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Metabolism of Chlorobiphenyls in Soil

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Polychlorinated biphenyls (PCBs) are spread widely in the environment, but the fate of PCBs in soil has received little consistent research attention. Unlike the chemically related organochlorine insecticides, PCBs were never applied intentionally to large land areas. Interest in disposing of sewage sludge on agricultural land is increasing and sludges from many industrial cities may contain PCBs (Furr et al. 1976). Thus, it is important to understand the fate of PCBs in soils if sludges are to be handled properly.

Pal et al. (1980) reviewed the fate of PCBs in soil-plant systems. The limited research on metabolism of PCBs in soil indicates that PCB congeners with the fewest chlorine atoms are the most likely to disappear from soils and other microbial systems. Metabolism of more highly chlorinated congeners in PCB mixtures is slow or nonexistent. Hydroxylated chlorobiphenyls were identified as soil metabolites of 2,2'-chlorobiphenyl (Moza et al. 1976) and 2,4'5-chlorobiphenyl (Moza et al. 1979). Methoxy metabolites of the latter compound were found also, but there were no metabolites of 2,2',4,4',6-chlorobiphenyl. Chlorobiphenyl metabolism in microbial cultures has been studied more extensively than metabolism in soil. Hydroxychlorobiphenyls, meta cleavage products, and chlorobenzoic acids were microbial metabolites of chlorobiphenyls, but no metabolites were found if there were two or more chlorines on each ring of the biphenyl (Furukawa and Matsumura 1976, Ballschmiter et al. 1977, Furukawa et al. 1979). Clark et al. (1979) found that rates of parent compound disappearance decreased with increased chlorination. Unidentified polar metabolites also were reported in most soil and microbial metabolism studies. A deficiency of all studies was the use of open systems and it is not possible to distinguish between metabolism and volatilization as routes of compound loss.

This study was carried out to compare the metabolism of selected chlorobiphenyls in soil. Radiolabeled compounds and closed incubation systems were used to determine complete carbon balances.

MATERIALS AND METHODS

Biphenyl and seven chlorobiphenyls used in this study are listed in Table 1. The uniformly ^{14}C-labeled compounds were more than 98%

Table 1. The chlorination, molecular weights, specific activities and soil concentrations of the biphenyls used in the study

Chlorination	Molecular Weight	Specific Activity, µCi/µM	Soil Concentration, µg/g
None	154.2	15.91	0.19
2	188.5	11.84	0.32
3	188.5	10.06	0.38
4	188.5	18.05	0.21
2,2',5,5'	292.0	6.30	0.93
2,2',4,4'	292.0	11.46	0.51
2,2',3,3',5,5'	360.9	10.59	0.68
2,2',4,4',5,5'	360.9	14.06	0.51

pure and were supplied in a small volume of benzene (Pathfinder Laboratories, St. Louis, Missouri)[1]. The benzene solutions were diluted to 10 mL with hexane to provide a stock solution that contained about 25 µCi/mL. Portions of the stock solutions were diluted with ethanol to provide a dosing solution that contained about 1 µCi/mL.

The Flanagan silt loam used in the study contained 5.8% sand, 69.0% silt and 25.2% clay. Organic matter was 3.0%, cation exchange capacity was 20.3 meq/100 g and moisture content at 1/3 bar was 37.6%. The soil had been stored at 5°C for about one year before use. It was air dried, sieved and mixed before use. Air dry soil in amounts equivalent to 50 g dry soil was placed in flasks and 1 mL of each dosing solution (about 1 µCi) was pipeted over the soil. The solvent was allowed to evaporate before the soils were mixed by gentle shaking. Water was added to bring total moisture contents to 70% field capacity. There were four replicates for each compound and a set of untreated soils were included to determine background levels of radioactivity.

Incubations were carried out in a system through which a stream of air was drawn continuously. The air was drawn from a tube that contained 0.25 N KOH and through a second tube that contained distilled water before it was introduced into the flask that contained the soil. Air that exited the flask was drawn to a tube that was fitted with a polyurethane foam filter (Analabs, New Haven, Connecticut) in order to trap volatilized compounds and then to a tube that contained 0.1 N KOH to trap $^{14}CO_2$. Flasks and tubes were were glass, but they were connected by plastic tubing (Tygon, Norton Plastics and Synthetics, Akron, Ohio). Rates of air flow were

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[1] Mention of a tradename or product does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that might also be suitable.

