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Formation and Fate of Bound Residues of [^{14}C]Benzene and [^{14}C]Chlorobenzenes in Soil and Plants

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Outdoor experiments with [^{14}C]hexachlorobenzene, [^{14}C]pentachlorobenzene, [^{14}C]1,2,4-trichlorobenzene, and [^{14}C]benzene in soil–crop systems indicate that the formation rate of bound residues in soil and plants, expressed as bound residues in percentage of total residue in the sample, decreases with increasing number of chlorine in the molecule and, thus, with increasing chemical stability. The time course of formation and fate of bound residues in soil and plants is characterized by a very slow decrease of residue levels in soil, indicating that biodegradation of bound residues hardly exceeds their reformation from the parent compound during one vegetation period, and by a decrease of residue levels in plants. The portion of bound residues as compared to the total residue increases with time, indicating that bound residues are more persistent than the parent compounds and their soluble metabolites; benzene is an exception. Cress plants, in general, contain less bound residues than do barley plants. Again, benzene is an exception. In deeper soil layers, soil-bound residues occur also. The ratio between bound and extractable residues does not differ to a larger extent between the soil layers.

INTRODUCTION

Nonextractable (or bound) chemical residues in soil and plants have been known for decades. However, intensive discussion concerning them started only in recent years when it became obvious that all chemicals lead to some strongly adsorbed or covalently bound portions in the biosphere, and that in many cases this portion exceeds the portion that is extractable and thus detectable by normal analysis. Nonextractable (or bound) pesticide residues have been defined by the IUPAC Pesticide Commission as "chemical species originating from pesticides used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues" (Klein and Scheunert, 1982).

That the tendency of a chemical to form bound residues in soil and plants depends upon chemical structure characteristics of the molecule has been reviewed recently (Klein and Scheunert, 1982). It is generally adopted today that this tendency is positively related to the susceptibility of the molecule to biological degradation and conversion and/or to its chemical reactivity in general. However, information on this topic has been obtained mostly by comparison of experiments differing in experimental conditions, like soil type, pesticide concentration in soil, and climatic conditions.

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The time course of formation of bound residues in soil has been studied in the laboratory (Lichtenstein *et al.*, 1977); information obtained under outdoor conditions is limited. Likewise, information is limited on the time course of formation of bound residues in plants; this topic is very complex since levels of bound residues in the plant mass are governed not only by the interactions between formation and degradation rates of bound residues in plants but also by the uptake kinetics of the parent compound or/and metabolites from the soil.

Although data are available on the dependence of the occurrence of soil-bound chemical residues upon soil depth, they also have not been obtained under comparable environmental conditions.

The study presented here was carried out to investigate the influence of chemical structure characteristics on the formation rate of bound residues in soil and plants, the time course of formation in soil and plants, and the occurrence of bound residues in deeper soil layers for a group of model compounds under identical experimental outdoor conditions. Since aromatic compounds probably constitute the bulk of pesticide-derived chemicals in agricultural soils, knowledge of the influence of substituents of the aromatic ring on the formation of bound residues is an essential precondition to assess total soil burden of bound chemicals after pesticide application. The fact that aromatic rings are also major constituents of natural humic substances gives further emphasis to the importance of aromatic compounds in the chemical composition of soil. Therefore, a series of aromatic compounds comprising a wide range of chemical reactivity and biological persistence, including benzene, 1,2,4-trichlorobenzene, pentachlorobenzene, and hexachlorobenzene, was selected for this study. Besides its suitability as a model substance, hexachlorobenzene is interesting with respect to its use as a seed dressing (Food and Agriculture Organization of the United Nations, World Health Organization, 1970). Pentachlorobenzene has been shown to be a metabolite of the insecticide lindane in soil and plants (Kohli *et al.*, 1976) and of the fungicide pentachloronitrobenzene in soil (Beck and Hansen, 1974) and plants (Begum *et al.*, 1979). 1,2,4-Trichlorobenzene has also been identified as a conversion product of lindane in plants (Kohli *et al.*, 1976), and benzene has been reported to be a conversion product of lindane in anaerobic soil (Beland *et al.*, 1976).

