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Accumulation of Chlorinated Benzenes in Earthworms

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Chlorinated benzenes are widespread in the environment. Hexachlorobenzene, pentachlorobenzene and all isomers of dichlorobenzenes, trichlorobenzenes, and tetrachlorobenzenes, have been detected in fish, water, and sediments from the Great Lakes (Oliver and Nicol, 1982). They probably entered the water as leachates from chemical waste dumps and as effluents from manufacturing (Oliver & Nicol, 1982). Hexachlorobenzene and pentachlorobenzene are commonly present in Herring gull (Larus argentatus) eggs from the Great Lakes, and some of the isomers of trichlorobenzene and tetrachlorobenzene are occasionally detected at low concentrations (Hallett and Norstrom, 1982). Hexachlorobenzene, which was formerly used as a fungicide, has been the most thoroughly studied chlorinated benzene, and has been detected in many species (Courtney, 1979; Dobson & Howe, 1986; Menzie, 1986). Its use as a fungicide in the United States was canceled in 1984. Since about 1975 hexachlorobenzene has been formed mainly in the production of chlorinated solvents. (Menzie, 1986). It is highly persistent in the environment (Courtney, 1979) and some species are poisoned by hexachlorobenzene at very low chronic dietary exposures. As little as 1 ppm in the diet of mink (Mustela vison) reduced the birth weights of young (Bleavins et al., 1984) and 5 ppm in the diet of Japanese quail (Coturnix coturnix japonica) caused slight liver damage (Vos et al., 1971).

The chlorinated benzenes are relatively simple molecules, and their chemical properties may be understood in relation to their molecular structure. As the number of chlorine atoms benzene ring increases, the vapor pressure and solubility decrease, and the boiling point and octanol water partition coefficient increase (Mackay et al., 1992). Several investigators (Könemann, 1979; Lord et al., 1980 Oliver, 1987) have noted that aquatic bioconcentration factors of chlorinated benzenes increase with the octanol water partition coefficients.

(Van Gestel et al. (1991) showed that increased chlorination of benzenes was correlated with increased octanol/water partition coefficients, increased adsorption to organic matter, and increased toxicity to earthworms. Bioaccumulation factors have also been shown to increase with the degree of chlorination of chlorinated benzenes in rainbow trout (Salmo gairdneri, Oliver & Niimi, 1983), guppies (Poecilia reticulata, Könemann, 1979; Opperhuizen et al., 1988), and aquatic worms (Tubifex tubifex, Limnodrilus hoffmeisteri, Oliver, 1987). Opperhuizen et al. (1988) also concluded that bioaccumulation increased with chlorination, but they suggested that the octanol water partition approach is misleading for thermodynamic reasons.

Two previous studies on chlorinated benzenes in earthworms have emphasized kinetics and partitioning. Lord et al. (1980) included hexachlorobenzene and 1,2,3,5-tetrachlorobenzene in their study of the mechanisms of pesticide uptake by earthworms. Belfroid et al. (1994) developed a biphasic model of elimination of chlorinated benzenes and a toxicokinetic model relating concentrations in earthworm lipids to concentrations predicted in interstitial soil water. My study was designed to help evaluate long-term hazards to terrestrial wildlife from contaminated soil. Lumbricus terrestris, an important agricultural species, was analyzed whole (ingested soil included) after long exposure to the test medium recommended by the European Economic Community (1985) for testing toxicity of pesticides to earthworms; Monochlobenzene and the dichlorobenzene were not included in the study, because they were thought to be the least likely of the chlorinated benzenes to accumulate in earthworms.

This paper describes a long-term (26 week) experiment relating the concentrations of chlorinated benzenes in earthworms to 1) the length of exposure, and it describes three 8-week experiments relating concentrations of chlorinated benzenes in earthworms to 2) their concentration in soil 3) the soil organic matter content and, 4) the degree of chlorination.

