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Chlorobiphenyl Movement from Soil to Soybean Plants

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Soybean plants were grown in specially constructed pots to determine residue contamination in plant tops from surface or subsurface soil applied [^{14}C]-2,2',5-trichlorobiphenyl, -2,2',5,5'-tetrachlorobiphenyl, and -2,2',4,5,5'-pentachlorobiphenyl. Each compound was evaluated individually, and sufficient unlabeled compound was added to give a soil concentration in the range of 2-3 ppm. Plants were harvested at 52 days and divided into five parts for analysis: top stem, bottom stem, top leaves, bottom leaves, and seed pods. Detectable residues were found only on plants grown with surface-treated soil with most of the residues confined to the lower leaves. Concentration of residues in plants increased with increasing chlorination of the biphenyls. There was little loss of ^{14}C from subsurface applications but 20-30% was lost by volatilization from the surface applications. It was concluded that plants grown on polychlorinated biphenyl contaminated soil would not be contaminated by root uptake and translocation, but some foliar contamination could occur from vapor sorption.

Polychlorinated biphenyls (PCB's) are industrial compounds that have been manufactured for ~50 years but were only recognized as environmental pollutants ~15 years ago. The commercial PCB's are complex mixtures of chlorobiphenyl compounds that may have from one to ten chlorine atoms per molecule. The PCBs are lipophylic and their persistence in the environment is greater as the degree of chlorination increases. The persistence and solubility in fat of PCBs have led to their biomagnification in certain environmental food chains. Human food and animal feed contamination by PCB's has generally involved fish, applications employing unsealed sources, or accidents.

The fate of PCB's in soil and their possible uptake by plants are research areas that have received little attention because their uses did not involve application to agricultural land. Iwata and Gunther (1976) reported uptake of a 54% chlorine PCB by carrot roots, but 97% of the residue was in the peels. They also reported foliage concentrations ranging from 1 to 6% of the soil concentration. Moza et al. (1979) found that the uptake of a tri- and pentachlorobiphenyl was greater in the high-oil carrot than in the low-oil sugar beet. Suzuki et al. (1977) detected PCB (0.15 ppm) in soybean sprouts grown in soil which contained 100 ppm of PCB. Weber and Mrozek (1979) also reported PCB residues on soybean plants grown in the greenhouse in contaminated soil.

None of the cited studies unequivocally demonstrate plant uptake of PCBs'. The residues could have arisen from direct adsorption to the roots or surface adsorption to the aerial parts by volatilized PCB. The purpose of this study was to determine the residues of chlorobiphenyls in the aerial parts of the soybean plants grown with surface contaminated soil and with contaminated soil separated from the aerial plant parts by a vapor barrier.

MATERIALS AND METHODS

Three uniformly ring-labeled [^{14}C]chlorobiphenyls were used: 2,2',5-trichlorobiphenyl (Tr), 2,2',5,5'-tetrachlorobiphenyl (Te), and 2,2',4,5,5'-pentachlorobiphenyl (Pe). The labeled compounds, obtained from Mallinckrodt Chemical Co., St. Louis, MO, had purities of 98% and specific activities ranging from 9.87 to 9.91 μCi/μM. The corresponding unlabeled chlorobiphenyls obtained from Analabs, Inc., North Haven, CT, were used to provide final

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soil concentrations in the range of 2-3 μg/g.

Galestown sandy loam soil (*Psammentic hapludults*) with a pH value of 6.7, organic matter content of 5.2%, and a $^1/_3$-bar moisture tension at 15.6% soil moisture content was used. The soil contained 67.3% sand, 22.2% silt, and 10.5% clay. The air-dried soil was passed through a 2-mm mesh screen before use. Acetone solutions, containing 0.25, 1.00, or 0.50 μCi of Tr, Te, and Pe per kg of soil, respectively, were pipetted onto the surface of pre-weighed soil spread in shallow pans. The solvent was allowed to evaporate and a commercial soybean root nodulating innoculum was sprinkled on the surface. Control soil was prepared in the same manner by using only the solvent and inoculum. Each soil was thoroughly mixed and sampled before potting.

Soybeans [*Glycine max* (L.) Merr., "Clark"] were grown in modified pots which contained two layers of soil as described by Beall and Nash (1971). The subsurface layer of soil was separated from the surface layer by an air space and a water- and vapor-tight disk. Soybean seeds were sprouted in untreated vermiculite and were transplanted into the experimental pots when they had elongated sufficiently to pass through the center opening in the disk. The opening was provided with a glass sleeve to prevent contact between the stem and surface soil. Polyethylene film was wrapped around the stem and glass sleeve to complete the seal. A polyethylene cage, open at the top, was provided to confine vapors around the plant.

