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Fate of Chloroalkylene-9-^{14}C in Carrots, Sugar Beets, and Soil Under Outdoor Conditions

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Abstract. Immediately after application of chloroalkylene-9-^{14}C to soil (1.32 ppm, based on dry weight of soil in the upper layer of 0 to 10 cm) under outdoor conditions, carrots were sown; in the following year, sugar beets were grown. About 80% of the radioactivity applied volatilized within one vegetation period. Most of the remaining radioactivity was still in the upper soil layer; 0.8% had dispersed to a depth of 40 cm, and 3.3% was taken up by the carrot plants. In the second year, no more decrease of soil residues was observed; uptake by sugar beets was 0.1% of the applied radioactivity.

In the first year, the residues in the upper soil layer consisted of 41% unchanged chloroalkylene-9, 19% soluble metabolites, and 40% unextractable residues; the amount of unextractable residues rose to 68% in the second year. The following conversion products were characterized in the soil extracts: a monohydroxylated dichlorobiphenyl, a monomethoxylated dichlorobiphenyl, and two isomeric monohydroxylated, monoisopropylated dichlorobiphenyls; in carrot roots, a monomethoxylated dichlorobiphenyl was detected. Conjugates occurring in the soil yielded, after acid hydrolysis, a monohydroxylated dichlorobiphenyl among other compounds.

A series of potential substitutes for polychlorobiphenyls (PCBs) in commercial applications contain alkyl groups in lower chlorinated biphenyls, which are aimed to facilitate the biodegradation of the molecule. Two types of products have been synthesized for practical use, the chloroalkylenes (isopropylated chlorobiphenyls; Patent 1972) and alkylated chlorodiphenyl ethers (Anonymous 1975). Chloroalkylenes undergo conversions to phenols and other products, either by irradiation (Kotzias et al. 1977, Ruzzo et al. 1976) or by metabolism in different organisms (Begum et al. 1975, Moza et al. 1977). Since the chloroalkylenes contain the PCB-skeleton in their molecule, the investigation of their behaviour in agricultural soil and crops under outdoor conditions seemed to be necessary. This paper deals with the fate of chloroalkylene-9, a mixture of 2,4′-dichlorobiphenyl and its mono-, di-, and triisopropylated derivatives. The mixture was used in ^{14}C-labeled form (synthesized by Sandrock et al. 1978). Carrots were chosen as a model for accumulators of lipophilic xenobiotics. In the second

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year, sugar beets were used as a model for a root crop with high water and low oil content.

Materials and Methods

Plants were grown in a water-resistant plywood box (60 × 60 × 60 cm) with a perforated base. The box was placed in a metal tray to collect the leached water. The outside of the box was wrapped in aluminium foil to prevent temperature increase from direct sunlight. The bottom of the box (2.5 cm) was layered with pebbles of about 2.5 cm diameter which in turn were covered with a layer of well rotted peat. The box was filled with 160 kg of soil to one cm from the top and was kept in a large pit with the upper surface of the soil level with the surrounding ground. Analysis of the soil: sand, 52.2%; silt, 34.5%; clay, 13.3%; organic matter, 0.3%; pH 6.8. The soil was allowed to settle for about four weeks before treatment with chloroalkylene-9-^{14}C and sowing. Potassium dihydrogenphosphate and calcium ammonium nitrate were applied to soil as fertilizers at agricultural rates.

In 1975, 46.0 mg chloroalkylene-9-^{14}C in acetone was applied dropwise on the soil surface and was incorporated to a 10 cm depth (initial concentration 1.32 ppm in dry soil). Carrot seeds (''Rote Riesen'') were sown in three rows. Air temperature (mean of weekly maxima, 31°C; mean of weekly minima, 13°C) and rainfall (287 mm) were recorded during normal vegetation period (5 months).

The following year (1976), sugar beets were sown in the soil used for the experiment with carrots. After one month of germination, only four sugar beets were allowed to grow in the box for five months (the others were harvested prematurely and stored at −20°C until analyzed). Air temperature (28°C/ 8°C) and rainfall (199 mm) were recorded during the vegetation period (5 months). During both vegetation periods, the leached water was checked for radioactivity.

