Fate of Pentachlorophenol-^{14}C in Soil under Controlled Conditions

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After application of pentachlorophenol-^{14}C at 23 kg/ha to flooded rice soil in a plant growth chamber, 36.5% was recovered in soil after one growing period and 30.1% after two periods. After one growing period, the residues were 28.61% unidentified unextractable substances, 0.51% unchanged free pentachlorophenol, 0.61% conjugated pentachlorophenol, 1.67% free tetra- and trichlorophenols, <0.01% conjugated trichlorophenols, 0.33% anisoles, and 4.74% highly polar, mostly nonhydrolyzable compounds (percent of applied radioactivity). In the second growing period, the portion of unextractable residues in soil increased; the composition of the extractable radioactivity was similar to that of the first vegetation period.

Pentachlorophenol (PCP) is widely used in agriculture and industry as a fungicide, bactericide, herbicide, molluscicide, algicide, and insecticide (e.g., against wood-damaging insects). Until 1971, it was used extensively in Japan as a herbicide in flooded rice fields (Matsunaka and Kuwatsuka, 1975). Although this use has been restricted, information on the fate of pentachlorophenol in soil is important because of its other application fields—especially wood protection—and the resulting release into the environment. Several publications have reported conversion and degradation in soil, and a number of conversion products have been identified (Matsunaka and Kuwatsuka, 1975; Kuwatsuka and Igarashi, 1975; Ide et al., 1972; Murthy et al., 1979). However, in some cases the authors did not succeed in separating and identifying isomeric phenols; furthermore, exact quantitative data on the conversion products resulting in a complete mass balance of PCP in soil are missing. Therefore, phytotron long-term studies with PCP in flooded soil are described in this paper. Rice plants were grown for two vegetation periods; metabolites isolated from plants are reported elsewhere (Weiss et al., 1982).

MATERIALS AND METHODS

Apparatus. For the extraction of the soil of the first year, a large Soxhlet apparatus, with a capacity of 10 L (NGW Vielzweck-Extraktor 12, Normschliff Glasgeräte, Wertheim, Federal Republic of Germany), was used. The radioactivity in various extracts was measured in a liquid scintillation counter (Packard Tricarb, Model 3375 or 3380) with external standardization. Unextractable residues were determined by automatic combustion (Oxymat, Intertechnique, or Packard Oxidizer B 306). Thin-layer chromatographic (TLC) plates were scanned for radioactive substances on a scanner supplied by Berthold/Frieseke GmbH, Karlsruhe, Federal Republic of Germany. Gas–liquid chromatographic (GLC) analysis was performed on a Carlo Erba unit with an EC detector, fitted with a glass capillary column 50 m long coated with methyl silicone (SP 2100) (Carbowax 20M deactivated) and with a Hewlett-Packard integrator, Model 3388 A. The following conditions were used: temperature 135–220 °C, programmed at 1.5 °C/min; detector temperature 250 °C; carrier gas N_2 (0.26 mL/min). A gas chromatograph–mass spectrometer (GC–MS), LKB 9000, from LKB-Produkter, Bromma, Sweden, was used for mass spectrometry.

Reagents. PCP-^{14}C was synthesized in our Laboratory (Sandrock et al., 1978). Its purity was determined by thin-layer chromatography (solvent: petroleum ether–benzene–acetic acid, 2:2:1) and was found to be >99%.

A scintillation liquid based on dioxane was used for assaying extracts, water, and TLC zones. A toluene-based scintillator containing phenethylamine was used for assaying $^{14}CO_2$ from samples combusted in the Oxymat. For the oxidizer a Packard basic scintillator was used. Silica gel G (Macheray, Nagel u. Co.) was used for the preparation of TLC plates. Ready-made silica gel plates (Merck; 0.25 and 2 mm) and Al_2O_3 plates (Type E, aluminum oxide 60; 0.25 mm) were also used. For column chromatography, silica gel 60 (0.063–0.200 mm; 70–230-mesh ASTM; Merck) and Sephadex LH-20 (25–100 μm; Pharmacia Fine Chemicals) were used.

Authentic Samples. PCP was purchased from Riedel-DeHaen AG, Selze-Hannover, purity 99% (checked by TLC using as the solvent petroleum ether–benzene–acetic acid, 2:2:1). All three tetrachlorophenol isomers were purchased from Fluka AG, Buchs SG. 2,3,4-, 2,3,6-, and 3,4,5-trichlorophenols were from EGA Chemie KG. 2,3,5-Trichlorophenol was from Aldrich Chemical Co., Inc. 2,4,5-Trichlorophenol was from Merck-Schuchardt. 2,4,6-Trichlorophenol was from Hoechst. All three tetrachloroanisole isomers, all six trichloroanisole isomers, and pentachloroanisole were synthesized by methylation of respective phenols. For methylation of metabolites and authentic samples, diazomethane was freshly prepared from (*p*-tolylsulfonyl)methylnitrosamide and KOH in diethyl ether and then distilled.