MATERIALS AND METHODS

The test medium (European Economic Community, 1985) comprised 69.7% sand, 20% clay (pulverized kaolin), 10% sphagnum peat, and 0.3 % $CaCO_3$ (enough to adjust the pH to between 6 and 7). Chlorinated benzenes were purchased from the Aldrich Chemical Company, Inc. (Milwaukee, WI) and had minimum purities of 97% to 99%. Soil media were treated with either hexachlorobenzene or 1,2,4-trichlorobenzene in the 26-week long-term accumulation experiment (10 ppm). Thirteen replicates (plastic bucket containing soil and earthworms) were prepared for each chemical, to be terminated at two-week intervals. Soils were treated with either hexachlorobenzene or 1,2,4-trichlorobenzene in the second experiment, relating concentrations in earthworms to nine concentrations in soil (0 - 100 ppm, one replicate per concentration). Soils were treated with 10 ppm hexachlorobenzene in the third experiment, and the sphagnum peat content was varied from 0 to 32% (eight peat contents, one replicate per peat content). In the fourth experiment, soil was treated with one of each of the isomers of the chlorinated benzenes (trichlorobenzene through hexachlorobenzene) at 10 ppm, four replicates per chemical. In all experiments chlorinated benzenes were first dissolved in ethyl ether and mixed into the sphagnum peat, which was then mixed with the other ingredients. The moisture content of the soils containing 10% sphagnum peat was adjusted to 30% of the weight of the dry ingredients. To achieve a similar pH and moisture availability in media containing different amounts of sphagnum peat, I adjusted the amount of $CaCO_3$ and water accordingly. Three kilograms of medium were placed in each plastic bucket, covered with nylon mesh to allow aeration. Soil moisture was regulated by adding enough tap water to maintain the initial weight of the buckets. Earthworms were moved to fresh

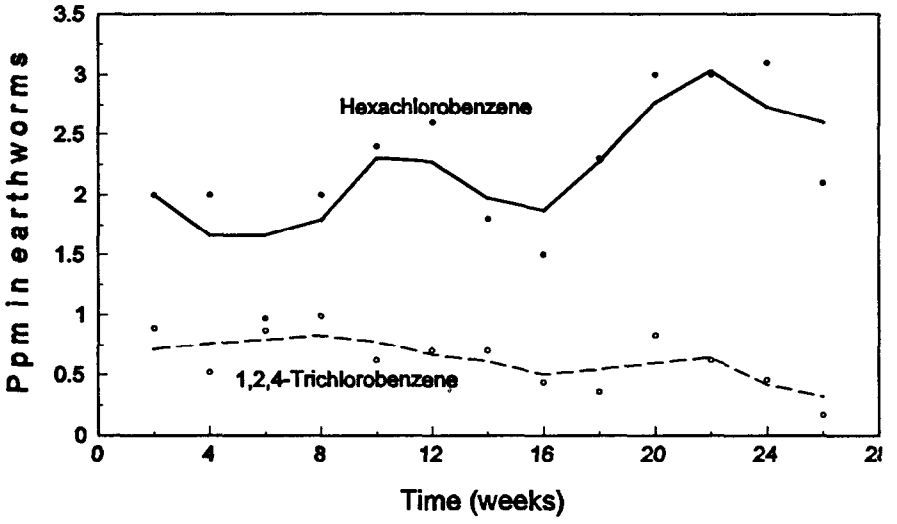

Figure 1. Concentrations (wet weight) of hexachlorobenzene and 1,2,4-trichlorobenzene in *Lumbricus terrestris* in artificial soil. The lines are drawn through 3-point (preceding point, point, following point) running averages .

soil medium at 8-week intervals in the long-term accumulation study, and were kept in the same medium throughout the 8 weeks of the other studies. Clitellate Lumbricus terrestris were purchased commercially, and acclimated to the experimental medium. Earthworms were rinsed in distilled water, blotted dry, and weighed. They weighed an average of 4 g each. Ten earthworms were put into each of the buckets, which were kept in a lighted chamber at 10°C. Earthworms were weighed and counted when the buckets were terminated. In the long-term study the buckets were terminated one at a time at two-week intervals and in the three other studies all buckets were terminated at eight weeks. Earthworms were rinsed and kept frozen until analyzed.

