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Problems Associated with the Purification of Pentachlorophenol for Biological Studies

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Pentachlorophenol (PCP) is widely used as a biocide and was the subject of an international symposium sponsored by the U.S. Environmental Protection Agency and the University of West Florida in June of 1977 (RAO 1979). Environmental concern about PCP and impurities in PCP has resulted in extensive research on the toxicological properties of these compounds (BENVENUE and BECKMAN 1976, and DOUGHERTY 1979). Knowledge of low concentrations of PCP impurities and PCP degradation products in stocks used in laboratory studies is essential for correct interpretation of PCP toxicity data. This information is of particular importance when conducting exposures to aquatic organisms in flow-through systems (CLEVELAND et al. in press). The relative toxicity and bioconcentration factors of contaminants in experimental stocks may be orders of magnitude greater than the parent compound (HUCKINS et al. in press).

We report the formation of small amounts of radiolabeled impurities during attempts to purify UL-^{14}C-PCP, using silica gel thin layer chromatography (TLC) and column chromatography. Several of these contaminants were apparently produced by coupling reactions of toluene or toluene impurities (used in cleanup procedures) and chlorophenols. The reaction may have been catalyzed during chromatography by active silica gel surfaces, or by heating at 40°C during subsequent rotary evaporation of radioactive fractions. Photolysis did not appear to play a major role in the formation of the chlorophenol derivatives, as yields of these compounds could not be correlated to lightning conditions. Impurities in PCP stock solutions were characterized by electron capture-gas chromatography (EC-GC) and electron impact gas chromatography-mass spectometry (EI-GC-MS).

MATERIALS AND METHODS

Radiolabeled Chemicals. Ring UL-^{14}C-labeled PCP was obtained from Pathfinder Laboratories, Inc., 11542 Fort Mims Drive, St. Louis, Missouri. The synthesis sequence consisted of ^{14}C-barium carbonate to benzene, chlorination to hexachlorobenzene, and finally dechlorination-hydroxylation to PCP. The purity and specific activity of the ^{14}C-PCP was 95.1% and 10.03 mCi/mM, respectively. The ^{14}C-PCP contained 3.6% of 2,3,5,6-tetrachlorophenol (TCP) and 1.2% of a dihydroxytetrachlorobenzene or a hydroxytetrachloroanisole (identified by GC-MS after methylation). A small amount of radioactivity (0.1%) also appeared to be associated with chlorophenol-solvent derivatives.

GC-MS. A Finnigan Model 4023 automated GC-MS system equipped with a quadrapole mass filter was used to identify PCP derivatives. The GC-MS was interfaced with a Nova 3, INCOS 3.1 data system. Source and GC-MS interface temperature were set at 250°C. The electron energy was maintained at 30eV and analyses were performed in the

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electron impact mode. The GC column was a 0.34 mm i.d. x 50m OV-17 glass capillary. Samples were temperature programmed from 100° to 180°C at 20°C/minute, 180° to 250°C at 2°C/minute and held at 250°C for 25 minutes.

Column Chromatography. Lobar silica gel 60 columns were obtained from E. Merck Laboratories, Inc., Elmsford, New York. A 3.7 cm i.d. x 44 cm column (63-125μm) was used for preparative separations and a 1.1. cm i.d. x 24 cm column (40-63μm) was used for a limited number of semi-preparative separations. A stepwise solvent gradient (all solvents were Burdick & Jackson nanograde, Muskegan, Michigan) was used for preparative LC separations which consisted of the following: 0-280 mL of 65% toluene and 35% ethyl acetate, 280-560 mL of 60% ethyl acetate, 35% toluene and 5% methanol, and 560-1500 mL of 10% methanol in ethyl acetate.

RESULTS AND DISCUSSION

In our laboratory new radiolabeled standards are normally examined by GC, TLC, LC, and GC-MS before they are used in biological studies. Analysis of several different lots of UL-^{14}C-PCP resulted in the detection of low levels of ^{14}C-impurities. These radiolabeled PCP standards had been added to purified PCP (99%) and an industrial PCP composite (88%) for flow-through exposures of fathead minnow (Pimephales promelas). The identification of labeled and non-labeled contaminants in these PCP stocks (CLEVELAND et al. in press) was important because the exposures were designed to detect biotransformation products and possible differences in PCP metabolism caused by PCP-related impurities in industrial grade PCP. The ^{14}C-UL-PCP added tothe non-labeled purified PCP (99%) was only 95.1% pure and had a yellow color when concentrated to volumes of 1 mL or less. Analysis of ^{14}C-impurities in the purified PCP stock, separated by silica gel column chromatography, revealed 2,3,5,6-TCP, tetrachlordimethoxybenzene (after methylation, 810-820 mL fraction, fig. 1) and small amounts of yellow unknowns in the 200-300 mL fractions (0.1%). To eliminate these impurities, the purified PCP stock solution was cleaned up by multiple 1 mL sample passes through a preparative Lobar (silica gel) column. The elution zones corresponding to ^{14}C-PCP (870-1000 mL fractions) were combined and concentrated on a rotary evaporator. At a volume of 3 mL a brilliant yellow color was observed in the composite and for that reason 1 mL of the 3 mL stock was again chromatographed on the preparative Lobar column. The yellow impurities were relatively non-polar and appeared to move with the 65% toluene and 35% ethyl acetate mobile phase (^{14}C-peak for this solvent system was 258-268 mL). The amount of these radioactive residues increased from 0.1% during the first separations to 1.8% of the total residue. However, radioactive impurities were generally reduced in the other silica gel fractions.

