

concentrations were also observed in the roots of plants grown in S-deficient solutions and in the greens of plants from K-deficient solutions.

Significant decreases in the concentrations of both Dyfonate and Dyfoxon in the roots were observed with plants grown in solutions deficient in K, Ca, or Mg. The concentrations of Dyfoxon were significantly decreased by deficiencies of all elements except nitrogen.

Analysis of the nutrient solutions after 8 days of plant growth showed that 2.7 to 13.3% of the recovered radioactivity was water soluble, compared to 2.1% immediately after treatment with [¹⁴C]Dyfonate. This indicates that either water-soluble ¹⁴C metabolites are released from the plant roots into the nutrient solutions or that exoenzymes metabolized the Dyfonate in the nutrient solutions.

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Fate of Aldrin-¹⁴C in Sugar Beets and Soil under Outdoor Conditions

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Aldrin-¹⁴C has been applied to soils under outdoor conditions in Germany (2.9 kg/ha) and England (3.2 kg/ha) and sugar beets have been grown. Upon harvest, more than 95% of the total radioactivity recovered from the plants and more than 40% of the radioactivity recovered from the soils was due to metabolites, mainly dieldrin and a group of hydrophilic metabolic products. The main hydrophilic compound was identified as dihydrochlordene-¹⁴C dicarboxylic acid (1,2,3,4,8,-

8-hexachloro-1,4,4a,6,7,7a-hexahydro-1,4-endo-methylene-indene-5,7-dicarboxylic acid); additionally, photodieldrin and two minor acidic compounds were detected. Between 52 and 58% of the total residue in the surface soils was aldrin, but the percentage of aldrin decreased with increasing depth. Only very low residues were detected in the deeper soil layers in England, whereas more radioactivity was found in the soil samples in Germany.

In a previous paper (Klein *et al.*, 1973) we reported upon outdoor soil application studies of aldrin-¹⁴C in potatoes carried out in 1969 at two locations in Germany and England. These outdoor studies have been set up to obtain an approach to field conditions, since radioisotope studies can not be carried out under real conditions. The simulated field conditions, however, give information which is not easily obtainable with small-scale indoor experiments. For potatoes, it was shown that more than 60% of the total radioactivity which was recovered in plants and soil after the harvest was due to metabolites, mainly dieldrin and a group of hydrophilic metabolic products, the main compound of which was identified as dihydrochlordene-¹⁴C dicarboxylic acid (Figure 1). The same compound was detected in the leaching water draining from the experimental box at a depth of 60 cm (Klein *et al.*, 1973; Moza *et al.*, 1972).

In the same year, experiments with other crops were conducted in Birlinghoven, Germany, and Sittingbourne, U. K., under the same conditions. The full results obtained from experiments with sugar beets are described in this paper.

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APPARATUS AND REAGENTS

The apparatus for radioactive counting, glc, and mass spectrometry, as well as the reagents, was the same as described in the previous paper (Klein *et al.*, 1973).

PROCEDURE

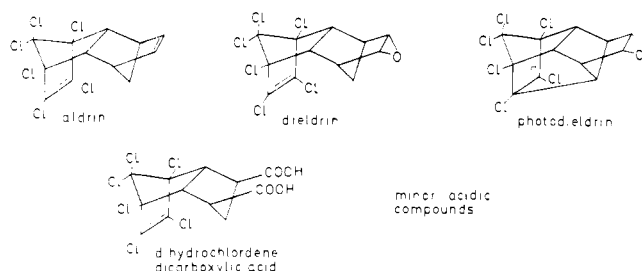
The sugar beets were grown in Birlinghoven, Germany, and Sittingbourne, U. K., in the open air in boxes 60 × 60 × 60 cm, constructed from water-resistant plywood. The base of the boxes contained holes to permit the drainage of excess water. The boxes were sunk into a pit such that the upper surface of the soil was level with the surrounding ground. The soils in the boxes were typical for sugar beet growing in the two locations. Fertilizers were applied as in agricultural practice. The aldrin-¹⁴C was applied as a diluted 30% emulsifiable concentrate using commercial surfactants; application rate and specific activity of aldrin-¹⁴C are given in Table I. The concentrate was diluted 100 times with water before application. The insecticide was incorporated in the soil to a 10-cm depth; then 40 sugar beet seeds were sown. The plants were thinned out to 20 when they were 5-cm high, and to 4 at the six-leaf stage. Air temperature, humidity, and pressure, as well as rainfall, were recorded during the vegetation period. A summary of the climatic conditions, the analyses of both soils, and further cultural details are included in Table I.