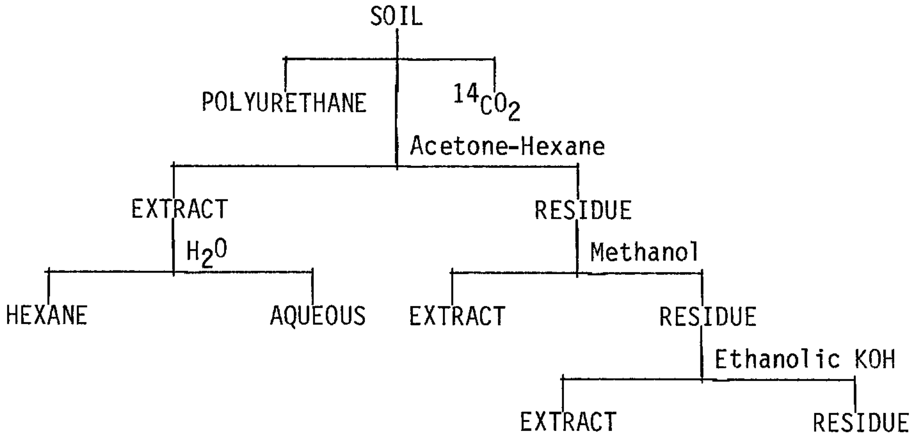

Figure 1. The partitioning of ^{14}C from labeled chlorobiphenyls during and after incubation with soil.

not maintained precisely, but needle valves were used to keep flow rates at a minimum and consistent amoung replicates.

Incubations were carried out at 28°C for 98 days in a constant temperature room. Solutions for trapping $^{14}CO_2$ were sampled and counted twice per week initially, but only once per week when it was found that $^{14}CO_2$ evolved at a low rate. Trapping solutions were replaced with new solutions after each sampling. Polyurethane filters were extracted with hexane in a soxhlet apparatus at the end of incubation and extracts were counted to determine amounts of trapped activity. Samples of soil were extracted by the sequential scheme outlined in Fig. 1. Hexane, aqueous and methanol extracts were counted for ^{14}C and concentrated before analysis by thin layer chromatography (TLC). Soils were air dried and activity that remained after methanol extraction was measured by counting $^{14}CO_2$ after combustion. Soils were then extracted with 5% KOH in ethanol (w/v). The extract was concentrated before TLC.

Precoated silica gel plates (E. Merck, Darmstadt, Germany) were used for TLC. Plates were developed with n-heptane or an 85/15 (v/v) mixture of acetone and chloroform. Locations of activity on TLC plates were determined by autoradiography and spots were recovered by scraping to measure the quantitative distribution of activity. Standards of parent compounds were included in the TLC, but we did not have labeled standards of potential metabolites.

RESULTS AND DISCUSSION

Evolution $^{14}CO_2$ from all compounds followed a similar pattern with time, but quantities of $^{14}CO_2$ evolved from the compounds varied by several orders of magnitude. Rates of $^{14}CO_2$ evolution from three representative compounds are in Fig. 2. Amounts of $^{14}CO_2$ from a compound depended upon the degree and position of chlorination. In general, $^{14}CO_2$ evolution began at low rates and the rates began to

