1-Hydroxy-2,3-Epoxychlordene in Oregon Soil Previously Treated with Technical Heptachlor

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Gas-chromatographic analyses of residues and degradation products of technical heptachlor weathered 4 years in an Oregon soil revealed an unknown component in layers below the soil surface. The degradation product was either missing or minor in the surface samples and in all samples taken after 1 or 2 years of weathering. In addition, it was not apparent on chromatograms of extracts of four other soils in test. Because the gas-chromatographic peak attributed to the unknown was larger than the peaks of residual heptachlor and gamma-chlordane, the work described in this paper was undertaken to isolate and identify the unknown component.

Methods and Materials

Soil samples were taken from test plots established to determine initial penetration of the soil by technical heptachlor at five field locations. The installation, locations, soil properties, and analytical methods have been previously reported (1,2).

The soil extracts and their fractions were analyzed with several gas chromatographs with different columns and operated under various conditions. Qualitative comparisons were made with standards analyzed under the same conditions and with samples spiked with standards. Column packings included 3% DC-200 on 100/120 mesh Gas Chrom Q,[1/] 3% SE-30 on 60/70 mesh Chromport XXX, 11% (QF-1 plus OV-17) by weight on 80/100 mesh Gas Chrom Q, and 9% QF-1 on 100/120 mesh Gas Chrom Q.

Extraction *p*-values (3,4) were determined for insecticides giving specific peaks by single distribution between 5-ml volumes of hexane or pentane and acetonitrile. The results were compared with those of standards partitioned with the same solvents and at the same temperature.

1/ Mention of a company or trade name does not imply endorsement by the U.S. Department of Agriculture.

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The major components in certain Oregon soil extracts were fractionated on columns of silica gel (powder, suitable for chromatograph use, Baker Analyzed Reagent). The solvents were either hexane followed by mixtures of hexane and isopropyl alcohol or pentane followed by mixtures of pentane and diethyl ether.

Mass spectra were obtained on a Consolidated Electrodynamics Corporation Mass Spectrometer, Model 21-110B, using the direct introduction system. Peaks were identified by the use of perfluorokerosene as a reference.

Results and Discussion

An unknown peak, not appearing on the chromatograms of soil extracts from other locations, occurred in the chromatograms from the Oregon soil extracts. The peak was most prominent in chromatograms of extracts of soils sampled at the 2- to 4-inch layers. The exact amount could not be determined, as a standard was not available at the time of analysis. The retention time of the unknown component was only slightly more than that of heptachlor epoxide on the 3% DC-200 column. However, as a result of its tailing, the unknown peak was more poorly resolved from the gamma-chlordane peak than was a standard heptachlor epoxide peak.

Gas chromatograms of an extract of the Oregon soil sampled 1 to 2 inches below the surface have eight peaks (Fig. 1A,B). Peaks 2, 4, 6, 7, and 8 were identified tentatively as heptachlor, 1-hydroxychlordene, gamma-chlordane, alpha-chlordane, and nonachlor. Peaks 1 and 3 (shoulder on peak 4) were small, but the fifth peak was larger than the residual heptachlor and gamma-chlordane peaks and therefore was considered to be significant enough to warrant isolation and identification.

A 100-ml aliquot of the extract (hexane) was fractionated on a 10-g silica gel column (15 mm I.D.). Components corresponding to peaks 1, 2, 6, 7, and 8 were either not retained on the column or readily eluted from it with 100 ml pentane (Fig. 1C). Additional pentane (300 ml) eluted a component with a retention time on the 3% DC-200 column slightly less than that of peak 5 and corresponding to heptachlor epoxide (Fig. 1D). Mixtures of pentane and diethyl ether eluted the more polar components corresponding to peaks 3, 4 (1-hydroxychlordene), and 5 (Fig. 1E,F). Although the heptachlor epoxide peak was overlapped and hidden by peak 5 in the chromatograms of the unfractionated soil extract (Fig. 1A,B), the amount of heptachlor epoxide present in the extract was quite small compared with that of the unknown. The two components were separated on the 11% (QF-1 plus OV-17) column, but then the unknown and gamma-chlordane peaks were not resolved.