Application and Climatic Conditions. A Kick-Brauckmann vessel (Kick and Grosse-Brauckmann, 1961) and a 7-L plastic vessel were filled with 8 and 7 kg of sandy soil, respectively. The composition of soil was clay 7.4%, silt 7.0%, sand 85.6%, and humus 1.1%, pH 5.7. Although it is not possible to simulate natural conditions (especially degree of oxygenation in flooded soil) completely in the laboratory, efforts were made to maintain the conditions as natural as possible. Rice plants were allowed to grow in a phytotron (Vötsch) under flooded conditions at a day and night temperature of 24 °C/18 °C, with a 16-h photoperiod at 22-klx light intensity and a humidity of 80% maximum and 65% minimum. When the plants were 3 months old, 171.7 mg of PCP-^{14}C, specific activity 0.25 mCi/mmol, was dissolved in a mixture of 3 mL of diethyl ether and 37 mL of acetone and applied dropwise onto the soil of the two pots (20 mL in each pot); thus, the total application rate was 23 kg of PCP/ha (corresponding to that in agricultural practice on rice fields). After 7 months and 16 days the plants were harvested. The soil in the Kick-Brauckmann vessel was stored at −20 °C for 5 months and then used for rice growing for a second vegetation period. The initial concentration in the second vegetation period corresponded to 8.4 kg/ha. The soil was sown again with 43 seeds of rice. After 5 weeks the soil was flooded and the experiment was continued in a phytotron under

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0021-8561/82/1430-1191$01.25/0

Table I. Thin-Layer Chromatography of Soil Extracts, One or Two Vegetation Periods after Application of PCP [Silica Gel, Chloroform-Cyclohexane-Acetic Acid (9:9:2)]

TLC zone no.	R_f	%, based on the radioact in each extract	
		1st year	2nd year
1	0	19.9	18.7
2	0.52	76.9	76.4
3	0.86	3.2	4.9

the conditions as given above.

Extraction of Soil and Radioactivity Measurement. Before extraction, the water was removed from the soil by sucking with a large pipet and checked for radioactivity. The moist soil of one of the experiments of the first year was extracted totally with methanol in a large Soxhlet apparatus; from the soil of the second year, only an aliquot was extracted in a normal small Soxhlet apparatus. Radioactivity in the extracts was counted by a liquid scintillation counter. The unextractable ^{14}C in the extracted soil was measured by liquid scintillation after oxidation to $^{14}CO_2$.

For analysis of rice plants, see Weiss et al. (1982). No detectable radioactivity was left at the walls of the containers at the end of the experiments.

Characterization of Extractable Residues. The extracts were evaporated with a rotary evaporator, and the aqueous residues were removed by freeze-drying. The dry residues were dissolved in a minimum amount of methanol. Aliquots were applied to thin-layer plates (self-coated with silica gel G; Macheray & Nagel) and developed with chloroform–cyclohexane–acetic acid (9:9:2) as solvent. The radioactive zones were localized with a scanner and determined by scraping off 1-cm zones that were extracted with 15-mL portions of dioxane-based scintillation liquid and counted in a liquid scintillation counter (Table I).

The remaining methanol extracts were evaporated to dryness and then subjected to liquid–liquid partition with a diethyl ether–water system, first at pH 10 and then at pH 1. By this procedure, separation into neutral organic-soluble substances (fraction A), acidic (fraction B) substances, and water-soluble substances (fraction C) was achieved (Table II).

Isolation and Identification of Individual Conversion Products. Since the thin-layer chromatograms (Table I) and the partition behavior (Table II) were similar for both soil extracts, isolation of conversion products was carried out only for the soil of the first year.

The fractions obtained by liquid–liquid partition (Table II) were precleaned by column chromatography with silica gel or Sephadex. Further purification and isolation of conversion products were achieved by thin-layer chromatography with the following solvents: chloroform–cyclohexane–acetic acid (9:9:2); dichloromethane–methanol (9:1); benzene–ethyl acetate–acetone (12:7:1); hexane–benzene (1:1); hexane; benzene.

Highly polar radioactive fractions were hydrolyzed with 9 N H_2SO_4 at 90 °C for 9 h. Then, the hydrolyzed products were extracted from the aqueous phase with diethyl ether, methylated with diazomethane, and chromatographed by TLC.