MATERIALS AND METHODS

The experiments were conducted under field-like outdoor conditions, as described earlier (Scheunert *et al.*, 1977). The plants were grown in boxes, 60 × 60 × 60 cm, constructed from water-resistant plywood. The bases of the boxes contained holes to permit the drainage of excess water which was collected in a metal splash tray. The boxes were wrapped in aluminum foil on the outside to prevent temperature increases due to sunlight. The bottoms of the boxes were packed with gravel (25 mm in diameter), and the stones were covered with a 25-mm layer of sand. The boxes were filled with the same soil to 1 cm from the top. The soil was allowed to settle for 1 month before planting. The box was sunk into a large pit so that the upper surface of the soil was at the same level as the surrounding ground. [^{14}C]Hexachlorobenzene, [^{14}C]pentachlorobenzene, [^{14}C]1,2,4-trichlorobenzene, and [^{14}C]benzene were mixed with the corresponding inactive compounds and incorporated in the soil, resulting in a soil concentration of about 2 ppm in the upper

layer (0–10 cm depth). In one-half of each box, barley grains were sown, and cress was sown in the other part of the box. This outdoor experimental setup has been shown to yield residue data which are within the range of field value variations (Scheunert *et al.*, 1977).

At specific time intervals, samples of both plant species as well as soil samples of the corresponding root zones were taken and analyzed for radioactivity. After the last harvest, soil samples were taken at definite depths (0–5, 5–10, 10–20, 20–30, and 30–40 cm), in order to assess the influence of soil depth upon the level of soil-bound residues.

For the determination of bound residues, the plants were homogenized in methanol with an Ultra-Turrax and then extracted with methanol in a Soxhlet for 48 hr. Fresh soils were also extracted in a Soxhlet for 48 hr. All unextractable residue data presented in this paper refer to this extraction method, with the exception of some soil samples containing [^{14}C]benzene residues, which were extracted with cold methanol; this is noted in the respective tables. After the extraction, the radioactivity in the extracts was determined by liquid scintillation counting. Unextractable radioactivity left in plant and soil samples was determined by combustion of the samples, followed by liquid scintillation counting of $^{14}CO_2$ trapped in a suitable scintillation cocktail.

RESULTS AND DISCUSSION

Influence of Chemical Structure on the Formation of Bound Residues in Soil and Plants

Figure 1 gives comparative data for unextractable residues in soil and plants, one vegetation period after the application of [^{14}C]hexachlorobenzene, [^{14}C]pentachlorobenzene, [^{14}C]1,2,4-trichlorobenzene, and [^{14}C]benzene into the soil, in micrograms equivalent to the parent compound per gram dry soil or plant material.

The formation of bound residues in soil and plants is negatively correlated to the number of chlorine atoms in the molecule (Klein and Scheunert, 1982). Contrary to this observation, Fig. 1, which presents absolute bound residue levels in terms of ppm, demonstrates a weak decrease of bound residues in soil with decreasing chlorine content of the chemicals and a strong decrease in plants. However, it should be kept in mind that these four substances are more or less volatile and that volatilization is a process which competes very effectively both with the process of binding in soil and with the uptake by plants; its rate may exceed both soil-binding rates and rates of plant uptake followed by binding in plants. Therefore, absolute concentration figures in this case are not suitable for comparative assessment of binding capacity of chemicals to soils or plants. The ratio between bound and soluble residues is a better way to compare the tendency of chemicals to form unextractable residues in soils or plants.

Therefore, Fig. 2 gives the same data as Fig. 1, not in terms of concentration figures but as bound residues in percentage of total residues in the respective sample. This figure shows a strong negative correlation between the degree of chlorine substitution of benzene and binding in soils and plants, with the exception of the value for benzene in barley, which does not fit this scheme. However, we should keep in mind that it is questionable whether benzene-derived ^{14}C taken up by plants is still the unchanged parent compound. It is probable that not benzene itself is

