Fate of Aldrin-^{14}C in Maize, Wheat, and Soils under Outdoor Conditions

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Maize has been grown in soils treated with aldrin-^{14}C at locations in Germany (2.9 kg/ha), England (3.0 kg/ha), Spain (3.0 kg/ha), and the United States (3.0 kg/ha), and wheat has been grown in Germany (2.9 kg/ha) and England (3.2 kg/ha). Additionally, aldrin-^{14}C has been applied to wheat seeds at locations in Germany and England (0.1% of the weight of grain). At harvest, residues did not exceed 0.01 ppm in the grain. The main radioactive products identified by glc–mass spectra in soils and in the plants of all locations were dieldrin and dihydrochlordene dicarboxylic acid (1,2,3,4,8,8-hexachloro-1,4,4a,6,7,7a-hexahydro-1,4-endomethyleneindene-5,7-dicarboxylic acid). Unchanged aldrin, photodieldrin, unknown acidic compounds, and an unknown nonpolar metabolite were detected in trace amounts. In wheat soil, up to 5% of the recovered radioactivity was due to photoaldrin. The quantitative data for aldrin and metabolites were different for the four locations.

In two previous papers, we reported outdoor studies with aldrin-^{14}C in potatoes (Klein *et al.*, 1973) and sugar beets (Kohli *et al.*, 1973), following soil application in 1969 at a location in Germany and England. In the same year, aldrin-^{14}C was applied to soils, and maize was grown in Birlinghoven, Germany, Sittingbourne, U. K., Sevilla, Spain, and Modesto, Calif., under the same experimental conditions. Furthermore, in Germany and England, wheat was grown in aldrin-^{14}C-treated soils, and at both locations, seed dressing was used additionally for the same crop. The rationale for these experiments was the same as described previously. The results are described in this paper.

APPARATUS AND REAGENTS

Apparatus for radioactive counting, glc, and mass spectrometry, as well as the reagents, were the same as described previously (Klein *et al.*, 1973).

PROCEDURE

Plant Growing and Application of Aldrin-^{14}C. Detailed information on experimental work and on weather data during the experiment is given in the previous paper (Klein *et al.*, 1973). Soil types included: Germany, loess; England, sandy clay loam; Spain, alluvial silty clay; U. S., local Californian soil.

Application rates for maize were: Germany, 2.9 kg/ha; England, Spain, and U. S., 3.0 kg/ha. Twenty maize seeds were sown into one box each in the four countries; the seedlings were thinned to 10 plants at 10 days from emergence and to 4 plants at 30 days from emergence. Varieties used included: Germany, Velox; England, Kelvedon 59 a; Spain, Prades 850; U. S., Dekalb.

Soil application rates for wheat were: Germany, 2.9 kg/ha; England, 3.2 kg/ha. Wheat seeds (117) were sown in each box. For seed application, 117 seeds for each box were treated individually with a known amount of an acetone solution of aldrin-^{14}C to produce an overall concentration of 0.1% aldrin on the weight of grain. Varieties used included: Germany, Heines Koga; England, Kloka.

Working Up of Plant Material and Soil. Intervals in time between planting and harvest were as follows: maize, Germany, May 19–Oct 23; maize, England, May 6–Oct 3; maize, Spain, July 3–Oct 21; maize, U. S., May 16–Aug 15; wheat, Germany, April 2–Aug 21; wheat, England, April 30–Aug 19. For the maize experiments, roots, stems, leaves, husk, grain, and core were analyzed separately. For wheat, roots, low stems (*ca.* 8 cm high), straw, husk, and grain were analyzed separately. Random soil samples of *ca.* 750 g were taken at depths of 0–10, 10–20, 20–40, and 40–60 cm from the surface, immediately after harvest. Methanol was used as an extraction solvent for all samples in order to extract the polar metabolites as well as the parent compound.

