# Isolation and Identification of Three Unreported Photodieldrin-$^{14}C$ Metabolites in Soil

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Fifteen months after application of photodieldrin-$^{14}C$ to soil (5 ppm of dry weight) in the open air, the following conversion products were identified by TLC, GLC, and mass spectrometry: the bridged isomer of dihydrochlordene dicarboxylic acid (1,7,8,*exo*-9,10,10-hexachlorotetracyclo-[5.2.1.0$^{2,6}$.0$^{4,8}$]decane-3,5-*exo,exo*-dicarboxylic acid), amounting to about 0.5% of recovered radioactivity, a methoxylated derivative of this acid, amounting to about 0.5% of recovered radioactivity, and a bridged isomer of *trans*-4,5-dihydroxy-4,5-dihydroaldrin (3,*exo*-4,5,6,6,7-hexachloro-11,12-*trans*-dihydroxypentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{5,9}$]dodecane), amounting to about 1% of recovered radioactivity.

Photodieldrin (Figure 1, structure I) is known to be a derivative of dieldrin formed by uv irradiation (Bird et al., 1961) as well as by sunlight on dieldrin-treated plants (Roburn, 1963; Weisgerber et al., 1970; Kohli et al., 1972, 1973b), on dieldrin-treated soil (Weisgerber et al., 1970; Kohli et al., 1972, 1973b), on aldrin-treated plants (Weisgerber et al., 1970, 1974; Klein et al., 1973; Kohli et al., 1973c), and on aldrin-treated soil (Lichtenstein et al., 1970; Kohli et al., 1973a,c; Weisgerber et al., 1974). According to Matsumura et al. (1970; Matsumura, 1972) photodieldrin is not only a photolysis product but also a metabolite formed from dieldrin by soil microorganisms; according to Patil et al. (1972), it is formed from aldrin and dieldrin by marine microorganisms.

Only little knowledge is available on the fate of photodieldrin in the environment and in living organisms. In rats and in several fresh water animals, it is converted to "Kleins metabolite" (Figure 1, structure II), a pentachloroketone in which a "keto" oxygen has been introduced into the chlorinated part of the molecule (Baldwin and Robinson, 1969; Klein et al., 1970; Daily et al., 1972; Georgacakis et al., 1971). This photodieldrin metabolite has also been detected in rat and mouse urine as an aldrin–dieldrin metabolite (Datta et al., 1965; Damico et al., 1968; Klein et al., 1968a,b; Matthews et al., 1971; Richardson et al., 1968; Baldwin et al., 1972). This fact suggests that photodieldrin might represent an intermediate of dieldrin metabolism in mammals. In insects, photodieldrin is converted to the pentachloroketone also (Khan et al., 1969; Zimmer, 1970, 1971).

The fate of photodieldrin in soil is of special importance because dieldrin is used mainly as a soil insecticide. As far as we know, no metabolites of photodieldrin in soil have been identified thus far. This paper deals with the identification of unreported photodieldrin metabolites in soil, isolated after 15 months of exposure in the open air.

## EXPERIMENTAL SECTION

**Apparatus.** In extracts and TLC zones, radioactivity was counted using Packard liquid scintillation counters (Tri-Carb-Model 3380 or 3375) with external standardization. Thin-layer chromatograms were examined qualitatively on chromatogram scanners from Berthold-Frieseke GmbH, Karlsruhe, one of which was fitted with a dot printer. Unextractable radioactivity was determined with an automatic oxidizer Oximat, Intertechnique.

Gas chromatography was performed on a Packard unit, Series 7400, with electron capture and flame ionization detectors and fitted with a glass column (diameter, 4 mm; length, 1.65 m) packed with 1% $OV_1$ on Chromosorb W (AW-DMCS), 80–100 mesh. The carrier gas was nitrogen (40 ml/min). An auxiliary Packard fraction collector 852 was used with anthracene tubes for the collection of radioactive effluent.

Mass spectra were taken after gas chromatography using a GC–MS LKB 9000, from LKB Produkter, Bromma, Sweden; data processing was performed with an IBM calculator, Model 1130, plotter Model 1627. The GLC conditions were as described above except that the carrier gas was helium.