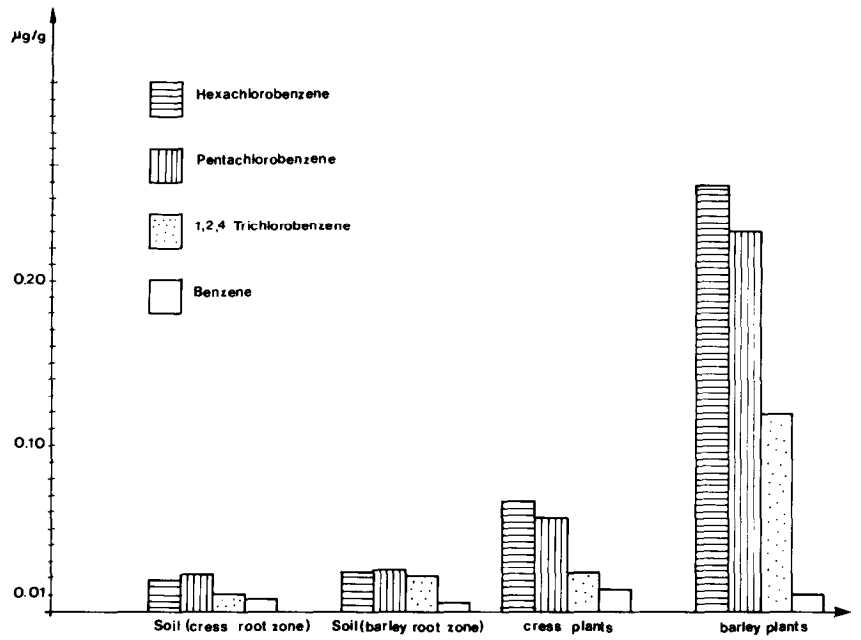

FIG. 1. Unextractable radioactive residues in soil and plants, one vegetation period after the application of [^{14}C]hexachlorobenzene, [^{14}C]pentachlorobenzene, [^{14}C]1,2,4-trichlorobenzene, and [^{14}C]benzene into the soil (in μg equivalent to the parent compound per g dry soil or plant material).

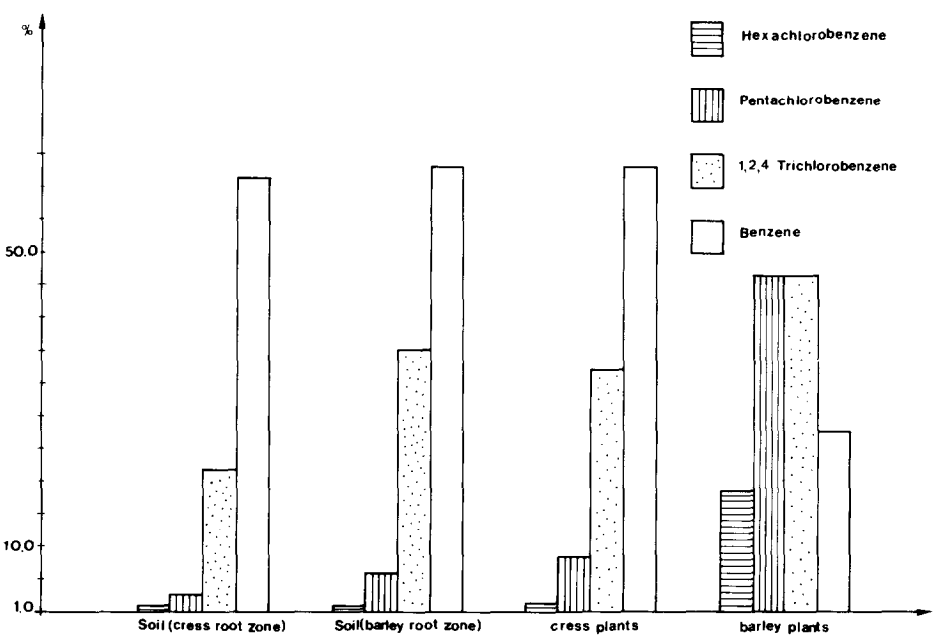

FIG. 2. Unextractable radioactive residues in soil and plants, one vegetation period after the application of [^{14}C]hexachlorobenzene, [^{14}C]pentachlorobenzene, [^{14}C]1,2,4-trichlorobenzene, and [^{14}C]benzene into the soil (in % of total ^{14}C of the respective sample).

absorbed by plants, but some degradation products of benzene in soil, which, of course, may have quite other plant-binding properties than benzene. The fact that cress plants do not show this irregularity is in accordance with the fact that the lipid content of this plant is up to 2% of dry mass. This means that these plants could have, besides the normal uptake route via the conduction channels, another possibility to take up unchanged lipophilic chemicals from soils and to accumulate them in oil cells, where they are not accessible to metabolism. It is interesting that the formation of plant-bound residues by chlorinated benzenes, a metabolic process, is much lower in cress than in barley, the differences being highest for hexachlorobenzene, a compound with very low water solubility, and lowest for 1,2,4-trichlorobenzene, with a higher water solubility. The formation of bound residues from benzene, however, is higher in cress than in barley.

Influence of Time on the Level of Bound Residues in Soil and Plants

In the following section, the time course of formation and fate of bound residues in soil and plants are discussed for each chemical separately.

