Metabolism of 2, 2'-Dichlorobiphenyl-^{14}C in Two Plant-Water-Soil-Systems

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Since residues of PCB have been found in water (1) and in aquatic plants (2) it is of interest to study their metabolism in aquatic plants. Recently, we reported on metabolism of 2, 2'-dichlorobiphenyl-^{14}C after foliar applicati in a marsh plant (3). It was shown that the compound was partly metabolize to monohydroxy derivatives which were mostly conjugated. This paper pres results of two studies with the same dichlorobiphenyl, on its conversion in ecosystems of aquatic plants growing submerged in water and rooted in soil

Experimental.

Application and Working Procedure.

Two higher plant species, Ranunculus fluitans and Callitriche spec., were selected for the study. Ranunculus fluitans and Callitriche spec., which gr submerged in water, were collected, and recultured under phytotron condit (day and night cycle 18/6 hrs., temp. 25°/8°C, relative humidity 65/95 %) rooted in soil and submerged each in about 2 l of tap water. After one week 2, 2'-dichlorobiphenyl-^{14}C (4), spec. activity 2.0 mC/mmol, dissolved in a minimum quantity of acetone, was applied to the water medium (13.7 ppm i Ranunculus water and 14.5 ppm in Callitriche water, respectively). The water level in the experiment jars was kept constant, and the jars were coo with flowing tap water.

Two parallel blank experiments were carried out, one with tap water in contact with the same soil as used for the plant experiment, and or with the river water where the plants had been collected.

Four weeks after application of the compound, water, plants, a soils were worked up. The plants were homogenized and extracted continuo with methanol for 48 hours. From a spiked homogenate, more than 95 % of radioactivity were recovered using this extraction method. Soils were soxh first with ether and then with methanol for 48 hours respectively. The wate after acidification to pH 2, was extracted continuously with ether.

The radioactivity of the extracts was determined by liquid scintillation counting (Tri-Carb 3380 and 3375, Packard; liquid scintillator based on dioxane). For further analysis, the extracts were concentrated

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with a rotary evaporator at room temperature and separated on TLC (10 % ethylacetate in benzene). The TLC-layers were cut into 1 cm zones which we counted in a liquid scintillation counter (liquid scintillator based on dioxane). Unextractable residues were determined by automatic combustion and liquid scintillation counting ("Oximat", Intertechnique, scintillator based on toluene).

Isolation of Metabolites.

For the isolation of metabolites from the water where the plants had been grown, the concentrated ether extracts were separated on TLC into four radioactive zones (10 % ethyl acetate in benzene, Tables 2 and 3). Each of th zones was examined for metabolites.

Zones I and II were purified several times on TLC, methylated with distilled diazomethane in ether, repurified, and examined by GLC/MS.

Zone III was resolved into 4 radioactive components on TLC (plate run first with 25 % benzene in n-hexane and then with benzene). Each of the active components from this zone was examined by GLC, methylated with distilled diazomethane, rechromatographed on TLC, and examined by GLC/MS.

Zone IV, after purification, was subjected to GLC/MS and turned out to be the unchanged parent compound.

For the isolation of metabolites from the plants, the methanolic extract were concentrated and separated on TLC (Silica gel, 10 % ethylacetate in benzene), into 4 radioactive zones (Tables 2 and 3). Zones 2 and 3 occurred only in small amounts and were not investigated further. Zone 1 was separat into two main zones by further TLC. The less polar zone was repurified, methylated with distilled diazomethane in ether, and repurified on TLC. The hydrophilic zone was hydrolyzed with 9 N H_2SO_4 at 40°C for 24 hours. The solution was extracted with ether. The ether extract was separated on TLC (5 % CH_3 OH in CH_2Cl_2) into two radioactive compounds which were further purified on TLC, methylated with diazomethane in ether, and repurified on TLC.

GLC of the purified products was performed with Packard gaschromatc graph, series 7400, with EC-detector, glass column, 200 cm, 0.4 cm in dia meter, OV_1 1 % on chromosorb G-AW-DMCS, 80-100 mesh, carrier gas nitrogen, flow rate 50 ml/min, injector and detector temp. 220°C and 240°C respectively. Column temperature 185°C, if not otherwise stated. The peaks were collected with a fraction collector attached to the GLC-apparatus in tubes containing anthracene. The tubes were checked for radioactivity by liquid scintillation counting. The mass spectra were taken with a gaschromat graph-mass spectrometer LKB 9000 from LKB-Producter, Bromma, Swede

Results and Discussion.

