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Fate of Radiolabeled Sodium Pentachlorophenate in Littoral Microcosms

Matthew F. Knowlton[1] and James N. Huckins[2]

[1]*School of Forestry, Fisheries, and Wildlife, University of Missouri, Columbia, MO 65211 and* [2]*Columbia National Fisheries Research Laboratory, U.S. Fish and Wildlife Service, Route 1, Columbia, MO 65201*

Pentachlorophenol (PCP) and its sodium salt are widely used as fungicides and bactericides for wood and wood products (ARSENAULT 1967). It is toxic to a number of aquatic organisms (GOODNIGHT 1942; CARDWELL et al. 1976; PRUITT et al. 1977) and over 1 million pounds a year have been used in the U.S.A. (U.S. International Trade Commission 1976). In recent years PCP has been involved in several major spills that resulted in the contamination of freshwater lakes and streams (PIERCE & VICTOR 1978).

The littoral zones, typically the first areas of lakes to be contaminated, are of vital importance to waterfowl and many aquatic organisms. However, little is known about the fate of potentially harmful chemicals in these ecosystems.

The purpose of this study was to delineate the fate of PCP in microcosms simulating some of the characteristics of littoral zones. The microcosms consisted of turbulent, well oxygenated sediment/water interfaces with heavy growths of rooted macrophytes. They were exposed to a single application of 41 µg/L of UL-^{14}C-sodium pentachlorophenate. Radioactive residues in water and plants were monitored through time, and residues accumulated by macrophytes were characterized.

MATERIALS AND METHODS

Reagents and Apparatus Uniformly ring-labeled ^{14}C-sodium pentachlorophenate was purchased from Pathfinder Laboratories Inc., 11542 Fort Mims Drive, St. Louis, MO. Its specific activity was 11.67 mCi/mM and the purity was >95% based on silica gel thin layer chromatography (TLC). A Beckman LS-3133-T liquid scintillation system (LS) from Beckman Instruments Inc., Fullerton, CA, was used for counting liquid ^{14}C-samples and a Harvey Biological Materials Oxidizer (BMO) from R. J. Harvey Instruments Corp, Hillsdale, NJ, was used with the LS for determining ^{14}C-residues in solid samples. A CO_2 coulometer from Coulometrics Incorporated, Wheat Ridge, CO, was used to determine carbon content of selected samples. Nanograde or high performance liquid chromatography (HPLC) grade solvents were purchased from Burdick and Jackson, Muskegon, MI, and were used in cleanup and analytical procedures. TRI-SIL 'Z', obtained from Pierce Chemical Co., Rockford, IL, was used to silylate carbohydrates. A column,

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0.9 cm i.d. X 25 cm, packed with Zorbax® BP (C18, 7-8 μ) from DuPont Co., Wilmington, DE was used for HPLC separations. The HPLC solvent systems were combinations of methanol and water or acetonitrile and water. Gel permeation chromatography (GPC) was done on columns 2.5 cm i.d. X 28 cm, of ≤400 mesh Bio-Gel P-2 (aqueous) or 200-400 mesh Bio Beads S-X3 (organic) from Bio-Rad Labs, Richmond, CA. The solvent systems were water (Bio-Gel P-2) and 50:50 (v:v) methylene chloride and cyclohexane (Bio Beads S-X3) and the flow rates were adjusted to 2-2.5 mL/min for both columns.

Biogenic and PCP samples were analyzed with a Varian 3700 gas chromatograph (GC) equipped with electron capture (EC) and flame ionization detectors (FID). The GC column was 2 mm i.d. X 1.8 m packed with 3% OV-17 on Chromosorb W HP; the effluent was directed through a Scientific Glass Engineering outlet splitter system (glass lined), permitting simultaneous EC/FID (1:9, v:v, split) analysis.

A Finnigan model 4023 automated GC-MS system equipped with a quadrapole mass filter was used to examine purified ^{14}C-sugars from a 62 day macrophyte sample. The analysis was performed in the electron impact mode and the GC column was a 25 m SE-30 glass capillary.