Hexachlorobenzene. Figure 3 shows soil- and plant-bound residues of hexachlorobenzene in micrograms per gram dry weight at different time intervals. Since hexachlorobenzene is known as a very persistent compound, the levels of unextractable residues in soil, resulting from metabolic processes, are as low as expected. There is no distinct time dependence for soil-bound residues, indicating some kind of equilibrium between formation and degradation of bound residues. In barley, there is also a very slow decrease of bound residues although the decrease of total

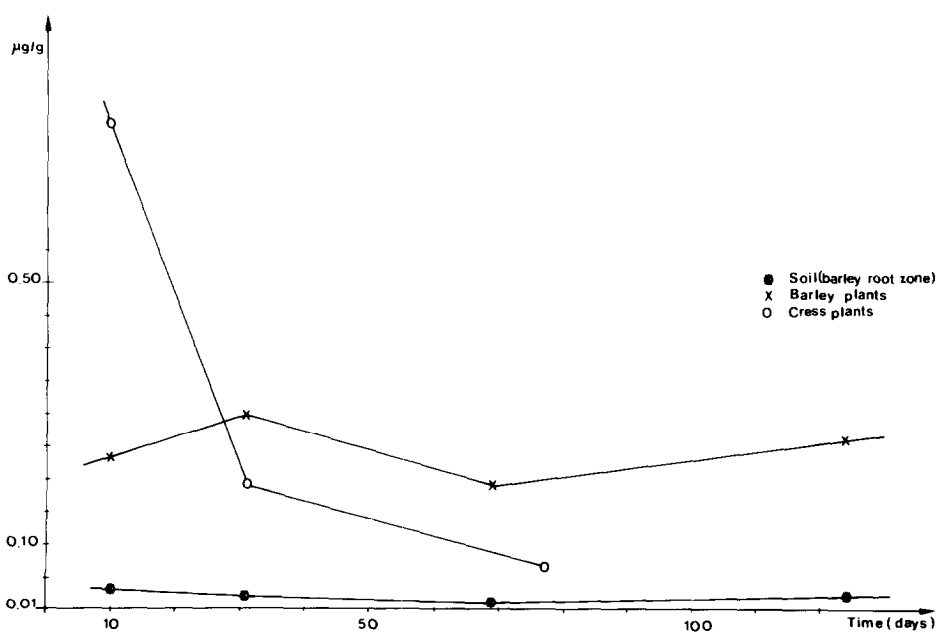

FIG. 3. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]hexachlorobenzene into the soil (in µg equivalent to the parent compound per g dry soil or plant material).

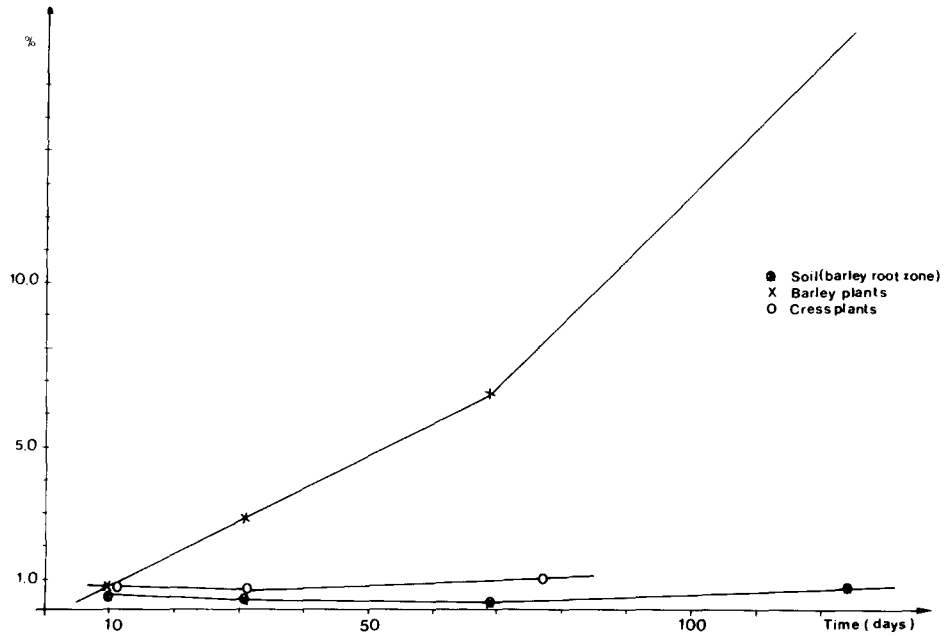

FIG. 4. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]hexachlorobenzene into the soil (in % of total ^{14}C of the respective sample).

residues including extractable radioactivity is very steep (E. Topp, unpublished work). In cress the unextractable residues decrease by one order of magnitude within the growing period of 79 days. In Fig. 4, the same data are expressed as relative figures, i.e., as bound residues in terms of percentage of total residue in each sample. The increase of the bound residues with time is less pronounced for soil and cress plants, but higher than one order of magnitude for barley. It seems that barley, similar to wheat (Scheunert *et al.*, 1983), is able to metabolize this persistent compound to a considerable extent by enzymatic activity.