Residue Analysis and Isolation of Conversion Products. Residue data were obtained by radioactivity measurements. Isolation of the nonpolar compounds was performed by repeated tlc. For isolation of the hydrophilic metabolites, liquid–liquid extractions and methylation were included in the isolation procedure. The identification was achieved by comparison of chromatographic data and of mass spectra with reference substances.

RESULTS AND DISCUSSION

Maize Experiments. *Identification of Conversion Products.* In all maize and soil samples, most of the radioactivity was due to conversion products. They were identified by comparison of chromatographic data and mass spectra with those of reference compounds. The conversion products were qualitatively identical with those found in potatoes and sugar beets (Klein *et al.*, 1973; Kohli *et al.*, 1973). The main conversion products were *dieldrin* and a group of highly hydrophilic metabolites. The major compound of this hydrophilic group was found to be *dihydrochlordenedicarboxylic acid* (1,2,3,4,8,8-hexachloro-1,4,4a,6,7,7a-hexahydro-1,4-endomethyleneindene-5,7-dicarboxylic acid); it constitutes at least two-thirds of the hydrophilic group. A nonpolar compound (metabolite X) with an R_f value between that of aldrin and dieldrin was found in some soil samples, especially those from the Sittingbourne experiment. *Photodieldrin* was detected mostly in leaves. Part of the radioactive residues in soil samples and some crop samples was not extractable with organic solvents.

Quantitative Residue Measurements. Table I shows the residues of aldrin and its conversion products in maize and soil samples at four locations. In the last column of each location, the sum of residue concentrations of aldrin and its metabolites in each sample is recorded. It was highest near the application site (upper soil layer at 0–10 cm from surface and roots). It decreased, in the soil layers, with increasing depth. In the plants, it decreased in the sequence roots, leaves, stems, and cobs. The radio-

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Table I. Residues of Aldrin-^{14}C and Its Conversion Products in Maize and Soil following Soil Application in Birlinghoven, Germany, Sittingbourne, U.K., Sevilla, Spain, and Modesto, Calif. (Expressed as Equivalent Parts per Million of Aldrin)

	Germany							England						
Sample	Aldrin	Metabolite X	Dieldrin	Photodieldrin	Hydro. metab. (extr.)	Unextracted residue	Total residue	Aldrin	Metabolite X	Dieldrin	Photodieldrin	Hydro. metab. (extr.)	Unextracted residue	Total residue
Roots	0.30	n.d.[a]	3.64	n.d.	2.42	0.84	7.20	0.19	n.d.	1.49	0.06	0.69	0.03	2.46
Leaves	0.01	<0.01	0.03	0.02	0.08	<0.01	0.15	<0.01	n.d.	0.01	n.d.	0.06	0.03	0.10
Stem	<0.01	n.d.	0.03	<0.01	0.01	<0.01	0.04	<0.01	n.d.	0.02	n.d.	0.01	<0.01	0.03
Husks	<0.01	n.d.	<0.01	<0.01	0.01	0.02	0.04	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01
Grain	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	0.01	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01
Core	<0.01	n.d.	<0.01	n.d.	0.01	<0.01	0.01	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01
Soil, 0–10 cm from surface	0.78	n.d.	0.55	0.02	0.07	0.16	1.58	1.30	0.11	0.72	n.d.	0.19	0.30	2.62
Soil, 10–20 cm from surface	0.18	<0.01	0.16	n.d.	0.04	0.06	0.45	<0.01	n.d.	<0.01	<0.01	0.01	0.05	0.06
Soil, 20–40 cm from surface	0.03	n.d.	0.04	n.d.	0.02	0.03	0.12	<0.01	<0.01	<0.01	n.d.	<0.01	0.03	0.04
Soil, 40–60 cm from surface	<0.01	n.d.	0.01	n.d.	0.01	0.03	0.05	<0.01	n.d.	<0.01	n.d.	<0.01	0.02	0.02
	Spain							U. S.						
Roots	0.29	0.03	4.16	n.d.	0.71	0.01	5.20							
Leaves	<0.01	n.d.	0.01	<0.01	0.03	<0.01	0.05	0.03	n.d.	0.06	0.05	0.23	0.03	0.40
Stem	<0.01	n.d.	0.01	n.d.	0.01	n.d.	0.02	<0.01	n.d.	0.05	n.d.	0.02	<0.01	0.08
Husks	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	n.d.	<0.01	<0.01	0.02	<0.01	0.03
Grain	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01
Core	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01
Soil, 0–10 cm from surface	0.83	0.04	0.60	n.d.	0.30	0.44	2.21	0.50	n.d.	1.17	n.d.	0.25	0.30	2.22
Soil, 10–20 cm from surface	0.02	<0.01	0.04	n.d.	0.03	0.05	0.15	0.01	n.d.	0.01	n.d.	0.02	0.08	0.12
Soil, 20–40 cm from surface	0.01	n.d.	0.02	n.d.	0.01	0.02	0.06	<0.01	n.d.	<0.01	n.d.	0.02	0.02	0.04
Soil, 40–60 cm from surface	<0.01	<0.01	0.01	n.d.	0.01	0.02	0.04	<0.01	n.d.	<0.01	n.d.	<0.01	0.03	0.04