Working up of Plant Material and Soil

There was a normal growth and yield of plants at the time of harvest. Soil samples (approximately 1 kg each) were taken at depths of 0–10, 10–20, 20–30 and 30–40 cm immediately after harvest. Each sample was obtained by mixing 20 cores taken with an auger. These samples were stored at −20°C until analyzed. The moisture content of soil samples was determined by drying to a constant weight in a vacuum desiccator at room temperature. The roots and leaves were analyzed separately. The roots and leaves were homogenized and extracted continuously with methanol for 48 hrs. respectively. Soil samples were soxhleted with methanol for 48 hr.

The radioactivity in extracts and in the leaching water was determined by counting in a liquid scintillation counter (Packard, Tri-Carb Mod. 3375 or 3380, scintillator based on dioxane; Table 1). Unextracted radioactivity in soil and plants was determined by combustion (Oxymat, Intertechnique),

Table 1. Distribution of radioactivity in soil, carrots and sugar beets (in % of applied radioactivity) after soil treatment with chloroalkylene-9-^{14}C

Experiment	Soil				Leached water	Crop root	Leaves	Weeds	Total
	0–10	10–20	20–30	30–40 cm					
Carrots (1st Year)	16.6	0.5	0.2	0.1	nd[a]	3.2	0.1	0.1	20.8
Sugar Beets (2d Year)	21.3	0.7	1.6	1.0	0.1	0.1	<0.1	—	24.8

[a] nd = not detected

followed by trapping $^{14}CO_2$ in a toluene-based scintillator containing phenethylamine, and liquid scintillation counting (Packard, Tri-Carb 2425). The total radioactivity of each sample was obtained by addition of extracted and unextracted radioactivity (Table 1).

For the determination of the ratio of chloroalkylene-9 and its conversion products, the individual extracts were concentrated in a rotary evaporator. The extracts were subjected to TLC analysis on silicagel plates in benzene/ethyl acetate (9:1). Zones of one cm were removed from the plates, and the radioactivity of each zone was counted in a liquid scintillation counter (Packard, Tri-Carb 3375 or 3380, scintillator based on dioxane).

Isolation and Identification of Conversion Products

The methanolic extracts of soil samples (0–10, 10–20, 20–30 and 30–40 cm) were combined and concentrated. The combined concentrated extract was separated into four radioactive zones on preparative silicagel plates (20 × 20 cm) using benzene/ethyl acetate, 9:1 (Table 2). Zone IV, which moved with the front on the plate, was found to be chloroalkylene-9 upon TLC. After repeated TLC purifications it was comparable to an authentic sample analyzed by gas chromatography (GC).

Zones II and III were combined together and prepared for the isolation of the metabolites. The main metabolite (60% of these zones, Rf 0.27 in hexane), after further purification on TLC, was subjected to gas chromatograph-mass spectrometer (GC-MS) analysis. Another group of substances in these zones was difficult to separate on a TLC plate. Other analytical methods for the separation of these compounds were not used because of an insufficient quantity of sample material. This group of compounds was subjected to GC-MS analysis after methylation with freshly prepared diazomethane.

The polar fraction (Zone I) was hydrolysed with 9 N H_2SO_4 at 70°C for 8 hr, diluted with water and extracted with ether. The hydrolysate, on TLC examination, was found to be a mixture of substances. The major one (50%) was purified on TLC and methylated with CH_2N_2 and subjected to GC-MS.

The methanol extract of carrots was analyzed similarly to the soil extracts. The leaves' extract did not contain enough radioactivity for isolation and identification of conversion products. In the second year crop (sugar beets), the isolation and identification of the conversion products in various extracts was not undertaken, because the chromatographic behavior was the same as in the case of the first year crops (carrots).

Results and Discussion

Balance of Total Radioactivity

In the first year of study (1975), 17.4% of the total applied radioactivity was found in the soil while 3.3% was taken up by carrots (Table 1). The radioactivity in the soil was dispersed to a 40 cm depth; no radioactivity was found in the leaching water. The recovery was 20.8%, which means that 79.2% of the applied activity was lost by volatilization. The loss of radioactivity can be attributed to physical factors and/or to microbial influences and plant evaporation, rather than to the application method, since the compound solution was not applied by spraying. Since PCBs were detected in air samples (Bidleman and Olney, 1974), it is possible that the volatilized radioactivity included the unchanged 2,4′-dichlorobiphenyl (a major compound of the technical product); to our knowledge, nothing is known on the volatilization of phenolic derivatives that are formed either by irradiation or by metabolism. The major residues were found at the application site (soil, 0 to 10 cm depth, 16.6% of the applied radioactivity). In the second year (1976), 24.6% of the applied radioactivity was found in the soil, 0.1% in sugar

beets and 0.1% in the leaching water (Table 1). Within one year's time, water-soluble radioactive substances had reached leaching water. The total amount recovered was 24.8%. Although each soil sample was a mixture of 20 cores taken with an auger, the higher value of residues obtained in the second year is probably due to inhomogeneous distribution of the radioactive compounds in the heavy soil that was treated.