Pentachlorobenzene. The levels of bound residues of pentachlorobenzene in soil (Fig. 5) are similar to those of hexachlorobenzene. Barley and cress exhibit a pronounced decrease of bound residue levels during the vegetation period, which parallels the decrease of total residue levels including extractable radioactivity (E. Topp, unpublished work). Both plants probably take up the chemicals mainly in their first growing stage; during further growth, the residues decrease. This might be due rather to "dilution" as a consequence of an increase in plant mass, offsetting the small uptake from soil in later growth stages, than to biodegradation. "Relative" bound residues, expressed as percentage of total residue of each sample, show a positive correlation with time for soil and both plant species (Fig. 6).

1,2,4-Trichlorobenzene. Bound residues of 1,2,4-trichlorobenzene, expressed as micrograms equivalent to the parent compound per gram dry weight (Fig. 7), in soil apparently do not change with time; in both plant species bound residues decrease with time. The relative data expressed as bound residues in percentage of total residue present in each sample increase with time (Fig. 8).

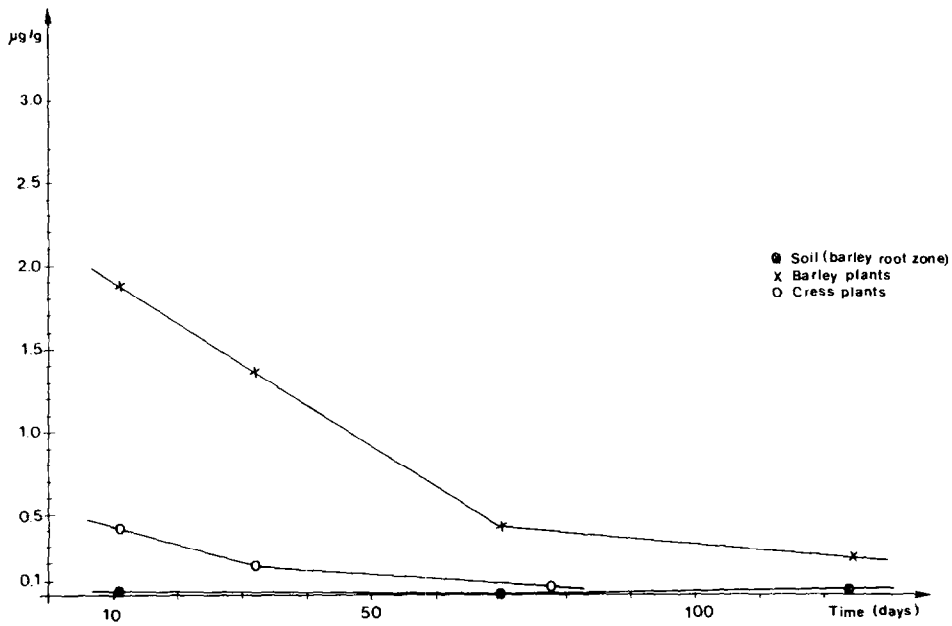

FIG. 5. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]pentachlorobenzene into the soil (in µg equivalent to the parent compound per g dry soil or plant material).

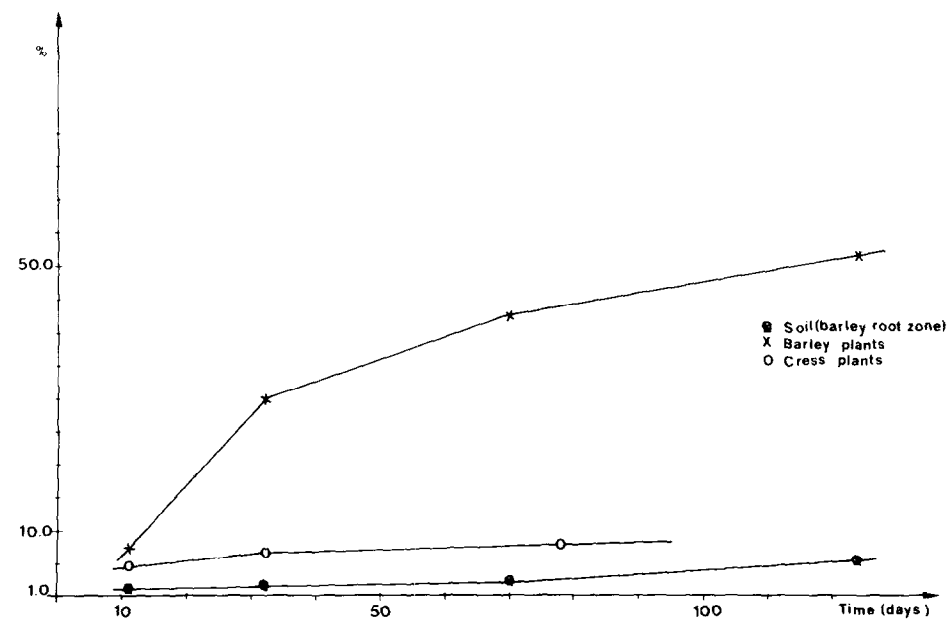

FIG. 6. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]pentachlorobenzene into the soil (in % of total ^{14}C of the respective sample).