Quantitative analyses.

27.3 % of the applied radioactivity could be accounted for upon analyzing the plants, soil, and water from the experiment with Ranunculus fluitans, and 26.4 % from the experiment with Callitriche spec.. In the blank experiment with river water, 12.7 % were recovered, and in the blank experiment with tap water and soil, 13. 1 %.
The distribution of radioactivity in the samples from the two plant experiments is given in Table 1. From this table it is apparent that the uptake of the PCB is higher by Ranunculus fluitans as compared to Callitriche spec.. The percentages of radioactivity detected in both plant species demonstrate a high concentration factor, at least as compared to the aqueous medium. Ranunculus fluitans concentrated the radioactive compounds from 0.37 ppm in the water (at time of working up) up to 301 ppm in the plants, and Callitriche from 0.30 ppm up to 86.7 ppm, corresponding to concentration factors of 814 and 289, respectively. The soils, too, adsorbed a considerable amount of radioactive products from the water, and concentrated them up to 2.3 and 2.9 ppm (concentration factors 6 and 10). Thinlayer chromatography of the individual extracts showed four radioactive zones, the percentages of which are given in Table 2 for the experiment with Ranunculus fluitans, and in Table 3 for Callitriche spec..

The conversion of 2, 2' -dichlorobiphenyl was less than 1 % in the blank experiment with river water. Analysis of the tap water in contact with soil showed a conversion of 15.9 % zone I, 3.0 % zone II, and 6.6 % zone III; in this soil, the total conversion was only 1.2 %. These results indicate that the metabolites detected in the water medium, partly are formed by soil microorganisms, and released into the water. However, the portion of metabolites formed by the plants is considerable since the total metabolism rates shown in Tables 2 and 3 are higher than in the blanks. This is in good agreement with the conversion which was found after foliar application of the same PCB-isomer to Veronica beccabunga (3).

Identification of metabolites.

In the water where the plants had been cultured, the following metabolites were identified or characterized in the 4 zones of Tables 2 and 3.

Zone I: Analyses carried out so far with this fraction suggest it to be a mixture of compounds; the major one (60 % of the zone) is probably a fully dechlorinated product.

Zone II: This polar substance was methylated with diazomethane, GLC/MS-analysis gave a mol peak at 282 ($C_{14}H_{12}O_2Cl_2$) with 2 Cl-Cluster. Because of the very small quantity and impurities, fragmentation was inconclusive. Probably, the compound is the dimethyl-ether of a dihydroxy derivative of 2, 2' -dichlorobiphenyl.

Zone III: This zone was found to be a mixture of four radioactive substances when subjected to further TLC separation. The amount of the least polar substance was insufficient for mass spectrometric analysis The radioactive substance next to it in polarity (14 % of zone III), in GLC, showed a broad curve characteristic for a polar compound. Methylation of th compound changed the broad curve into a well defined peak (R_t 17.2 min. at 150°C). Mass spectrometry analysis gave a spectrum with 2 Cl-Cluster, mo peak at 252 ($C_{13}H_{10}OCl_2$, 100 %). The fragments are at 237 (M^+-CH_3, 7.6 % 217 (M^+-Cl, 3.6 %), 209 (M^+-CH_3-CO, 22.0 %), 202 (M^+-Cl-CH_3, 8.4 %), 174 (M^+-Cl-CH_3-CO, 4.3 %), 173 (M^+-CH_3-CO-HCl, 17.0 %), and at 139 (M^+-2 Cl-CH_3-CO, 27.6 %). The fragments of this compound suggest that it is a methyl ether of a mono-hydroxy derivative of dichlorobiphenyl. The third substance (21 % of zone III), which was somewhat more polar than the second one, had the same mass spectrum with differences in peak intensities only: mol peak at 252 (100 %), fragments at 237 (2.4 %), 217 (11.5 %), 209 (31.7 %), 202 (18.6 %), 174 (4.7 %), 173 (13.7 %), and 139 (32.8 %), and in GLC-retention time (R_t 19.4 min. at 150°C). These differences suggest that they are isomers. Both were also found as conjugate in plant leaves after foliar application (3).