The uptake of radioactivity by carrot roots (3.2% of the applied radioactivity) was much higher than by sugar beet roots (0.1%). The difference is greater than expected from the differences between the corresponding soil concentrations. This agrees with the fact that carrots, due to their oil content, tend to take up lipophilic soil constituents.

Conversion and Residues

The radioactivity recovered from soils and plants consisted of unchanged chloroalkylene-9, of soluble conversion products and of unextractable radioactive residues (with organic solvent). One growing season after application of chloroalkylene-9 to soil (0–10 cm depth), 40.7% of the radioactivity present was unchanged chloroalkylene, 18.9% was soluble metabolites, and 40.4% was unextractable residues. In the second year, the unextractable residue was 67.9%. Thin-layer chromatography of the individual extracts (silicagel, benzene/ethylacetate, 9:1) showed four radioactive zones. Tables 2 and 3 show the concentrations of these zones in carrots, sugar beets, and soil, together with chloroalkylene-9 and unextractable residues. For the plant samples, ppm are based on fresh weight; for soil samples, ppm are based on dry weight determined by drying in a vacuum desiccator at room temperature to constant weight.

Identification of Metabolites

Identification of metabolites was carried out with the soil and carrot extracts of the first vegetation period.

Table 2. Residues of chloroalkylene-9-^{14}C and metabolites in carrots and soil (ppm equivalent to chloroalkylene-9, based on dry weight for soil and fresh weight for plants; see text), TLC, solvent 10% ethyl acetate in benzene

Sample	Zone I (conjugates)	Zone II (phenols and phenolethers)	Zone III	Zone IV (chloroalkylene-9)	Unextrac.[a]	Total
Soil (0–10 cm)	0.011	0.003	0.022	0.078	0.077	0.19
Soil (10–20 ″)	<0.001	<0.001	<0.001	<0.001	0.005	0.006
Soil (20–30 ″)	<0.001	<0.001	<0.001	<0.001	0.002	0.002
Soil (30–40 ″)	<0.001	<0.001	<0.001	<0.001	0.001	0.002
Carrots	0.090	0.024	0.187	0.632	0.021	0.954
Leaves	0.139	0.016	0.015	0.079	0.071	0.320
Weeds	0.853	0.013	0.021	0.005	0.248	1.14

[a] unextractable with organic solvent

Table 3. Residues of chloroalkylene-9-^{14}C and metabolites in sugar beets and soil (ppm equivalent to chloroalkylene-9, based on dry weight for soil and fresh weight for plants; see text), TLC, solvent 10% ethyl acetate in benzene

Sample	Zone I (conjugates)	Zone II (phenols and	Zone III phenolethers)	Zone IV (chloroalkylene-9)	Unextrac.[a]	Total
Soil (0–10 cm)	0.012	0.002	0.018	0.052	0.173	0.26
Soil (10–20 ″)	0.001	nd[b]	<0.001	0.002	0.005	0.008
Soil (20–30 ″)	0.007	<0.001	0.002	0.007	0.002	0.018
Soil (30–40 ″)	0.002	nd	0.002	0.005	0.003	0.012
Sugar Beets	0.024	nd	<0.001	0.006	0.002	0.032
Leaves	0.001	0.003	0.001	0.009	0.014	0.028

[a] unextractable with organic solvent
[b] nd = not detected

In the soil extract, a major substance (metabolite A, 60% of zone II and zone III, Table 2), Rf 0.27 (plate run with n-hexane), gave, on GC-MS analysis, a molecular ion at m/e 252 ($C_{13}H_{10}OCl_2$) with fragment ions at 237 and 209. This fragmentation is typical of methyl ethers of hydroxychlorobiphenyls.