[a] n.d., none detected.

Table II. Residues of Aldrin-^{14}C and Its Conversion Products in Wheat and Soil following Soil and Seed Application in Birlinghoven, Germany, and Sittingbourne, U.K. (Expressed as Equivalent Parts per Million of Aldrin)

	Germany, soil application							England, soil application						
Sample	Aldrin	Metabolite X	Dieldrin	Photodieldrin	Hydro. metab. (extr.)	Unextracted residue	Total residue	Aldrin	Metabolite X	Dieldrin	Photodieldrin	Hydro. metab. (extr.)	Unextracted residue	Total residue
Roots	0.07	n.d.[a]	0.79	0.02	0.18	0.05	1.11	0.57	n.d.	2.92	0.02	0.82	0.31	4.64
Low stems								0.10	n.d.	0.69	0.02	0.11	<0.01	0.92
Straw	<0.01	n.d.	0.07	<0.01	0.04	<0.01	0.12	0.02	n.d.	0.11	<0.01	0.05	<0.01	0.18

Husks	<0.01	n.d.	0.01	<0.01	0.01	<0.01	0.03	0.01	n.d.	0.01	<0.01	0.02	<0.01	0.04
Grain	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	0.01
Soil, 0–10 cm	1.09	n.d.	0.52	n.d.	0.09	0.17	1.87	2.00	0.11	0.42[b]	0.05	0.14	0.15	2.87
from surface														
Soil, 10–20 cm	0.45	n.d.	0.21	n.d.	0.05	0.07	0.78	<0.01	<0.01	<0.01	n.d.	<0.01	0.05	0.05
from surface														
Soil, 20–40 cm	0.09	n.d.	0.05	n.d.	0.02	0.02	0.18	<0.01	<0.01	<0.01	<0.01	<0.01	0.05	0.05
from surface														
Soil, 40–60 cm	<0.01	n.d.	<0.01	n.d.	<0.01	0.01	0.01	<0.01	n.d.	<0.01	<0.01	<0.01	0.02	0.03
from surface														
							England, seed application							
Roots	0.03	0.03	0.51	0.02	0.05	<0.01	0.64	0.63	n.d.	2.88	0.13	0.58	0.26	4.48
Low stems								0.02	n.d.	0.35	0.02	0.06	<0.01	0.45
Straw	<0.01	n.d.	0.10	0.01	0.02	<0.01	0.13	<0.01	n.d.	0.10	0.01	0.02	<0.01	0.13
Husks	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	<0.01
Grain	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	<0.01
Soil, 0–10 cm	0.02	n.d.	0.05	n.d.	0.02	0.02	0.11	0.05	0.01	0.03	<0.01	0.01	0.02	0.12
from surface														
Soil, 10–20 cm	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
from surface														
Soil, 20–40 cm	<0.01	n.d.	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
from surface														
Soil, 40–60 cm	<0.01	<0.01	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	<0.01
from surface														