Table I. Cultural Details for Sugar Beets Grown with Aldrin-¹⁴C Treatment

Growth Parameter	Details for site indicated	
	Sittingbourne, Kent, U. K.	Birlinghoven, Westphalia, Germany
Variety	Kleinwanzlebener E	Kleinwanzlebener E
Date of sowing	April 30th, 1969	April 2nd, 1969
Depth of sowing	2 cm	2 cm
Fertilizer (as kg/ha)		
K ₂ O	150 (as KCl)	96 (as KH ₂ PO ₄)
P ₂ O ₅	96 (as "super")	143 (as KH ₂ PO ₄)
N	40 + 40	40 + 40 (as Kalkammonsalpeter)
Application rate of aldrin- ¹⁴ C	3.2 kg/ha (117 mg)	2.9 kg/ha (103 mg)
Specific activity of aldrin- ¹⁴ C	5.6 nCi/ μ g	8.2 nCi/ μ g
Date of harvest	November 5th, 1969	October 8th, 1969
Watering	As necessary, near 5 l. weekly in dry weather	Little watering needed
Soil		
Texture	Sandy clay loam	Sandy loam
Organic matter	2.0%	3.5%
Sand	65%	67.3%
Silt	7.2%	16.7%
Clay	25.8%	12.5%
pH	7.4	8.1
Total rainfall during crop growth, mm	290	554
Range of mean maximum daily temperature, °C	13-26	9-29

Table II. Residues of Aldrin-¹⁴C and Its Conversion Products in Sugar Beets and Soil following Soil Application (Expressed as Equivalent ppm of Aldrin)

Sample	Germany						England					
	Aldrin	Metabolite X	Dieldrin	Hydrophilic metabolites (extr) + photo-dieldrin	Unextracted residue	Total residue	Aldrin	Metabolite X	Dieldrin	Hydrophilic metabolites (extr) + photo-dieldrin	Unextracted residue	Total residue
Peel	0.03	<0.01	0.51	0.15	<0.01	0.70	0.01	0.03	0.26	0.16	<0.01	0.46
Peeled beets	<0.01	<0.01	<0.01	0.05	<0.01	0.06	<0.01	<0.01	<0.01	0.04	<0.01	0.05
Leaves	<0.01	<0.01	0.01	0.03	<0.01	0.04	<0.01	<0.01	<0.01	0.05	<0.01	0.05
Soil, 0-10 cm from surface	0.87	0.02	0.68	0.10	0.21	1.88	1.28	0.07	0.69	0.18	0.28	2.50
Soil, 10-20 cm from surface	0.38	0.01	0.28	0.05	0.06	0.78	0.01	<0.01	0.01	0.01	0.10	0.13
Soil, 20-40 cm from surface	0.08	<0.01	0.07	0.02	0.03	0.21	<0.01	<0.01	<0.01	<0.01	0.03	0.04
Soil, 40-60 cm from surface	0.01	<0.01	0.01	0.02	0.01	0.05	<0.01	<0.01	<0.01	<0.01	0.02	0.03

**Figure 1.** Formula of aldrin and its conversion products.

At harvest time, the total crop resulting from four sugar beet plants was examined. The plant growth and yield were normal. The total crop was worked up. Peeled beets, peels, and leaves 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 the harvest. Methanol was used as extraction solvent for all samples in order to extract the polar metabolites as well as the parent compound. Most of the sugar which had been coextracted from the plant samples crystallized when the extracts were concentrated. The extracts were filtered and the sugar was washed several times with methanol. The radioactivity remaining in the sugar was

determined after combustion. It was less than 5% of the total radioactivity present in the extract.

Residue data were obtained by radioactive measurements. Isolation of the nonpolar compounds was performed by repeated tlc. For the 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 compounds.

Further experimental details are described in the previous paper on metabolism in potatoes (Klein *et al.*, 1973).

RESULTS AND DISCUSSION

Identification of Metabolites. In all plant samples and in most of the soil samples, the main part of the radioactivity was due to conversion products. The following substances were identified or characterized.

Dieldrin (Figure 1) constituted the main metabolite in the upper soil layers and in the beet peel of both experiments. It was identified by comparison with authentic dieldrin in several tlc solvent systems and by glc at various temperatures.

Both in soil and plant extracts a compound (Metabolite X) was found in small concentrations which, on tlc, had an R_f value between that of aldrin and dieldrin. Further purification proved unsuccessful due to its instability.