<u>Microcosms</u> Three aquaria were constructed from pairs of 3.7 L glass jars glued (Dow Corning® Silicone Rubber Sealant) mouth-to-mouth. The bottoms were removed from the upper jars. About 0.5 L of sediment (black sapropel from a small pond near Columbia, MO) and 6 L of well water (alkalinity 237 mg/L; SANDERS, 1980) were placed in each aquarium. Water was aerated and circulated by compressed air bubbled from a 2 mm i.d. glass tube that opened near the soil surface, and the microcosms were illuminated 14 hours a day (Sylvania Gro-Lux lamps). Evaporative water loss (about 1%/day) was replaced every two weeks of the exposure. Ambient temperature was 18-22° C.

The microcosms were allowed to stabilize 37 days before chemical treatments, permitting the growth of macrophytes, mostly <u>Potamogeton foliosus</u> and <u>Najas guadalupensis</u>, from seeds or turions in the sediment. About 11.7 μCi of UL-^{14}C-sodium pentachlorophenate or 41 μg/L was then added to the water of each microcosm, and samples of water and whole plants (minus roots) were collected regularly during the next 180 days. Macrophyte samples were typically 5-100 mg (dry weight), taken from an estimated average standing crop of 2 g. The plants were not visibly affected by PCP during the exposure and there were no indications that removing plant samples affected macrophyte or algal growth. After the exposure, microcosms were disassembled and samples of water, soil, plants and residues from the tank walls were stored at -25° C prior to analysis.

<u>Radiometric Analysis</u> Aliquots (≤1 mL) of microcosm water and organic extracts were counted with a mixture of toluene, Triton

X-100, and Fluoralloy® cocktail (Beckman Instrument Co., Fullerton, CA) in glass or plastic scintillation vials. Solid samples were lyophilized or air dried and processed by BMO. The $^{14}CO_2$ evolved was trapped and counted in Harvey Carbon-14 Cocktail. Counts of samples were corrected for background, quench, and losses through the oxidation procedure. The ^{14}C-residues are generally reported as nCi.

Residue Analysis Lyophilized samples of plants were ground with 10 times their weight of anhydrous sodium sulfate and placed in columns for solvent extraction. The samples were eluted (<5 mL/min) with 200 mL of a mixture of methylene chloride, isopropanol, and toluene (70:20:10, v:v:v, solvent I) and then with 200 mL of methanol (solvent II). After extraction, the sodium sulfate was removed from selected plant samples with hot water (100 mL, 40° C) and the remaining tissues were analyzed for neutral carbohydrates.

The solvent I extract of selected samples was chromatographed on the Bio-Beads S-X3 GPC system. Fractions containing radioactivity were concentrated and methylated with diazomethane at 40° C for 1 hour (SCHULTZ 1973). After methylation, radioactive residues were examined on EC/FID-GC.

Neutral carbohydrates were determined in extracted and non-extracted plants by a modification of a method published by SAWARDEKER et al. (1965). Ground plant samples (<0.5 g) were weighed into glass, Teflon-capped culture tubes, 1.6 o.d. X 15 cm. We added 3 mL of 72% H_2SO_4 (Ultrex, J.T. Baker Chemical Co.) to each sample and heated the sample at 40° C for 1 hour. Hydrolysis was completed by diluting each sample to 1 N with water and heating at 120° C for 2 additional hours. The sulfuric acid was neutralized with 4 N sodium hydroxide and the solution was concentrated to ≤1 mL volume by rotary evaporation in the presence of acetonitrile. The hydrolysate was cleaned up with Bio-Gel P-2 GPC. The GPC column was equipped with an omnifit on-column injector and the solvent used was HPLC grade water. Samples of 1 or 2 mL were injected and the flow rate was maintained at 1 mL/min. GPC fractions containing radioactive residues were concentrated to near dryness by rotary evaporation and silylated with an excess of TRI-SIL 'Z'. Silylated fractions were examined on EC/FID-GC for sugars and ^{14}C-PCP degradation products. Selected radioactive fractions from GPC separations were then concentrated to near dryness and chromatographed on C18-HPLC to reduce the possibility of ^{14}C-PCP degradation products coeluting with sugars. Subsequently, radioactive HPLC fractions were silylated again and re-examined on EC/FID-GC.