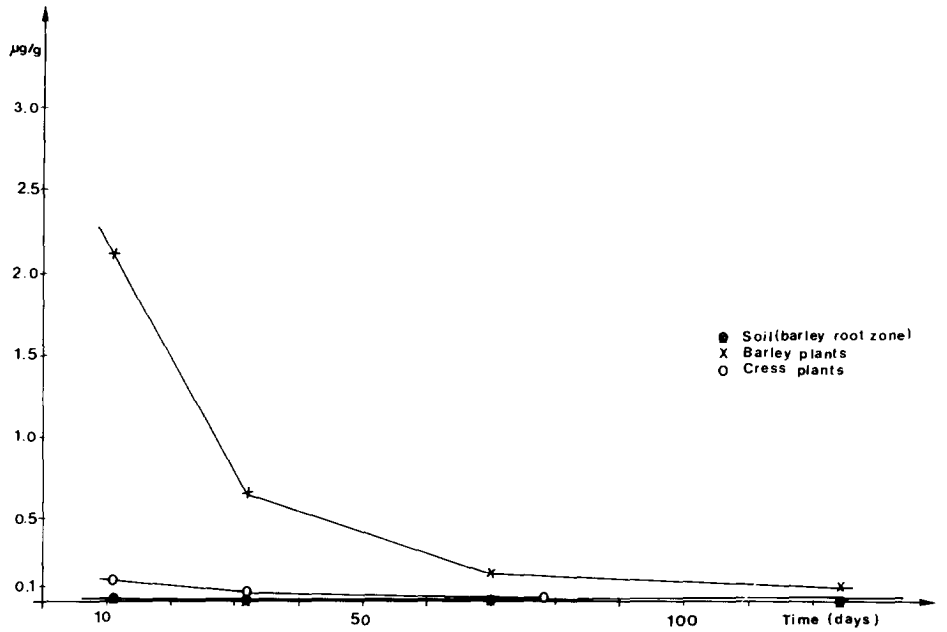

FIG. 7. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]1,2,4-trichlorobenzene into the soil (in μg equivalent to the parent compound per g dry soil or plant material).

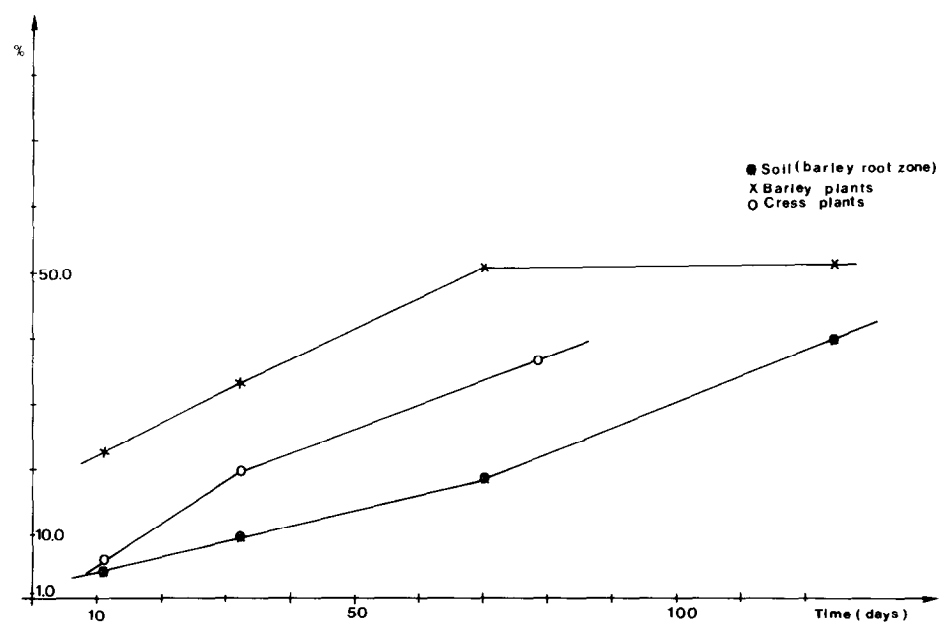

FIG. 8. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]1,2,4-trichlorobenzene into the soil (in % of total ^{14}C of the respective sample).

