ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 17, 157–166 (1989)

Kinetics of the Uptake of ^{14}C-Labeled Chlorinated Benzenes from Soil by Plants

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Received March 3, 1988

[^{14}C]Benzene, [^{14}C]1,2,4-trichlorobenzene, [^{14}C]pentachlorobenzene, and [^{14}C]hexachlorobenzene were applied to soils in outdoor lysimeters to a 10-cm depth (2 mg/kg dry soil); barley and cress plants were grown for one vegetation period and analyzed after varying time intervals. The bioaccumulation factors (concentration of radioactive substances in plants divided by that in soils) of barley were higher than those of cress, except for hexachlorobenzene. In barley, bioaccumulation factors increased with decreasing chlorine content of the molecules, except for benzene, whereas in cress hexachlorobenzene exhibited the highest bioaccumulation factor. The conversion ratios of chlorinated benzenes (percentage of conversion products based on total radioactivity in plants) were negatively correlated to the chlorine content of the molecules and, in barley, positively correlated with time; in general, they were higher in barley than in cress. The concentration of radioactive substances in the plants, as well as bioaccumulation factors, decreased with time, except for a slight increase in benzene-derived residues in barley after 125 days. This effect is due to growth dilution. The percentage of radioactivity in barley seeds, based on that in the whole plant, was negatively correlated to the chlorine content of the molecule.

INTRODUCTION

Benzene and its chlorinated derivatives are widespread environmental pollutants. Benzene is used as a parent compound for various industrial syntheses and as a solvent and fuel additive. It is emitted from certain landfills and occurs in air and water (Haag and Korte, 1982); from there it may be transported to the soil as a contaminant. 1,2,4-Trichlorobenzene, in addition to its industrial use as a solvent and as an intermediate in syntheses, is applied to soils as an insecticide (termites) and for the treatment of waste water channels (Römpps, 1973). Its formations from benzene during the chlorination of water (Höfler *et al.*, 1983) and from polyethylene and sodium chloride during waste incineration (Lahaniatis *et al.*, 1981) are further sources of its occurrence in the environment. Pentachlorobenzene and hexachlorobenzene are used as flame retardants and are formed as byproducts in various industrial processes and, also, during water disinfection by chlorine (Höfler *et al.*, 1983) and during waste incineration (Lahaniatis *et al.*, 1981). Pentachlorobenzene is formed in soil as a metabolite of the fungicide pentachloronitrobenzene (Kamal *et al.*, 1983) and of the insecticide lindane (Kohli *et al.*, 1976). Hexachlorobenzene was formerly used as a wheat seed dressing in agriculture (FAO/WHO, 1970) and has been identified as an impurity in five pesticides; 135 further pesticides have a potential for containing hexachlorobenzene. Twenty-six industrial compounds are known or suspected to be associated with hexachlorobenzene as a by-product (Tobin, 1986). Due to its chemical stability, it is a world-wide ubiquitous environmental contaminant.

0147-6513/89 $3.00

Although some data are available on the uptake of these contaminants by higher plants (Wallnöfer and Königer, 1974; Smelt and Leistra, 1974; Häfner, 1975; Scheunert *et al.*, 1983; Topp *et al.*, 1986), the kinetics and mechanisms of their uptake from soil into plants have not been investigated. However, information on this subject is essential with respect to the increasing number of contaminated areas with potential use for crop growing. For hexachlorobenzene and pentachlorobenzene, it was shown that the bioaccumulation factors (concentration in plants divided by concentration in soil) in barley decreased with time (Topp *et al.*, 1986). Although growth dilution was assumed to be the reason for this decrease, no further investigations were reported. In the present paper, the kinetics of the uptake of ^{14}C-labeled benzene, 1,2,4-trichlorobenzene, pentachlorobenzene, and hexachlorobenzene are presented.