Microcosm water samples (50 mL) were acidified with 1 mL of 5 N HCL and extracted with three 50 mL aliquots of methylene chloride. The extracts were combined, dried over 10 g of Na_2SO_4 and rotory evaporated to 1-2 mL volume in the presence of 1 mL of toluene. The extracts were transferred to glass culture tubes with Teflon-lined caps, evaporated to <1 mL with dry N_2, and brought to a volume of 10 mL with hexane. The samples were methylated with diazomethane as described earlier and the anisole of PCP was determined by EC/FID-GC.

The quantity of inorganic ^{14}C (CO_2 and HCO_3^-) in microcosm water resulting from the mineralization of ^{14}C-PCP, was estimated by the loss of $^{14}C-CO_2$ after acidification (pH <2, 0.2 N H_2SO_4) and bubbling of water samples with nitrogen for 1 minute. This procedure removed >99% of the ^{14}C added to control samples as $Na_2H^{14}CO_3$ but resulted in no measurable losses of ^{14}C-PCP spikes. Total inorganic carbon in water was estimated by potentiometric alkalinity titration (A.P.H.A., 1976) and the total carbon in samples of _P. foliosus_ was determined by using the CO_2 coulometer. These values were used to estimate $^{14}C/^{12}C$ ratios in water and macrophyte samples.

RESULTS AND DISCUSSION

The PCP disappeared from the water of the microcosms during the first 6 weeks of exposure (Fig. 1). Concentrations of inorganic ^{14}C in water samples increased during this period and subsequently declined. The total ^{14}C-residues in the water declined about 2% per day throughout the experiment (Fig. 1). Radioactive residues in macrophytes increased linearly between days 1 and 35 (Fig. 1), reaching concentrations over 700 times the initial concentration in the water (41 µg/L). Concentrations of ^{14}C-residues in macrophytes dropped rapidly after the eighth week of the experiment. About 14% of the radioactivity applied to the microcosms was accounted for in samples of macrophytes and filamentous algae collected during the exposure. At the end of the experiment, 40 to 43% of the radioactivity was present in benthic sediments and about 5% was recovered in scrapings of mineral precipitates and algae from the walls of the microcosms. Acidification of these scrapings resulted in the volatilization of 40+5% of the radioactivity present. About 40% of the applied radioactivity was not detected in microcosm components and was presumably lost to the atmosphere during the 180 day exposure period.

Analysis of a macrophyte sample (no seeds) from day 62 revealed only 0.2 µg/g of ^{14}C-PCP. We recovered 140 nCi/g (equivalent to 3.2 µg/g of PCP) of the ^{14}C-residues present in the 62 day sample using an efficient column extraction technique (HESSELBERG and JOHNSON, 1972) with solvents I and II. Biogenics coextracted with solvents I and II would include lipids, chlorophylls, carotenoids, free glucose, and glucose-containing oligosaccharides. The amount of extractable ^{14}C-residues in two composite samples of plants collected on days 42 and 50 and days 131 and 138 (equivalent to 3.2 and 3.7 µg/g PCP respectively) was similar to that in the 62 day sample. The GPC elution profile of the 62 day sample extract (PCP eluate, solvent I) suggested the presence of radiolabeled biogenic compounds or PCP bound to biogenics, because the extracted ^{14}C-residues (83.5%) eluted in the non-aromatic lipid fraction (0-55 mL).