Analysis of the early eluting yellow impurities by GC-MS revealed chlorophenol adducts of toluene and benzoic acid. The specific isomer composition of these compounds was not identified. Chlorophenol-toluene adducts were detected only after the sample was methylated with diazomethane (1 hr at 40°C). This observation suggested the presence of free hydroxyl groups in the non-methylated sample. Figure 2 shows differences in the total ion current of the sample before and after methylation. The tetrachlorophenylbenzoate peaked at scan 838 in the

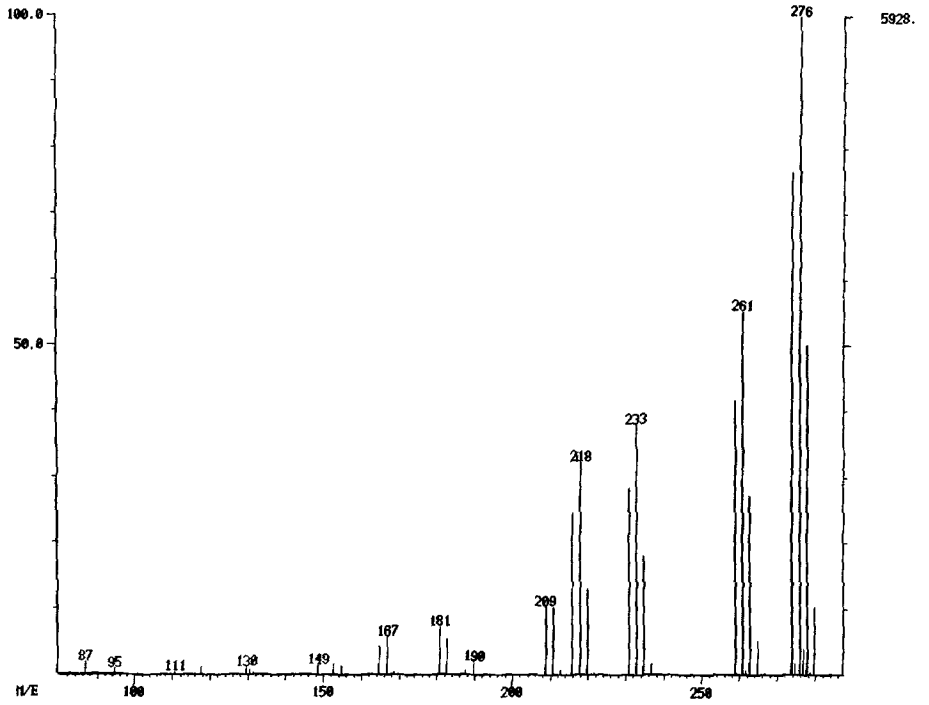

Fig. 1. Mass Spectra of a methylated 810-820 mL fraction from the Lobar separation of a purified ^{14}C-PCP stock sample. The presence of a tetrachlorodimethoxybenzene is indicated by the molecular ion at M/E 274, the proper tetrachloro isotope pattern, and the ion cluster at M/E 259.