**Reagents and Reference Compounds.** Photodieldrin-$^{14}C$ (specific activity, 0.3 $\mu$Ci/mg) was prepared by irradiation of dieldrin-$^{14}C$ in acetone for 5 hr with a medium-pressure mercury lamp and a quartz finger, and it was recrystallized from ethanol. Purity was better than 99%. The product was labeled uniformly within the chlorinated ring.

The bridged isomer of dihydrochlordene dicarboxylic acid was synthesized by oxidation of photodieldrin with $CrO_3$ in acetic acid. Its dimethyl ester was prepared by methylation with $CH_3I$ in the presence of $Ag_2O$.

The bridged isomer of *trans*-4,5-dihydroxy-4,5-dihydroaldrin was obtained by two different methods: first, by irradiation of *trans*-4,5-dihydroxy-4,5-dihydroaldrin (Hupe, 1973) and separation of the reaction mixture by preparative layer chromatography (plate 20 × 20 cm, 24 g of silica gel H from "Merck", Darmstadt; solvent, benzene–acetonitrile, 3:1); second, by hydrolysis of photodieldrin with sulfuric acid in a dioxane–water mixture. In the latter case, no chromatographic purification was necessary. Its dimethyl ester was prepared with $CH_3I$ in dimethyl sulfoxide, in the presence of NaH, following a method for methylating polysaccharides (Hakomori, 1964; Björndal et al., 1970).

For counting of radioactivity, a liquid scintillator based on dioxane was used for extracts and TLC zones. For absorbing and for counting $^{14}CO_2$ after combustion of samples with unextractable residues, a toluene-based scintillator containing phenethylamine was used.

Methylation of metabolites 1 and 2 was performed with diazomethane which was freshly prepared from *p*-tolylsulfonylmethylnitrosamide and KOH in diethyl ether and then distilled. Metabolite 3 was methylated according to Hakomori (1964).

For silylation of metabolite 3 and its monomethyl ether, samples containing about 1 $\mu$g were evaporated to dryness. *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (7 $\mu$l) (from Maccherey and Nagel, Germany) was added and the mixture was kept overnight.

For TLC, silica gel G from Merck was used for the preparation of plates. All solvents were distilled on a 1-m fractionating column.

**Procedure.** *Application and Work-Up Procedure.* An

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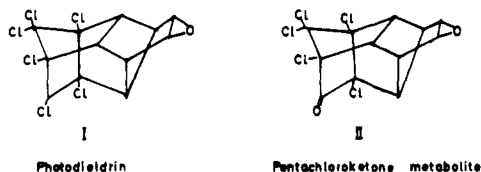


**Figure 1.** Structural formula of photodieldrin (I) and its known pentachloroketone metabolite (II).

aluminium tube (length, 50 cm; $\phi$, 11 cm) containing a screen at the bottom was filled with glass wool (about 35 g), charcoal (about 300 g), and then with soil (about 3.5 kg dry weight). Analysis of the soil showed: coarse sand, 50.7%; fine sand, 28.3%; silt, 10.1%; clay, 10.9%; organic matter, 2.9%; pH 7.0. The tube was sunk into the soil, in the open air, and allowed to settle for about 6 weeks. Then the tube content was levelled off at the surrounding ground. From the remaining tube content, the upper layer (0–10 cm depth) was removed and mixed thoroughly with 6.788 mg of photodieldrin-$^{14}C$ dissolved in a minimum amount of acetone, resulting in an overall initial concentration of 4.977 ppm (dry weight). Then this treated soil was put into the tube again.

Fifteen months later, the treated soil layer and the untreated soil layer were extracted separately with methanol in a soxhlet for 48 hr; the radioactivity of the extracts was determined by liquid scintillation counting. For determination of the amounts of metabolites present in the extracts, they were concentrated and separated by TLC on silica gel (solvent, cyclohexane–acetone (4:1)), and the zones were counted by liquid scintillation.

The radioactive residues in the soils, which were not extractable with methanol, were determined by combustion of aliquots followed by liquid scintillation counting. The extracted soils were then reextracted with 4% aqueous ammonia at room temperature, since, in former studies, we had observed that strongly adsorbed polar substances, e.g. dicarboxylic acids, are extracted by this method (Klein et al., 1973). After centrifugation and acidification, the aqueous solution was extracted with ether. The radioactivity of the ether and the aqueous medium was determined in a liquid scintillation counter, that of the soil again by combustion. TLC of the ether (cyclohexane–acetone, 4:1) showed that the radioactivity consisted nearly quantitatively of hydrophilic metabolites. In order to determine the radioactivity adsorbed by charcoal and glass wool, aliquots of them were burned, too, and subjected to liquid scintillation counting.