The identification of isolated conversion products was performed by comparison of R_f values, capillary GLC retention times, and mass spectra with authentic reference substances. The quantitative determination of conversion products was carried out by liquid scintillation counting of extracted TLC zones or—in the case of isomeric phenols or anisoles that could be separated only by GLC—by comparison of GLC peaks with those of authentic standard solutions using an integrator and calibration curves.

Table II. Liquid–Liquid Partition of Soil Extracts in Diethyl Ether–Water at Different pH Values (after Application of PCP-^{14}C to Soil)

partition fraction	group of substances	%, based on the radioact in each extract	
		1st year	2nd year
A (ether after partition at pH 10)	phenol ethers and other neutral or alkaline ether-soluble substances	36	25
B (ether after partition at pH 1)	phenols and other acidic ether-soluble substances	54	62
C (water fraction)	water-soluble substances	10	13

The mean recovery for each chromatographic step was about 90%; losses of about 10% (evaporation and extraction losses, etc.) could not be avoided. No chemical alteration of the identified metabolites occurred, as evident from identical chromatographical behavior between the purification steps.

Characterization of Bound Residues. For the unextractable residues bound to various soil constituents, the binding sites were localized by fractionation of the extracted soil according to Kearney (1976).

In order to obtain information also on mechanisms of binding, we isolated humic acids and fulvic acids from an untreated soil sample [method according to Kearney (1976)]. These soil fractions were treated with PCP-^{14}C and, after 10 days, extracted with hot methanol for 7 h.

Since alkali may cause chemical changes in the molecules of PCP metabolites, acidic hydrolysis was carried out to determine the chemical nature of bound residues. Extracted soil (50 g) was hydrolyzed with 80 mL of 6 N HCl for 1 h at 90–95 °C. After centrifugation, the aqueous hydrolysate was extracted 3 times with hexane–acetone (1:1) and then 2 times with diethyl ether. The extracts were methylated with diazomethane and analyzed for radioactive anisoles by TLC.

RESULTS

Mass Balance. After application of PCP-^{14}C to soil, 36.5% of the radioactivity applied was recovered in soil after one vegetation period and 30.1% after two periods. From the remaining 70%, 15.4% was taken up by rice plants (12.9% in the first year and 2.5% in the second year), and 55% was lost by volatilization. No radioactivity was detected in the flooding or leaching water. The chemical nature of the radioactivity lost by volatilization cannot be elucidated in an open experiment; however, in a closed aerated system (Kloskowski et al., 1981) it was shown that most of this radioactivity was due to carbon dioxide.

The major portion of the radioactivity left in soil was unextractable by organic solvents (28.6% of the applied radioactivity after the first growing period and 25.6% after the second period). The ratio of unextractable to extractable residues increased in the second year.

Characterization of Extractable Residues. The extracted radioactivity was separated into characteristic fractions by TLC and by liquid–liquid partition at different pH values. Table I shows the percentages of different TLC fractions of both extracts and Table II the percentages of partition fractions. Both tables show that the composition of the extractable radioactivity is similar for both years.