All earthworms in a bucket were cut into pieces and a sample of 5 g was added to a glass centrifuge tube containing 20 ml of extraction solvent (94% hexane-6% ethyl ether, by volume). The sample was extracted using a polytron, then centrifuged, and the supernatant saved. The extraction was repeated twice using 10 mL of the extraction solvent. The supernatants were combined and then cleaned up by florisil column chromatography, eluting with 200 mL of the extraction solvent. The volume was reduced to between 2 and 10 mL by rotary evaporation, and brought to a final volume of 10 mL with hexane. I quantified the chlorinated benzenes by electron capture gas chromatography using a Hewlett-Packard 5840 Gas Chromatograph (Column - J & W Scientific DB-17, 30 m, 0.25 mm id; Injection volume - 2 µl; Injection temp - 200°C; Detector temp - 325°C; carrier gas - helium [1.5 mL/m]; make-up gas - argon [95%] and methane [5%][40 mL/m]; Temperature program - 75°C for 1 m, ramp at 8°C/m to 200°C, 200°C for 10 m). To separate the peaks of the tetrachlorobenzene isomers I interrupted the temperature ramp at 111 °C for 14.5 m, before increasing the temperature 20°C per m to 200°C.

The method detection limits (U.S. Environmental Protection Agency, 1982) for the eight chlorinated benzenes were from 0.013 µg hexachlorobenzene/g of earthworm tissue to 0.20 µg 1,3,5-trichlorobenzene/g of earthworm tissue. Recoveries from spiked samples averaged from 91 to 99%. The average relative percent difference between samples analyzed in duplicate was 14%.

RESULTS AND DISCUSSION

Earthworm survival rates were high in the three 8-week experiments (97% in the 2nd, 99% in the 3rd, and 100% in the 4th experiment). Average weight losses per worm were 11% in the 2nd, 10% in the 3rd, and 9% in the 4th experiment. If the chemicals bad had an effect on weight loss or mortality, we would have expected the effects to be greatest in earthworms exposed to the highest concentrations of chlorinated benzenes. However, this was not the case - three earthworms died and weight loss was 11% at the three highest concentraytions (60, 80, 100 ppm) and two earthworms died and weight loss was 12% at the three lowest concentrations. In contrast, the addition of sphagnum peat affected weight loss. Weight loss was 22% at 0% peat, 16% at 0.5% peat, 9% at 1.0% peat, and lower than 7% at each of the five treatments containing more than 1% peat.

Mortality was 5.4% in both the hexachlorobenzene and trichlorobenzene long-term experiments. Weight changes in earthworms exposed to hexachlorobenzene were similar to those in earthworms exposed to trichlorobenzene. Earthworms from buckets terminated after 14 weeks had lost about 22% of their initial weights, and earthworms from buckets terminated after 26 weeks had lost about 49% of their initial weights. Although the artificial soil mixture was suitable for maintaining the earthworms at about 90% of their initial weight for 8 weeks, the large weight loss (49%) by the end of 26 weeks suggests the earthworms were unhealthy by the end of the long-term study. The average moisture content of the earthworms was 75%. The average acid-insoluble ash weight divided by the wet weight was 11%. This means that about 10% of the wet weight of earthworms was soil.

In the 26-week experiment, the concentration of 1,2,4 - trichlorobenzene in earthworms fluctuated only slightly about a mean of 0.63 ppm (Fig. 1). Although a statistically significant decrease can be demonstrated over the test (Pearson correlation coefficient, $r = -0.62$ $p < 0.05$), the decrease was minor. Hexachlorobenzene in earthworms snowed a cyclical trend that coincided with replacement of the media, and a slight but statistically significant tendency to increase from about 2 to 3 ppm over the 26 weeks ($r = 0.55$, $p < 0.05$).