*Isolation of Metabolites.* For isolation of metabolite 1, the ether obtained from ammonia extraction was concentrated and purified several times on TLC with silica gel. After developing the chromatogram twice with cyclohexane–acetone (4:1), a main fraction at the origin was observed consisting of metabolite 1, and a very small fraction was observed at $R_f$ 0.43 matching photodieldrin. The latter fraction was not analyzed further; the metabolite fraction was desorbed with methanol, applied on another TLC plate, and rechromatographed with benzene–ethyl acetate (1:1). Since the metabolite fraction did not move with this solvent, the chromatogram was re-run with petroleum ether–benzene–acetic acid (2:2:1). This resulted in a radioactive zone at $R_f$ 0.51 which was desorbed with methanol, methylated with diazomethane, and further purified on TLC with cyclohexane–acetone (4:1). The methylated product showed an $R_f$ value of 0.42. It was desorbed with benzene, concentrated to about 100 $\mu$l, and subjected to GLC. For GLC–MS, the solution was further concentrated, and the solution containing about 1.5 $\mu$g was completely injected.

For isolation of metabolites **2** and **3**, the methanol extract was concentrated and applied on three 20 × 20 cm preparative layer plates, each prepared with 24 g of silica gel H from Merck, Darmstadt. After developing with benzene–ethyl acetate (3:1), a major fraction matching photodieldrin ($R_f$ 0.84) and a minor fraction at the origin of the chromatogram, consisting of metabolites **2** and **3**, were obtained. The metabolic fraction was desorbed with methanol and rechromatographed with benzene–ethyl acetate (1:1) as solvent. While metabolite **2** remained at the origin of the chromatogram, metabolite **3** had an $R_f$ value of 0.59. Both were desorbed with methanol.

Metabolite **2** was rechromatographed with petroleum ether–benzene–acetic acid (2:2:1) ($R_f$ 0.42), desorbed with methanol, and methylated with diazomethane. The methylated product was purified on TLC with cyclohexane–acetone (4:1) and gave $R_f$ 0.30. After desorption with benzene, the product was concentrated to 200 $\mu$l and subjected to GLC. For GLC–MS, it was further concentrated to 10 $\mu$l, and 1 $\mu$l thereof was injected.

Metabolite **3** was methylated with methyl iodide and NaH in dimethyl sulfoxide, and then separated on TLC with cyclohexane–acetone (4:1). The chromatogram showed three radioactive zones: one at $R_f$ 0.10 corresponding to unreacted diol, one at $R_f$ 0.23 corresponding to its monomethyl ether, and one at 0.47, corresponding to the dimethyl ether. The unreacted diol was desorbed with methanol, evaporated to dryness, and silylated before GLC and GLC–MS. The monomethyl ether was desorbed with methanol; half of this methanol was evaporated to dryness, silylated, and subjected to GLC and GLC–MS; the other was evaporated to dryness and taken in 10 $\mu$l of benzene, 1 $\mu$l of which was used for GLC and the rest for GLC–MS. The dimethyl ether was desorbed with benzene, evaporated to about 10 $\mu$l, and subjected to GLC and GLC–MS like the monomethyl ether.

*Blank Experiments.* A sample of the photodieldrin-$^{14}C$ solution used for the soil experiments, containing 6.8 mg of photodieldrin, was applied on TLC. The very small radioactive fractions which did not match photodieldrin were separated and isolated in the same manner as the metabolites; they were checked by GLC and GLC–MS. In the case of impurities behaving comparably to metabolites 1 and 2 on TLC, methylation with diazomethane was included in the isolation procedure. Impurities similar to metabolite 3 were silylated before GLC.

## RESULTS AND DISCUSSION

**Quantitative Measurements.** Table I shows the results of quantitative analysis of soil treated with photodieldrin-$^{14}C$ in the upper layer (0–10 cm depth) and kept in the open air for 15 months. The table shows a total recovery of 84.5% of the applied radioactivity after 15 months; 15.5% has been lost by evaporation. A leaching of 30.2% of the applied radioactivity was detected in the 10–35-cm soil layer, while only a small amount (1.9%) was leached in greater depth and absorbed by charcoal. In the two soil layers, 11.8 and 21.1%, respectively, of the radioactivity present were due to metabolites and material nonextractable with organic solvents.

