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Isolation and Characterization of Palmitoylpentachlorophenol from Human Fat

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Pentachlorophenol (PCP) has been used widely in industry and agriculture since the 1930's. It has been used to control mold, mildew and termites in wood, and also as an herbicide in pineapple and sugarcane fields. Because of its use as an herbicide, PCP has been found in food and water samples. Additional sources of PCP in the environment include the degradation of phenoxyacetic acid herbicides and their contaminants, chlorination of water by disinfection plants, and as a result of industrial pulp bleaching (Ahlborg and Thunberg, 1980). Because of extensive usage and wide distribution, PCP has been found to be a fairly ubiquitous environmental contaminant.

The toxicity of PCP has been shown to be similar to that of other phenols, in that it binds to mitochondrial protein and thereby uncouples oxidative phosphorylation. This interference with the electron transport system explains the acute toxic effects of PCP, namely pyrexia, tachycardia and dyspnea (Hayes, 1982). PCP has been further shown by Arrhenius et al. to accumulate in the microsomes (1977a) and to inhibit the microsomal detoxification enzyme chain (1977b). These authors also suggested that PCP may act as a synergist for the carcinogenic action of aromatic amines. Such alterations in the body's detoxification enzyme systems may mediate a broad spectrum of chronic toxic effects.

A study by Leighty and Fentiman (1982) showed the in vitro esterification of PCP with palmitic acid in a rat liver coenzyme A fortified microsomal system. They speculated that the toxic effects of PCP may be the result of its esterification with fatty acids. The ester thus formed may intercalate into the lipid membrane and alter the function of the enzyme system.

The present study was undertaken to determine if palmitoylpentachlorophenol (PPCP) was present in human adipose tissue in detectable amounts, because we and others (Reynolds et al., unpublished results; Ohe, 1979; Shafik, 1973) have shown the presence of PCP in human adipose tissues.

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MATERIALS AND METHODS

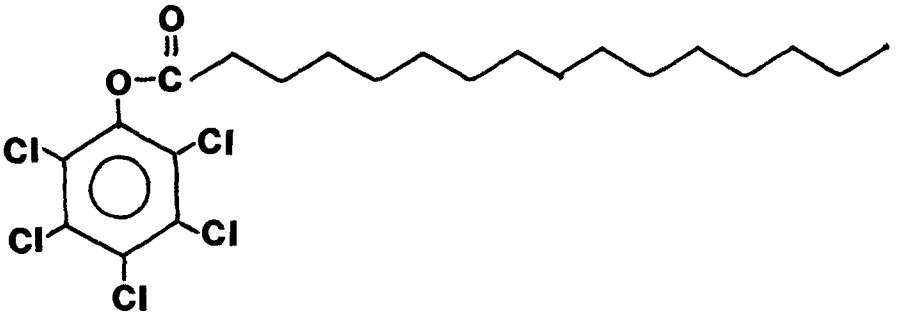
PCP, pyridine (both 99+% purity, "Gold Label"), and palmitoyl chloride (99% purity) were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin). The pyridine was dried over anhydrous potassium hydroxide before use. Other solvents and chemicals were used as received from Fisher Scientific Co. (Fairlawn, New Jersey) or MCB Manufacturing Chemists, Inc. (Cincinnati, Ohio), and were of reagent grade, except those used for high performance liquid chromatography (HPLC), which were of HPLC grade. Silica Gel GF thin layer chromatography (TLC) plates were obtained from Analtech, Inc. (Newark, Delaware) and were of 250 (analytical) or 1000 (preparative) micron thickness.

PPCP (Figure 1) was synthesized from pentachlorophenol and palmitoyl chloride in dry pyridine according to the method of Roberts and Simmons (1951). The product was recrystalized twice from hexane- methanol, yielding white spines with an uncorrected melting point of 75.5 - 76.5°C.

Human fat randomly obtained at autopsy from the anterior abdominal wall and frozen at -80°C was thawed and 4.75 g was homogenized with a Brinkman Polytron in 30 ml of hexane with 5 g of anhydrous sodium sulfate. The homogenate was centrifuged for 10 minutes at 1500 RPM and the supernatant was collected. The pellet was rehomogenized with an additional 30 ml of hexane and centrifugation was repeated. The combined supernatants were dried under nitrogen (weight 3.63 g).