MATERIALS AND METHODS

^{14}C-Labeled benzene was purchased from Amersham. ^{14}C-Labeled 1,2,4-trichlorobenzene (Attar *et al.*, 1982), pentachlorobenzene (Müller *et al.*, 1978), and hexachlorobenzene (Sandrock *et al.*, 1978) were synthesized in this Institute and purified by suitable chromatographic methods up to a purity of >99%. Before use, the ^{14}C-labeled chemicals were mixed with commercially available inactive compounds, resulting in an application rate of about 200 μCi per lysimeter and a concentration in soil of about 2 mg/kg dry soil in a 10-cm depth (benzene, 2.00 mg/kg; 1,2,4-trichlorobenzene, 2.02 mg/kg; pentachlorobenzene, 2.03 mg/kg; hexachlorobenzene, 2.20 mg/kg).

The composition of the soil used was as follows: particle size distribution: clay (<2 μm) 33.6%, silt (2–63 μm) 27.4%, sand (0.06–2 mm) 32.4%, coarse matter (>2 mm) 6.6%; organic C: 2.06%, pH 6.4.

Outdoor experiments simulating field conditions were carried out in lysimeters of 60 × 60 × 70 cm filled with soil and placed into a large pit such that the surface of the soils was at the same level as the surrounding ground. The soil was treated with ^{14}C-labeled chemicals, and barley and cress seeds were sown. Further details of these experiments are given in Klein *et al.* (1973) and in Scheunert *et al.* (1986). By the example of aldrin it was shown that residues in this experimental unit were within the variation limits of field experiments (Scheunert *et al.*, 1977).

Climatic conditions during the experimental period were determined as follows. For the determination of air temperature, relative air humidity, and atmospheric pressure, a meteorograph from Lambrecht (Göttingen, Federal Republic of Germany) was used; precipitations were recorded with a standardized apparatus (according to DIN 58667B) with a 200-cm^2 surface. After the application (middle of May), during the second half of May, mean maximal and minimal air temperatures were 8 and 22°C, respectively; in June they were 11 and 24°C; in July, 13 and 25°C; in August, 12.5 and 24.5°C; and in the first half of September, 12 and 23.5°C, respectively. The relative air humidity varied between 40 and 100%, air pressure between 970 and 990 mbar. During the experimental time, rainfall amounted to about 300 mm.

Barley plants, along with soil corresponding to the depth of the roots, were sampled after 10–12 (8 plants), 31–33 (8 plants), 69–71 (6–8 plants), and 124–125 (9–12 plants) days; cress plants with soil, after 10–12 (about 100–140 plants), 31–33 (about 100–140 plants), and 77–79 (200–260 plants) days. Except for the last harvest where the seeds were analyzed separately, all plants were analyzed on the whole.

Total ^{14}C residues in soils were determined after extraction of samples with methanol in a soxhlet for 48 hr. Plants were homogenized with an Ultra Turrax prior to

TABLE 1

RESIDUE CONCENTRATIONS AND BIOACCUMULATION FACTORS OF RADIOACTIVE SUBSTANCES IN BARLEY PLANTS AFTER TREATMENT OF SOIL WITH ^{14}C-LABELED CHLORINATED BENZENES UNDER OUTDOOR CONDITIONS

Substance applied	Time of exposure (days)	Concentration of ^{14}C (μg/g)[a]	Bioaccumulation factor[b]
Benzene	12	0.18	17
	33	0.02	2.3
	71	0.02	2.9
	125	0.03	4.6
1,2,4-Trichlorobenzene	11	9.3	36
	32	2.0	16
	70	0.35	5.8
	124	0.23	4.3
Pentachlorobenzene	11	26	8.4
	32	4.6	4.2
	70	0.96	1.8
	125	0.45	1.1
Hexachlorobenzene	10	29	4.8
	31	11	1.7
	69	2.9	0.62
	124	1.4	0.42

[a] Micrograms calculated as parent compounds, per gram dry plant matter.
[b] Concentration of ^{14}C, calculated as parent compounds, in dry plant matter, divided by concentration of ^{14}C in dry soil.

soxhlet extraction. Radioactivity in extracts was determined by liquid scintillation counting (Berthold/Frieseke Betaszint BF 8000) using a dioxane-based scintillation liquid. Unextractable ^{14}C was determined in a Packard Tri-Carb B 306 automatic sample oxidizer. $^{14}CO_2$ was absorbed in Packard Carbo-Sorb/Permafluor V 8:12 and counted in a liquid scintillation counter. Radioactivity per plant was calculated by dividing total radioactivity in each sample by the number of plants.