**Identification of Metabolites.** The following metabolites of photodieldrin were isolated in soil and identified.

*Metabolite 1* represented about 0.5% of the recovered radioactivity. On TLC, GLC, and in mass spectrometry, it was found to be identical (including fragmentation and peak height) with an authentic sample of the bridged isomer of dihydrochlordene dicarboxylic acid (1,7,8,*exo*-9,10,10-hexachlorotetracyclo[5.2.1.$0^{2,6}$.$0^{4,8}$]decane-3,5-*exo*,*exo*-dicarboxylic acid; Figure 2, metabolite 1). The mass spectrum is shown in Figure 3. The typical $Cl_6$ pattern of the molecular peak (*m/e* 454) indicates that the metabolite is not a dechlorination product of photodieldrin. The fragment $M^+ - OCH_3$ (*m/e* 423), which is partly mixed with $M^+ - Cl$ (*m/e* 419), and the fragment $M^+ -$

**Table I. Residues of Photodieldrin-$^{14}C$ and Its Metabolites after Soil Application**

| Sample | % of applied radioact. | % of recovered radioact. | Concn, ppm equiv to photodieldrin | %[a] of metabolites + residues unextractable with solvent |
|---|---|---|---|---|
| Treated soil (0–10 cm from surface) | 52.3 | 62.0 | 3.599 | 11.8 |
| Untreated soil (10–35 cm from surface) | 30.2 | 35.7 | 0.834 | 21.1 |
| Charcoal | 1.9 | 2.2 | 0.406 | |
| Glass wool | 0.1 | 0.1 | 0.104 | |
| Total | 84.5 | 100.0 | | |

[a] Based on the radioactivity of each sample.

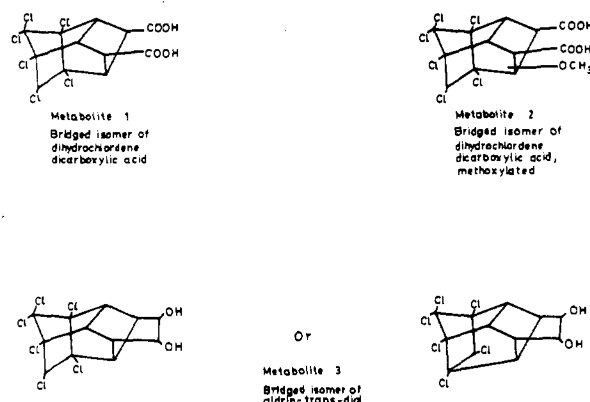


**Figure 2.** Structures of photodieldrin metabolites in soil.

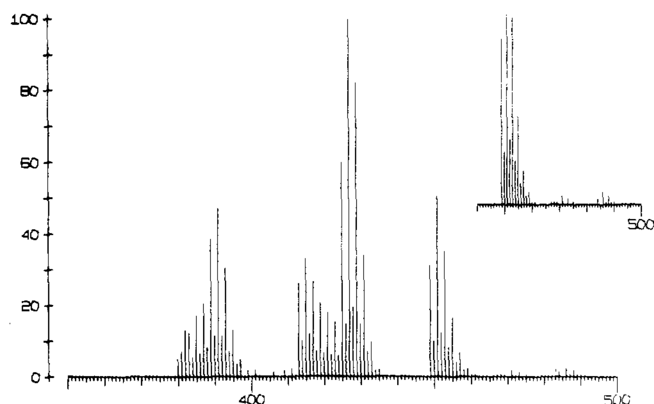

**Figure 4.** Mass spectrum of metabolite **2** of photodieldrin in soil (bridged isomer of dihydrochlordene dicarboxylic acid, methoxylated), after methylation.