Also in small concentrations, a substance was found in soil and plant samples which was identified as photodieldrin (Figure 1). The identification was performed by comparison of R_f values in various tlc solvent systems, of the glc retention times, and of the mass spectrum with an authentic sample of photodieldrin. It was not measured separately; in Table II, it is recorded together with "Hydrophilic metabolites."

In leaves and peeled beets as well as in the deepest soil samples, the main portion of the recovered radioactivity was accounted for by a group of hydrophilic substances consisting of a main product and two minor products. The major product (more than 60% of this group) was found to be dihydrochlordene dicarboxylic acid (1,2,3,4,8,8-hexachloro-1,4,4a,6,7,7a-hexahydro-1,4-endomethylene-indene-5,7-dicarboxylic acid, Figure 1). Its identity was confirmed by cochromatography of the metabolite with an authentic sample of the free acid, as well as of the dimethyl esters on tlc. Glc analysis showed that the dimethylated metabolite had the same retention time as the dimethyl ester of the reference compound. Further confirmation of the identity of the metabolite was obtained by comparison of the mass spectra, which also showed definitely that the metabolite was not the bridged photoisomer acid.

Previously, the same metabolite was detected in these laboratories in potatoes, soil, and leaching water after soil application of aldrin-¹⁴C (Klein *et al.*, 1973; Moza *et al.*, 1972). Work on the further breakdown of this compound is in progress.

The minor hydrophilic metabolites in plants and soil were not examined in detail, but they also possess acidic properties and will be investigated further.

Some radioactivity remained in the samples after extraction with methanol, mostly in the soils from England, as indicated in Table II. This radioactivity was not extractable by any organic solvent. It probably consists of polar metabolites; also, upon extraction with dilute ammonia or sodium hydroxide, half of this radioactivity was isolated and identified as dihydrochlordene dicarboxylic acid by tlc, glc, and mass spectrometry.

Quantitative Residue Measurements. The residues of aldrin and its conversion products, as well as the total residues in all samples, are shown in Table II. The residues, in ppm, are based on fresh weight of the plant samples. With the soil samples, the residues are based on dry weight.

In the experiments in both countries, the concentration of radioactive products (ppm) was highest in the upper soil layer (0-10 cm from surface) where the radioactivity had been applied. It decreased with increasing depth. In plant samples, the radioactivity was higher in the peel and lower in leaves and peeled beets. The total residues in the peeled beets were 0.06 ppm in the sample from Germany and 0.05 ppm in the sample from England; in both cases, the radioactivity was mostly due to hydrophilic metabolites (0.05 ppm in the German sample and 0.04 ppm in the English sample).

The residues of the radioactive products at the soil surface were higher in England than in Germany, whereas the deeper soil layers (10-60 cm from the surface) contained only very small residues in England and more radioactivity in the German experiment, indicating a greater leaching of radioactivity in the German soil used. This was likely to be due to higher rainfall in Germany (Table I) and to the soil type.

Conversion Rates. The total conversion of aldrin to metabolites amounted to more than 40% of the total radioactivity present in the soil samples and to more than 95% in the plant samples. The percentages of unchanged aldrin in the total residues were highest in the two upper soil layers (0-10 and 10-20 cm from surface), namely 52% of the total residue in the German surface sample and 58% of the total residue in the English surface sample. Although the absolute amount of hydrophilic metabolites decreases with soil depth, the portion of hydrophilic metabolites, including unextracted material, in relation to that of the aldrin increases and constitutes nearly half of the total residue in the deepest soil layer. In the plant samples, aldrin amounted to less than 5% of the total residue; in the beet peels most of the radioactivity was due to dieldrin, whereas in the peeled beets and the leaves more than 80% of the total radioactivity was due to hydrophilic metabolites.

CONCLUSIONS

It is shown that after soil application of aldrin, hydrophilic breakdown products may occur in peeled beets in concentrations which may exceed the concentrations of aldrin and dieldrin. Consequently, we expect these same breakdown products to occur in all samples grown in agricultural practice following aldrin application. Whereas aldrin and dieldrin residues are determined routinely by established analytical methods, these hydrophilic products escape analytical detection because of their physical and chemical properties. Thus, it is possible that only less than half of the total residue from aldrin application is detected by general residue analysis.

However, although these experiments were undertaken outdoors, the persistence of aldrin was greater than is found following normal agricultural practice. The residue levels found in the present work may, therefore, be somewhat higher than in normal field samples.

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