Benzene. The levels of bound residues derived from benzene in soil and plants (Fig. 9) are very low at the first sampling (12 days) and decrease further during the vegetation period, in spite of the fact that benzene is a readily biodegradable compound. Indeed, both volatilization and total degradation to carbon dioxide are so fast that only low residues, both bound and soluble, are left. In cress, the levels of bound residues are higher than in barley; this is in contrast to the three chlorinated benzenes discussed above. The unextractable residue portion is between 55 and 68% of total residues in soil and 27 and 100% in plants (Fig. 10) and does not show any distinct time dependence, neither for soil nor for plants. This means that both kinds of residues are formed and degraded at a comparable rate and bound residues derived from benzene are hardly more persistent than extractable residues. In cress, the portion of unextractable residues as compared to total residues is higher than in barley; this is in contrast to the three chlorinated benzenes discussed above.

Influence of Soil Depth on the Level of Bound Residues in Soil

After the harvest of all plants in these experiments, soil samples were taken at specific depths (0–5, 5–10, 10–20, 20–30, 30–40 cm), in order to assess the influence of soil depth on the level of soil-bound residues of benzene and its chlorinated derivatives. Table 1 shows the levels, in percentage of initially applied radioactivity, for the four test compounds and the six different depths. The table shows the presence of bound residues to a depth of 40 cm for hexachlorobenzene, 1,2,4-

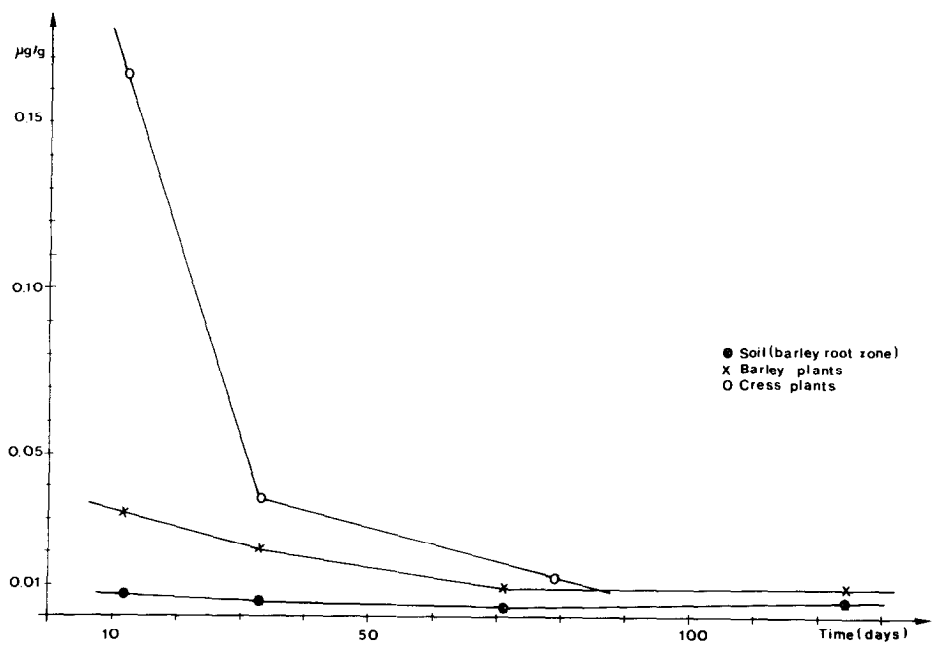

FIG. 9. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]benzene into the soil (in µg equivalent to the parent compound per g dry soil or plant material).