[a] n.d., none detected. [b] Including 5% of radioactivity as photoaldrin.

activity in the kernels was, at all locations, near the lowest quantitative detection limit (0.01 ppm). The results of the four local experiments are in line with each other, with the exception of a greater leaching of radioactivity in the German soil, and a higher radioactivity in some U. S. plant samples. These differences are probably due to local climatic and soil factors, as well as to the intervals in time between planting and harvest.

In the upper soil layer (0–10 cm from the surface), aldrin (Table I, first column of each location) represents one-half to one-quarter of the total radioactive residues; in the plant samples and in some deeper soil layers, the amounts of dieldrin (third column) and of the hydrophilic metabolites (fifth column) exceed aldrin by far. It is evident that the hydrophilic metabolites may leach in soil and may be translocated to the aerial parts of the plants. The leaching and translocation, however, are small for aldrin and dieldrin (Table I). For instance, the leaching of aldrin and dieldrin in the German soil is less than 10% down to 40 cm depth; in the other soils, it is much less or even negligible.

Wheat Experiments. *Soil Application.* The first part of Table II shows the radioactive residues in wheat and soils following soil application. After application of aldrin-^{14}C to soil, the same radioactive products were detected in wheat crops and soil as in the experiments with maize. Additionally, photoaldrin was found in the combined soil samples from England in concentrations of about 5% of the total radioactive residue, and in trace amounts in the other location. Photoaldrin was not determined separately in each soil layer. The total radioactive residues decreased, as in the maize, sugar beet, and potato experiments, from the site of application (upper soil layer and roots) to the deeper soil layers and to the plant tops. The radioactivity in the grains was near the limit for quantitative measurements (0.01 ppm).

In the crop samples from England, total residues were slightly higher than in the respective samples from Germany; on the other hand, a greater leaching of radioactivity occurred in the German soil than in the English one, demonstrated by higher radioactivity in deeper soil layers in Germany than in England. Unchanged aldrin (first column of each location, Table II) represents the major portion of radioactivity in all upper soil samples. The radioactivity in the deeper soil layers of the English experiment was mostly unextractable (sixth column) and probably due to hydrophilic compounds, as considered previously. In most of the plant samples, dieldrin (third column) was the main radioactive compound; the hydrophilic metabolite group (fifth column) constitutes a minor portion. Photodieldrin (fourth column) and metabolite X (second column) occurred in even smaller amounts; the small quantities of photoaldrin are included in the dieldrin column (third column of Table II).

Seed Application. Seed application resulted in the conversion of aldrin to dieldrin, to a group of hydrophilic metabolites including dihydrochlordene dicarboxylic acid, and to small amounts of photodieldrin, the unknown metabolite X, and unextractable residues. The residues of radioactive products in wheat and soil after seed treatment with aldrin-^{14}C are shown in the second part of Table II. The total residues (last column) were lower than after soil application; in the grains and the deeper soil layers, the residues were below the quantitative detection limit (<0.01 ppm). In most samples, dieldrin was the main radioactive compound (third column).

CONCLUSIONS

As described in a previous paper on aldrin-^{14}C application to soil (Kohli *et al.*, 1973), hydrophilic metabolites were detected in the edible parts of sugar beets, not looked for in any routine analyses before, and exceeding the residues of aldrin and dieldrin. In this paper, such residues were detected, too, in most of the wheat, maize, and soil samples, but in the case described here, only trace amounts were found in the edible parts, the grains. Similarly, the total residues in wheat grain were negligible after *seed treatment*, but residues occurred in straw, low stems, roots, and soil.