A 2 × 4 factorial experimental design was used. The four treatments were control, Tr, Te, and Pe in either of the two soil layers. All treatments were replicated 4 times and the pots were randomly arranged on a greenhouse table. Both layers of soil were watered as required and care was exercised to prevent splashing of soil onto the stems and lower leaves.

The plants were harvested at 52 days when growth reached the top of the polyethylene cylinder. The plants were cut at the cotyledonary node and the length of stem measured. The plants were divided into lower stem, upper stem, lower leaves, upper leaves, and seed pod. The plants were not mature enough to separate the seed from the pods. The roots were not collected. The plant parts were weighed, chopped, and immediately frozen. Before analyses, the plant parts were ground in a Waring blender with dry ice to provide a uniform material for sampling. Soil samples were also obtained at the end of the study and stored frozen until analyzed. Possible vapor sorption by the polyethylene cage was not determined.

Total ^{14}C in soil and plant samples was determined by combustion in a stream of O_2. The $^{14}CO_2$ was dried by

Table I. Relative Concentration of ^{14}C Extracted from Fresh Tissue of Soybean Plants Exposed to Treated Surface Soil

plant part	% of soil concentration[a]		
	Tr[b]	Te	Pe
upper stem	ND[d]	0.1 ± 0.1	<0.2
lower stem	0.4 ± 0.2	0.7 ± 0.2	0.5 ± 0.1
upper leaves	<0.4	1.2 ± 1.1	0.6 ± 0.4
lower leaves	5.2 ± 3.5	7.3 ± 4.1	11.5 ± 5.5
seed pods	<0.4	0.5 ± 0.1	<0.2
whole plant	1.1 ± 0.7	1.8 ± 0.8	1.8 ± 0.9
detection limit[c]	0.4	0.1	0.2

[a] Each value in the mean ± standard deviation of four replicates. Actual soil concentrations were 2.08, 3.56, and 2.69 µg/g for Tr, Te, and Pe, respectively. [b] Abbreviations used: Tr, 2,2′,5-trichlorobiphenyl; Te, 2,2′,5,5′-tetrachlorobiphenyl; Pe, 2,2′,4,5,5′-pentachlorobiphenyl. [c] Concentration equivalent to a net count twice the standard deviation of the background count. [d] ND = none detected.

passing through a column of anhydrous $CaSO_4$ and trapped in 10 mL of monoethanolamine–2-methoxyethanol (1:7 v/v). A 5-mL aliquot of the trapping solution was assayed for radioactivity by liquid scintillation counting. Results from the control soils and plant materials were used for background correction.

Soil and plant samples were also extracted and cleaned up in order to determine concentration of Tr, Te, and Pe by gas–liquid chromatography (GLC). The thawed soil, premoistened with 0.2 M NH_4Cl, was shaken with hexane–acetone (1:1) to extract the chlorobiphenyls (Woolson, 1973). Plant material was extracted with petroleum ether–ethyl ether (1:2) by using a Polytron homogenizer.

The extracts were cleaned up with an activated Florisil column by using petroleum ether–ethyl ether (97:3) for elution. Quantitation was by electron-capture GLC (Fries et al., 1973). The cleaned up extracts were also counted for ^{14}C. Average ^{14}C recoveries from fortified soil samples determined immediately after fortification were 89.8, 96.9, and 95.5% for Tr, Te, and Pe, respectively. All values were corrected for recovery.

RESULTS AND DISCUSSION

Plant Residues from Surface Application. The relative distribution of the three chlorobiphenyls on plants grown with exposure to treated surface soil is shown in Table I. The ^{14}C determined by the extraction method was used for quantitation because, at the levels used, this method was ~1 order of magnitude more sensitive than either the GLC method or the combustion method which required small samples. The values are expressed as percent of initial soil concentration to facilitate comparisons among compounds.

The pattern of residue distribution was similar for all compounds. Highest residues occurred in the lower leaves, detectable residues occurred in the upper leaves and lower stem, while residues were generally not detected in the upper stem and seed pods. In general, the concentration in the lower leaves was at least 10 times the concentration in any other plant part. The higher concentrations on the lower leaves are consistent with what would be expected if the mechanism of contamination is by volatilization from the soil surface and redeposition on the plant. The lower leaves have more surface area per unit weight than the lower stems and are closer to the source of contamination than any other plant part. The average residue level in the lower leaves relative to the soil residue level increased with increasing chlorination of the compound, but the large standard deviations preclude a definite conclusion.

