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Environmental Contamination and Toxicology

Accumulation of Methylmercury in the Earthworm, *Eisenia foetida*, and its Effect on Regeneration

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The environmental contaminants mercury and methylmercury cause teratogenic, nervous, and renal damage in mammals (Venugopal and Luckey 1978). Methylmercury interferes with the normal development of tadpoles (Chang et al. 1974) and with the regeneration of fins in fish (Weis and Weis 1978) and limbs in crabs (Callahan and Weis 1983). Because mercury is highly toxic, it has been measured in a wide variety of wildlife and their foods. Earthworms provide an appropriate model for evaluating the environmental hazards of metals in soil (Ireland 1983), and they are also excellent organisms for studying the process of regeneration (Moment 1950, 1953, 1979). Two studies (Siegel et al. 1975, Helmke et al. 1979) have found that concentrations of mercury in earthworms were higher than those in the soil where they lived. This study investigates the accumulation of methylmercury in the earthworm, Eisenia foetida (Savigny), and its effect on regeneration after excision of the caudal end.

MATERIALS AND METHODS

Eisenia foetida, whose common names include the barnyard, manure, and tiger worm, was chosen as an experimental organism because its physiology and ability to regenerate segments have been well studied. Some other species of earthworms, such as Lumbricus terrestris, do not have the same ability to regenerate segments (Edwards and Lofty 1972). Although E. foetida is native to Europe and Asia, it is now widely distributed and may be found in North and South America, Africa and Australia (Reynolds 1977). It rarely inhabits agricultural soils, but it is common in compost piles, soils in barnyards, and in similar disturbed sites rich in organic matter. Most specimens had contrasting light and dark stripes across each segment that are typical of the zebra form, or the subspecies foetida. When the caudal section is excised, segments regenerate in a predictable manner (Moment 1950, 1953, 1979); if one group of earthworms is cut at a particular segment and a second group is cut at a point 10 segments to the posterior, then the second group will regenerate, on the average, 10 fewer segments. The number of regenerated segments is independent of the earthworm's size, state of sexual development, and number of segments. The proliferation of new segments is

visible in histological sections about three weeks after excision and three weeks later the earthworms have grown to their normal lengths.

Methylmercury chloride was dissolved in water and added to petri dishes containing 30 g of commercial "Baccto" potting soil (60% water content) to yield the following treatments of methylmercury (wet wt): controls (A) - 19 dishes; 1 ppm (B) - 20 dishes; 5 ppm (C,D,E) - 60 dishes; 25 ppm (F) - 25 dishes; 125 ppm (G) - 20 dishes. Two earthworms were added to each petri dish and kept at 25°C. All earthworms were adult size and most had a well developed clitellum. Previous tests have shown that regeneration is similar in clitellate and aclitellate earthworms. After six weeks survivors were anesthetized in 0.2% aq. chloretone (Chlorobutenol) and cut under a dissecting microscope between the 50th and 51st segments. The posterior ends were discarded. Earthworms from treatments A, B, and C were returned to the same soils, earthworms from treatment D were placed in control "Baccto" potting soil, and earthworms from treatment E were placed in a "barnyard" soil taken from the site where the earthworms were collected.

After an additional six weeks, earthworms were anesthetized and the number of regenerated segments counted. A representative sample of earthworms from the control and 5 ppm groups were fixed in Bouin's solution, sectioned, stained with eosin and hematoxylin, and examined under an oil immmersion lens of a light microscope. The rest of the earthworms were kept for three days on moist tissue paper and then cut into pieces 1 - 2 cm long. Soil remaining in their intestines was flushed out with distilled water from a syringe with a hypodermic needle. The pieces were frozen until they were chemically analyzed. Earthworms from treatments C, D, and E were pooled to give an adequate sample size.

Samples of the soil from the petri dishes and of the earthworms were digested (Monk 1961), and then total mercury was determined by cold vapor atomic absorption (Hatch and Ott 1968) with a Coleman model MAS-50 mercury analyzer. Recoveries averaged 90% and the lower limit of reportable concentrations was 0.02 ppm.

The numbers of segments regenerated by earthworms from the different treatment groups were compared statistically. After determining that the data were normally distributed and that the variances were homogeneous, we analyzed them with an F-test and separated means with the use of Bonferroni's test.