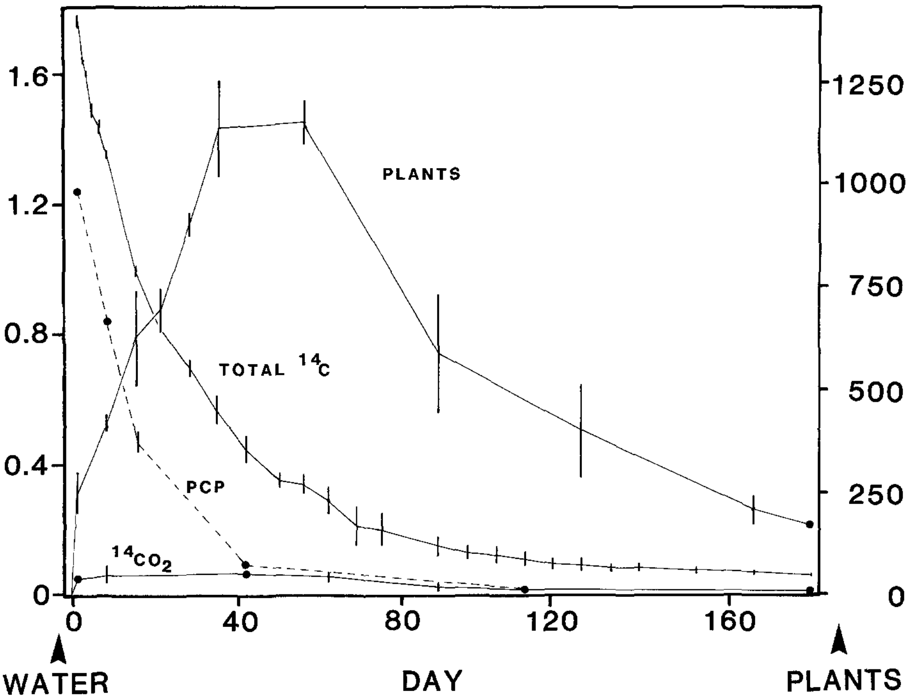

Figure 1. Concentrations (nCi/mL) of total ^{14}C, ^{14}C-PCP, and ^{14}C-CO_2 in microcosm water and total ^{14}C in plants (nCi/g) following treatment. Vertical bars represent the range of values of the microcosms.

A significant portion of the non-extractable (organic solvent) ^{14}C-residues in the 62 day macrophyte sample (equivalent to 3.1 µg/g PCP) was recovered upon acid hydrolysis of tissues, using a procedure developed for the analysis of neutral carbohydrates in agricultural crops (SAWARDEKER et al. 1965). Neutralized hydrolysates of ^{14}C-residues were fractionated on Bio-Gel P2; the GPC elution profiles of these ^{14}C-residues corresponded closely with ^{14}C-D-glucose standard. Selected samples were further purified by HPLC and fractions (HPLC or GPC or both) containing ^{14}C-residues were silylated by using TRI SIL 'Z'. FID-GC analysis of the silylated fractions revealed a number of carbohydrate peaks whose centroid paralleled the peak of ^{14}C-residues on HPLC (Fig. 2). Subsequent GC-MS analysis of the HPLC fraction containing the peak of ^{14}C-residues also indicated the presence of sugars.

Figure 2. FID-GC analysis of purified and silylated radioactive residues in a 62 day macrophyte hydrolysate. Peaks 1 and 4 appeared to be carbohydrates (GC-MS analysis) but were not identified. Peaks 2, 3, and 5 were D-Fructose, α-D-Glucose, and β-D-Glucose, respectively.

Direct BMO analysis of ^{14}C-residues in a 62 day macrophyte sample indicated the presence of 1.08 μCi/g, or radioactivity equivalent to 24.7 μg/g of PCP. Summation of the ^{14}C-residues in solvent extracts and the acid hydrolysate of a similar 62 day macrophyte sample examined earlier revealed only 275 nCi/g or radioactivity equivalent to 6.3 μg/g of PCP. Consistently higher

^{14}C-residues were detected by BMO analysis of macrophyte samples than could be recovered by solvent extraction or acid hydrolysis, suggesting the presence of substantial amounts of bound or incorporated radioactive residues. The presence of bound or incorporated pesticidal residues in plants has frequently been reported (KAUFMAN et al. 1976).

