Accumulation of Toxaphene in Earthworms Exposed to Fortified Soil

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Introduction

Prior to its ban by the US EPA in 1982, followed by other countries, more than 1 million tons of toxaphene had been used globally (1). Commercial toxaphene is a complex mixture of more than 600 chlorobornanes and related compounds (2). It replaced DDT after DDT was banned, and was used on cotton, soybeans, peanuts and other crops to kill insects. Toxaphene is acutely and chronically toxic to aquatic organisms and to wildlife, bioaccumulates, and is a major contaminant in environmental biological samples (2). Its occurrence has been extensively documented and it can be found in specimens from all trophic levels (2). Nevertheless, little is known of its actual uptake and potential bioaccumulation by organisms from contaminated soil. In this study we report the uptake of toxaphene in earthworms.

Materials and Methods

Soil and Test Organisms
Soil was prepared by Pelagia AB, Sweden, according to OECD's guidelines for testing of chemicals. The soil contained 10% peat, 70% sand and 20% kaolin clay, based on weight (3) ("the test soil"). An aliquot corresponding to 0.1 µg toxaphene/g test soil, wet weight (ww), was dissolved in acetone and added to 37 g of sand per kg of test soil. The acetone was gently evaporated, the sand was then mixed with the test soil and left to equilibrate for 10 days. Adult earthworms (*Eisenia foetida*) with clitellum weighing 0.33 to 0.87 g were used throughout the study.

Toxicity Screening
The toxicity of toxaphene was screened by exposing earthworms to a commercial toxaphene (Ultra Scientific, N. Kingstown, RI, USA) on filter paper for two days. This screening established the maximum concentration that could be used in the test soil to which the earthworms would be exposed. Prior to any exposure to toxaphene, all worms were kept on wet filter paper for 5 hours (4) to allow them to clear their guts. Nine worms were exposed to concentrations of 0, 3.7, 19, 37, 190, or 3700 ng/cm^2. All worms survived exposure at these levels, but the worms exposed to the highest concentration became rubber-like and dense.

Worm Exposure Protocol

The worms were placed in a two litre glass bottle containing one kg of the fortified test soil (ww), and plugged with a polyurethane foam plug (PUF). Worms (n= 5, pooled), fortified test soil (20-28g ww) and one PUF were analyzed prior to exposure, after one week of exposure, and after four weeks of exposure. Prior to extraction, all worms were kept on wet filter paper for 24 hours to allow them to clear their guts.

Extraction and Clean-up

One hundred nanogram of an internal standard, $^{13}C_{12}$-labelled PCB#180 (2,2′,3,4,4′,5,5′), was added to the samples prior to extraction. The worms were homogenized with anhydrous sodium sulfate and were extracted using 100 mL of acetone/*n*-hexane (5/2, v/v) and 100 mL *n*-hexane/ diethyl ether (9/1, v/v), and the lipid were weighed after complete solvent removal. The PUF and 20-28 g (ww) of test soil material were Soxhlet-Dean-Stark extracted for 24 hours with toluene. All samples were then cleaned up using deactivated (1.2% water, w/w) Florisil columns from which the analytes were eluted into three fractions (5). The first fraction was eluted with *n*-hexane; the second with *n*-hexane/ methylene chloride (85/15, v/v); and, the third with *n*-hexane/methylene chloride (1/1, v/v). The elution windows on Florisil were confirmed by GC-ECD analyses. The first two fractions were combined, toluene was added, and the solvent volume was reduced to 100 μL by evaporation, prior to analysis by GC-MS.

GC-MS analysis

One to two microliter aliquots of the extracts were on-column injected into a 25 m SE-54 gas chromatography column and analyzed for toxaphene components using a VG Tribrid mass spectrometer operated under electron-capture negative ion chemical ionization (ECNI; argon buffer gas) conditions. Selected ion monitoring (SIM) was used for homologue analyses, using the M-Cl fragment ions at m/z 307, 341, 375, 409 and 443, for the hexa- through decachloro-bornanes, respectively (6). Additionally, the M^- ions for the hexachlorobornanes and $^{13}C_{12}$-PCB#180 were monitored at m/z 342 and m/z 404 and 406, respectively. A detailed description of the instrumental conditions and identification procedures are described elsewhere (7). The quantification was performed using a five component standard solution (Ehrenstorfer, Augsburg, Germany). The response factors of the chlorobornanes P32 (a hepta-), P26 (an octa-), P50 (a nona-), and P69 (a decachlorobornane) were used for all isomers in their respective homologue groups. Because this standard mixture lacks hexachlorobornanes, the response factor of these compounds was extrapolated from the response of the hepta- through nonachloro congeners, using a semi log plot of the responses versus the number of chlorine atoms.