The conversion ratios in the methanol extracts of the plants of the benzene experiment were not determined due to very low radioactivity. Conversion ratios of the chlorinated derivatives were determined as follows. In order to avoid volatilization losses of the unchanged parent compounds during the concentration of the water-containing methanol extracts, the parent compounds were transferred to *n*-hexane by shaking the methanol extracts three times with *n*-hexane (1:1). Both the dried hexane and the methanol extracts were evaporated to dryness in a rotary evaporator with a vacuum at 30–40°C. The residues of both extracts were combined, dissolved in *n*-hexane, and extracted with 0.1 *N* NaOH (50 ml hexane solution three times with 20 ml 0.1 *N* NaOH), resulting in an aqueous NaOH solution containing polar metabolites (phenolic conversion products and water-soluble conjugates) and in a hexane solution containing parent compounds and nonpolar metabolites (such as anisoles). The radioactivity of both solutions was determined in a liquid scintillation counter.

TABLE 2

RESIDUE CONCENTRATIONS AND BIOACCUMULATION FACTORS OF RADIOACTIVE SUBSTANCES IN CRESS PLANTS AFTER TREATMENT OF SOIL WITH ^{14}C-LABELED CHLORINATED BENZENES UNDER OUTDOOR CONDITIONS

Substance applied	Time of exposure (days)	Concentration of ^{14}C (μg/g)[a]	Bioaccumulation factor[b]
Benzene	12	0.22	10
	33	0.04	2.3
	79	0.02	1.9
1,2,4-Trichlorobenzene	11	2.3	11
	32	0.27	3.3
	78	0.07	1.5
Pentachlorobenzene	11	8.7	2.1
	32	2.9	1.4
	78	0.68	0.74
Hexachlorobenzene	10	96	15
	31	24	5.5
	77	6.6	2.6

[a] Micrograms, calculated as parent compounds, per gram dry plant matter.
[b] Concentration of ^{14}C, calculated as parent compounds, in dry plant matter, divided by concentration of ^{14}C in dry soil.

The nonpolar hexane fractions were separated into parent compounds and nonpolar metabolites. For 1,2,4-trichlorobenzene, gas–liquid chromatography was used. The hexane extract was dried with Na_2SO_4 and injected into a Hewlett-Packard Model 3880A gas chromatograph, capillary column glass, 12 m, stationary phase OV101; N_2 40 ml/min; detector ^{63}Ni ECD; temperature: column 50–200°C, program 3°C/min; injector 260°C; detector 250°C. The retention time was 13.8 min. For pentachlorobenzene and hexachlorobenzene, thin-layer chromatography was used (plates 5 × 20 cm, ready-coated with Merck (Darmstadt) silica-gel 60 F_{254}; layer thickness 0.25 mm; solvent *n*-hexane; Rf values for pentachlorobenzene, 0.75, and for hexachlorobenzene, 0.80).

