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Studies with 2,4′,5-Trichlorobiphenyl-^{14}C and 2,2′,4,4′,6-Pentachlorobiphenyl-^{14}C in Carrots, Sugar Beets, and Soil

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2,4′,5-Trichlorobiphenyl-^{14}C (1.28 kg/ha) and 2,2′,4,4′,6-pentachlorobiphenyl-^{14}C (1.12 kg/ha) were applied each to soil in a lysimeter-type box under outdoor conditions, and carrots were grown. In the following year, sugar beets were grown without retreatment. For the trichlorobiphenyl, only 32.5% of the applied radioactivity was recovered in soil and plants after the first season; 67.5% was lost due to volatilization, uptake by carrot plants was 3.1% of the applied radioactivity. The radioactivity remaining in the soil was partly dispersed to a depth of 40 cm and consisted of 78.7% trichlorobiphenyl, 1.6% soluble conversion products, and 19.7% unextractable residues; in the second year, total recovery as well as the portion of unchanged parent compound decreased. Uptake by sugar beets was only 0.2%. The soluble conversion products in plants and soil were identified as oxygenated metabolites. For the pentachlorobiphenyl, total recovery was 58.5%, and loss due to volatilization 41.5%, uptake by crops 1.4% (after first season), and conversion below 1%; no metabolites were identified.

Polychlorinated biphenyls (PCB's) are still widely distributed pollutants. The fate of these compounds in different ecosystems has attracted much interest. The metabolic conversion of PCB's by various organisms to phenols or their methyl ethers is well known (Block and Cornish, 1959; Hutzinger et al., 1972, 1974; Moza et al., 1973, 1974, 1976a; Herbst et al., 1976; Safe et al., 1974, 1975; Goto et al., 1974a,b; Greb et al., 1975a,b; Lay et al., 1975; Sundström et al., 1975). Some of the phenols are reported to be more toxic than the parent compounds (Yamamoto and Yoshimura, 1973; Yoshimura and Yamamoto, 1973). The accumulation and formation of phenols of a lower chlorinated biphenyl in food crops (Moza et al., 1976b) led us to investigate the total balance of accumulation and the conversion of higher chlorinated isomers in agricultural soil and crops.

The present paper records the balance and conversion of 2,4′,5-trichlorobiphenyl and 2,2′,4,4′,6-pentachlorobiphenyl in carrots (accumulators for lipophilic xenobiotics) in the first year and in the second year sugar beets (a root crop with low oil and high water content).

EXPERIMENTAL SECTION

Apparatus. Packard liquid scintillation counters (Tri-Carb Model 3380 and 3375) with external standardization were used for assaying radioactivity in various extracts. The ^{14}C in insoluble soil and plant residues was determined by liquid scintillation counting after the sample was oxidized to $^{14}CO_2$ in an Oximat (Intertechnique). Radioactive substances were located on TLC plates by scanning, using a scanner supplied by Berthold-Frieseke GmbH, Karlsruhe. An LKB Model 9000 GLC–MS, from LKB Produkter, Bromma, Sweden, was used for mass spectrometry. A 2.0 m × 0.4 cm i.d. glass column packed with 1% OV-1 on Chromosorb W-AW-DMCS 80–100 mesh was used for gas chromatographic separation. The column was programmed from 180 to 240 °C at a rate of 4 °C/min and helium was used as a carrier gas. The mass spectra were recorded at 70 eV.

Reagents. 2,4′,5-Trichlorophenyl-^{14}C (99% pure) and 2,2′,4,4′,6-pentachlorobiphenyl-^{14}C (99.6% pure) synthesized in this laboratory (Sandrock et al., 1978) were uniformly labeled. A scintillation solution based on dioxane was used for assaying various extracts and TLC zones. A toluene based scintillator containing phenethylamine was used for collection and counting $^{14}CO_2$. Thin-layer chromatographic plates were coated with silica gel G (Merck); ready made silica gel plates (Merck, 0.25 mm thick) were also used. For column chromatography silica gel Woelm 0.063–0.2 mm was used. For derivatization of metabolites, diazomethane was prepared from *p*-tolylsulfonylmethylnitrosamide and KOH in diethyl ether.