The other compounds (together about 40% of zone II and III) were identified by GC-MS analysis after methylation with CH_2N_2, as methyl ether of hydroxy-2,4′-dichlorobiphenyl (metabolite B), and two isomers of methyl ether of hydroxymonoisopropyl derivative (metabolites C and D). The fragmentation of these compounds was in accordance with the usual fragmentation of methyl ethers of hydroxychlorobiphenyls. The formation of these products suggests that an oxygen atom is introduced in the molecule of 2,4′-dichlorobiphenyl and monoisopropyl 2,4′-dichlorobiphenyl, giving hydroxy-products which are methylated with CH_2N_2 to their corresponding ethers.

The polar fraction (zone I) was a group of conjugates which, upon hydrolysis, were shown to contain, among other unidentified substances, an oxygenated compound (the major one) of 2,4′-dichlorobiphenyl (GC-MS analysis). Comparison with the free hydroxylated product identified was not possible because of an insufficient amount.

Carrot roots extract contained among many unidentified substances a methoxyderivative of 2,4′-dichlorobiphenyl (identified by GC-MS analysis). This compound was comparable by TLC to metabolite A isolated from soil extract. The metabolites from carrot leaves could not be identified due to the small amounts available, but they should be the same as those in carrot roots. In Figure 1, the formula of identified conversion products of chloroalkylene-9 in soil and carrots are depicted.

The metabolites from the second year's crop (sugar beets) and soil were not isolated and identified, because the TLC behavior of the extracts was the same as that of the extracts of the first year crop. Contrary to carrots, in sugar beets the metabolite residues exceeded those of the unchanged chloroalkylene, as observed for 2,2′-dichlorobiphenyl (Moza et al., 1976).

The figures in Table 2 and 3 indicate that carrots, in spite of their high lipid content, accumulate the unchanged chloroalkylene-9 (0.632 ppm) from soil only

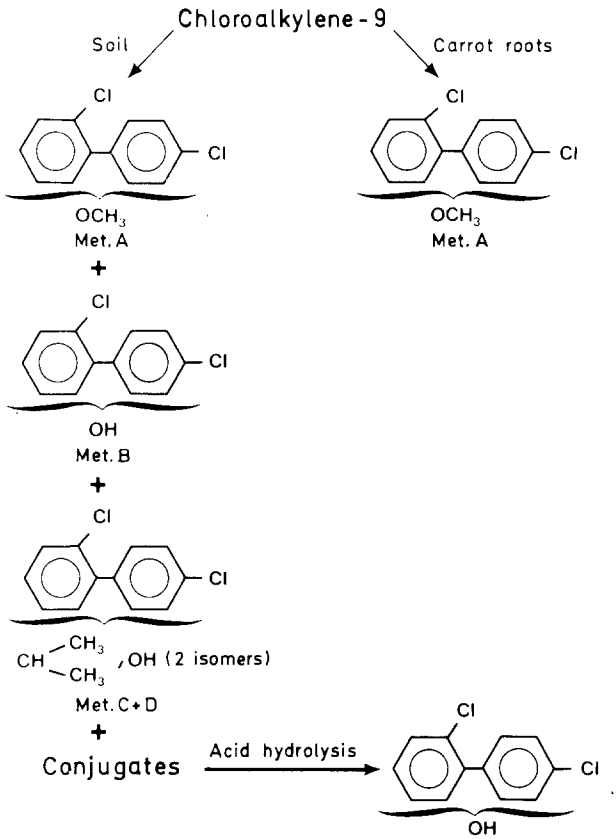

Fig. 1 Conversion of chloroalkylene-9 in carrots and soil

by a factor of 8, while sugar beets do not accumulate and even contain much lower residue concentrations than the surrounding soil (for beets based on fresh weight; for soil on dry weight).

Conclusion

Chloroalkylene-9 is volatilized rapidly from soil. It is converted to phenolic substances, both in soil and carrots, as well as to unextractable residues which occur preferably in the soil and the plant leaves. The major residues in carrots is unchanged chloroalkylene-9 (0.632 ppm), whereas in sugar beets hydrophilic metabolites are most prevalent (0.024 ppm). Compared to isomers of commercial PCBs, the residues in soil after one growing season are lower than those of 2,2'-dichlorobiphenyl. After the second year, however, the residues of chloroalkylene-9 in soil are comparable or even higher than those of 2,2'-dichlorobiphenyl. Residues in carrots and sugar beets were higher for chloroalkylene (Moza et al., 1976). Comparisons between chloroalkylene-9 and tri- and pentachlorobiphenyl will be made in further studies.

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