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Application of Dehydrochlorination to the Determination of Toxaphene in Soil and Identification of the Major Gas Chromatographic Peak

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The determination of toxaphene in environmental samples is a formidable analytical problem. This is due not only to the complexity of the pesticide itself, but also to possible alteration of its components by environmental degradation. Interferences of other chlorinated pesticides and PCBs can further complicate the measurement of this pesticide. An alternative technique of analysis for toxaphene is dehydrochlorination. Dehydrochlorination with alcoholic potassium hydroxide to produce a simplified gas chromatographic pattern having a major analytical peak has been reported (GOMES 1977). Other laboratories have modified this procedure (Personal Communication, Colo. Epidemiologic Studies Program). Dehydrochlorination has been used for analysis of toxaphene in certain crop samples (DOLAN et al. 1974) and in selected biological samples (ARCHER and CROSBY 1966).

We applied the dehydrochlorination technique to determination of toxaphene in soil at 1.0, 0.5 and 0.1 µg/g. Combined gas chromatography-mass spectrometry (GC-MS) was used to characterize the major constituent of the analytical peak after separation by high performance liquid chromatography.

MATERIALS AND METHODS

Apparatus*

A Tracor 222 gas chromatograph, equipped with a linearized Ni-63 electron capture detector was used. The gas chromatographic column was 1.8 m x 4 mm, i.d. borosilicate glass, packed with 1.5% OV-17/1.95% OV-210 on 80-100 mesh Gas Chrom Q.

The column was operated at 210°C or 195°C with a nitrogen flow rate of 60 ml/min. Other instrument parameters were: detector 350°C; inlet 235°C; transfer line 235°C.

A Varian Model 2700 gas chromatograph (GC) coupled directly to a Varian 311A mass spectrometer (MS) was also utilized. The GC was equipped with a 30 m x 0.25 mm i.d. SE-30 WCOT glass capillary column capable of resolutions between 60,000 to 90,000 effective plates. The MS was equipped with a combination chemical ionization

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(CI) and electron impact (EI) ion source which was operated in the EI mode.

The GC/MS parameters were: GC parameters--injection port temperature, 260°C; GC transfer line into the MS ion source was maintained at 260°C; the capillary column temperature was maintained at 80°C for on column splitless injection technique; GC oven temperature was rapidly raised to 140°C exactly six minutes after injection of sample and then programed at a rate of 5°C/min to 250°C. MS parameters--Ion source temperature, 240°C; acceleration voltage, 3kV; electron energy, 70 eV; filament emission, 1 mA; mass resolution, 1000 or 10,000; multiplier gain, 10^5.

A Willems Ploytron Type PT20 (Brinkman Instruments) was used for extraction of soil samples.

Reagent and Materials

Toxaphene, 68.5% chlorine, analytical reference standard
Hexane, pesticide grade
Methanol, pesticide grade
Toluene, pesticide grade
Potassium hydroxide, reagent grade
Diethyl ether with 2% alcohol, analytical grade
Sodium sulfate, reagent grade
Florisil, held at 130°C for 24 hr prior to use
Deionized water, hexane extracted.

Procedure

Extraction and Cleanup

Approximately 10 g of soil fortified with toxaphene was transferred to a 100 ml round bottom tube. Water corresponding to 10% of the weight was added to the sample. The sample was extracted with 20 ml methanol-toluene (1:1) on the Polytron blender for 2 min and then placed in a centrifuge to separate the solvent and soil particles. The organic phase was transferred to a 100 ml round bottom flask. The extraction and transfer of solvent was repeated twice more. The solvent was evaporated to 5-10 ml with a warm water bath (60°C) and a vacuum rotary evaporator. Further evaporation to 0.5 ml was done with a warm water bath and a nitrogen stream. The sample was then eluted through a Kontes chromaflex column, (size 22) containing 1.6 g Florisil with 1.6 g sodium sulfate on top, with 30 ml 1% methanol in hexane. Due to variation in Florisil activity the elution pattern may vary; therefore, the analyst should determine the elution characteristics of a toxaphene standard.

