Bioconcentration of Toxaphene by Microorganisms

Doris F. Paris, David L. Lewis, and John T. Barnett

Environmental Research Laboratory
U.S. Environmental Protection Agency
Athens, Ga. 30601

Toxaphene, an organochlorine insecticide, has been shown by HOLMSTEAD et al. (1974) to be a mixture of about 177 polychlorinated 10-carbon compounds. The components were identified as polychlorobornanes, polychlorbornenes, and polychlorotricyclenes with 6 to 10 chlorine atoms per component. Field studies have shown toxaphene to be persistent - remaining in lakes for several years after its application in fish eradication programs (TERRIERE et al., 1966; JOHNSON et al., 1966).

Because of its persistence, toxaphene may be concentrated in the biota in environmental waters. HUGHES (1970), in his studies of toxaphene persistence in treated lakes, investigated the accumulation of toxaphene in the aquatic environment. He found that various fishes and sediments selectively accumulated different fractions of toxaphene. TERRIERE et al. (1966) and JOHNSON et al. (1966) also reported that fish selectively accumulated certain fractions of toxaphene.

Microorganisms can sorb pesticides (JOHNSON and KENNEDY, 1973; PARIS and LEWIS, 1976), be consumed, and the sorbed pesticide can move up the food chain. Studies were therefore undertaken to measure the extent of sorption of toxaphene by different aquatic microorganisms (algae, bacteria, and fungi).

A second factor examined was selective sorption of the various toxaphene fractions.

MATERIALS AND METHODS

Test Medium

A saturated solution of toxaphene was prepared by stirring the insecticide into basal salts medium (PAYNE and FEISAL, 1963) and sterilized by filtering through a sterile 0.22-micron Millipore filter. Duplicate pesticide solutions with varying concentrations were

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prepared by aseptically diluting the filtrate with sterile basal salts medium.

Microorganisms

The bacteria and the fungus used in our studies were isolated from natural aquatic systems -- Flavobacterium harrisonii, from a stream containing the effluent of a citrus processing plant, and Bacillus subtilis and Aspergillus sp., from a stream containing the effluent of a chicken processing plant. The alga, Chlorella pyrenoidosa 395, was from the Starr collection, University of Indiana.

Procedures

Inocula for the sorption studies were cultured as follows:

- Bacteria were incubated for 24 hours at 28°C in nutrient broth.
- Fungi were incubated for 72 hours at 28°C in basal salts medium containing glucose.
- Algae were incubated under continuous light (170 ft-c) for 200 hours at 15°C in Bensen and Fuller medium containing Hutner's trace elements (HUTNER, 1950).

Bacteria and algae were harvested by centrifugation and washed three times in sterile dilution water prior to inoculation in test medium. Liquid cultures of fungi were decanted and the liquid was replaced with basal salts medium containing the desired concentration of toxaphene.

Dry weights of bacteria and algae were determined by transferring washed cells to tared beakers and oven drying at 90°C. Dry weights of fungi were determined by filtering the culture first through tared pre-filters, then through tared 0.22-micron Nuclepore filters, and drying to a constant weight at 90°C.

The sorptive properties of the microorganisms were determined by incubating the organisms in basal salts medium with varying concentrations of toxaphene (0.002-0.09 mg/l) on a gyratory shaker at 28°C. Organism concentrations ranged from 100 to 1000 mg dry weight per liter. At each sampling time the cultures of bacteria and algae were centrifuged and the pesticide remaining in the supernatant was measured by gas liquid chromatographic analysis of the organic solvent extract. The fungal cultures were removed from the

shaker and allowed to settle for one minute; samples of the supernatant in the flask were analyzed as above.

Water Solubility -- To show the relative solubilities of the various toxaphene fractions, sides of the lower portion (below 1000-ml mark) of two 2000-ml Erlenmeyer flasks were coated with one ml of acetone containing 0.1 mg and 5 mg of toxaphene, respectively. Coating of the flasks was continued until the acetone evaporated. To each flask one liter of sterile distilled water was added and the solutions were stirred on a magnetic stirrer for 120 hours. Samples were taken every 24 hours, and centrifuged one hour at 15,000 rpm in a Sorvall RC-2 centrifuge prior to glc analysis.

Glc analysis -- Supernatants of the cultures were extracted with 2,2,4-trimethylpentane (isooctane) and analyzed using a Tracor MT-220 gas liquid chromatograph equipped with a high temperature Nickel-63 electron capture detector. A 0.3-meter glass column (4mm ID) containing 80/100 mesh Gas Chrom Q with 3% silicone SE 30 liquid phase was used at a column oven temperature of 190°C for toxaphene determinations. Toxpahene was then quantitated by measurement of the resulting wide base asymmetrical peak, as done by ARCHER and CROSBY (1966). All measurements were made within a linear response range. A two-meter glass column (4mm ID) containing 80/100 mesh gas Chrom Q with 5% OV-17 liquid phase was used at a column oven temperature of 210°C for the separation of toxaphene into 14 different fractions. All quantitations were made by comparison of peak areas using a planimeter. Recoveries from spiked water samples ranged from 77 to 91%.