Procedure. *Plant Growing.* Plants were grown in water-resistant plywood boxes (60 × 60 × 60 cm), one for the experiment with 2,4′,5-trichlorobiphenyl-^{14}C and one for the experiment with 2,2′,4,4′,6-pentachlorobiphenyl-^{14}C. The boxes had perforated bases (to drain the excess water) and were placed in metal trays. They were wrapped with alumina foil to exclude heating by sunlight. About 2.5 cm of washed gravel covered with a layer of well-rotted turf were placed at the bottom of each box. Each box contained 160 kg of soil, 1 cm from the top, and was kept in a large pit with the upper surface at the soil level of the surrounding ground. Air temperature, humidity, and rainfall were recorded during vegetation periods. Table

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Table I. Cultural Details for Carrots and Sugar Beets Grown with 2,4',5-Trichlorobiphenyl-^{14}C and 2,2',4,4',6-Pentachlorobiphenyl-^{14}C

soil analysis	climate	1st year	2nd year	application	PCB-Cl_3[a]	PCB-Cl_5[b]
sand 52.2% silt 34.5% clay 13.3% organic matter 0.3% pH 6.8	rainfall, mm	287	119	rate of application	1.28 kg/ha (46.01 mg)	1.12 kg/ha (40.2 mg)
	temp °C (max)	31	28	sp act.	1.3 mCi/mmol	1.82 mCi/mmol
	temp °C (min)	13	8			

[a] 2,4',5-Trichlorobiphenyl-^{14}C. [b] 2,2',4,4',6-Pentachlorobiphenyl-^{14}C.

I records the summary of the climatic conditions as well as the analysis of soil. The soil in each box was allowed to settle for 4 weeks before application of the chemicals. Fertilizers (KH_2PO_4 and NH_4NO_3) were applied to the soil as in local agricultural practice.

2,4',5-Trichlorobiphenyl-^{14}C and 2,2',4,4',6-pentachlorobiphenyl-^{14}C were applied separately to two boxes in acetone solution dropwise on the soil surface and were thoroughly incorporated to a depth of 10 cm into the soil just prior to seeding. The application rate and specific activity of trichlorobiphenyl and pentachlorobiphenyl are also given in Table I. Three rows of carrot plants ("Rote Riesen") were grown in each box. After 4 months of vegetation period, the crops were harvested and stored at −20 °C until analyzed.

The next season, sugar beets were sown in the same boxes. After 1 month of germination, only four beets were allowed to grow for a period of 6 months. The other sugar beets were harvested prematurely and stored in a freezer until analyzed. During both vegetation periods, water drained from the boxes was collected and assayed for radioactivity.

Processing of Plant Material and Soil. There was a normal growth and yield of plants at harvest. Soil samples (800–1000 g) were taken at depths of 0–10, 10–20, 20–30, and 30–50 cm immediately after harvest. Each sample was obtained by mixing 20 cores taken with an auger. Analysis of individual cores of soil was not possible due to low levels of radioactivity present. The roots and leaves were analyzed separately. 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.

Soil samples were extracted continuously for 48 h with methanol. The finely chopped plant material was thoroughly homogenized and Soxhleted with methanol for 48 h. Quantitative determinations of ^{14}C in various extracts and leaching water were made by liquid scintillation counting. The data obtained were corrected by counting blanks of inactive extracts prepared in the same manner as the active ones. Insoluble ^{14}C in soil and plant residues was determined by the combustion of 200–250-mg samples after extraction and drying in a vacuum desiccator at room temperature. For the quantitative determination of 2,4',5-trichlorobiphenyl-^{14}C and 2,2',4,4',6-pentachlorobiphenyl-^{14}C, and their degradation products, the individual extracts were concentrated in a rotary evaporator. They were subjected to TLC analysis on silica gel plates in benzene/ethyl acetate (9:1). TLC zones of 1 cm were scraped off and suspended in dioxane scintillator for measuring radioactivity.

Isolation and Characterization of 2,4',5-Trichlorobiphenyl-^{14}C Metabolites. The combined methanolic extract of *soil samples* (0–50 cm) after concentration was applied on preparative silica gel plates (20 × 20 cm, 24 g of silica gel), and the plates were developed in hexane. The main radioactive zone (R_f 0.62 in hexane) was scraped off from the plate and eluted with ethyl acetate. This radioactive material after purification was found to be the parent compound upon GLC and GC–MS analysis. Because of low levels of radioactivity, the other peaks on the radiochromatograph were not well defined, hence the rest of the plate was scraped off and the radioactivity from the gel was eluted with methanol; after concentration it was rechromatographed on a small silica-gel plate (5 × 20 cm). Chromatography on the silica-gel or Al_2O_3 (neutral) plate did not resolve this zone which seemed to be a mixture of radioactive substances. After methylation with CH_2N_2 and many TLC purifications, this zone (R_f 0.62 in hexane, silica-gel plate) was subjected to GC–MS analysis and identified.