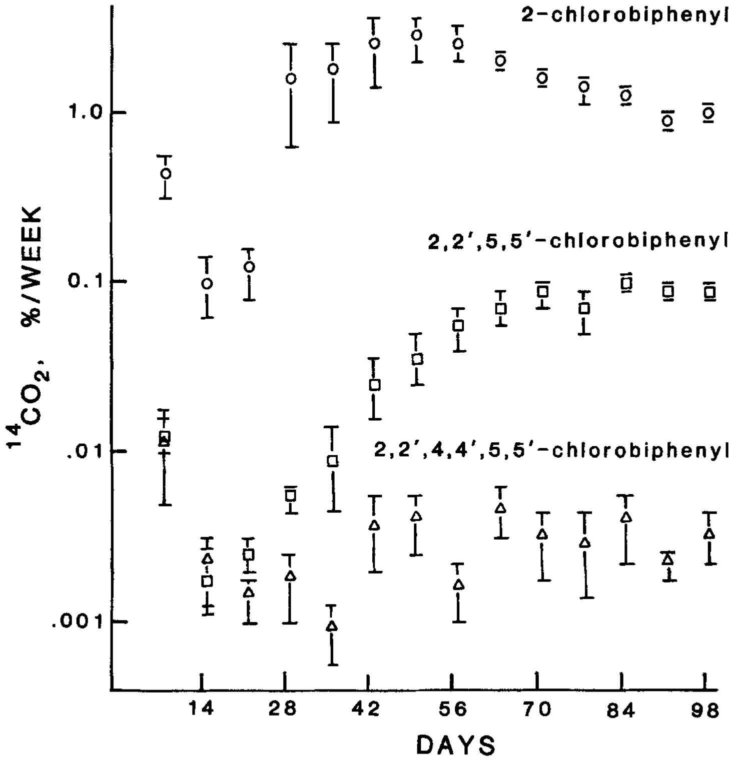

Figure 2. Evolution of $^{14}CO_2$ from selected chlorobiphenyls by weekly periods. Each point is the mean and standard error of four values.

increase at about three weeks after incubation began. The rates increased and reached maximums at about six weeks for biphenyl and the monochlorobiphenyls which are represented in Fig. 2 by 2-chlorobiphenyl. Rates of $^{14}CO_2$ evolution from these compounds began to decrease at about eight weeks. The decreases could reflect decreased amounts of substrates available for metabolism.

The two tetrachlorobiphenyls and the hexachlorobiphenyl that was not chlorinated in the para position evolved $^{14}CO_2$ at rates that were about one order of magnitude lower than the rates for biphenyl and the monochlorobiphenyls. These rates are represented by 2,2'5,5'-chlorobiphenyl (Fig. 2). Maximum CO $_2$evolution was not reached until about ten weeks and was declining only modestly when the study ended. The remaining compound, 2,2',4,4',5,5'-chlorobiphenyl produced little $^{14}CO_2$ (Fig. 2).

Recoveries of original ^{14}C in CO_2, extracts and extracted soils after 98-day incubations are in Table 2. Values for the polyurethane filters are not included because they contained no activity. The range amoung compounds for some of these values varied over several orders of magnitude. Therefore, all data were transformed

Table 2. Recovery of original ^{14}C after incubating biphenyl and chlorobiphenyls in soil for 98 days (%).

Compound	Evolved CO_2	Hexane extract	Aqueous extract	Methanol extract	Extracted soil	Total
Biphenyl	14.1^{a}	38.1^{b}	1.3^{a}	0.9^{b}	17.6^{ab}	72.0^{d}
2	19.7^{a}	30.3^{c}	1.2^{a}	0.8^{b}	14.2^{b}	66.2^{e}
3	16.5^{a}	41.8^{b}	1.3^{a}	1.3^{ab}	16.3^{ab}	77.2^{cd}
4	16.1^{a}	42.0^{b}	1.3^{a}	1.4^{ab}	19.3^{a}	80.1^{bc}
2,2',5,5'	0.7^{b}	81.1^{a}	0.9^{ab}	1.5^{ab}	3.8^{cd}	88.0^{ab}
2,2',4,4'	0.4^{b}	82.6^{a}	0.5^{b}	2.8^{a}	3.5^{c}	89.8^{a}
2,2',3,3',5,5'	0.8^{b}	80.3^{a}	0.8^{b}	2.3^{a}	2.9^{d}	86.8^{ab}
2,2',4,4',5,5'	0.1^{c}	90.0^{a}	0.3^{c}	2.6^{a}	1.4^{e}	94.3^{a}

[abcde] Values in the same column with a common superscript are not significantly different ($P < 0.05$)

to logarithms for statistical analysis by single classification analysis of variance and Duncan's multiple range test (SAS 1982).

Substantial $^{14}CO_2$ was evolved from biphenyl and the monochlorobiphenyls. Differences in quantities of $^{14}CO_2$ produced these four compounds were not statistically significant. Less than 1% of the original activity was evolved as $^{14}CO_2$ from the tetra- and hexachlorobiphenyls and in the case of 2,2',4,4',5,5'-chlorobiphenyl, $^{14}CO_2$ production was often not greater than background. Since the total $^{14}CO_2$ evolved in 98 days from the tetra- and chlorobiphenyls was less than the level of potential impurities in the starting material (2%), it is possible that the $^{14}CO_2$ evolution represents metabolism of impurities rather than metabolism of parent compounds. However, both the tetrachlorobiphenyl and the hexachlorobiphenyl with chlorines in the para positions evolved less $^{14}CO_2$ than the corresponding isomers with no chlorine in the positions.