Whereas a comparison of these data for hydrophilic metabolites with those found in real field experiments is not possible, the aldrin and dieldrin data are in line with former field data, in the case of soil application. The residues found after seed application, however, were somewhat higher than in agricultural practice, for, with radioactive compounds, it was not possible to use exactly the same application method as in practical seed dressing.

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Metabolism of Chlordane in Rats

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The fate of HCS-3260-^{14}C (3:1 *cis*-chlordane and *trans*-chlordane) and the individual isomers was studied in rats. Single oral doses of the compounds were almost completely (>90%) eliminated after 7 days. Females excreted more of the dose, 5–6%, in the urine than did the males, 2–3%. *cis*-Chlordane was eliminated more rapidly, 70% after 24 hr, than the trans isomer, 60% after 24 hr. Approximately 15% of the radiocarbon in the 0–24-hr feces was as the administered compound, but none was detected in the urine. The metabolites in the excreta were formed predominantly by dechlorination and various degrees of hydroxylation of the cyclopentane ring. Levels of residues in the fat of rats after being fed 1, 5, and 25 ppm of HCS-3260-^{14}C in the diet for 56 days were approximately three times the parts per million level in the diet. In liver, kidney, brain, and muscle, the levels were $\frac{1}{8}$, $\frac{1}{10}$, $\frac{1}{25}$, and $\frac{1}{50}$ that of the concentration in the feed. Oxychlordane was the major ^{14}C residue in the tissues, ranging from 50% of the radiocarbon in the kidney to about 90% in the fat. Feeding *trans*-chlordane gave higher residue levels in the tissues than *cis*-chlordane, the increase being primarily in higher oxychlordane concentration. Oxychlordane was the most persistent residue in the tissues after the chlordane was removed from the diet.

Although chlordane is one of the older and most commonly used chlorinated hydrocarbon insecticides, its metabolism in animals has received little attention until recently. This stems, in part, from the fact that technical chlordane is composed of a complex mixture of components including the cis and trans isomers of the insecticide. Furthermore, ^{14}C-labeled pure *cis*- and/or *trans*-chlordane has not been generally available. Poonawalla and Korte (1971) did investigate the metabolism of *trans*-chlordane-^{14}C in rabbits, demonstrating that the compound was rapidly metabolized and excreted.

Lending new interest to the fate of chlordane in animals was the development of a high purity chlordane by Velsicol Chemical Corp. This product is composed of 98+% of a 3:1 mixture of *cis*- and *trans*-chlordane and is designated as HCS-3260 by the manufacturer. This designation will be used to denote high-purity chlordane in this report. The components of HCS-3260 and technical chlordane as determined by gas–liquid chromatography (glc) are illustrated in an earlier report from our laboratory (Dorough *et al.*, 1972).

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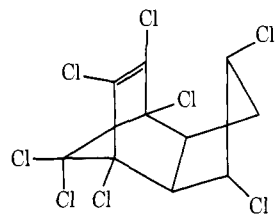
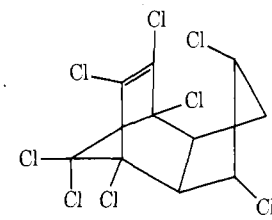


cis-chlordane



trans-chlordane

One metabolite whose significance appears to increase with each new report on the fate of chlordane is oxychlordane (1-*exo*,2-*endo*-4,5,6,7,8,8a-octachloro-2,3-*exo*-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene). This metabolite is formed from both *cis*- and *trans*-chlordane in animals and indications are that it is a persistent storage product (Schwemmer *et al.*, 1970; Polen *et al.*, 1971; Street and Blau, 1972; Dorough and Hemken, 1973).

In the current paper, a study of the fate of HCS-3260-^{14}C and of the individual pure isomers in rats is presented. The data reflect the fate of the materials in animals treated with single oral doses and in animals fed the insecticides in the diet.

METHODS AND MATERIALS

Chemicals. Radioactive materials used in this study were as follows: HCS-3260-^{14}C, 3:1 mixture of *cis*- and