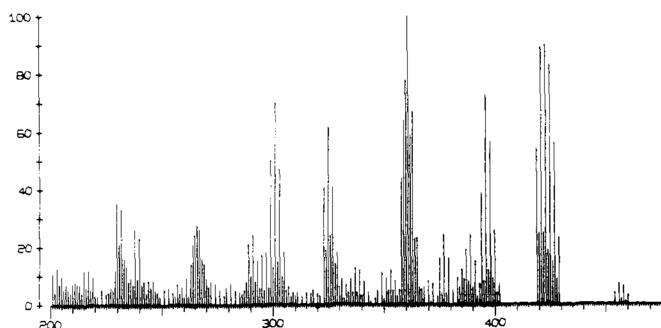

**Figure 3.** Mass spectrum of metabolite **1** of photodieldrin in soil (bridged isomer of dihydrochlordene dicarboxylic acid), after methylation.

$CH_3COOH$ (*m/e* 394) prove the presence of carboxylic groups in the molecule. Contrary to the unbridged isomer, there are no retro-Diels–Alder fragments at *m/e* 270 and 235; as a consequence of the increased chemical stability of the bridged compound, the formation of fragment ions at *m/e* 359 ($M^+ - Cl - CH_3COOH$), *m/e* 394, and *m/e* 419 is favored. Furthermore, the bridge is demonstrated by the following fact. A fragment at *m/e* 368, which is easily formed from the unbridged isomer by the loss of a $COOCH_3$ group and two neighboring C atoms ($M^+ - CH_2CHCOOCH_3$), is observed only in traces for this metabolite. Metabolite 1 was strongly adsorbed to soil particles. It could be extracted only with dilute ammonia, not with organic solvents.

*Metabolite 2* (Figure 2; about 0.5% of the recovered radioactivity) was, following its chromatographic properties and mass spectrum, a derivative of the bridged dihydrochlordene dicarboxylic acid, with a methoxy group introduced into the molecule. A reference compound could not be synthesized; nevertheless, the mass spectrum of the metabolite isolated from soil and methylated (Figure 4) gives evidence for this structure. It shows a molecular weight of 484 with a typical $Cl_6$ pattern. There are fragments analogous to metabolite 1, e.g. $M^+ - Cl$ (*m/e* 449), which is partly mixed with a small fragment $M^+ - OCH_3$ (*m/e* 453), and $M^+ - COOCH_3$ (*m/e* 425). It is noteworthy that the fragment $M^+ - Cl$, in relation to $M^+ - OCH_3$, is favored with increasing substitution or bridge formation: it is relatively small for the unbridged acid, it equals the $OCH_3$ fragment for the bridged acid (metabolite 1), and it is strong for the $OCH_3$ substituted bridged acid (metabolite 2). The fragment *m/e* 413 ($M^+ - Cl - HCl$) does not have the typical appearance of a $Cl_4$ cluster, since the intensity of the isotopic peak *m/e* 413 + 4 is increased by the peak *m/e* 417 ($M^+ - Cl - CH_3OH$). The same applies to the fragment *m/e* 389 ($M^+ - COOCH_3 - HCl$), a $Cl_5$ fragment which is also altered by the fragment *m/e* 385 ($M^+ - Cl - 2CH_3OH$) + 4.

An additional fragment 469 ($M^+ - CH_3$), which does not occur for metabolite 1, indicates the presence of the $OCH_3$ group; the fact that an analogous fragment does not exist in the mass spectrum of metabolite 1 suggests that this fragment does not arise from a $COOCH_3$ group. Since only microgram quantities of the purified metabolite were available, other identification methods, e.g. NMR or ir, could not be applied. Contrary to metabolite 1, metabolite 2 was extractable with organic solvent and was not found in the ammonia extract obtained after the methanol extraction.

*Metabolite 3* (Figure 2; about 1% of the recovered radioactivity) was also extractable with methanol. It was identical with a synthetic sample of a bridged isomer of *trans*-4,5-dihydroxy-4,5-dihydroaldrin (3,*exo*-4,5,6,6,7-hexa-

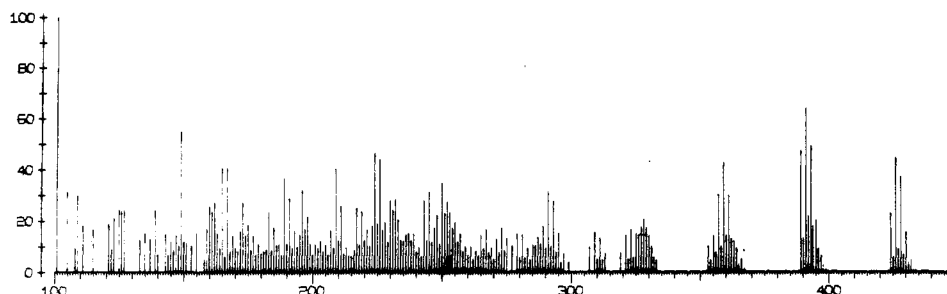


**Figure 5.** Mass spectrum of metabolite **3** of photodieldrin in soil (bridged isomer of aldrin–*trans*-diol), after methylation.