The methanol extract of *carrot roots* was concentrated, and the remaining residue was extracted with a methanol–diethyl ether mixture (1:1) several times. Eighty-five percent of the radioactivity present in the extract was recovered by this treatment, which separated the radioactivity from a thick mass of biological byproducts. The methanol–ether extract after concentration was worked like the soil extract. The metabolites were identified after methylation with freshly prepared diazomethane. Likewise, the methanol extract of *carrot leaves* after concentration was extracted with a methanol–ether mixture (1:1). More than 80% of the radioactivity present in the extract was recovered. The concentrated methanol–ether extract was analyzed like the carrot root extract. The polar fraction (10%) of the extracts having low levels of radioactivity was not subjected to any chemical or instrumental analysis. Also, isolation and identification of conversion products from sugar beets and from leaching water was not undertaken due to insufficient amounts. Since the quantity of conversion products of *2,2',4,4',6-pentachlorobiphenyl* in soil, carrots, and sugar beets (approximately 1%) is small in relation to the parent substance, identification of these products was also impossible.

RESULTS AND DISCUSSION

Balance of Radioactivity. In the case of 2,4',5-trichlorobiphenyl-^{14}C, only 32.5% of the ^{14}C applied to the soil was recovered in the first year (1975). 3.1% of the applied activity was taken up by the carrots, within 4 months. The radioactivity in the soil was dispersed to a depth of 40 cm while no radioactivity was found in the leaching water drained from the bottom of the box. More than 67% was lost by volatilization. The loss of radioactivity was due to the physical nature of the compound and/or to microbial influences rather than to the application method. Bidleman and Olney (1974) have reported the presence of trichlorobiphenyl in air samples, so the loss of trichlorobiphenyl-^{14}C due to volatilization is highly possible. The loss of phenols or phenolic derivatives, the main conversion products formed either by metabolism or

Table II. Distribution of Radioactivity in Carrots, Sugar Beets, and Soil (in Percent of Radioactivity Applied) after Soil Treatment with 2,4′,5-Trichlorobiphenyl-^{14}C

exp. with	soil (cm) 0-10	10-20	20-30	30-45	leached water	roots	leaves	weeds	total recov.
carrots (1st year)	28.6	0.3	0.3	0.1		2.7	0.4	0.1	32.5
sugar beets (2nd year)	26.3	1.2	0.5	0.4	0.2	0.2	<0.1		28.8

Table III. Distribution of Radioactivity in Carrots, Sugar Beets, and Soil (in Percent of Radioactivity) after Soil Treatment with 2,2′,4,4′,6-Pentachlorobiphenyl

exp. with	soil (cm) 0-10	10-20	20-30	30-45	leached water	roots	leaves	weeds	total recov.
carrots (1st year)	49.9	4.8	0.8	0.4		1.2	0.2	1.2	58.5
sugar beets (2nd year)	46.8	4.9	2.0	0.4	0.1	0.1	<0.1		54.3

Table IV. Residues of 2,4′,5-Trichlorobiphenyl-^{14}C and Metabolites in Carrots and Soil[a]

sample	zone I	zone II	zone III	zone IV (parent compd)	unext.	total
soil						
0-10 cm	0.002	0.001	0.002	0.249	0.059	0.313
10-20 cm	<0.001	<0.001	<0.001	0.001	0.001	0.002
20-30 cm	<0.001	<0.001	<0.001	0.001	0.001	0.002
30-45 cm	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
carrots	0.084	0.006	nd[b]	0.705	0.063	0.858
leaves	0.038	0.023	0.034	1.149	0.054	1.298
weeds	0.202	0.014	0.046	1.518	0.113	1.894

[a] Parts per million equivalent to 2,4′,5-trichlorobiphenyl; TLC, solvent: benzene/ethyl acetate (9:1). [b] Not detected.

irradiation due to volatilization is unknown to us. The major residues remained at the application site (0–10 cm depth; see Table II).

In the second year, total recovery was 28.8%; again most of the radioactivity was at the application site. Uptake by sugar beets was 0.2%. In case of 2,2′-dichlorobiphenyl, Moza et al. (1976b) observed that carrot roots accumulated more of the radioactivity, due to their high oil content which takes up lipophilic substances, in contrast to sugar beets; this holds good with 2,4′,5-trichlorobiphenyl also (2.7% by carrot roots and 0.2% by sugar beets).