Table II. Relative Concentration of GLC-Detected Chlorobiphenyls, Extractable ^{14}C, and ^{14}C Total in Lower Leaves of Soybeans Exposed to Treated Surface Soil

compd[b]	% of soil concentration[a]		
	plant extract		plant ^{14}C by combustion
	GLC	^{14}C	
Tr	5.2 ± 2.2	5.2 ± 3.5	7.1 ± 3.5
Te	6.7 ± 3.8	7.3 ± 4.1	8.8 ± 5.5
Pe	7.7 ± 3.0	11.5 ± 5.5	18.3 ± 9.9

[a] Each value is the mean ± standard deviation of four replicates. [b] Abbreviations used: Tr, 2,2′,5,5′-tetrachlorobiphenyl; Pe, 2,2′,4,5,5′-pentachlorobiphenyl.

The lower leaves were the only plant parts to consistently have high enough residues to be detectable by all methods used in this study. A comparison of results of the three methods is shown in Table II. The values obtained by combustion were consistently greater than the values obtained by either of the other two methods. However, the amount of activity involved in our combustion analyses was small because the samples for combustion were only one-tenth the size of those used for extraction and the large standard deviations preclude a definite conclusion on whether the differences were real. The finding may be analytical artifact or reflect incomplete extraction. The possibility of metabolic conversion of the parent compound cannot be ruled out. Moza et al. (1979) has reported a dihydroxylated derivative of 2,4′,5-trichlorobiphenyl in the leaves of carrots grown in treated soil. The amount of ^{14}C used in our study was insufficient to allow investigation of possible metabolites.

Plant Residues from Subsurface Application. No detectable residues were found in any of the aerial plant parts when the plants were grown in soil treated with the chlorobiphenyls and a vapor barrier was provided between the treated soil the atmosphere surrounding the plant. Suzuki et al. (1977) grew soybean sprout in PCB-treated soil with an experimental system similar to ours but with a less rigorous vapor barrier. Plant residues of 0.15 ppm of Aroclor 1242 were found when sprouts were grown on soil containing 100 ppm. This plant concentration relative to soil concentration would have been undetected under our experimental conditions. The limits of ^{14}C detection with our most sensitive method (extraction) are shown in Table I. Therefore, one cannot rule out the possibility that there could be some uptake and translocation of PCB by plants. If more [^{14}C]chlorobiphenyl had been used, it is possible that root uptake and translocation would have been detected. However, this potential route of contamination is so small that it would be of little practical significance in any scenario involving PCB application to agricultural soil.

Loss from Soil. The recovery of the chlorobiphenyls in soil at the end of the experiment is summarized in Table III. There were substantial losses from the surface soil by volatilization. In general, the rate of loss decreased with increasing chlorination as one would predict if the saturation vapor pressures of the compounds in our study follow the general finding that saturation vapor pressures of PCB's decrease with increasing chlorination (Junge, 1977). The loss from the subsurface soil was much less than that from surface soil. This would be expected because the subsurface soil was present in an essentially enclosed system.

Moza et al. (1979) applied a tri- and a pentachlorobiphenyl to soil in a lysimeter box under outdoor conditions. They reported volatility losses of 67.5 and 41.5%, respectively, during the first year after application. Clearly,

Table III. Recovery of GLC-Detected Chlorobiphenyls, Extractable ¹⁴C, and Total ¹⁴C in Soil at the End of the Experiment

		% recovery[a]		
		soil extract		soil ¹⁴C by combustion
compd[b]	location	GLC	¹⁴C	
Tr	surface	69 ± 6	72 ± 8	75 ± 12
	subsurface	82 ± 7	93 ± 10	81 ± 9
Te	surface	73 ± 11	75 ± 10	84 ± 6
	subsurface	96 ± 2	99 ± 2	94 ± 11
Pe	surface	73 ± 12	79 ± 13	87 ± 6
	subsurface	91 ± 9	94 ± 6	95 ± 9

[a] Each value is the mean ± standard deviation of four replicates. [b] Abbreviations used: Tr, 2,2′,5-trichlorobiphenyl; Te, 2,2′,5,5′-tetrachlorobiphenyl; Pe, 2,2′,4,5,5′-pentachlorobiphenyl.

Table IV. Growth of Soybean Plants Exposed to Soils Containing Chlorobiphenyls[a]

	height, cm		fresh weight, g	
compd[b]	surface	subsurface	surface	subsurface
control	59.9	47.3	32.5	25.9
Tr	57.5	50.5	32.3	27.3
Te	57.0	50.8	26.3	32.5
Pe	48.3	52.8	24.1	29.5

[a] No significant differences. Each value is a mean of four replications. The standard deviations were ±6.6 cm for height and ±5.6 g for fresh weight. [b] Abbreviations used: Tr, 2,2′,5-trichlorobiphenyl; Te, 2,2′,5,5′-tetrachlorobiphenyl; Pe, 2,2′,4,5,5′-pentachlorobiphenyl.

volatilization can be a significant route of PCB dissipation from surface soil and will be greater outdoors where there is air movement.