RESULTS AND DISCUSSION

Differences in Bioaccumulation between Plant Species

In Table 1, the residue concentrations and bioaccumulation factors of radioactivity in barley plants, derived from [^{14}C]benzene, [^{14}C]1,2,4-trichlorobenzene, [^{14}C]-pentachlorobenzene, and [^{14}C]hexachlorobenzene, are presented; in Table 2 are those of radioactivity in cress plants. It can be seen that the bioaccumulation factors (expressed as concentration of ^{14}C, equivalent to parent compounds, in dry plant matter divided by concentration of ^{14}C in dry soil) are higher for barley than for cress, except for hexachlorobenzene. These differences might be due to differences in the chemical nature of plant constituents, which gives the cress plant a higher affinity for lipophilic xenobiotics. Analogous differences between barley and cress have been

TABLE 3

CONVERSION RATIOS OF ^{14}C-LABELED CHLORINATED BENZENES IN BARLEY PLANTS (IN % OF TOTAL RADIOACTIVITY PRESENT IN PLANTS) AFTER UPTAKE FROM SOIL (2 mg/kg DRY SOIL) UNDER OUTDOOR CONDITIONS

Substance applied	Time of exposure (days)	% Of total radioactivity present in plants		
		Parent compound[a]	Polar metabolites	Bound residues
Benzene	12	82.2		17.8
	33	n.d.[b]		100
	71	49.3		50.7
	125	72.4		27.6
1,2,4-Trichlorobenzene	11	19.5	53.3	27.2
	32	5.5	58.4	36.1
	70	5.3	36.2	58.5
	124	6.4	25.2	68.4
Pentachlorobenzene	11	65.4	25.4	9.2
	32	25.7	39.2	35.1
	70	14.5	39.9	45.6
	125	13.8	28.1	58.1
Hexachlorobenzene	10	95.6	3.6	0.8
	31	93.8	3.4	2.8
	69	88.3	5.2	6.5
	124	66.3	13.7	20.0

[a] Including small amounts of nonpolar metabolites.
[b] n.d., none detected.

observed in laboratory experiments, where chlorinated benzenes such as 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3,5-tetrachlorobenzene, and pentachlorobenzene had higher bioaccumulation factors in barley than in cress, whereas hexachlorobenzene and other very lipophilic chemicals such as kepone, dieldrin, 2,4,6,2′,4′-pentachlorobiphenyl, and *p,p′*-DDT were accumulated more in cress (Topp, 1986). Smelt and Leistra (1974) also found significant differences for the uptake of hexachlorobenzene between different plant species. They tested seven species and found the highest bioaccumulation factors in carrot and grass roots.

Differences in Bioaccumulation between Chemicals

In barley (Table 1), concentration of radioactive residues in plants increased with the increasing number of chlorine in the molecule; in relation to soil residues (= bioaccumulation factor, right column), they decreased, with the exception of the unsubstituted benzene which did not fit this correlation. In cress (Table 2), there was a strong positive correlation between chlorine content of molecules and radioactive residues in plants, the difference for one more chlorine atom being nearly one power of 10. In terms of bioaccumulation factors, hexachlorobenzene, also, had the highest value. Similar correlations were observed in laboratory experiments (Scheunert, 1986). In Table 2, pentachlorobenzene exhibits the lowest bioaccumulation factor. The reason for this lower bioaccumulation potential is not known.

TABLE 4

CONVERSION RATIOS OF ^{14}C-LABELED CHLORINATED BENZENES IN CRESS PLANTS (IN % OF TOTAL RADIOACTIVITY PRESENT IN PLANTS) AFTER UPTAKE FROM SOIL (2 mg/kg DRY SOIL) UNDER OUTDOOR CONDITIONS

Substance applied	Time of exposure (days)	% Of total radioactivity present in plants		
		Parent compound[a]	Polar metabolites	Bound residues
Benzene	12	25.2		74.8
	33	n.d.[b]		100
	79	32.2		67.8
1,2,4-Trichlorobenzene	11	12.4	81.0	6.6
	32	42.2	36.4	21.4
	78	33.2	26.0	40.8
Pentachlorobenzene	11	89.1	4.1	6.8
	32	78.6	12.3	9.1
	78	86.7	3.6	9.7
Hexachlorobenzene	10	99.2		0.8
	31	99.3		0.7
	77	97.7	1.0	1.3

[a] Including small amounts of nonpolar metabolites.
[b] n.d., none detected.