chloro-11,12-*trans*-dihydroxypentacyclo[6.4.0.0$^{2,10}$.0$^{3,7}$.0$^{5,9}$]dodecane), which was synthesized by two methods: by hydrolysis of photodieldrin and by irradiation of aldrin–*trans*-diol. In addition to the TLC comparison of the free diols, GLC and mass spectrometric comparisons were made of the following derivatives: the monomethyl derivative, the dimethyl derivative, the monomethyl monosilyl derivative, and the disilyl derivative. The mass spectrum of the dimethyl derivative is presented in Figure 5. It shows a molecular peak at $m/e$ 424 with a typical $Cl_6$ pattern, and fragments at $m/e$ 389 ($M^+ - Cl$) and at $m/e$ 353 ($M^+ - Cl - HCl$). The latter is partly mixed with the fragment $m/e$ 357 ($M^+ - Cl - CH_3OH$). The base peak at $m/e$ 101 is a fragment consisting of the two $OCH_3$ groups with their attached C atoms and one neighboring tertiary C atom ($CHOCH_3CHOCH_3CH$).

Due to the asymmetry resulting from the bridged skeleton, and the additional asymmetry resulting from the diol group, two stereoisomeric forms of the bridged diol are possible, as shown in Figure 2. Each of these two structures shown represents a pair of optic antipodes; thus, a total of four isomeric bridged aldrin–*trans*-diols should exist. In order to decide unequivocally which of these structures is that of metabolite **3**, methods like determination of optic activity and X-ray crystallographical analysis are needed. This was not possible with a few micrograms of isolated metabolite.

**Blank Experiments.** Although the photodieldrin-$^{14}C$ used in these experiments was more than 99% pure, the impurities were separated, isolated, and checked by GLC–MS. In GLC, none of the impurities matched any of the identified metabolites; likewise, none of the impurities gave mass spectra identical with those of any metabolites. Thus, the three metabolites identified above have really been formed in the soil and are not due to by-products in the photodieldrin solution used.

**Unidentified Radioactive Products.** Although the combined soil samples contained about 15% of radioactive substances other than photodieldrin, only a total of 1.5% of these products (percent of total radioactivity in the sample) was identified. Most of these products other than photodieldrin were not extractable with organic solvents.

It is known from various publications that pesticides may be converted, in soils or plants, to products which are fixed in soil or plant–cell wall complexes and, therefore, are not extractable either with methanol or with other organic solvents. Such residues have been reported for different chemical classes, especially for fungicides or herbicides containing N (Chin et al., 1973; Yih et al., 1968; Bakke et al., 1972), but also for metabolites of organochlorines (Klein et al., 1973; Kilzer et al., 1974). The complex-forming substances were lignins in the case of plants (Chin et al., 1973; Yih et al., 1968); in the case of soil, humic acids seem to be complex formers. The bound residues are released when the complex is destroyed; for soil, alkali has been successful in some cases (Klein et al., 1973; Kilzer et al., 1974). Indeed, when dilute ammonia was used for reextraction of the methanol-extracted soil containing bound photodieldrin metabolites, part of the unknown substances were dissolved, but they could not be extracted from the ammonia with ether in either alkaline or acidic medium, showing that they were fully water soluble. All attempts to convert these materials to less polar, volatile derivatives (acidic or alkaline hydrolysis, methylation, acetylation) have not been successful.