There was a trend towary greater recovery of total ¹⁴C than extractable ¹⁴C or GLC-detectable chlorobiphenyls. Methanol extraction following hexane extraction did not yield additional ¹⁴C. The amount of activity remaining in the soil after extractions was insufficient to attempt further characterization.

Plant Growth. The average heights and weights of the soybean plants expose to the chlorobiphenyls is presented in Table IV. None of the treated groups differed significantly from the controls.

Weber and Mrozek (1979) reported significant inhibition of soybean growth on soils containing 1000 ppm of Aroclor 1254. Nonsignificant reductions were reported with concentrations as low as 1 ppm. Our work with soil concentrations of chlorobiphenyls ranging from 2.08 to 3.69 ppm fails to confirm any growth reductions at low concentrations of a few ppm. We are not aware of any other study reporting phytotoxicity of PCB's, but this does rule out the possibility of a phytoxic component of Acroclor 1254 that has not been studied.

CONCLUSIONS

There was no evidence of root uptake and translocation of chlorobiphenyls by soybean plants at the levels used in this study. The results do not rule out the possibility that some uptake occurs, but the quantity would be so small that it would not be important under the soil concentrations that would occur from the application of PCB-contaminated sewage sludge to agricultural land (Environ-

mental Protection Agency, 1979).

Significant residues did occur through the mechanism of volatilization and redeposition when the plants were exposed to surface-contaminated soil. Most of the residue was on the lower leaves. The protected pots would not represent the air movement or plant growth characteristics that would occur in the field. In the field, air movement would favor revolatilization from the plant while the denser leaf canopy would favor greater absorption. Thus, the specific numerical relationships between soil and plant levels cannot be directly applied to the field situation.

The is a third potential mechanism for the movement of PCB's from soil to aerial plant parts under field conditions. It is possible that PCB-laden dust could deposit on the plant. Iwata and Gunther (1976) reported carrot foliage PCB concentrations that were 2–6% of the soil concentrations. They suggested that much of the residue was from adhering soil dust but they did not provide definite evidence.

Our study was limited to chlorobiphenyls with three to five chlorine atoms per molecule whereas many of the PCB residues in the environment may have six or more chlorine atoms per molecule. The magnitude of plant residues and soil losses were related to the degree of chlorination. One might speculate that the trend toward higher plant residues with greater chlorination would not continue indefinitely with increases in chlorination. The less chlorinated PCB's have higher saturation vapor pressures (Junge, 1977) and are more likely to volatilize from soil, but the more chlorinated PCB's have lower solubility in water and are more lipophilic (Hutzinger, 1974) which should enhance absorption and retention by cuticle waxes. At some point in increasing chlorination the lower volatilization rate from soil should become the controlling factor. The failure to find residues of the chemically related, but less volatile, polybrominated biphenyls in orchard grass grown in soil that contained 100-ppm residues (Jacobs et al., 1976) tends to confirm this conclusion.

LITERATURE CITED

Beall, M. L., Jr.; Nash, R. G. Agron. J. 1971, 63, 460.
Environmental Protection Agency Fed. Regist. 1979, 44, 53 438.
Fries, G. F.; Marrow, G. S.; Gordon, C. H. J. Agric. Food Chem. 1973, 21, 117.
Hutzinger, O.; Safe, S.; Zitko, V. "The Chemistry of PCBs"; CRC Press: Cleveland, OH, 1974; p 16.
Iwata, Y.; Gunther, F. A. Arch. Environ. Contam. Toxicol. 1976, 11, 523.
Jacobs, L. W.; Chou, S.; Tiedje, J. M. J. Agric. Food Chem. 1976, 24, 1198.
Junge, C. E. In "Fate of Pollutants in the Air and Water Environment"; Suffet, I. H., Ed.; Wiley: New York, 1977; pp 7–25.
Moza, P.; Scheunert, I.; Klein, W.; Korte, F. J. Agric. Food Chem. 1979, 27, 1120.
Suzuki, M.; Aizawa, N.; Okano, G.; Takahushi, T. Arch. Environ. Contam. Toxicol. 1977, 5, 353.
Weber, J. B.; Mrozek, E. Bull. Environ. Contam. Toxicol. 1979, 23, 412.
Woolson, E. A. J. Assoc. Off. Anal. Chem. 1973, 57, 604.