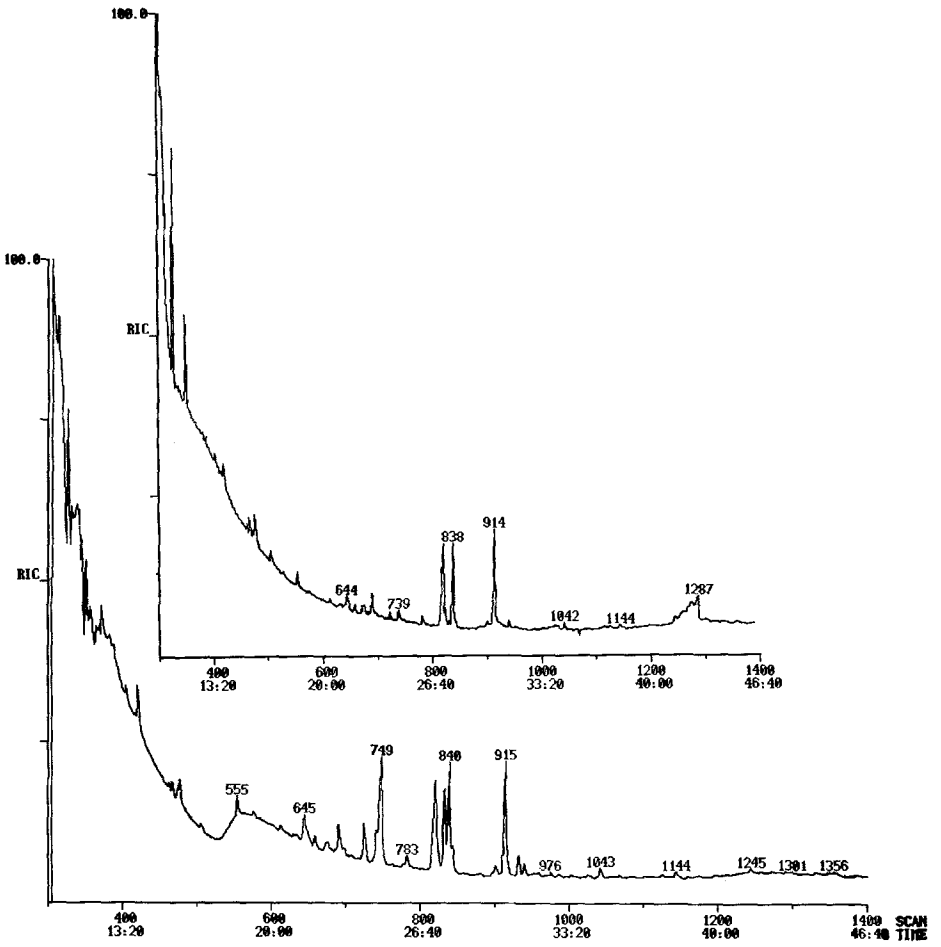

Figure 2. Total ion current of non-methylated (top trace) and methylated (bottom trace) 258-268 mL Lobar fraction of a purified ^{14}C-PCP stock sample. Peaks at scans 838 (top) and 840 (bottom) are the phenybenzoate adduct. Trichlordimethoxydiphenylmethane and tetrachloromethoxydiphenylmethane were present in the methylated sample at scans 726 and 749. The other peaks observed were hydrocarbons or pthalates.

non-methylated sample and scan 839 in the methylated sample. The peaks at scans 820 and 914 of the non-methylated sample were due to a hydrocarbon and a phthalate respectively. After methylation chlorinated peaks were observed at scans 726 and 749 which appeared to be trichlorodimethoxydiphenylmethane and tetrachloromethoxydiphenylmethane respectively. The peaks not identified in the methylated sample were hydrocarbons and scan 915 revealed a phthalate similar to that in scan 914 of the non-methylated sample.

The chlorophenol-toluene adducts had molecular ions at M/E 334 (tetrachloro-) and M/E 330 (trichloro-) and the spectra are shown in figure 3. These compounds clearly exhibit the expected chlorine isotope patterns for the tetrachloro- (M/E 334, 336, and 338) and the trichloro- (M/E 330, 332, and 334) phenols. The loss of chlorines (M/E 264 and 266) is clearly shown in the tetrachloromethoxydiphenylmethane spectra. both spectra have an intense M/E 91 peak of the tropylium ion, which is typical of a toluene fragment. The mass