The nature of the fourth compound from this zone could not be elucidated.

Zone IV was found to be identical with standard 2, 2' -dichlorobiphenyl by TLC and GLC analysis.

In the plants, only zone I was sufficient for GLC/MS analysis. It consisted of an unknown product and of several conjugates. These, after acid hydrolysis, yielded two monohydroxy derivatives which turned out to be identical to those isolated from the water.

Conclusions.

Under the experimental conditions used, 2, 2' -dichlorobiphenyl proved to be non-persistent. It is metabolized (Fig. 1) to at least two monohydroxy derivatives and probably one dihydroxy derivative and a dechlorinated product. The monohydroxy derivatives occur in free form, as well as conjugated in th plant tissues.

Table 1. Distribution of Radioactivity in Plants, Water, and Soil, 4 Weeks after Application of 2, 2' -Dichlorobiphenyl-^{14}C to Water (in % of applied radioactivity).

Experiment	Ether extract of water	Ether extract of soil	Methanol extract of soil	Methanol extract of plants	Unextractable radioactivity, sum of all fractions	Total radioactivity
Ranunculus fluitans	2.2	6.2	0.2	17.7	1.0	27.3
Callitriche spec.	1.9	15.0	0.6	7.6	1.3	26.4

Table 2. Conversion of 2, 2' -Dichlorobiphenyl-^{14}C in Water, in Ranunculus fluitans, and in Soil, 4 Weeks after Application to Water (in % of the radioactivity of each sample). TLC, solvent: 10 % ethylacetate in benzene.

Sample	Zone I	Zone II	Zone III	Zone IV (unchanged PCB)
Ether extract of water	54.6	1.1	10.7	33.6
Methanol extract of plants	2.6	0.4	0.4	96.6
Ether extract of soil	1.8	0.1	1.9	96.1
Methanol extract of soil	18.2	2.0	7.5	72.2

Table 3. Conversion of 2, 2' -Dichlorobiphenyl-^{14}C in Water, in Callitriche spec., and in Soil, 4 Weeks after Application to Water (in % of the radioactivity of each sample). TLC, solvent: 10 % ethylacetate in benzene.

Sample	Zone I	Zone II	Zone III	Zone IV (unchanged PCB)
Ether extract of water	42.6	2.4	7.2	47.7
Methanol extract of plants	12.0	0.5	2.4	85.0
Ether extract of soil	2.0	4.3	n.d.	93.6
Methanol extract of soil	10.8	0.7	4.8	83.6

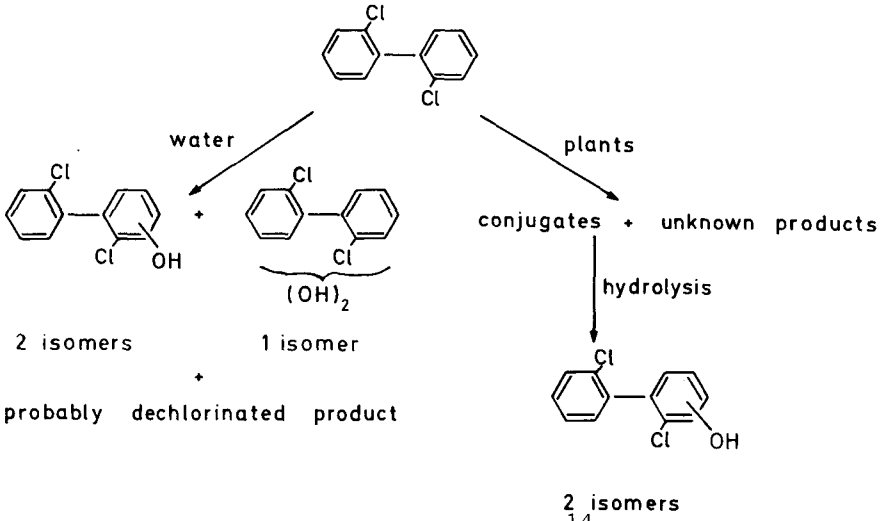

Fig. 1. Metabolism of 2, 2'-Dichlorobiphenyl-^{14}C in Water and Plants.

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