Quantities of ^{14}C that were not extracted from soil with the various solvents were positively related to quantities of ^{14}C that were recovered as CO_2. Unextracted ^{14}C in soil was 15 to 20% of the the original activity with biphenyl and the monochlorobiphenyls. In contrast, less than 3% of the original activity of the tetrachlorobiphenyls and hexachlorobiphenyls was unextracted from soil.

Greatest recoveries of ^{14}C were in hexane extracts of all compounds. Quantities recovered from biphenyl and monochlorobiphenyls were 30-40% of the original activity and quantities recovered from other compounds were 81-90% of the original. Quantities of ^{14}C that partitioned into the aqueous phases of acetone-hexane extracts were small for all compounds. However, a larger percentage of the activity was recovered in this phase from biphenyl and the monochlorobiphenyls than from the other compounds. The value for

Table 3. R_f's of metabolites of biphenyl and monochlorobiphenyls on thin layer chromatograms developed with 85/15 acetone-chloroform

Compound	Parent R_f	Metabolite R_f[1]
Biphenyl	0.67	0.32 0.37
2-monochlorobiphenyl	0.68	0.33 0.49
3-monochlorobiphenyl	0.65	0.34 0.49
4-monochlorobiphenyl	0.63	0.45 0.51

[1] All chromatograms also had activity at the origin.

2,2',4,4'5,5'-chlorobiphenyl was significantly lower than all other compounds. The trend with methanol extracts was opposite to the trend with the aqueous phase of the acetone-hexane extracts. Least activity was extracted from biphenyl and the monochlorobiphenyls.

Total recovery of ^{14}C was high (87 to 94% of the initial activity) with the tetra- and hexachlorobiphenyls. Total recovery of ^{14}C from biphenyl and the three monochlorobiphenyls was lower (66 to 80%). It is probable that lower recoveries of activity from biphenyl and monochlorobiphenyls were caused in part by volatilization of the parent compounds. It was intended originally that volatilization would be measured by trapping the compounds on the polyurethane filters within the airstream leaving the flasks that contained soil. However, polyurethane filters did not contain radioactivity at levels that exceeded background. In a follow-up investigation of the incubation system, it was found that the plastic tubing that connected flasks to tubes with polyurethane filters adsorbed parent compounds volatilized from flasks that did not contain soil. However, volatilized compounds were recovered from polyurethane plugs when plastic tubing was replaced with glass tubing. Vapor pressures of biphenyl and the monochlorobiphenyls are greater than those of the tetra- and hexachlorobiphenyls (Hutzinger et al. 1974). Thus, it is probable that lower recoveries of biphenyl and monochlorobiphenyls was due to volatilization and adsorption of the compounds on the plastic tubing.

Hexane extracts of all compounds contained only the parent compounds and no metabolites when examined by TLC. The situation was different when aqueous and methanol extracts were examined. Parent compounds and low levels of polar metabolites that remained at the origin were found with all compounds. There were no other metabolites of the tetra- and hexachlorobiphenyls, but two metabolites were found for each of the other compounds. The R_f values of biphenyl, the monochlorobiphenyls and their metabolites are in Table 3. Metabolite R_f values were always much less than parent compound R_f values which indicates that the metabolites were more polar. There was no change in the metabolite picture after the more rigorous extraction of the soils with ethanolic KOH. The small amount activity of the metabolites and the lack of samples of potential metabolites precluded further metabolite identification, but our results are consistent with previous reports of soil metabolism of chlorobiphenyls (Moza et al. 1976, Moza et al. 1979).

Our work utilized one soil under one set of artificial conditions. Therefore, it is unlikely that rates of metabolism and loss found here would have a direct application to environmental conditions, but the relative ranking of the compounds should be maintained under most conditions. We used fewer compounds than were used in most studies of microbial metabolism of chlorobiphenyls (Furukawa and Matsumura 1976, Ballschmiter et al. 1977, Furukawa et al. 1979, Clark et al. 1979). However, we have extended the previous work to show that losses of parent compounds from soil can occur through metabolism and volatilization, and that CO_2 is one of the major metabolites. Both processes occured most readily with the less chlorinated biphenyls. Volatilization (inferred by difference) was the most significant route of loss with all compounds in this study, but the relative losses by the two routes under environmental conditions might be considerably different.

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