Indentification of the Major Metabolic Product of Heptachlor Epoxide in Rat Feces

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Heptachlor is a widely used chlorinated hydrocarbon insecticide which has been primarily used as a soil insecticide and for seed treatments. As one of the chlorinated cyclodiene insecticides, it is known to persist for long periods of time (1) in the environment either as heptachlor or as an oxidized form, heptachlor epoxide (2,3). The latter compound is regarded as the actual toxic form of heptachlor (4). In the past, researchers have found that when heptachlor is fed to rats (5) or cows (6,7), only heptachlor epoxide could be detected in the tissues and milk.

Not much is known about the metabolic fate of heptachlor or heptachlor epoxide except for production of the 1-hydroxy derivative of heptachlor epoxide (1-exo-hydroxy-2,3-epoxychlordene, commonly referred to as 1-OH chlordene epoxide as in Fig. 1) by combined action of microorganisms and physical action (8) and chromatographic evidence indicating the formation of the above metabolite in rats (9).

Since the metabolic fate of this environmentally occurring pesticide is of concern, we have made an attempt to study the metabolism of heptachlor epoxide in the rat.

Heptachlor epoxide (99% pure) was fed to 4 male albino rats by impregnating their normal diet (dog food cubes) with the insecticide by the aid of corn oil at a level of 10 ppm by weight, and feeding them ad libitum. Each rat consumed about 5 mg of heptachlor epoxide in 30 days. Feces and urine were collected separately for a period of 30 days. The test animals did not show any recognizable sign of ill effects during that period.

Approximately 250 gm of dry feces were ground up and extracted with 1 liter each of acetone, hexane and diethyl ether. The extracts were combined, concentrated, and the residue was picked up in acetonitrile. The method used for cleaning with hexane washing and a Florisil column was identical to that published before (10). The column eluates were spotted on a thin-layer plate with silica gel G and developed with a

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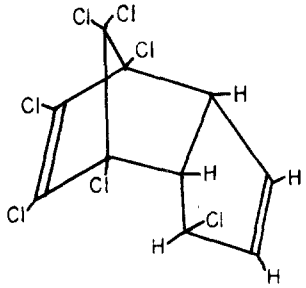
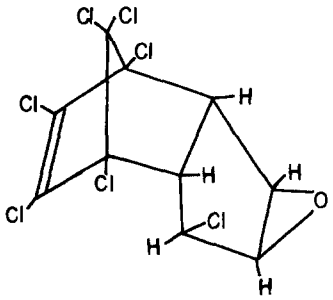
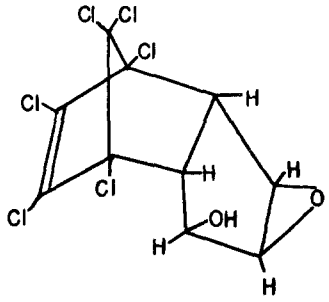


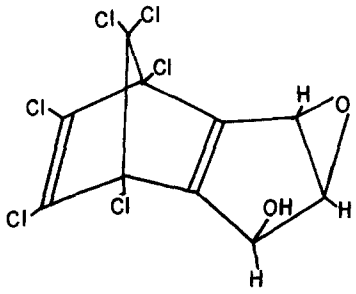
HEPTACHLOR



HEPTACHLOR EPOXIDE



1-OH CHLORDENE EPOXIDE



FECAL METABOLITE

Figure 1.

solvent mixture of cyclohexane 3 and acetone 1. Upon treatment of the plate with a silver nitrate agent, two major spots each were clearly observed among the chromatograms from some of the column eluate fractions: the upper spot corresponding to heptachlor epoxide (R_f = 0.67) and the lower unknown metabolite spot with R_f value of 0.41. By using a preparative thin layer (1 mm thick) chromatographic method with the same solvent system and another solvent mixture (methylene chloride 1 and CCl_4 1), the metabolite was isolated and was crystallized from a solvent mixture of cold n-hexane and acetone. Approximately 5 mg of the major metabolite in a white crystal form was collected from two independent experiments. Quantitative assessment of the metabolic activity of heptachlor epoxide indicated that a rat fed 5

mg of this insecticide at a 10 ppm level during a 30-day period excreted 950 µg of the fecal metabolite and 66 µg of heptachlor epoxide in the feces.

Thin layer chromatographic comparison of this metabolite with the authentic 1-hydroxy-2,3-epoxychlordene revealed that these two compounds had similar R_f's but could easily be resolved on several solvent systems: the major metabolite, therefore, cannot be 1-hydroxy-2,3-epoxychlordene.

The infrared spectrum of the major metabolite showed distinct peaks at 2.95, 6.3 and 11.5 µ indicating the presence of -OH, ClC=CCl and an epoxy ring.

The mass spectrum of the metabolite indicated that its parent mass (P) was 366 (as Cl-35) with P+2, P+4 and P+6 peaks distinctly showing the presence of 6 chlorine atoms. Other major peaks were at 331 (P-Cl), 309 (P-CHO,-C=O) and 303 (P-Cl,-C=O) indicating the route of fragmentation was through formation of keto-form isomer (enol-keto isomerization) at the 1 position. By comparing this spectrum to that of 1-hydroxy-2,3-epoxychlordene (P=368) it was concluded that the former compound is actually a dehydrogenated (-2H) derivative of the latter.

The nuclear magnetic resonance spectrum of the major metabolite showed only 4 protons at δ =3.00 (-O<u>H</u>), 3.56, 3.65 (both epoxyhydrogens) and 4.12 ppm (<u>H</u>COH). The spectrum for 1-hydroxy-2,3-epoxychlordene showed almost identical peaks at δ =2.90 and 4.16 ppm in addition to unresolved massive (equivalent of 4 protons) peaks ranging from 3.35 to 3.75 representing two epoxyhydrogens and two hydrogens at the ring-junctions.

Upon hydrogenation of the major metabolite through PtO_2, a new peak corresponding to 1-hydroxy-2,3-epoxychlordene was detected in a gas chromatographic system. Oxidation of the major metabolite through a CrO_3-H_2SO_4 system (11) yielded a ketone which was detected by infrared spectroscopy. The balance of evidence indicates that the structure of the major metabolite of heptachlor epoxide from the rat feces must be the structure shown in the illustration.

Such a metabolic product could be formed if the parent compound should be first dehydrochlorinated and then hydroxylated with subsequent rearrangement of the double bond position. While the exact metabolic processes to produce this particular compound remain unknown at this stage, the presence of a large quantity of this compound in rat feces is clearly

established. The toxicological implication resulting from the presence of such a metabolic product should be studied in the future.

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