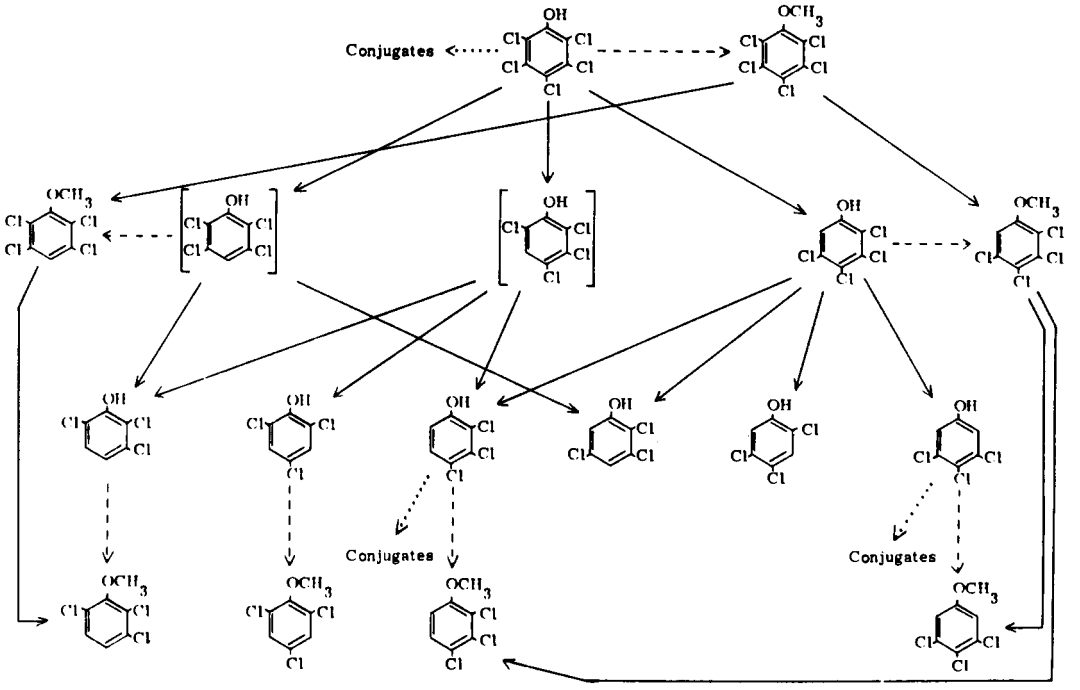

Figure 1. Conversion pathways of PCP in rice soil. Brackets = hypothetical intermediate; solid arrow = dechlorination; dashed arrow = methylation; dotted arrow = conjugation.

Table I demonstrates also that none of these radioactive TLC zones matches that of unchanged PCP (R_f 0.57); it may be concluded that PCP is present in the soil extract only in minor amounts as part of zone II.

Isolation and Identification of Individual Conversion Products. Further analysis of the partition fractions A–C (Table II) was carried out only with the extract of the first year, since qualitative differences between the two extracts seemed to be minimal. Separation of the extract by column chromatography and TLC including derivatization procedures (see Materials and Methods) revealed that the radioactivity consisted of numerous conversion products (Table III). All isomers of chlorinated anisoles could be separated clearly by the capillary GLC method used.

Unchanged free parent compound was present only in very low amounts (0.51% of the applied radioactivity); conjugated PCP occurred in a similar order of magnitude (0.61%). Conjugated lower chlorinated phenols (2,3,4- and 3,4,5-trichlorophenol) were detected only in minimal concentrations (<0.01%). The group of free trichlorophenols isolated comprised all six existing isomers, 3,4,5-trichlorophenol being the main metabolite. From the three existing tetrachlorophenols, only the isomer dechlorinated in the ortho position (2,3,4,5-tetrachlorophenol) was detected.

Besides reductive dechlorination and conjugation, methylation was also found to be a conversion reaction of PCP in soil. Four trichloroanisole isomers, two tetrachloroanisole isomers, and pentachloroanisole were identified.

The major portion of extractable residues of PCP in soil (4.25% of applied radioactivity) consisted of polar, unidentified substances that were mostly not hydrolyzable.

Characterization of Bound Residues. The binding sites of unextractable residues in soil were determined by fractionation of the soil [method according to Kearney (1976)]. It turned out that the binding sites in the soil were the humin fraction counting 10.9% of applied radioactivity, the humic acid fraction (11.9%), and the fulvic acid fraction (5.8%). After incubation of PCP-^{14}C with isolated humic acid and fulvic acid fractions for 10 days, no binding of radioactivity was observed. Acidic hydrolysis of the extracted soil did not yield any identifiable chemical product.

DISCUSSION

The results of these experiments show that PCP disappears rapidly from soil: partly by volatilization (including mineralization) and partly by conversion reactions that mostly result in the formation of unextractable residues in soil. The identified conversion products indicate at least four different reaction mechanisms: (1) reductive dechlorination; (2) methylation; (3) conjugation; (4) incorporation into insoluble macromolecules. The conversion pathways concluded from the products identified are shown in Figure 1.

Reductive Dechlorination. Reductive dechlorination of PCP in soil resulting in trichloro-, dichloro-, and monochlorophenols has been reported in the literature (Ide et al., 1972; Kuwatsuka, 1972; Kuwatsuka and Igarashi, 1975; Murthy et al., 1979). Contrary to Ide et al. (1972), who identified dichloro- and monochlorophenols as conversion products of PCP, the results of our experiments suggest that the dechlorination probably stops at the three-chlorine stage or that any formed lower chlorinated derivatives are incorporated immediately into soil constituents, either in an unchanged form by nonhydrolyzable bonds or in a form of unknown degradation products after further chemical reactions. This agrees well with studies of Fragiadakis (1980), who, after application of 2,4,6-trichlorophenol-^{14}C to soil, did not identify any lower chlorinated phenols or anisoles but found 90% of the residues in the form of unextractable radioactive materials.