For isolation of larger quantities of the components, additional extracts were prepared of the Oregon soil remaining from previous analyses. By rechromatographing fractions on silica gel columns and varying the solvents and amount of silica gel,

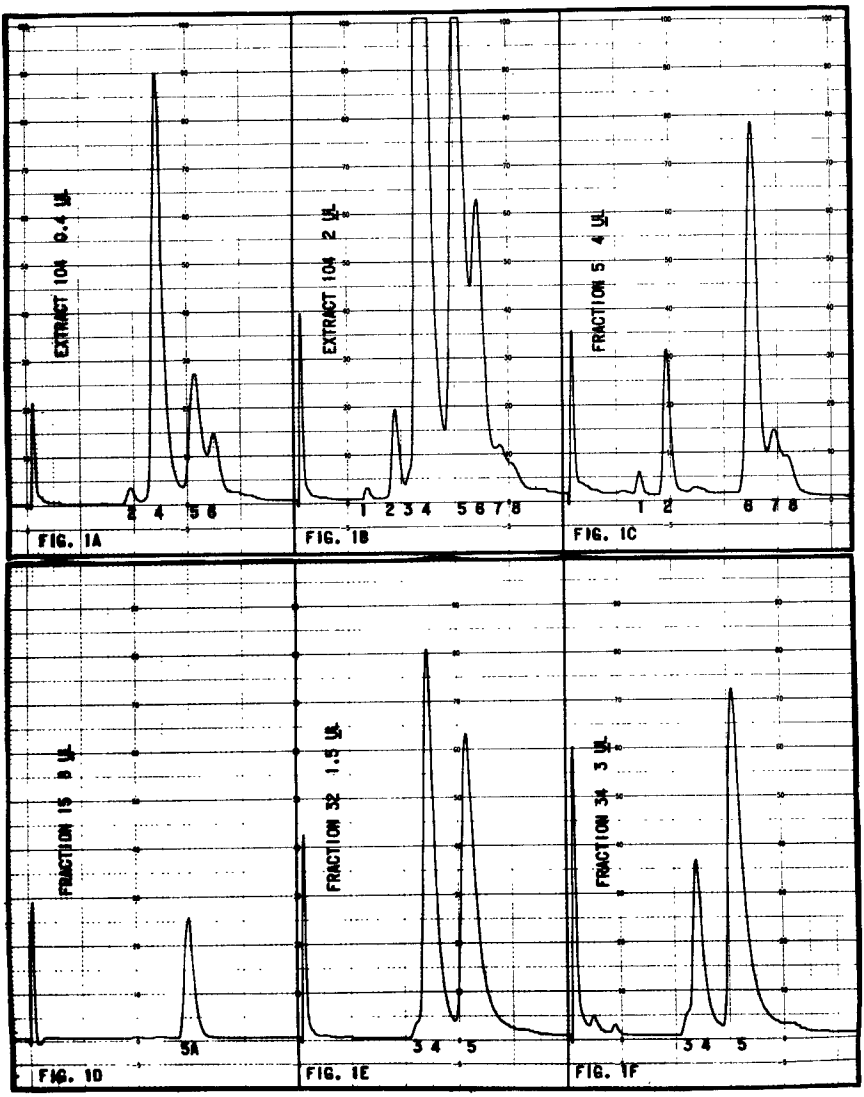

Figure 1. Gas chromatograms (3% DC-200 column) of Oregon soil extracts and fractions of the extract through a silica gel column: (A) 0.4 ul of original extract; (B) 2.0 ul of original extract to show minor peaks; (C) fraction 5, eluted with pentane; (D) fraction 15, eluted with pentane; (E) fraction 32, eluted with 90:10 pentane:diethyl ether; (F) fraction 34, eluted with 85:15 pentane:diethyl ether.

fractions consisting mainly of one component were obtained. Hexane and isopropyl alcohol which were used in preliminary experiments were replaced by pentane and diethyl ether in later experiments. The silica-gel column fractions were analyzed on several columns in different gas chromatographs. In all cases, peaks 2, 4, 5A, 6, 7, and 8 corresponded to standards of heptachlor, 1-hydroxychlordene, heptachlor epoxide, gamma-chlordane, alpha-chlordane, and nonachlor.