In the case of 2,2′,4,4′,6-pentachlorobiphenyl-^{14}C, 58.5% was recovered in the first year (1975). The percentage of applied radioactivity found in the soil was 55.9, while 1.4% was taken up by carrots (Table III). No radioactivity was found in leaching water, though the soil layer 30–50 cm in the box contained low levels of radioactivity. Forty-one and a half percent of the applied radioactivity was lost due to volatilization. Since pentachlorobiphenyl has been found in air samples (Bidleman and Olney, 1974), it is highly possible that the volatilized radioactivity included unchanged 2,2′,4,4′,6-pentachlorobiphenyl. The volatilized radioactivity was not trapped for identification, which would have disturbed the natural condition of the system. The major residues of this compound were also found at the application site (0–10 cm).

In the second year (1976), 54.1% of the applied radioactivity was found in soil, 0.1% in sugar beets, and 0.1% in leaching water (Table II). Within a lapse of 1 year, radioactivity had reached the leached water. The total amount recovered was 54.3% and 45.7% loss of radioactivity by volatilization. Uptake of radioactivity in this case was again higher with carrots (1.4%) than with sugar beets (0.1%), which agrees with the general behavior of PCB's.

Conversion and Residues. The extracts of soils and plants were found to contain, besides trichlorobiphenyl and pentachlorobiphenyl, respectively, soluble metabolites and radioactive residues unextractable with organic solvents. One vegetation period after application of trichlorobiphenyl and pentachlorobiphenyl in two boxes separately to the soil (0–10 cm depth), 78.7% of the radioactivity present in the soil to a depth of 45 cm was 2,4′,5-trichlorobiphenyl, and 91.0% of the radioactivity was pentachlorobiphenyl. 1.6% and <1.0% were soluble metabolites of trichlorobiphenyl and pentachlorobiphenyl, respectively. Unextractable radioactive residues were 19.7% in the experiment with trichlorobiphenyl and 8.0% in the experiment with pentachlorobiphenyl. In the second year unextractable residues in the soil treated with trichlorobiphenyl fell to 15.5 and 7.3% in the soil treated with pentachlorobiphenyl, whereas the soluble metabolites rose to 8.0 and 1.0%, respectively. Thus, in both cases the total conversion has increased during the second year.

The soil samples from the second year after hot methanol extraction were divided into humin, humic acid, and fulvic acid fractions by the method reported by Kearney (1976). In the case of 2,4′,5-trichlorobiphenyl-^{14}C, out of 15.5% of unextractable radioactivity, 13.28% was found in the humin fraction, while 0.62 and 2.22% was found in the humic acid and fulvic acid fractions, respectively. Out of 7.3% of unextractable radioactivity, 3.06% was found in humin, 1.34% in humic acid, and 2.9% in fulvic acid fractions, respectively, in the case of 2,2′,-4,4′,6-pentachlorobiphenyl-^{14}C. Katan et al. (1976) have shown that pesticides and their conversion products can be bound to soil components and further Bollag et al. (1977) have shown that fungal enzymes polymerize phenols. It is assumed that phenols, the main metabolites of PCB's, are polymerized in soil and the oligomeric products are bound to soil components.

The soluble metabolite fraction from 2,2′,4,4′,6-pentachlorobiphenyl and 2,4′,5-trichlorobiphenyl was resolved into various radioactive zones on silica gel TLC plates. The concentration of these metabolites, together with the starting material and the unextractable residues, is shown in Tables IV and V for trichlorobiphenyl and Tables VI and VII for pentachlorobiphenyl. For the plant samples, parts per million are calculated on fresh weight; for soil sample, parts per million are based on dry weight. The tables show that radioactive residues are only relevant to the soil with a depth of 10 cm and to the crops, and that

Table V. Residues of 2,4',5-Trichlorobiphenyl-^{14}C and Metabolites in Sugar Beets and Soil[a]

sample	zone I	zone II	zone III	zone IV (parent compd)	unext.	total
soil						
0–10 cm	0.01	0.004	0.004	0.240	0.042	0.300
10–20 cm	<0.001	<0.001	<0.001	0.008	0.003	0.011
20–30 cm	<0.001	<0.001	<0.001	0.003	0.001	0.004
30–45 cm	<0.001	<0.001	<0.001	0.002	0.001	0.003
sugar beets	0.006	<0.001	<0.001	0.039	0.008	0.053
leaves	0.003	0.003	0.001	0.008	0.002	0.019

[a] Parts per million equivalent to 2,4',5-trichlorobiphenyl; TLC, solvent: benzene/ethyl acetate (9:1).