Dehydrochlorination

The sample was reduced in volume to 0.5-1.0 ml and quantitatively transferred with hexane to a 50 ml round bottom flask. Approximately 25 ml of a 43% KOH-methanol solution was added. A

condenser was attached and the sample was refluxed for 1 hr at 92°C ± 2°. At the end of the reaction period, the sample was quantitatively transferred to a 125 ml separatory funnel containing 20 ml water and 15 ml hexane. The toxaphene derivative was partitioned into the hexane phase by vigorously shaking the separatory funnel for 2 min. The phases were allowed to separate and the hexane was transferred to a clean tube. The partitioning was repeated using 15 ml hexane. The hexane phases were combined and evaporated to about 5 ml with a nitrogen stream and a warm water bath (60°C). The sample was then eluted through a column (300 mm x 20 mm) containing 100 mm Florisil with 2.5 cm of sodium sulfate on top. The first fraction of 150 ml hexane was discarded, the toxaphene derivative was then eluted with 200 ml 6% diethyl ether in hexane. The elution pattern of the derivative should be verified using a dehydrochlorinated toxaphene standard. The solvent was evaporated to an appropriate volume on a steam bath using a Kuderna-Danish evaporator. An injection was made into a gas chromatograph and the sample was quantified by comparison with derivatized toxaphene standards.

RESULTS AND DISCUSSIONS

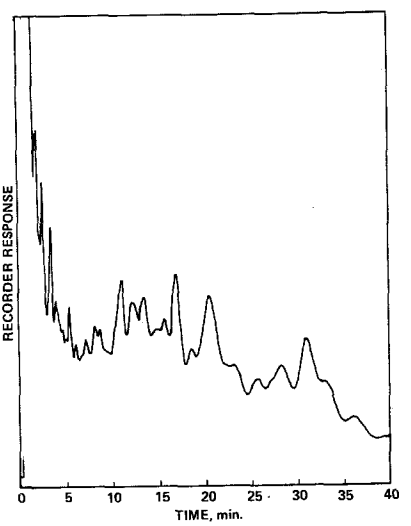

Fig. 1 Gas Chromatogram of a soil sample extract fortified with 0.1 µg/g toxaphene. Injection: 5 µl/3 µl (16.7 mg soil equivalent). Column: 1.5% OV-17/1.95% OV-210 on Gas Chrom Q, 1.8 m x 4 mm i.d. borosilicate glass. Oven temp: 210°C. N_2 flow rate 60 ml/min.

Fig. 1 indicates the characteristic gas chromatographic pattern of toxaphene at 0.1 µg/g in soil. With proper cleanup, measurement of toxaphene should be feasible down to at least 0.05 µg/g. The sample is quantified by comparison of the major peaks with those of a standard. Care should be used to consider any possible effects caused by "weathering" of the sample.

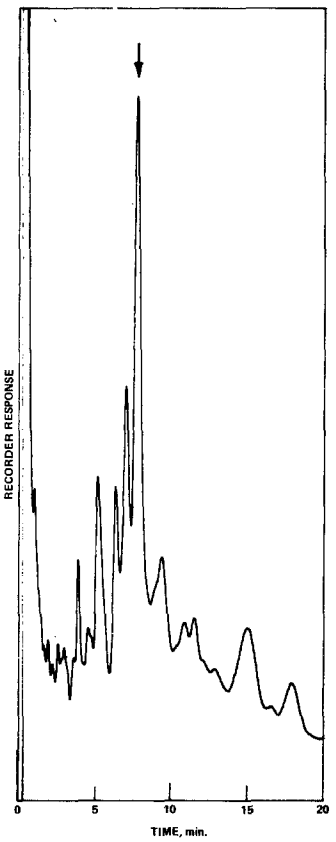

Fig. 2 Gas chromatogram of a dehydrochlorinated toxaphene standard. Injection: 2.5 ng. Oven temp: 195°C. Other conditions same as Fig. 1.

