl- and dieldrin-exposed worms generally decreased with increasing concentrations of each chemical (Table 3). Upon removal of the chemical from the worms' environment, those exposed to the highest concentrations of chemical showed the greatest recovery growth rates. At the end of the 16 weeks, the data from Figs 1 and 2 were analyzed and there was no significant difference in the final weight of the worms for any of the concentrations of both chemicals tested, including controls.

Cocoon production was severely hindered by the presence of carbaryl or dieldrin (Table 3). Removal of either chemical permitted cocoon production to begin after 8 weeks for the higher concentrations. At 25 mg carbaryl kg^{-1} and at 25, 50 and 100 mg dieldrin kg^{-1}, a similar number of cocoons were produced as in the controls. It is apparent that the worms were able to start reproduction after the inhibiting chemical was removed.

These results strongly suggest that even though the population density may not be immediately affected (i.e. increased mortality) exposure to sublethal concentrations results in reproductive changes that reduce the succeeding population density. Carbaryl or dieldrin at 50 mg kg^{-1} reduced the cocoon production by >50% which means that the populations would be significantly reduced in 2–3 generations (Table 1).

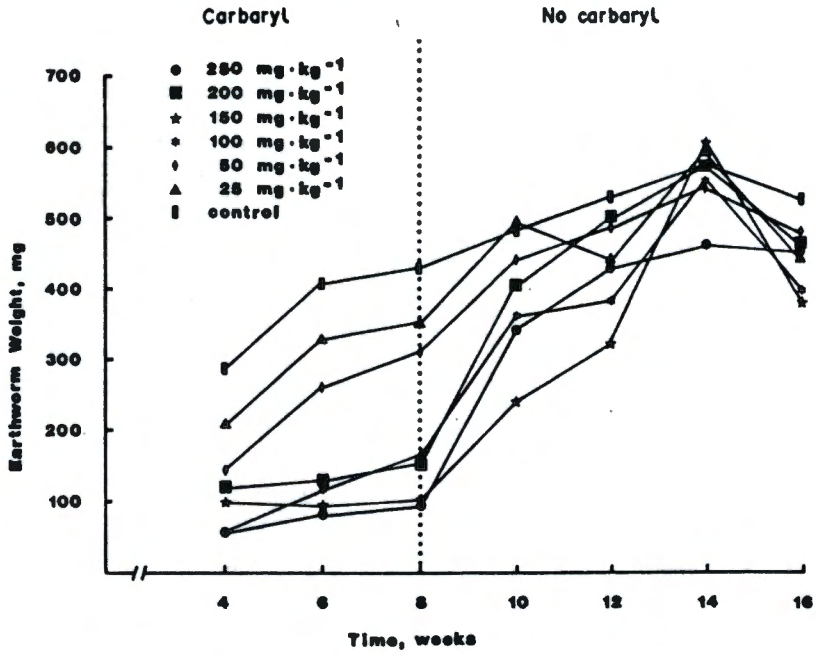

Fig. 1. The growth of *E. fetida* in the presence and absence of various concentrations of carbaryl. $N = 10$.

The sublethal effects of pesticides on earthworms can be very disruptive of important life history events such as growth and reproduction (this research) and nervous system functioning (Drewes and Vining, 1984) which could result in effects on important soil processes. Although recovery of reproduction and nervous system functioning were observed after dieldrin exposure (Drewes and Vining, 1984), more permanent damage has resulted from exposure to benomyl (Drewes *et al.*, 1987), suggesting that recovery may not occur in some cases.

Our research suggests that more consideration should be given to evaluating sublethal effects under field conditions to determine ecological implications of toxic materials. It is obvious that LC_{50} estimates should be considered a first approximation of toxic effect and that more refined tests (e.g. growth and reproduction) should be used to fully evaluate toxic substances.

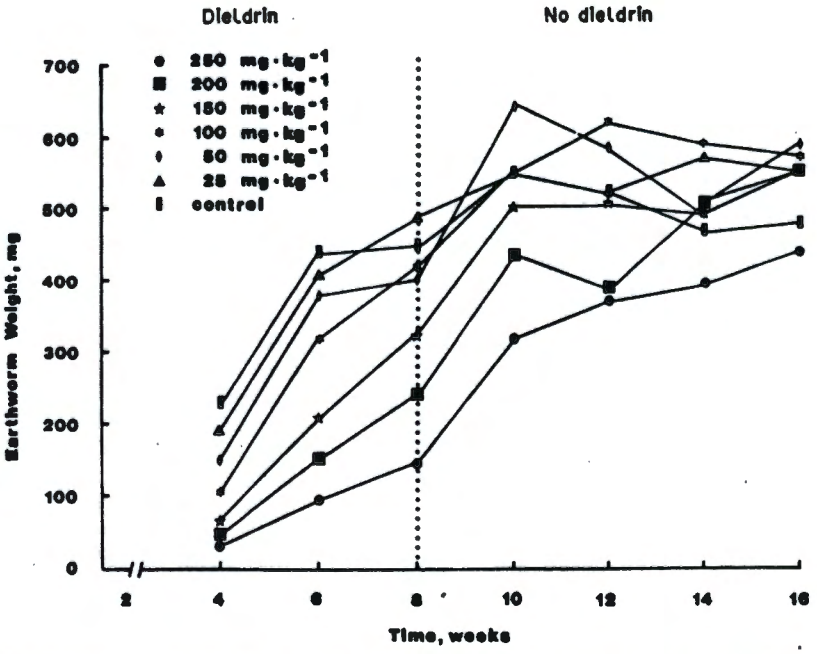

Fig. 2. The growth of *E. fetida* in the presence and absence of various concentrations of dieldrin. $N = 10$.

Table 3. Growth rates and cocoon production for *E. fetida* grown in the presence and absence of carbaryl or dieldrin ($N = 10$)

Concentration (mg kg^{-1})	Carbaryl					Dieldrin				
	Growth (mg day^{-1})		Cocoon production (cocoon's worm^{-1})			Growth (mg day^{-1})		Cocoon production (cocoon's worm^{-1})		
	0–8 weeks carbaryl	8–16 weeks no carbaryl	0–8 weeks carbaryl	0–16 weeks no carbaryl	Total	0–8 weeks dieldrin	8–16 weeks no dieldrin	0–8 weeks dieldrin	8–16 weeks no dieldrin	Total
Control	7.5[a1]	1.8[a]	7.7[a]	18.1[ab]	25.8[a]	7.9[a]	0.5[a]	8.4[a]	19.4[ab]	27.8[a]
25	6.1[b]	2.0[a]	3.1[b]	22.4[a]	25.5[a]	8.8[a]	1.4[a]	4.6[b]	24.1[a]	28.7[a]
50	5.4[b]	3.0[ab]	1.2[b]	12.7[c]	13.9[b]	7.1[a]	3.2[b]	2.1[b]	20.1[b]	22.2[b]
100	2.7[cd]	4.1[b]	0	10.0[c]	10.0[b]	7.3[a]	2.7[b]	0	23.8[a]	23.8[a]
150	1.6[d]	5.2[bc]	0	13.5[b]	13.5[b]	5.5[b]	4.3[bc]	0	14.9[bc]	14.9[b]
200	2.5[c]	5.5[c]	0	13.7[b]	13.7[b]	4.1[b]	5.7[c]	0	15.2[bc]	15.2[b]
250	1.4[d]	6.4[c]	0	9.6[c]	9.6[b]	2.3[c]	5.4[c]	0	11.6[c]	11.6[b]

[1]Means with a common letter in the same column are not significantly different, $P < 0.05$. $N = 10$ for all points.

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