Conversion Ratios

The radioactive residues in plants were extracted, and both the extracted and the unextractable radioactivities were determined. The extracted radioactivity was separated by chromatographic methods, and the conversion ratios were established. In Tables 3 and 4, the amounts of parent compounds, polar metabolites, and bound residues, expressed as percentages of total radioactivity present in plants, are recorded. Nonpolar metabolites were also detected; since their amount was very low (0–3.3%), they are included in the columns of parent compounds. Only in the case of 1,2,4-trichlorobenzene in cress did they amount to 14% of total radioactivity present in plants. Since the aim of this study was primarily to investigate the uptake kinetics of parent compounds, the chemical nature of metabolites was not established. According to previous work (Zhou *et al.*, unpublished), the nonpolar metabolite fraction of pentachlorobenzene in barley contains pentachloroanisole. For benzene and, to a smaller extent, for 1,2,4-trichlorobenzene, radioactive residues in plants may also contain ^{14}C assimilated from $^{14}CO_2$ formed in soil by mineralization; in closed aerated laboratory systems it was shown that [^{14}C]benzene formed 62% $^{14}CO_2$ within 1 week, and [^{14}C]1,2,4-trichlorobenzene, 0.5% (Scheunert and Korte, 1986).

From Tables 3 and 4, it is apparent that the ratios of conversion of chlorinated benzenes to polar metabolites in barley were negatively correlated to the chlorine content of the molecules; in barley, they increased with time. In general, they were higher in barley than in cress. For the unsubstituted benzene, the differentiation be-

TABLE 5

ABSOLUTE AMOUNTS OF RADIOACTIVITY IN BARLEY PLANTS, TAKEN UP FROM SOIL TREATED WITH ^{14}C-LABELED CHLORINATED BENZENES (2 mg/kg DRY SOIL) UNDER OUTDOOR CONDITIONS

Substance applied	Time of exposure (days)	Nanograms radioactivity per plant[a]			
		Parent compound[b]	Polar metabolites	Bound residues	Total ^{14}C
Benzene	12	2.75		0.596	3.35
	33	n.d.[c]		9.21	9.21
	71	27.6		27.5	54.2
	125	78.2		29.8	108
1,2,4-Trichlorobenzene	11	36.7	100	51.1	188
	32	20.0	212	131	363
	70	49.1	335	542	926
	124	47.8	188	511	747
Pentachlorobenzene	11	395	153	55.6	604
	32	417	635	569	1621
	70	389	1071	1224	2684
	125	258	524	1084	1866
Hexachlorobenzene	10	680	25.6	5.69	711
	31	4419	160	132	4711
	69	8824	520	649	9993
	124	3973	821	1198	5992

[a] Calculated as parent compounds.
[b] Including small amounts of nonpolar metabolites.
[c] n.d., none detected.

tween parent compound and conversion products in the extracts could not be performed due to very low radioactivity present in the extracts.

Time Course of Bioaccumulation

When considering the time course of residues in the plants, it is obvious that the concentration of radioactive substances in dry plant matter, as well as bioaccumulation factors, decreased with time, except for a slight increase in benzene-derived residues in barley after 125 days (Tables 1 and 2). This effect is due to growth dilution, since the absolute amounts of radioactive substances in plants increased with time (Tables 5 and 6, last columns) except for the three chlorinated benzenes in barley between Days 70 and 125. In this period, plant growing declines, and the loss of radioactive compounds by evapotranspiration probably exceeds further uptake from soil.

When looking at the absolute amounts of parent compounds, of extracted metabolites, and of bound residues, the increase in bound residues is most remarkable (Tables 5 and 6, columns 5). It is obvious that the uptake of radioactive substances from soil, as well as their conversion and binding in plants, continues during the growth of the plants; however, continuing increase in plant mass results in a decrease in residue concentration.