RESULTS AND DISCUSSION

Earthworms treated with 25 ppm or 125 ppm methylmercury did not survive the length of the study. Survival rates after 12 weeks were high in the three other groups (97 % in control group, 92 % in 1 ppm group, and 79 % in 5 ppm groups). All surviving earthworms in the control and 1 ppm groups regenerated, but 29 %

Table 1. Regeneration of segments in Eisenia foetida after exposure to methylmercury and being cut between segments 50 and 51

Methylmercury concn in soil	No. worms *	No. segments regenerated ** mean	SD	SE
A Control (12 wk)	37	37.7a	7.5	1.2
B 1 ppm (12 wk)	37	33.6a	7.0	1.1
C 5 ppm (12 wk)	25	11.8b	6.3	1.3
D 5 ppm (6 wk), control (6 wk)	25	19.6c	8.4	1.7
E 5 ppm (6 wk), barnyard soil (6 wk)	17	22.3c	11.9	2.9

* Includes only earthworms with at least 52 segments.
** Means not sharing the same letter differ significantly from each other by Bonferroni's test ($p < 0.05$).

of the earthworms in the 5 ppm groups either healed without regenerating or autotomized their tail end, leaving 50 or fewer segments.

Regeneration of segments varied among treatment groups (F-test, $p < 0.05$). Earthworms kept in the 5 ppm soil for either 6 (D,E) or 12 (C) weeks regenerated significantly fewer segments than did the earthworms kept in the control (A) or the 1 ppm (B) soil (Table 1). The earthworms that were moved from the 5 ppm soil to uncontaminated soil (D,E) regenerated significantly more segments than did the earthworms that were kept in the 5 ppm soil (C), but the kind of soil used (D vs. E) had no effect. Although the earthworms exposed to 1 ppm (B) regenerated fewer segments than did earthworms from the control group (A), the difference was not significant at the 0.05 level ($0.1 > p > 0.05$).

Examinations of histological sections did not reveal any differences from controls in either the newly regenerated or old tissue of the muscles (which make up the major volume of the body wall), epithelium, gut, chlorogogue, or cells of the neural ganglia, where changes might have been expected.

It is not known how methylmercury interferes with regeneration in E. foetida. In rats, methylmercury inhibited regeneration of liver tissue after partial hepatectomy by interfering with DNA synthesis (Chen and Mottet 1980). However, the neurotoxic effects of methylmercury (Chang 1977) might inhibit regeneration. Massaro and Schrank (1959) showed that cholinesterase inhibitors, lithium (a nerve inhibitor), barbituric acid (inhibitor of RNA synthesis) and iodoacetic acid (glycolysis inhibitor) also inhibit regeneration in E. foetida. Carcinogens have also been shown to interfere with normal regeneration in E. foetida (Andrews 1974).

Mercury concentrations in uncontaminated soil are generally lower than the concentration in the control soil used in this study, and much lower than the concentrations in the treated soils. Soils from the eastern United States had mean concentrations of 0.05 to 0.10 ppm Hg (Wiersma and Tai, 1974). In a polluted environment, however, near a chlor-alkali plant, the soil contained 3.8 ppm Hg (Bull et al., 1977). Although it is possible that soils in very contaminated sites might have mercury concentrations that would be lethal to earthworms, this is probably not a serious environmental concern. Survival of earthworms exposed to 5 ppm and then cut was 79 %. If there is a hazard from soil contaminated with methylmercury it is probably a hazard to predators of earthworms rather than to the earthworms themselves. Table 2 shows the concentrations detected in the soils and earthworms.

Table 2. Concentrations of mercury in composite samples of Eisenia foetida and in soils treated with methylmercury

Methylmercury treatment	Hg in soil ppm (wet wt)*	Hg in worms ppm (wet wt)
A Control	0.63	13
B 1 ppm	1.3	27
C,D,E 5 ppm	3.8	85

*Concentrations adjusted to a water content of 60%.

The ratios of the mercury concentration in the earthworms to that in the soils were similar for the three treatments, ranging from 21 to 22. This study demonstrates that under laboratory conditions earthworms may accumulate concentrations of mercury that would be considered hazardous to wildlife eating earthworms. As little as 3 ppm methylmercury in feed interfered with reproduction of mallard ducks (Heinz, 1974) and produced lesions in the nervous tissues of their ducklings (Heinz and Locke, 1976). However, uptake of mercury or methylmercury may be different under field conditions and the hazards cannot be evaluated until mercury concentrations have been measured in earthworms collected from a variety of sites. There are only a few published reports on mercury concentrations in earthworms; concentrations ranging from 0.50 ppm to 0.66 ppm Hg (dry wt) were reported in earthworms living in soil containing less than 0.02 ppm Hg (Helmke et al. 1979), 0.39 ppm Hg (dry wt) in earthworms from soil containing 0.014 Hg (dry wt, available fraction; Siegel et al., 1975) and 1.3 ppm Hg (wet wt) in earthworms from a polluted soil near a chlor-alkali plant that contained 3.8 ppm Hg (dry wt; Bull et al., 1977).

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