Table VI. Residues of 2,2',4,4',6-Pentachlorobiphenyl-^{14}C and Metabolites in Carrots and Soil[a]

sample	zone I	zone II	zone III	zone IV (parent compd)	unext.	total
soil						
0–10 cm	0.001	<0.001	<0.001	0.441	0.044	0.486
10–20 cm	0.001	<0.001	<0.001	0.033	0.005	0.039
20–30 cm	<0.001	<0.001	<0.001	0.005	0.002	0.007
30–45 cm	<0.001	<0.001	<0.001	0.002	0.001	0.003
carrots	0.072	0.009	0.006	0.407	0.009	0.503
leaves	0.022	0.003	0.005	0.426	0.016	0.472
weeds	0.273	0.037	0.075	12.024	<0.01	12.409

[a] Parts per million equivalent to 2,2',4,4',6-pentachlorobiphenyl; TLC, solvent: benzene/ethyl acetate (9:1)

Table VII. Residues of 2,2',4,4',6-Pentachlorobiphenyl-^{14}C and Metabolites in Sugar Beets and Soil[a]

sample	zone I	zone II	zone III	zone IV (parent compd)	unext.	total
soil						
0–10 cm	0.013	0.007	0.002	0.409	0.026	0.457
10–20 cm	0.001	<0.001	<0.001	0.040	0.005	0.047
20–30 cm	0.001	<0.001	<0.001	0.015	0.002	0.018
30–45 cm	<0.001	<0.001	<0.001	0.002	0.001	0.003
sugar beets	<0.001	<0.001	<0.001	0.025	0.002	0.027
leaves	0.002	0.001	<0.001	0.002	0.003	0.008

[a] Parts per million equivalent to 2,2',4,4',6-pentachlorobiphenyl; TLC, solvent: benzene/ethyl acetate (9:1).

only the unchanged PCB's are important as residues. However, even small quantities of conversion products need a chemical identification because of their possible toxicological or ecotoxicological effects.

The last columns of Tables IV–VII show the "unextractable residues". This term means that these residues could not be extracted from moist samples by continuous methanol extraction for 48 h and that other organic solvents were likewise inefficient. Since the free parent PCB's are fully extractable by these methods, it is anticipated that the unextractable residues represent the sorbed parent compound or conversion products.

Chemical Nature of Soluble Metabolites of 2,4',5-Trichlorobiphenyl. Identification of metabolites was carried out with soil extracts and carrot and carrot leaf extracts. A major radioactive zone (from combined zones II and III, Table IV) of the soil extract of the first year after methylation (90%, R_f 0.62 plate run in hexane) was subjected to GC–MS analysis. Four radioactive compounds A–D (Figure 1) in the sequence of elution were separated. A molecular ion at 344 ($C_{15}H_{11}O_3Cl_3$) and the occurrence of masses at 329 ($M^+ - CH_3$) and 301 ($M^+ - COCH_3$) with chlorine isotope distribution associated with the ions suggest that the molecule contains OCH_3 and $OCOCH_3$ groups (compound A). Mass spectrum of B showed a molecular ion at m/e 302 ($C_{13}H_9O_2Cl_3$). The fragments at 287 ($M^+ - CH_3$), 243 ($M^+ - CH_3 - (C{=}O) - CH_3 - H$) and 252 ($M^+ - CH_3 - Cl$) show that a hydroxy and a methoxy group were introduced into the parent molecule. The molecular ion at m/e 286 ($C_{13}H_9OCl_3$) and ions resulting from the loss of CH_3 (m/e 271), CH_3Cl (m/e 236) and CH_3CO (m/e 243) demonstrate that the compound C is a methoxy derivative of trichlorobiphenyl. The mass spectrum of compound D was identified by the characteristic isotope distribution pattern for three chlorine atoms at molecular ion at 302 ($C_{13}H_9O_2Cl_3$). The fragments were comparable to the fragments of compound B. The behavior on the GLC column suggests that compounds B and D are isomers. Since hydroxylation of PCB's is the major metabolic pathway, it is assumed that 2,4',5-trichlorobiphenyl gets mono- and dihydroxylated, these are acetylated in soil (compound A), totally or partially methylated by CH_2N_2 upon analysis (compounds B, D, and E). The partial methylation of the dihydroxy derivative is due to steric effects.