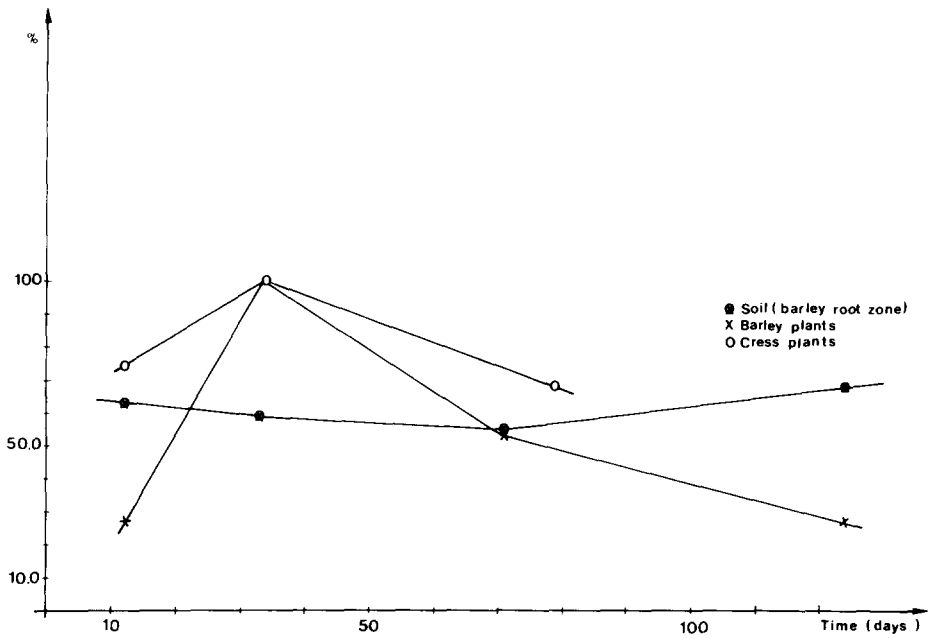

FIG. 10. Time course of soil- and plant-bound radioactive residues after application of [^{14}C]benzene into the soil (in % of total ^{14}C of the respective sample).

trichlorobenzene and benzene. The difference in concentration between the top soil and the lowest soil layer is not very great. If the distribution rate of bound residues in soil is expressed in terms of residues below 10 cm as a percentage of total residue in soil, then we will find 51.0% for hexachlorobenzene, 32.7% for pentachlorobenzene, 71.2% for 1,2,4-trichlorobenzene, and 63.5% for benzene.

The formation of soil-bound residues in soil layers below the treated 0- to 10-cm top soil layer depends upon the migration rate of the parent compound, which in turn depends on its water solubility, and upon the formation rate of bound residues

TABLE 1

DISTRIBUTION OF BOUND RESIDUES IN DIFFERENT SOIL LAYERS, 126 DAYS AFTER TREATMENT OF SOIL WITH [^{14}C]BENZENE AND CHLORINATED [^{14}C]BENZENES (IN % OF APPLIED RADIOACTIVITY)

Soil depth (cm)	Hexachlorobenzene	Pentachlorobenzene	1,2,4-Trichlorobenzene	Benzene[a]
0–5	0.32	0.43	0.23	0.16
5–10	0.16	0.23	0.17	0.11
10–20	0.32	0.32	0.38	0.20
20–30	0.09	n.d.	0.37	0.15
30–40	0.09	n.d.	0.24	0.12
Soil, total	0.98	0.98	1.39	0.74

Note. n.d. = not detected.

[a] Residues after cold extraction with methanol.

TABLE 2

BOUND RESIDUES IN DIFFERENT SOIL LAYERS, 126 DAYS AFTER TREATMENT OF SOIL WITH [^{14}C]BENZENE AND CHLORINATED [^{14}C]BENZENES IN RELATION TO EXTRACTABLE RESIDUES (BOUND RESIDUES IN % OF TOTAL RADIOACTIVITY OF EACH SAMPLE)

Soil depth (cm)	Hexachlorobenzene	Pentachlorobenzene	1,2,4-Trichlorobenzene	Benzene[a]
0–5	0.8	2.0	23.0	100.0
5–10	0.9	2.1	19.3	100.0
10–20	1.2	4.3	28.5	100.0
20–30	1.1	n.d.	35.0	100.0
30–40	0.7	n.d.	38.0	100.0

Note. n.d. = not detected.
[a] Residues after cold extraction with methanol.

which is influenced, as discussed above, by its biodegradability. Both parameters—water solubility and biodegradability—are highest for benzene and lowest for hexachlorobenzene. Consequently, benzene should give higher bound residues in deeper soil layers than the other three test substances. In fact, the experiments described here show that this is not true; the percentage of bound residues in deeper soil layers as compared to the top soil layer is highest for 1,2,4-trichlorobenzene and lowest for pentachlorobenzene. It should be considered that in top soil volatilization and total biodegradation to carbon dioxide may overcome leaching processes. In deeper soil layers, total biodegradation is also a competitive process so that a compound with lower water solubility and higher biological persistence (1,2,4-trichlorobenzene) may form higher levels of soil-bound residues in deeper soil layers than a nonpersistent compound with a higher water solubility (benzene). The formation of soluble metabolites in soil, which may have water solubilities and biological persistences quite different from those of the parent compound, further complicates the prediction of leaching and binding processes.

The ratio between soluble and bound residues, expressed as bound residues in percentage of total radioactivity in each sample (Table 2), shows no marked differences between various soil depths.

CONCLUSION

It may be concluded that the strong relationship between chemical structure of chlorinated benzenes and binding processes in soil and plants, as documented by these experiments, gives evidence for the participation of chemical reactions in the binding processes. The elucidation of the type of reactions and binding mechanisms needs further research.

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