Concentrations of chlorinated benzenes in Eisenia andrei were reported to reach equilibria with artificial soils within seven days (Belfroid et al., 1994). In aquatic worms (Tubifex tubifex and Limnodrilus hoffmeister) the half-life of hexachlorobenzene was 27 days, whereas half-lives of the dichlorobenzenes through pentachlorobenzene were less than 5 days (Oliver, 1987). Hexachlorobenzenes reached equilibrium in rainbow trout (Salmo gairdneri) (Oliver and Niimi, 1983) in a minimum

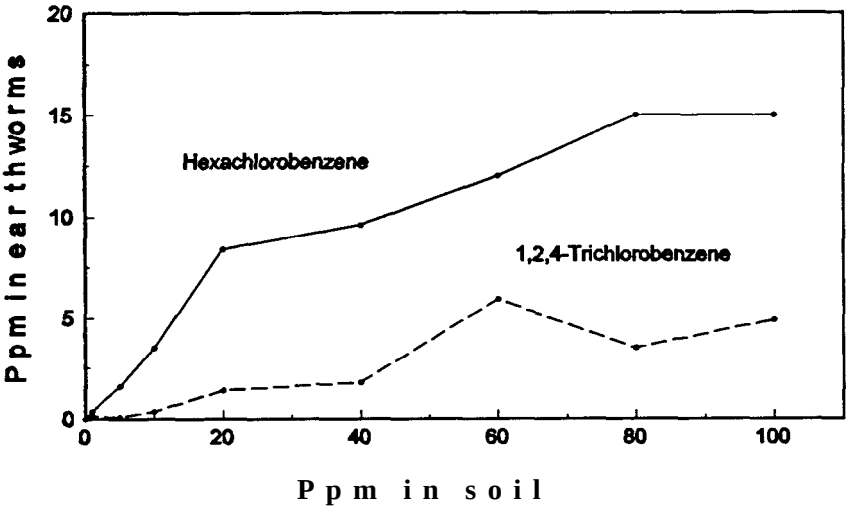

Figure 2. Concentrations (wet weight) of hexachlorobenzene and 1,2,4-trichlorobenzene in *Lumbricus terrestris* exposed for 8 weeks.

of 119 days. In a study by Lord et al. (1980), concentrations of hexachlorobenzene in earthworms increased slightly over 8 weeks, although the authors did not state whether they considered the increase significant. In our study, the concentrations measured at 2 weeks (2.0 ppm), 8 weeks (2.0 ppm) or the average for the 26 weeks (2.2 ppm) were similar.

Concentrations of both trichlorobenzene and hexachlorobenzene in earthworms increased as the concentrations in the soil increased (Fig. 2). The most surprising result of this study was the relatively low concentrations in earthworms compared to those in soils. The average concentration of each of the six isomers of trichlorobenzene and tetrachlorobenzene in earthworms was only about 1 ppm (Table 1); the similarity of the means suggests that isomeric structure did not affect accumulation. The low concentrations of chlorinated benzenes detected in earthworms (Table 1) limit the conclusions that might otherwise have been drawn from the data. Hexachlorobenzene was detected at the highest concentrations in earthworms, followed by pentachlorobenzene, as would have been expected from the octanol water partition coefficients (Table 1).

The concentration of organic matter in soil had a prominent effect on hexachlorobenzene concentrations in earthworms (Fig. 3). Hexachlorobenzene concentrations decreased steadily from 9.3 ppm in earthworms kept in soil without any peat moss added to about 1 ppm in soil containing 16 or 32% organic matter.

The concentration factors of chlorinated benzenes (0.088 - 0.27) calculated from Table 1 are low compared to the concentration factors of organochlorines reported in other studies. Wheatley and Hardman (1968) for example, concluded from 90 paired values of field data on organochlorines that when soil concentrations were about 10 ppm the concentration factor was about 1. Two features of the experimental design of this study contributed to the low concentration factors.

Table 1. Concentrations of trichlorobenzene (TCB), tetrachlorobenzene (TeCB), pentachlorobenzene and hexachlorobenzene in earthworms exposed for 8 weeks to soils containing 10 ppm of these chemicals.