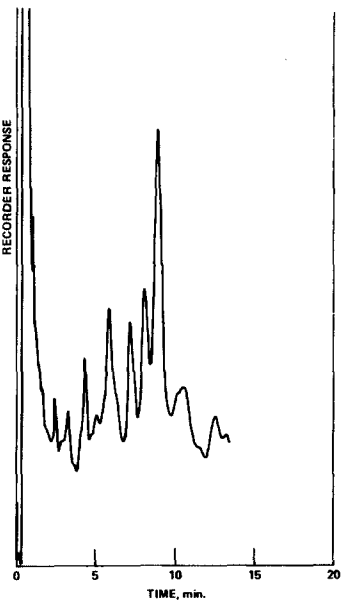

Fig. 3 Gas chromatogram of a dehydrochlorinated soil sample extract fortified with 0.1 µg/g toxaphene. Injection: 5 µl/2 ml (2.5 mg soil equivalent. Other conditions same as Fig. 2.

Fig. 2 illustrates the gas chromatogram of a dehydrochlorinated toxaphene standard indicating the major analytical peak. The

reaction results in sharper and earlier eluting peaks than for intact toxaphene. By measuring the response for one peak rather than for several, sensitivity is increased. Amounts of toxaphene from 10 ng to 100 µg were derivatized and a linear relationship was observed to exist between the intensity of the analytical peak and the amount of toxaphene derivatized. This technique was applied to analysis of soil by fortifying with toxaphene at levels of 1.0, 0.5 and 0.1 µ/g.

Fig. 3 depicts the gas chromatogram of a fortified soil sample after dehydrochlorination. In addition to providing an alternative means of quantification, the procedure could be useful for confirmation of toxaphene. Polychlorinated biphenyls and other organochlorine pesticides are altered during the reaction or removed during the Florisil elution so that interferences from these compounds are prevented. The reaction conditions must be carefully controlled in order to obtain reproducible data.

Table 1 summarizes the recovery data obtained from the fortified samples. At these levels of fortifications there were no problems with cleanup or sensitivity.

Table 1

RECOVERY OF TOXAPHENE FROM FORTIFIED SOIL SAMPLES

Toxaphene added, ppm	Toxaphene recovered, percent	
	Before Dehydrochlorination	After Dehydrochlorination
1.0	87.1	84.2
1.0	92.7	87.3
1.0	93.7	83.3
Average	91.2	84.9
0.5	86.5	73.3
0.5	83.4	69.7
0.5	88.3	71.2
Average	86.1	71.4
0.1	74.8	74.0
0.1	71.6	68.1
0.1	82.8	71.2
Average	76.4	71.1

Efforts to apply this method to determination of biologically incorporated toxaphene in rat adipose tissue were unsuccessful. There was an obvious interference at the retention time of the analytical peak. Certain toxaphene components have been reported

to undergo structural alterations in the rat metabolic system (SALEH and CASIDA, 1978 and OHSAWA, et al, 1975, 1977). Any change or alteration of toxaphene components could affect the production or intensity of the analytical peak.

A dehydrochlorinated toxaphene standard was fractionated by high performance liquid chromatography. A fraction was collected which corresponded with the retention time of the analytical peak using our usual gas chromatographic column (OV-17/OV-210). This fraction was characterized by capillary column GC-MS. Fig. 4 shows the mass spectrum of the major component comprising the analytical peak. This analysis also indicated a number of minor toxaphene components in the fraction. High resolution mass spectrometric determination of the elemental composition of the molecular ion and fragment ion are consistent with the assignment of this constituent as a polychlorinated bornadiene with the general formula $C_{10}H_8Cl_6$.

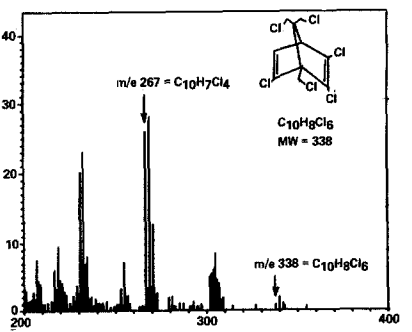

Fig. 4 Mass spectrum and tentative structure of the major component from the analytical peak of a dehydrochlorinated toxaphene standard.

Although useful for quantitative determination of toxaphene when extensive metabolic or other structural changes have not occurred, the routine application of the dehydrochlorination reaction to biological samples would require further careful investigation.

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