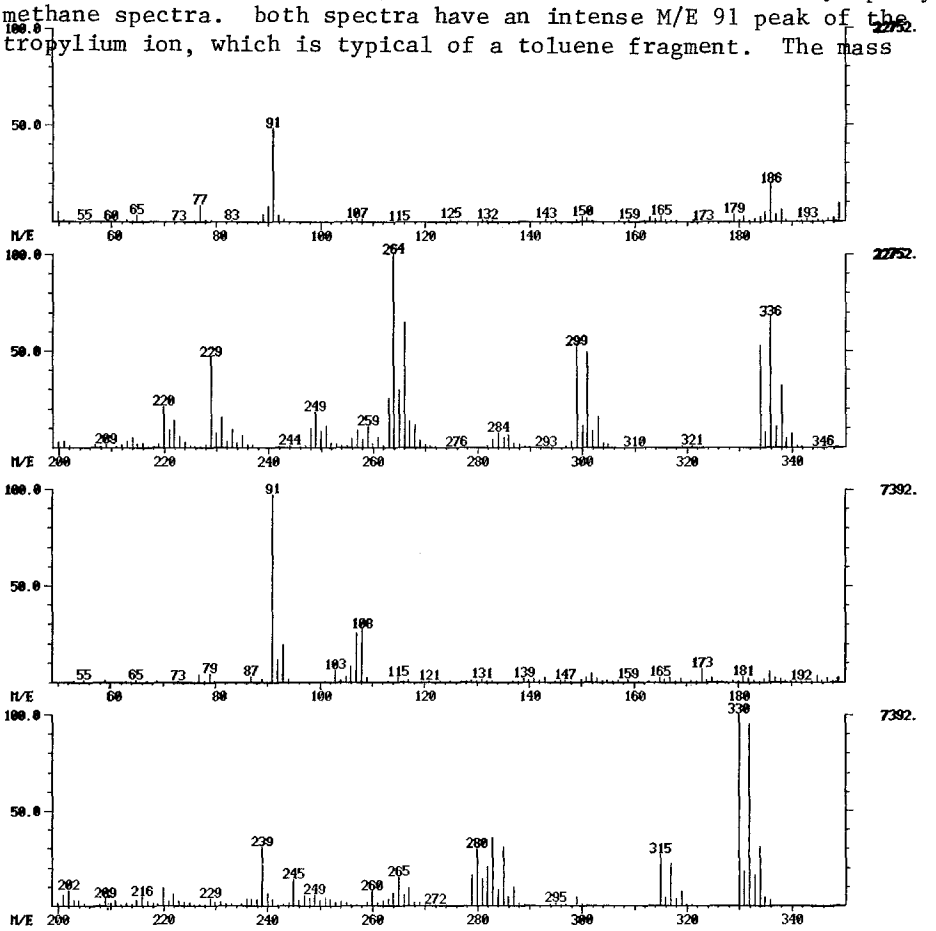

Fig. 3. Mass Spectra of scans 748 to 750 and 725 to 728 of the methylated 258-268 Lobar fraction of purified ^{14}C-PCP stock. The total ion current of the top spectra peaked at scan 749 and the lower spectra peaked at scan 726 which were tetrachloromethoxydiphenylmethane and trichlorodimethoxydiphenylmethane respectively.

spectrum of the tetrachlorophenylbenzoate (figure 4) has a molecular ion cluster centered at M/E 334 and exhibits both the expected M+ isotope ion plus the $(M - H)^+$ isotope cluster. The fragment at M/E 105 is typical of the (C_7H_5O) ion associated with aromatic esters.

The mechanisms governing the formation of the chlorophenol-toluene and -benzoic acid adducts were not elucidated. However, the apparent rise in the concentrations of these compounds during silica gel chromatography and subsequent rotary evaporation does suggest that surface or thermally mediated free radical reactions may have been resposible for their formation. The presence of benzoic acid as a solvent impurity or from the oxidation of toluene appeared to be a prerequisite for the formation of the tetrachlorophenylbenzoate adduct.

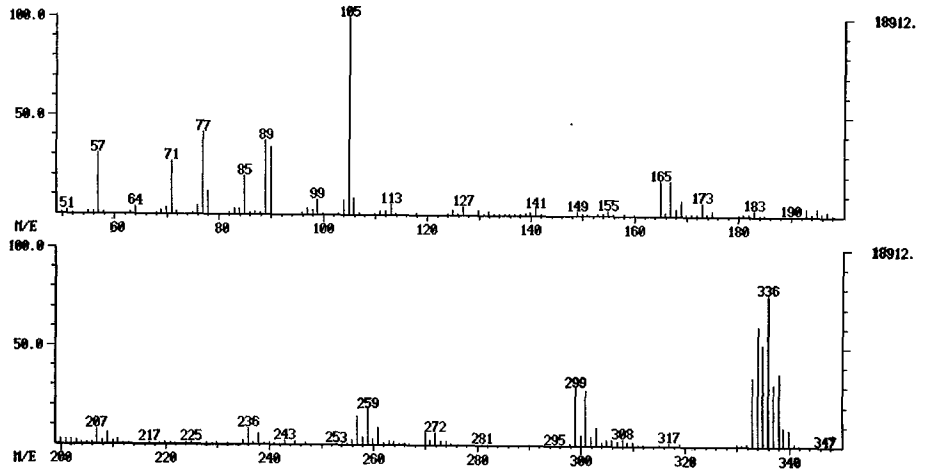

Figure 4. Mass spectra of the tetrachlorphenylbenzoate. Represents scan 839 of the methylated 258-268 mL Lobar fraction of a purified ^{14}C-PCP stock sample.

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