TABLE 6

ABSOLUTE AMOUNTS OF RADIOACTIVITY IN CRESS PLANTS, TAKEN UP FROM SOIL TREATED WITH ^{14}C-LABELED CHLORINATED BENZENES (2 mg/kg DRY SOIL) UNDER OUTDOOR CONDITIONS

Substance applied	Time of exposure (days)	Nanograms radioactivity per plant[a]			
		Parent compound[b]	Polar metabolites	Bound residues	Total ^{14}C
Benzene	12	0.132		0.392	0.524
	33	n.d.[c]		1.33	1.33
	79	1.02		2.16	3.18
1,2,4-Trichlorobenzene	11	0.582	3.80	0.310	4.69
	32	3.28	2.83	1.66	7.77
	78	3.52	2.76	4.32	10.6
Pentachlorobenzene	11	16.6	0.763	1.26	18.6
	32	58.8	9.20	6.81	74.8
	78	91.0	3.78	10.2	105
Hexachlorobenzene	10	174		1.40	175
	31	745		5.25	750
	77	1005	10.3	13.4	1029

[a] Calculated as parent compounds.
[b] Including small amounts of nonpolar metabolites.
[c] n.d., none detected.

Transport of Residues to Plant Seeds

From the mature plants (after 124–125 days for barley, after 77–79 days for cress), the seeds were removed and analyzed separately for radioactive substances. For barley (Table 7), radioactive residues were lower in seeds than in the whole plants, except

TABLE 7

RADIOACTIVITY IN MATURE SEEDS OF BARLEY PLANTS GROWN IN SOIL TREATED WITH ^{14}C-LABELED CHLORINATED BENZENES (2 mg/kg DRY SOIL) UNDER OUTDOOR CONDITIONS

Substance applied	^{14}C in seeds			
	μg/g[a]	Bioaccumulation factor[b]	% Of total ^{14}C in plant	ng[c]/plant
Benzene	0.220	3.32	33.77	36.5
1,2,4-Trichlorobenzene	0.054	2.74	9.78	73.1
Pentachlorobenzene	0.057	0.52	4.40	82.1
Hexachlorobenzene	0.028	0.30	0.80	47.9

[a] Micrograms, calculated as parent compounds, per gram dry seed matter.
[b] Concentration of ^{14}C, calculated as parent compounds, in dry seed matter, divided by concentration of ^{14}C in dry soil.
[c] Calculated as parent compounds.

for benzene. However, for benzene-derived ^{14}C residues, the identity with unchanged benzene was not demonstrated; the occurrence of ^{14}C assimilated from $^{14}CO_2$ formed by mineralization in soil cannot be excluded. The bioaccumulation factors in seeds were lower than those in whole plants for all ^{14}C-labeled test chemicals. Radioactive residues, bioaccumulation factors, and the percentage of radioactivity in seeds, based on ^{14}C in total plants, were highest for benzene and lowest for hexachlorobenzene. Thus, the uptake of residues into seeds is negatively correlated to the chlorine content of the molecules and positively to water solubility and vapor pressure; both of these substance properties contribute to the transport of chemicals to the plant tops by pathways within and outside the plants. For cress, the correlation of residues in seeds with molecular properties is not clear. Except for 1,2,4-trichlorobenzene, transport of residues from the base of the plants to the seeds, expressed as percentage of radioactive residues in seeds based on total radioactivity in plants, was higher in barley than in cress. The absolute amounts of radioactive substances in seeds (Table 7, last column) depend on several factors that superimpose each other, such as root uptake, translocation within the plant, and transport of volatilized residues from soil to plant tops, resulting in values that are only indirectly correlated to the chemical structure of the chemicals.

CONCLUSION

It may be concluded that chlorinated benzenes are taken up by barley and cress plants during the whole growing period; however, the uptake is over-compensated by a higher increase in plant mass, resulting in a decrease in residue concentrations and in bioaccumulation factors. The substance amounts taken up into the plants depend both on the chemical structure and on the plant species.

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