Results and Discussion

Like the toxicity screening, the bodies of the worms became rubber-like and dense following the exposure to the fortified test soil treated with toxaphene. The chlorobornane pattern in the fortified test soil remained unchanged during the four week period and was the same as that of the technical mixture. The chlorobornane concentrations in the fortified test soil also remained unchanged during the experiment. The PUFs contained less than 1/10 000 th of the toxaphene in the test soil, predominantly lower chlorinated homologues, thus indicating negligible volatilization of toxaphene from the test soil.

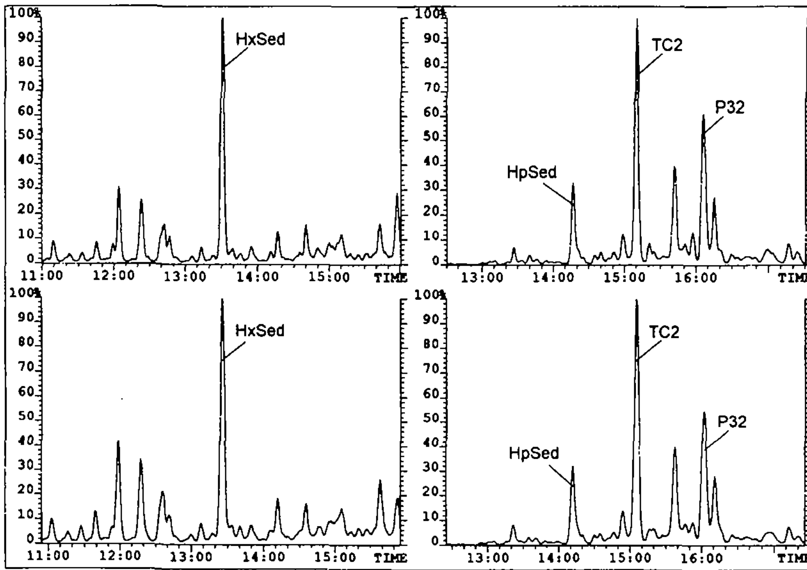

Figure 1: ECNI SIM chromatograms (m/z 307, left-side panels; m/z 343, right-side panels) showing elution of hexa- and heptachlorobornanes from the test soil (upper panels) and from *Eisenia foetida* after 4 weeks of exposure (lower panels).

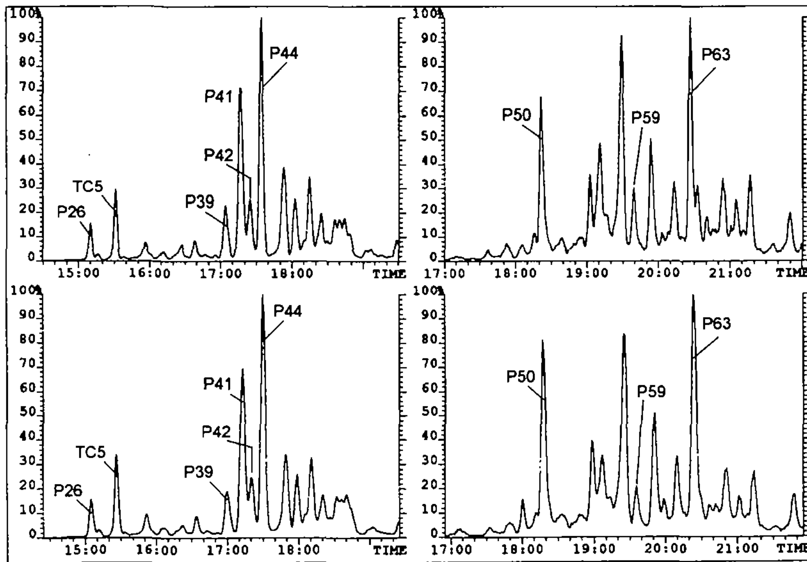

Figure 2: ECNI SIM chromatograms (m/z 377, left-side panels; m/z 413, right-side panels) showing elution of octa- and nonachlorobornanes from the test soil (upper panels) and from *Eisenia foetida* after four weeks of exposure (lower panels).