The combined zones II and III (Table V) of *carrot* extract showed a single zone of radioactive substances (R_f 0.42) in hexane on scanning after methylation with CH_2N_2. This peak on GC–MS analysis was shown to consist of a monomethoxy and dimethoxy derivative of trichlorobiphenyl. The monomethoxy derivative was identified by a mole peak at m/e 286 ($C_{13}H_9OCl_3$) and the fragments were identical with metabolite C isolated from soil. The mass spectrum of the dimethoxy derivative compound E showed a molecular ion at m/e 316 ($C_{14}H_{11}O_2Cl_3$). The ions at 301, 285, and 273 suggest that two oxygen atoms are introduced into the molecule, giving a dihydroxy product, which was methylated upon analysis. It is assumed that this dihydroxy product is sterically different from that in soil since methylation was completely achievable.

In *carrot leaves*, the main metabolite identified was a dihydroxy derivative of trichlorobiphenyl (R_f 0.07, in hexane). The mass spectrum of the methylated product

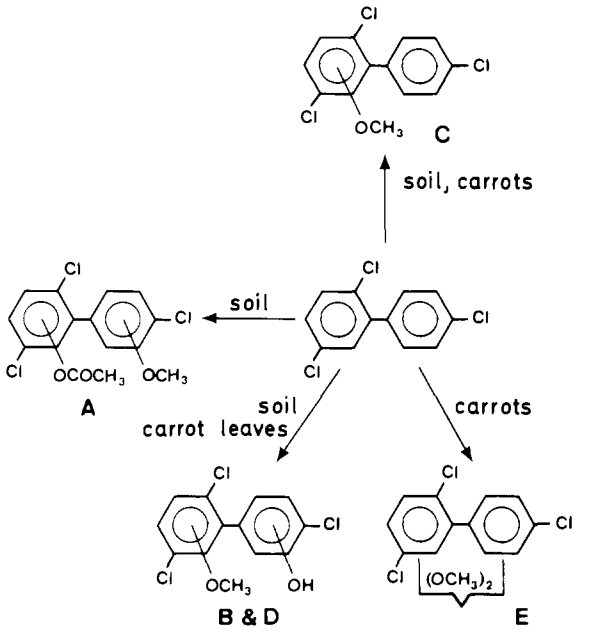

Figure 1. Methylated conversion products of 2,4′,5-trichlorobiphenyl in soil, carrots, and carrot leaves.

(R_f 0.62, in hexane) was similar to the MS of metabolite B. TLC behavior of this compound suggests that it is an isomer of the compound isolated from carrots.

Two isomers of a phenol were the main metabolites identified in the *soil extract from the second year*. The disappearance of the dihydroxy and acetoxy derivatives is probably due to its diffusion in the plant and/or in the *leaching water*, or to further degradation resulting in unidentified polar (zone I of Table V) or unextractable products; also, complete microbial degradation cannot be excluded.

Due to low levels of radioactivity in the *sugar beet extract* and *leaching water*, isolation and identification of the conversion products was not undertaken. The same applies to the conversion products of *2,2′,4,4′,6-pentachlorobiphenyl*. Figure 1 shows the proposed formula of the conversion products of 2,4′,5-trichlorobiphenyl in soil, carrots, and carrot leaves.

Accumulation of Residues in Plants. Carrots accumulate the unchanged 2,4′,5-trichlorobiphenyl from the soil by a factor of 2.8 (Table IV). Sugar beets on the other hand show no bioconcentration of 2,4′,5-trichlorobiphenyl or its metabolites (Table V).

The figures in Tables V and VI show that neither carrots nor sugar beets show any biomagnification of 2,2′,4,4′,6-pentachlorobiphenyl.

CONCLUSIONS

2,4′,5-Trichlorobiphenyl volatilizes more rapidly than 2,2′,4,4′,6-pentachlorobiphenyl from soil. The conversion of 2,4′,5-trichlorobiphenyl to soluble as well as to unextractable metabolites is higher than that of 2,2′,4,4′,6-pentachlorobiphenyl. This agrees well with the general behavior of polychlorinated biphenyls showing an increasing persistence with increasing number of chlorine atoms. Uptake of residues by crops on the other hand is higher for trichlorobiphenyl. The trichlorobiphenyl isomer is metabolized to phenolic products (Figure 1). Metabolism of pentachlorobiphenyl is only insignificant.

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