Purified sugars extracted from plants (no seeds) collected on day 62 contained 3.3 µCi/g. These results indicated that some ^{14}C-PCP had been degraded to ^{14}C-CO_2 and that photosynthetic fixation of ^{14}C-CO_2 had occurred. Although the exact proportions of ^{14}C present in the photosynthate and the amounts of biotransformed ^{14}C-PCP in plants were not determined through time, their relative proportions may be estimated from changes in the ratio of ^{14}C to ^{12}C in plants and changes in the ^{14}C:^{12}C ratio of inorganic C (CO_2, HCO_3^-) in the water through time (Fig. 3). Photosynthetically incorporated materials would have a ^{14}C:^{12}C ratio equal to that in the C being fixed. The total ^{14}C:^{12}C ratio in plants should increase with plant growth until near the maximum ^{14}C:^{12}C ratio of the available inorganic C, and decrease with further growth. This pattern clearly emerges from the data of

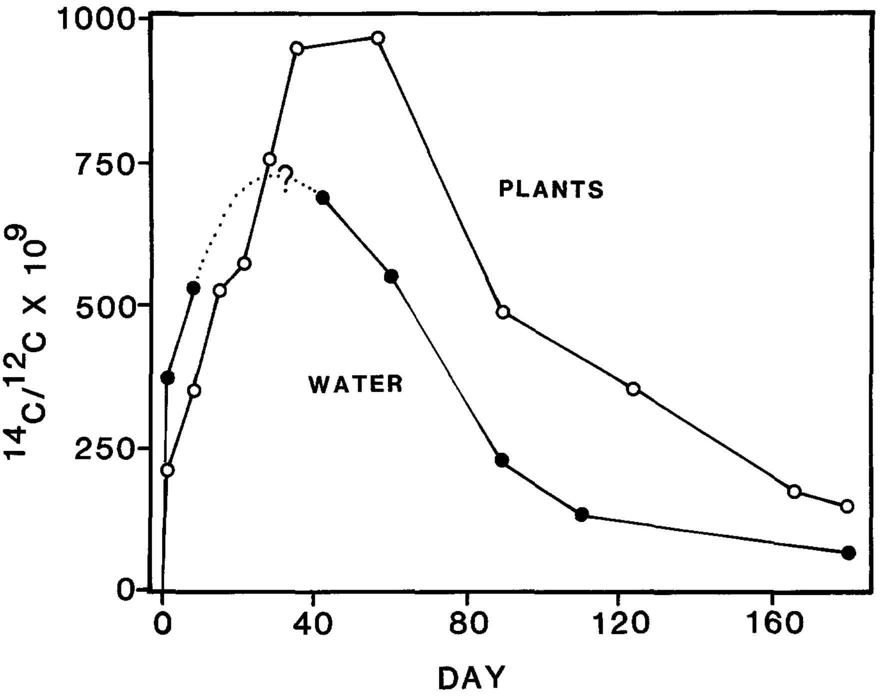

Figure 3. Ratio of $^{14}C/^{12}C$ in microcosm plants and $^{14}C/^{12}C$ inorganic carbon (CO_2, HCO_3^-) in water.

this experiment (Fig. 3), in which the rise and fall of inorganic ^{14}C:^{12}C ratios in the water paralleled the ^{14}C:^{12}C ratios of the macrophytes. The occurrence of greater ^{14}C:^{12}C ratios in the macrophytes than in water during most of the experiment suggests the presence of nonphotosynthetically incorporated ^{14}C-residues.

CONCLUSIONS

Degradation of PCP to CO_2 has been demonstrated in several laboratory experiments in which soil or microorganisms derived from soil were used (KAUFMANN 1978). In view of the final residue distribution and the detection of ^{14}C-CO_2 in this experiment, it appears that microorganisms may have been responsible for the degradation and subsquent mineralization of PCP. Earlier, Knowlton (1981) found that ^{14}C-PCP added to soils used in this experiment degraded in less than 9 weeks, and that 2,3,4,5-tetrachlorophenol was one of the main degradation products. Although ^{14}C-PCP was accumulated by macrophytes, this pathway accounted for only a small percentage of the total loss of ^{14}C-PCP from the water. The binding to or photosynthetic incorporation of ^{14}C-PCP residues into plant proteins and lignin may account for a large share of the radioactive residues observed in macrophyte samples.

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