Table 1. Chlorobornane concentrations in worms (ng/g lipid) after 0, 7 and 28 days of exposure; modelled C_{max} concentrations; first-order constants (k, d^{-1}); and, bioaccumulation factors (BAF).

	Hexa	Hepta	Octa	Nona	Deca
Worms (Day 0)	<2	<2	<2	<2	<2
Worms (Day 7)	3200	5800	2700	310	60
Worms (Day 28)	4000	8800	4300	510	90
C_{max} (worm)	4320	8887	4270	496	91
k	0.222	0.150	0.132	0.128	0.153
BAF	20	23	20	13	8

The results of the worm analyses indicate the absence of toxaphene in the unexposed worms, and the rapid uptake by the worms of toxaphene from the fortified test soil. The concentrations in the worms increased with time of exposure, Table 1. In Figures 1 and 2, we compare chromatograms from worm and soil after four weeks of exposure.

It is reasonable to assume that the chlorobornane concentrations increase steadily up to a certain maximum concentration (C_{max}), the equilibrium concentration after infinite time. This concentration is a function of the bioaccumulation factor of individual chlorobornanes. The chlorobornane concentrations in the worms were fitted to a model assuming an exponential increase (first-order kinetic) according to the equation

$$C(t) = C_{max} \times (1 - e^{-kt})$$

where C(t) represents the concentrations for the hexa- through decachloro congeners measured after 0, 7 and 28 days, and k represents the rate (d^{-1}) constant for this increase. Curves were fitted to the data using a least-square function.

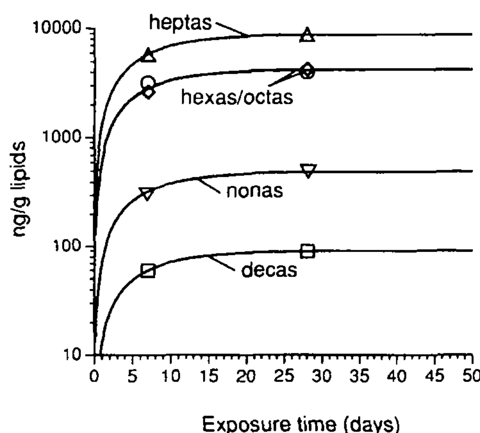

Figure 3. Accumulation of chlorobornanes in earthworms exposed to fortified test soil.

The values for k ranged from 0.128 d^{-1} to 0.227 d^{-1} (see Table 1), with an average of 0.154 d^{-1}. The maximum concentrations (C_{max}) calculated for the worms were 4320 (hexa), 8887 (hepta), 4270 (octa), 496 (nona), and 91 (deca) ng/g lipid. The model suggests that the worms reach 95 % of the estimated final concentrations within 28 days. The time to reach 50% of C_{max} is 4.5 days, based on the average rate constant of 0.154 d^{-1} indicated by the data.

As shown by the chromatograms, Figures 1 and 2, there are no significant differences in the isomer profiles in the worms and those in the test soil. However, the homologue pattern changed during the experiment. The bioaccumulation factor (BAF) is defined as BAF= C_{max}/C_{soil}, where C_{soil} are the test soil concentrations expressed in ng/g organic matter calculated from loss of ignition data. The BAFs range from 8 to 23 based on lipid in worm and organic matter in the test soil, which is very similar to the range found for PCBs, viz. 7 (hepta-) to 22 (tetrachloro-biphenyls) (8).

The worms accumulated the chlorobornanes from the fortified test soil treated with toxaphene. The model used to evaluated the data suggests that the worms reach steady state within a few weeks. Further, the worms appear to lack a capacity to metabolize the compounds because the chlorobornane pattern in the worms is similar to the chlorobornane pattern in the fortified test soil. The chlorobornane pattern in the fortified test soil was conserved throughout the study and no obvious degradation was seen for these compounds.

Acknowledgement

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