Chemical	Concn in earthworms (ppm wet wt)		OW partition coefficient. [1]
	Mean	Standard deviation	
1,3,5- TCB	0.88	0.12	4.2
1,2,4-TCB	0.90	0.18	4.0
1,2,3-TCB	1.1	0.14	4.1
1,2,3,5-TeCB	0.72	0.13	4.68
1,2,4,5-TeCB	0.8	0.20	4.5
1,2,3,4-TeCB	0.78	0.30	4.5
Pentachlorobenzene	1.7	1.0	4.9
Hexachlorobenzene	2.7	0.78	5.5

[1] The octanol water partition coefficient for 1,2,3,5-TeCB was from Lord et al. (1980), and the others were from Oliver (1987)

The first of these was the choice of a species that lives in permanent burrows for a long-term study. Lord et al. (198)) suggested that earthworms such as L. terrestris remain in contact only with the soil lining its burrow, and that as a chemical is absorbed by the earthworm the concentration in the soil decreases. They showed that stirring laboratory soil treated with dieldrin increased dieldrin concentrations in earthworms. As shown in Fig. 1, concentrations of hexachlorobenzene increased for a few weeks when earthworms were moved to containers of fresh soil after 8 and 16 weeks, and then decreased. The fresh soil had been treated with hexachlorobenzene at the same time as the old soil, but because it did not contain burrows, I suspect the earthworms came into greater contact with the hexachlorobenzene.

The high organic matter content of the soil media used in this study also reduced the concentration factors. Hexachlorobenzene concentration factors were about 1 (0.75 to 0.94) in the four media containing from 0% to 2% sphagnum peat, which is closer to what has been reported previously. Lord et al. (1980) found a concentration factor for hexachlorobenzene of about 2 or 3 for Lumbricus terrestris in a soil with an organic matter content of 2.6%.

Davis (1971) demonstrated that earthworms accumulate DDT and dieldrin most efficiently from soils low in organic matter. Within the range of 2 to 16% sphagnum peat in my study, the concentration factor was inversely proportional to the per cent sphagnum peat. The highest concentration of hexachlorobenzene was detected in earthworms from soil containing no sphagnum peat; presumably these earthworms ingested little of this soil and the hexachlorobenzene was absorbed by the earthworms mainly through their skin. Lord et al. (1980) suggested a theoretical means of predicting the accumulation of chemicals by earthworms from soil; for most

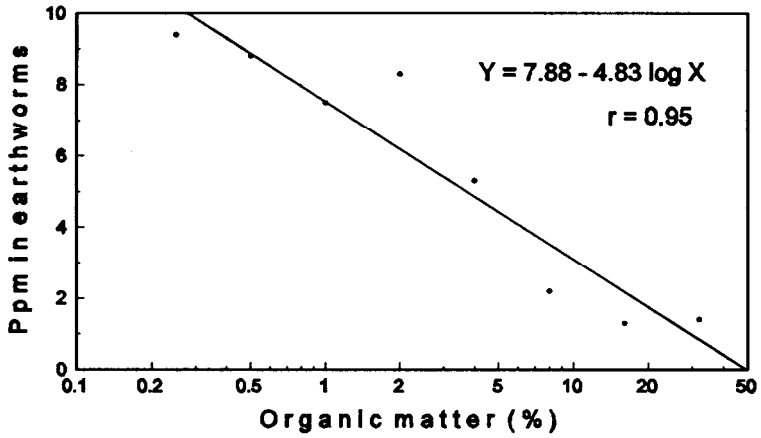

Figure 3. Concentrations (wet weight) of hexachlorobenzene in *Lumbricus terrestris* exposed to 10 ppm in soils containing from 0 to 32% sphagnum peat. (0% peat is illustrated as 0.25% on this log scale.)

chemicals other than relatively polar ones, a decrease in the soil organic matter content from 10% to 1% should be accompanied by a ten-fold increase in concentrations in earthworms. This was explained by partitioning of chemicals among earthworm tissues, soil water, and soil organic matter. In this study, hexachlorobenzene concentrations in earthworms increased six-fold as the percent sphagnum peat content decreased from 16% to 2%. Van Gestel and Dis (1988) showed that toxicity of chemicals to earthworms decreased with increased organic matter content.

The soil medium recommended by the European Economic Community has a relatively high content (10%) of peat compared to the organic matter content in most agricultural soils. Consequently, the concentration factors observed in the artificial soil are probably lower than would be generally observed under field conditions. The dependence of organochlorine accumulation on soil organic matter means that movement of organochlorines into terrestrial food chains at contaminated sites could be reduced by amending the soil with organic matter. This is practical mainly in soils where the organic matter content is very low.

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