## CONCLUSION

These results show that photodieldrin is slowly hydrolyzed in soil to form metabolite **3**. The two dicarboxylic acids, metabolites **1** and **2**, originate from oxidative ring cleavage analogous to the formation of dihydrochlordene dicarboxylic acid from aldrin, dieldrin, and aldrin–*trans*-diol (Klein et al., 1973; Kohli et al., 1973b,c; Weisgerber et al., 1974; Kilzer et al., 1974). In the case of metabolite **2**, methoxylation occurred before or after ring cleavage. The bridged diol (metabolite **3**) seems to be a precursor of these acids since, in the case of aldrin, the oxidative ring cleavage occurs much easier with the *trans*-diol than with the epoxide (Kohli et al., 1973b; Kilzer et al., 1974). The known pentachloroketone which had been isolated from rats, insects, and other animals (Figure 1, structure II), has not been found in this study. Probably the enzymes catalyzing this rearrangement and dechlorination do not occur in significant amounts in microorganisms of normal soils. Upon comparison with the conversion of dieldrin, it may be concluded that photodieldrin is less persistent in soil and that it is subjected to further degradation.

## LITERATURE CITED


Bakke, J. E., Shimabukuro, R. H., Davison, K. L., Lamoureux, G. L., *Chemosphere* **1**, 21 (1972).
Baldwin, M. K., Robinson, J., *Nature (London)* **224**, 283 (1969).
Baldwin, M. K., Robinson, J., Parke, D. V., *Food Cosmet. Toxicol.* **10**, 333 (1972).
Bird, C. W., Cookson, R. C., Crundwell, E., *J. Chem. Soc.*, 4809 (1961).
Björndal, H., Hellerqvist, C. G., Lindberg, B., Svensson, S., *Angew. Chem.* **82**, 643 (1970).
Chin, W. T., Kucharczyk, N., Smith, A. E., *J. Agric. Food Chem.* **21**, 506 (1973).
Daily, R. E., Klein, A. C., Brouwer, E., Link, J. D., Braunberg, R. C., *J. Agric. Food Chem.* **20**, 371 (1972).
Damico, J. N., Chen, J. Y. T., Costello, C. E., Haenni, E. O., *J. Assoc. Off. Anal. Chem.* **51**, 48 (1968).
Datta, P. R., Laug, E. P., Watts, J. O., Klein, A. K., Nelson, M. J., *Nature (London)* **208**, 289 (1965).
Georgacakis, E., Chandran, S. R., Khan, M. A. Q., *Bull. Environ. Contam. Toxicol.* **6**, 535 (1971).
Hakomori, S., *J. Biochem. (Tokyo)* **55**, 205 (1964).
Hupe, J., Diplom-Thesis, Bonn, 1973.
Khan, M. A. Q., Rosen, J. D., Sutherland, D. J., *Science* **164**, 318 (1969).
Kilzer, L., Detera, S., Weisgerber, I., Klein, W., *Chemosphere* **3**, 143 (1974).
Klein, A. K., Dailey, R. E., Walton, M. S., Beck, V., Link, J. D., *J. Agric. Food Chem.* **18**, 705 (1970).
Klein, A. K., Link, J. D., Ives, N. F., *J. Assoc. Off. Anal. Chem.* **51**, 595 (1968a).
Klein, A. K., Link, J. D., Ives, N. F., *J. Assoc. Off. Anal. Chem.* **51**, 805 (1968b).
Klein, W., Kohli, J., Weisgerber, I., Korte, F., *J. Agric. Food Chem.* **21**, 152 (1973).

Kohli, J., Weisgerber, I., Klein, W., *Chem. Mikrobiol. Technol. Lebensm.* **1**, 149 (1972).
Kohli, J., Weisgerber, I., Klein, W., *Chemosphere* **2**, 125 (1973a).
Kohli, J., Weisgerber, I., Klein, W., Korte, F., *Chemosphere* **2**, 153 (1973b).
Kohli, J., Zarif, S., Weisgerber, I., Klein, W., Korte, F., *J. Agric. Food Chem.* **21**, 855 (1973c).
Lichtenstein, E. P., Schulz, K. R., Fuhremann, T. W., Liang, T/ T., *J. Agric. Food Chem.* **18**, 100 (1970).
Matsumura, F., *Environ. Qual. Saf.* **1**, 96 (1972).
Matsumura, F., Patil, K. C., Boush, G. M., *Science* **170**, 1206 (1970).
Matthews, H. B., McKinney, J. D., Lucier, G. W., *J. Agric. Food Chem.* **19**, 1244 (1971).
Moza, P., Mostafa, I., Klein, W., *Chemosphere* **3**, 255 (1974).
Patil, K. C., Matsumura, F., Boush, G. M., *Environm. Sci. Technol.* **6**, 629 (1972).
Richardson, A., Baldwin, M., Robinson, J., *J. Sci. Food Agric.* **19**, 524 (1968).
Roburn, J., *Chem. Ind. (London)*, 1555 (1963).
Weisgerber, I., Klein, W., Korte, F., *Tetrahedron* **26**, 779 (1970).
Weisgerber, I., Kohli, J., Kaul, R., Klein, W., Korte, F., *J. Agric. Food Chem.* **22**, 609 (1974).
Yih, R. Y., McRae, D. H., Wilson, H. F., *Science* **161**, 376 (1968).
Zimmer, M., in Institut für ökologische Chemie der GSF, Jahresbericht 1970, GSF-Bericht Ö 29.
Zimmer, M., Doctoral Dissertation, Bonn 1971.