Previously undetected components became apparent in certain fractions. Although these components could be breakdown products of the other components on the silica gel column, we believe they are minor components which were concentrated by being retained on the column and then eluted in specific patterns. Although in many cases a very large and sharp peak would be obtained for such components, the entire quantity of the specific component was too small for identification. Fractions that contained unusual components are being saved for possible reference materials for later studies.

The unknown component (peak 5) was postulated to be a degradation product of 1-hydroxychlordene, the major component in the Oregon soil after weathering for only 1 year. The elution of both the unknown and 1-hydroxychlordene from the silica gel column was similar and gas chromatographic peaks of both tended to tail. In a recent paper, Miles, Tu, and Harris (5) reported the epoxidation of 1-hydroxychlordene by soil microorganisms to 1-hydroxy-2,3-epoxychlordene. This hydroxy-epoxide has also been reported by Kaul *et al.* (6) as a metabolite in rats which were intravenously injected with heptachlor, and by Brooks and Harrison (7) as a metabolite of chlordene in the housefly, *Musca domestica* L. Brooks (8) reported that both 1-hydroxychlordene and 1-hydroxy-2,3-epoxychlordene were nontoxic when topically applied to adult female houseflies.

The retention time of the unknown on four gas chromatographic columns agreed with those of an authentic reference standard of 1-hydroxy-2,3-epoxychlordene furnished by Dr. Percy B. Polen of Velsicol Chemical Corporation. The *p*-values (3,4) obtained with pentane and acetonitrile also checked. Mass spectra were compared for the standard hydroxy-epoxide and the unknown. The parent peak end of the spectra of both known and unknown showed a group of peaks of similar relative intensities. The lowest of these had a m/e value of 368, corresponding to the formula $C_{10}H_6O_2Cl_6^{35}$. Higher m/e values corresponded to the various Cl^{35}-Cl^{37} combinations, with the m/e for $-Cl_6^{37}$ (380) being barely apparent. The data thus indicate that the component found in the Oregon soil which had been treated 4 years previously with technical heptachlor is 1-hydroxy-2,3-epoxychlordene.

For all soils except the Quincy loamy fine sand of Oregon, heptachlor and gamma-chlordane (trans-isomer present in technical heptachlor) are still the major components (Table 1). Essentially no heptachlor remained in the Oregon soil, and the major component was 1-hydroxychlordene. This degradation product of heptachlor was also found in the Hawaii (Makalapa clay) and Florida (Lakeland sand) soils. Relatively high values for heptachlor epoxide were found for the Hawaii and Missouri (Lebanon silt loam) soils. Only traces of 1-hydroxychlordene and heptachlor epoxide were detected in the soil from South Carolina (Cataula loamy sand).

TABLE I

Major insecticide residues (ppm) found in upper 1-inch layer of various soils weathered for 4 years after application of water emulsion of 0.5% technical heptachlor at 2 pt/sq ft

Field location	Heptachlor	γ-chlordane	1-OH-chlordene	Heptachlor epoxide
Florida	148.	90.1	13.6	1.9
Hawaii	425.	387.	41.0	95.9
Missouri	472.	191.	...*	29.5
Oregon	0.0	106.	238.	...
South Carolina	482.	142.	...	...

* Peak either missing or too small to calculate.

Research reported here is part of a field evaluation of chlorinated hydrocarbons[2/] for control of various species of termites in different soils and climates in the United States (1). After 1 year at the Oregon test site, much of the heptachlor was converted to 1-hydroxychlordene, the major component in the extracts prepared from the soil (2). After 4 years essentially no heptachlor remained, and a portion of the 1-hydroxychlordene had further degraded to 1-hydroxy-2,3-epoxychlordene. Thus, under certain conditions such as those found at the Oregon test site, a major pathway of heptachlor degradation in soil is hydrolysis to 1-hydroxychlordene, which then can be epoxidized. Epoxidation was probably effected by soil microorganisms (5). To our knowledge, 1-hydroxy-2,3-epoxychlordene has not been reported previously as a degradation product in heptachlor-treated soils.

2/ This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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