Metabolism of β-Dihydroheptachlor-^{14}C in Soil and by Microorganisms

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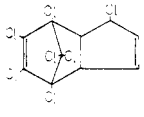
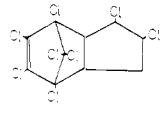
Metabolism of ^{14}C-β-dihydroheptachlor at 5- and 10-p.p.m. levels was studied in soil. Metabolic products (3 and 4%) were detected in 10 and 15 days, respectively. Metabolism by *Aspergillus niger* and *Penicillium urticae* also proceeded very slowly.

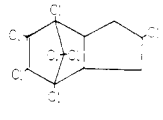
In the past several years it has been observed that most of the insecticides possessing the hexachlorobicyclopentadiene ring are metabolized in vivo to hydrophilic compounds without any change in the ring (Korte, 1965). Aldrin is metabolized in soil (Gannon and Bigger, 1958) and by microorganisms (Korte *et al.*, 1962) to dieldrin. Heptachlor is oxidized in vivo to its epoxide, and epoxides are normally more toxic to warm-blooded animals (Davidow and Radomski, 1953). As heptachlor is metabolized to its epoxide (Gannon and Decker, 1958; Perry *et al.*, 1958) at the position of the double bond in the monochlorocyclopentene ring, it became interesting to study the metabolic fate of a similar monochloro compound in which the monochloro ring is saturated. For this, the authors selected 2-*exo*-4,5,6,7,8,8-heptachloro-4,7-methano-3*a*,4,7,7*a*-tetrahydroindan, commonly known as β-dihydroheptachlor. This compound is known to have very low toxicity to warm-blooded animals (Büchel *et al.*, 1964).



Heptachlor



α-Chlordan



β-Dihydroheptachlor

The chlorine atom in position 1 in α-chlordan is replaced in vivo by a hydroxyl group, but very little is known about the fate of the chlorine atom in position 2 (Poonawalla and Ludwig, 1965). Hence it became all the more interesting to study the metabolism of β-dihydroheptachlor. To study the metabolism, the synthesis of ^{14}C-β-dihydroheptachlor with the label in the hexachlorocyclopentene ring was undertaken (Poonawalla and Korte, 1967). A sample of radiochemically pure ^{14}C-β-dihydroheptachlor was obtained with a specific activity of 4.4 mc. per mmole, m. p. 133° C.

MATERIALS AND METHODS

Standard Solution. A standard solution containing 20 mg. of ^{14}C-β-dihydroheptachlor in 10 ml. of ether was used for all subsequent experiments.

Culture Media. Fifty grams of glucose, 1 gram of asparagin, 0.5 gram of $MgSO_4 \cdot 7H_2O$, and 1.5 grams of KH_2PO_4 were dissolved in 1 liter of distilled water and sterilized for 30 minutes at 120° C. and 1.2 atm. This sterile medium supplies the minimum requirement for the growth of microorganisms (Schopfer, 1938).

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Paper Chromatography. Whatman No. 3 paper strips were immersed in 3% paraffin oil solution in ether and dried in air. Methanol-water (97 to 3) was used as the mobile phase.

Thin-Layer Chromatography. The procedure for thin-layer chromatography has been described (Poonawalla and Korte, 1967; Tschesche *et al.*, 1963).

Qualitative and Quantitative Determinations. All chromatograms were traced by a Friesecke-Hoepfner counter, type FH 49, a methane flow counter type FH 407 (5% efficiency), and a radiochromatograph type FH 452. The percentage of any component was calculated by determining the percentage of counts per minute of that component of a paper radiochromatogram.

Metabolism of ^{14}C-β-Dihydroheptachlor in Soil. One kilogram of fertile soil containing some decaying plant material was suspended in 5 liters of distilled water and stirred thoroughly. The solution was then filtered. To three 1-liter flasks, each containing 200 ml. of filtrate, 0.5 ml. of the standard solution of ^{14}C-β-dihydroheptachlor was added. Ether was evaporated by blowing a gentle stream of nitrogen over the surface for 10 minutes. The flasks were then incubated at 25° C. and stirred gently from time to time. Once every day for the next 10 days, paper and thin-layer chromatograms of the samples of this solution were made. Thus metabolism of ^{14}C-β-dihydroheptachlor at 5 p.p.m. was studied. The same experiment was repeated once again at the 10-p.p.m. level. At this concentration, about 3.0% of the metabolites could be detected. If an atmosphere of oxygen is maintained over the surface, the rate of metabolism is not affected (Table I) and metabolism proceeds very slowly.

Metabolism of ^{14}C-β-Dihydroheptachlor by Microorganisms. In five 1-liter flasks, 200 ml. of culture media were taken. Two of these were inoculated with *Asper-*

Table I. Metabolism of ^{14}C-β-Dihydroheptachlor in Soil

	10 P.P.M.	
Days	Air	Oxygen
7	2.3	2.0
8	2.5	2.5
9	2.1	2.5
10	3.0	2.8
11	x	2.8
12	x	3.5
13	x	3.5
14	x	3.8
15	x	4.0

The figures indicate percentage of the metabolic products detected. No metabolic products were detected at the 5-p.p.m. level.

gillus niger, two with *Penicillium urticae*, and one was used as a control. To each of the inoculated flasks, 0.5 ml. of a standard solution of ^{14}C-β-dihydroheptachlor was added. Care was taken to see that ether evaporated from the surface. The flasks were then incubated at 25° C. for 15 days. The solution was analyzed daily by paper and thin-layer chromatography. No metabolism was observed. After 15 days, the mycelium was filtered and homogenized in an ultraturrax homogenizer and extracted with ether. This contained nearly 25% of the applied radioactivity in either case. The filtrate was then extracted with ether and contained over 70% of the total radioactivity in the form of unchanged β-dihydroheptachlor. For *Aspergillus niger* and *Penicillium notatum*, 3 and 4.5% of the applied radioactivity were detected in the form of more hydropholic compounds.

Since the hydropholic metabolites were not found in the culture media, the insecticide apparently was taken up by the microorganisms and metabolized, and the metabolites were not excreted.

Since the metabolism of β-dihydroheptachlor in soil and by microorganisms proceeded very slowly and the mammalian toxicity is low, it has a considerable advantage over insecticides having a similar structure. Further studies on the metabolism of this interesting insecticide are in progress.

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