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# Fate of Alachlor and Propachlor in a Model Ecosystem

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Ring-$^{14}C$-labeled alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide] and propachlor (2-chloro-*N*-isopropylacetanilide) were studied in a model ecosystem designed by R. L. Metcalf and associates. These two herbicides were transformed into many compounds in water over the experimental period of 33 days. According to thin-layer chromatographic and radioautographic analyses, at least eight alachlor degradation products and seven propachlor degradation products were found in the water. Alachlor and propachlor constituted only 1.8 and 0.4%, respectively, of the radioactivity in the water at the end of the experiment. Thus, it appears that these two herbicides are labile under an aquatic environment. No parent compounds were detected in the organisms and there was no evidence to indicate that their metabolites or degradation products were magnified in the food chain.

Alachlor [2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide] and propachlor (2-chloro-*N*-isopropylacetanilide) are widely used for weed control in corn, soybeans, and many other crops. The mode of action of these $\alpha$-chloroacetamides was described by Jaworski (1969). Inhibition of protein synthesis is believed to be the general locus of interaction, although multiple sites of action are also indicated. Metabolism of propachlor in corn, sorghum, sugar cane, and barley has been reported by Lamoureux et al. (1971). At least three water-soluble metabolites are produced in each species during the first 6 to 24 hr following treatment. Compounds I and II were identified as the glutathione conjugated and $\gamma$-glutamylcysteine conjugated propachlor, respectively. Compounds I and II in corn seedlings were shown to be transitory metabolites, and they were not detected 72 hr following treatment.

At the present time, no data are available concerning these two compounds in the food chain. Recently a model ecosystem for the evaluation of pesticide biodegradability and food chain magnification has been developed (Metcalf et al., 1971). The model ecosystem has been used to evaluate several pesticides (Yu et al., 1974a,b, 1975; Sanborn and Yu, 1973; Booth et al., 1973; Isensee et al., 1973). Based on our past experience, good reproducibility was obtained with this model ecosystem. This paper reports the study of alachlor and propachlor in this model ecosystem and is part of our continuing effort to examine effects of pesticides in the environment.

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## MATERIALS AND METHODS

**Labeled Compounds.** Ring-$^{14}C$-labeled alachlor (specific activity 1.0 mCi/mmol) and propachlor (specific activity 4.0 mCi/mmol) were provided by Monsanto Co., St. Louis, Mo. Radiochemical purities were determined to be 99% by thin-layer chromatography and radioautography.

**Model Ecosystem.** The procedures described by Metcalf et al. (1971) were followed with some modification (Yu et al., 1974a). Fifty microcuries of ring-$^{14}C$-labeled alachlor (13.5 mg) or propachlor (2.65 mg) was applied to the base of the 7-day-old sorghum plants in the aquarium. These dosages approximated 2.7 and 0.5 lb/acre, respectively, for alachlor and propachlor based on the land area of the model ecosystem. Experiments were carried out in one aquarium for each compound.

**Sample Preparation.** The procedures, which have been described previously (Yu et al., 1974a), are essentially an acetone extraction for organisms and an ether extraction for water.

**Thin-Layer Chromatography (TLC).** Solvent extracts from water and organisms were analyzed on a silica gel F-254 aluminum plate (0.25 mm thickness, E. Merck AG.) developed in a solvent consisting of methanol–benzene (5:95, v/v).

## RESULTS AND DISCUSSION

Radioactivity in the water was monitored through the