In addition of the five trichlorophenols reported as soil metabolites in the literature (Ide et al., 1972; Kuwatsuka, 1972; Kuwatsuka and Igarashi, 1975), these experiments demonstrate the presence of the 3,4,5-trichlorophenol as a main soil metabolite.

From the three tetrachlorophenol isomers, only the 2,3,4,5 isomer resulting from ortho dechlorination was found. Para dechlorination was shown to occur also; however, the dechlorination product was not found in the

Table III. Qualitative and Quantitative Composition of Radioactivity in Rice Soil, One Vegetation Period after Soil Treatment with PCP-^{14}C

substance group	compd identified	% of applied radioact	
total residues		36.47	
bound residues		28.61	
total PCP	free PCP	0.51	1.12[a]
	conjugated PCP	0.61	
free lower chlorinated phenols	2,3,6-trichlorophenol	traces	1.67[a]
	2,4,6-trichlorophenol	traces	
	2,4,5-trichlorophenol	0.03	
	2,3,4-trichlorophenol	0.25	
	2,3,5-trichlorophenol	0.28	
	3,4,5-trichlorophenol	0.73	
	2,3,4,5-tetrachlorophenol	0.38	
conjugated lower chlorinated phenols	2,3,4-trichlorophenol	<0.01	<0.01[a]
	3,4,5-trichlorophenol	<0.01	
anisoles	3,4,5-trichloroanisole	<0.01	0.33[a]
	2,3,6-trichloroanisole	<0.01	
	2,4,6-trichloroanisole	0.02	
	2,3,4-trichloroanisole	0.06	
	2,3,5,6-tetrachloroanisole	0.02	
	2,3,4,5-tetrachloroanisole	0.14	
	pentachloroanisole	0.09	
unidentified conversion products	highly polar nonhydrolyzable compounds	2.70	4.74[a]
	highly polar hydrolyzable compounds	0.72	
	medium polar substances (R_f in benzene: 0.18–0.70)	0.83	
	substances in rinsing water of roots	0.49	

[a] Sum.

form of phenol but of the respective anisole. Products resulting from meta dechlorination were not isolated.

Methylation. Up to now, only pentachloroanisole was reported as a methylation product of PCP in soil (Murthy et al., 1979); no tetra- or trichloroanisoles were reported. Our results show that not only PCP but also its lower chlorinated degradation products may be methylated in soil, although it must be conceded that tetra- and trichloroanisoles could originate as well from pentachloroanisole by dechlorination. Since the toxic effects of phenols are mainly caused by the hydroxyl group, the methylation may be regarded as an inactivation process.

Conjugation. Conjugation is shown to occur in soil as another detoxication mechanism. Unchanged PCP as well as some of its lower chlorinated derivatives is conjugated. However, the percentage of conjugated phenols as compared to other conversion products is very small (Table III). The chemical nature of the conjugation partners was not established.

Incorporation into Insoluble Macromolecules. The unextractable radioactivity was bound in the humin as well as humic and fulvic acid fractions of the soil. The binding mechanisms probably are not adsorption or incorporation into complete humic acid macromolecules but chemical reactions during the formation of the macromolecules from lower molecular precursors. The chemical nature of the incorporated radioactivity could not be established. Since acidic hydrolysis failed to liberate chlorinated phenols, these are either incorporated by nonhydrolyzable bonds or the bound radioactivity is due to other chemical PCP degradation products that are not identifiable.

Table IV. Identification and Quantification of Residues of Pentachlorophenol-^{14}C in the Plant-Soil System, One Growing Period after Application to Soil (in Percent of Applied Radioactivity)

fraction	soil	rice plants	total
free PCP	0.51	0.14	0.65
conjugated PCP	0.61	0.06	0.67
total PCP	1.12	0.20	1.32
soluble metabolites (phenols, anisoles, conjugates, unidentified)	6.74	4.25	10.99
bound residues	28.61	8.45	37.06
sum	36.5	12.9	49.4

CONCLUSION

It may be concluded that PCP is not persistent in soil. It is partly volatilized or mineralized, partly degraded, and incorporated into soil constituents as unextractable residues. Uptake of residues from soil by rice plants (Weiss et al., 1982) occurs at a limited rate; these plant residues, as well as soil residues, consist mainly of unextractable substances. Unchanged free and conjugated PCP present in soil and plants after one growing season is less than 2% of the applied amount; soluble metabolites that were identified as chlorinated phenols, anisoles or conjugates also occur only in small concentrations (Table IV). Table IV shows that the major residues of PCP in soil as well as in plants are bound residues, the toxicological importance of which is not known.

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Received for review December 21, 1981. Accepted August 5, 1982.