Determination of peak areas for the resolved toxaphene fractions can be accomplished by dropping perpendicular lines from valleys to the baseline and measuring the enclosed areas with a planimeter. Peak areas thus obtained are not linear with respect to quantity of toxaphene and require more calibration. However, toxaphene component amounts are linear with peak areas obtained by drawing individual baselines from valley to valley of each fraction. Both procedures yield the same results but the latter was used in our studies.

RESULTS AND DISCUSSION

Analyses (glc) of extracts from whole cultures (medium and microorganisms) gave the same "fingerprint" chromatogram as the control, indicating that toxaphene was not degraded even after extended periods of time. The insecticide was also added to autoclaved cultures of bacteria, fungi, and algae which sorbed as much or a little more pesticide than the viable cells. Therefore, the observed sorption was not the result of a metabolically active process.

Time required for equilibrium was short for all the cultures -- 10 minutes for algae, 30 minutes for bacteria, and 2 hours for fungi. No further changes were detected over a 24-hour period.

Desorption of toxaphene was studied by harvesting bacterial cells that had reached equilibrium in the pesticide solution and resuspending them in medium containing no pesticide. Samples were centrifuged, and extracted, and the supernatant was analyzed for pesticide as described. Desorption equilibrium was achieved in as short a time as was equilibrium in the sorption studies and the distribution coefficients were the same.

For very low solute concentrations, sorption of a compound to microorganisms may often be adequately described by a distribution coefficient.

$$K_d = \frac{C_m}{C_w}$$

where K_d = distribution coefficient of toxaphene between microorganisms and medium;

C_m = mg of toxaphene sorbed per mg of microorganisms;

C_w = concentration of toxaphene in the medium (mg/mg) at equilibrium.

This equation was also used to calculate the partition coefficient of toxaphene between n-octanol and water; however, units in these calculations were mg/ml instead of mg/mg.

The measured distribution coefficients and partition coefficient are listed in Table 1. The water-octanol partition coefficient is included for comparison since the partition coefficient between organic solvents and water is widely used as an

indicator of bioaccumulation potential (NEELY et al., 1974; KURIHARA et al., 1973).

TABLE 1

Distribution Coefficients (K_d) of Toxaphene

Microorganisms	K_d*
Bacillus subtilis	$(3.4\pm0.5) \times 10^3$
Flavobacterium harrisonii	$(5.2\pm0.2) \times 10^3$
Aspergillus sp.	$(1.7\pm0.2) \times 10^4$
Chlorella pyrenoidosa 395	$(1.7\pm0.1) \times 10^4$
Average of axenic cultures	$(1.1\pm0.8) \times 10^4$
Field Sample (bacteria and algae)	$(6.6\pm0.2) \times 10^3$
n-octanol	$(3.3\pm2.5) \times 10^3$

*Mean and standard deviation for 12 determinations (only four for field sample).

The range of values for the axenic cultures is within a factor of three of the mean for the four cultures ($\bar{K}_d = 1.1 \times 10^4$). To determine if laboratory data on microbial sorption of toxaphene may be used to predict extent of sorption in a water column from a natural system, a water sample (pH 6.7) was collected from Chandler's pond near Athens, Georgia, for use in checking this point. Two liters of water were collected in sterile flasks. Upon return to the laboratory the sample was mixed well and allowed to stand for 15 minutes to allow large particles of debris to settle out. The supernatant containing microorganisms and suspended detritus was transferred to a sterile flask and was used for sorption studies. Microscopic examination of the supernatant showed the presence of green algae (Arthospira sp., Phytoconis sp., and a few Chlorella sp.), bacteria, ciliates, diatoms, and amorphous material. An aqueous solution of toxaphene was added to the pond water and equilibration was reached within one hour. The distribution coefficient (K_d) was found to be about one-half the average value for the axenic cultures. Such agreement between the laboratory and field data suggests that laboratory data may be used to predict

the extent of pesticide sorption in water columns of a pond or stream.

Factors Governing Sorption Ability

It is well known that an inverse relationship often exists between water solubility and extent of bioaccumulation (METCALF et al., 1975; LEOPOLD et al., 1960). A similar relationship exists between partition coefficients and solubility for organic solvent-water systems (VOERMAN, 1969; HANSCH, 1969). Thus, it was not surprising when earlier studies in our laboratory with microorganisms and carbaryl, diazinon, malathion, parathion, methoxychlor, and toxaphene, showed that only the latter two had distribution coefficients greater than 10 (PARIS et al., 1975). These two compounds are at least 50 times less soluble than the others. The distribution coefficients for methoxychlor and toxaphene were similar to those of JOHNSON and KENNEDY (1973) for methoxychlor and DDT with A. aerogenes and B. subtilis (K_d = 1.4 x 10^3 - 4.3 x 10^3). A characteristic common to all three of these pesticides is their low water solubility (DDT, 1 µgm/l; methoxychlor, 50 µgm/l; toxaphene 500 µgm/l). Thus, we concluded that if selective partitioning of toxaphene components occurs, the least soluble components should have the greatest tendency to accumulate.

Representative chromatograms (Figure 1) of the extracts from supernatants of cultures and controls indicated that the various toxaphene fractions are not sorbed to the same extent. The later eluting compounds (Regions II and III) sorbed to a greater extent than did the early eluting compounds of Region I. Although the specific components of each fraction were not identified, the later eluting compounds have higher boiling points and should have higher molecular weights than those eluting earlier. For compounds of a similar chemical type, those of higher molecular weight are generally less water soluble. This is demonstrated in Figure 2 where the area ratio in chromatogram A for Regions I, II, and III is 1:10:14, respectively; and in chromatogram B, 1:8:5. Flask B contained 50 times as much toxaphene as Flask A so there was a greater quantity of each fraction available for solubilization. The ratios show that Region I represents a proportionately larger area in chromatogram B than in chromatogram A because these components dissolved more than those in Region II and III. Thus, the inverse correlation between sorption and water solubility appears to hold.

Since the less soluble fractions of toxaphene appear to be selectively sorbed by microorganisms, they

would be expected to accumulate in biota and be passed up the food chain. Relative amounts in higher organisms will of course depend not only on the relative amount accumulated but also on the relative excretion rates. In any event, toxicity to higher aquatic life in the environment may differ from that expected if accumulation occurred only through the gills.

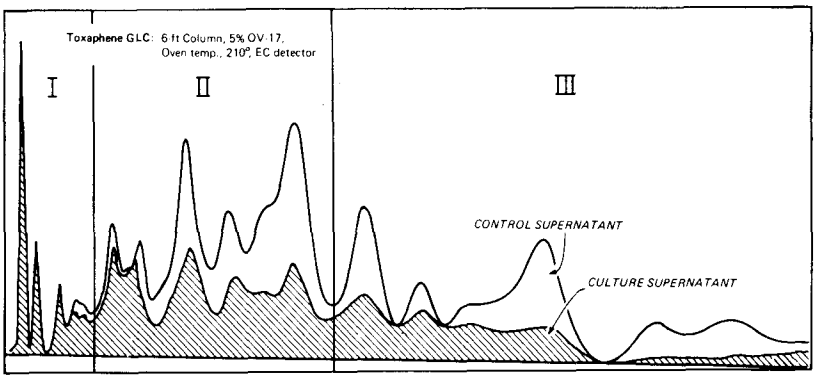

Figure 1. Chromatograms of the isooctane extracts from the supernatants of Chlorella pyrenoidosa culture and of the control.

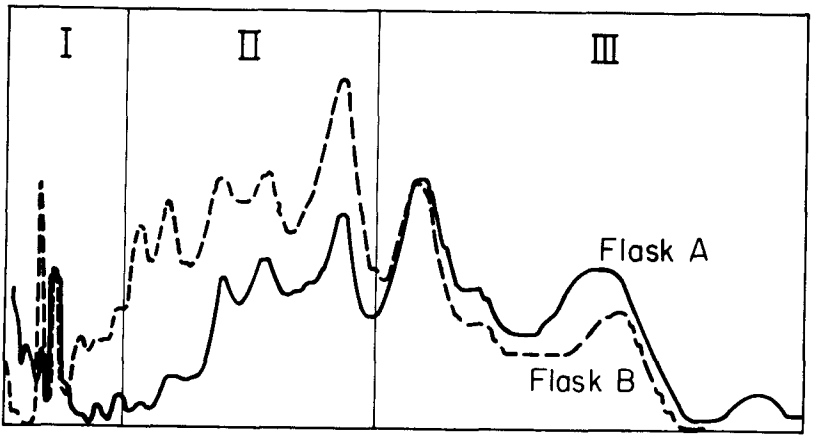

Figure 2. Chromatograms of isooctane extracts of water from flask (A) containing 0.1 mg toxaphene and one liter water (———) and flask (B) containing 5 mg toxaphene and one liter water (----).

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DISCLAIMER

Mention of commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

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