A portion of the extracted fat (0.91 g) was dissolved in 1.5 ml of hexane and applied to four preparative TLC plates activated overnight at 110°C. The plates were irrigated with hexane-acetic acid (49:1, v/v). The plates were removed, allowed to air dry and viewed under ultraviolet (UV) light. An area with Rf less than 0.76 and more than 0.38 was scraped from each plate. The scrapings were suspended, with agitation, in 150 ml of hexane and allowed to stand for 15 minutes. The suspension was suction filtered, and the silica gel was flushed with an additional 150 ml of hexane. The solvent was evaporated under reduced pressure, and the extracted material was transferred to a conical tube and evaporated to dryness under nitrogen (residue 2.0 mg.). Analytical TLC using 250 plates and the same solvent system as above was used to evaluate the clean up procedure.

HPLC was used for the final purification of PPCP from the fat sample. A 4.6 mm x 25 cm, 5 Altex Ultrasphere ODS reverse phase C_{18} column with a small Whatman CO:PEL ODS guard column was used on a Beckman 334 HPLC with model 165 UV detector at a wavelength of 210 nm. Separation was carried out using methanol-H_2O (99:1, v/v) at a flow rate of 2.0 ml/minute. The fat residue sample and PPCP standard were prepared as a 10 mg/ml and 1.0 mg/ml solutions in hexane. The fat sample was injected seven times in 100 µg quantities, and each time the UV absorbing



Palmitoylpentachlorophenol

Figure 1.

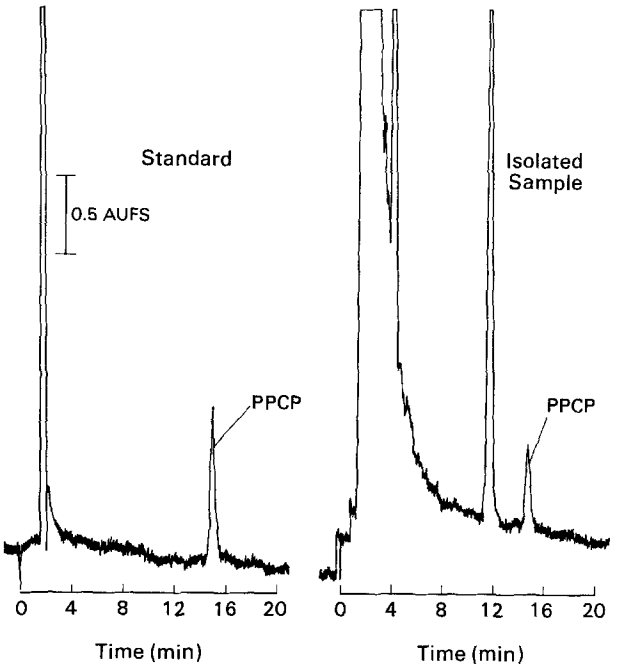


Figure 2. HPLC profile of PPCP standard and isolated material.

species with the same retention time as the PPCP standard was collected repeatedly using a Gilson fraction collector. The fractions were pooled, and the solvent was evaporated under nitrogen. The residue was subjected to solid probe Mass Spectrometry using a Finnigan 4000 instrument, with emission current 1.00 mA, electron energy 70 eV, source temperature gradually increased from 35° to 250°C in one minute, high vacuum pressure 4.9×10^{-5} torr, and where applicable the methane gas pressure was 0.22 torr. Analysis was carried out in both the electron impact (EI) and chemical ionization (CI) (methane reagent gas) modes. Selective ion monitoring of masses 503, 505 and 507 was carried out for the fat residue and the standard in the CI mode.

RESULTS AND DISCUSSION

TLC analysis of the fat residue following preparative TLC clean up showed a spot under UV light with Rf of 0.56, corresponding to the spot of PPCP standard with Rf of 0.52. HPLC analysis showed a peak at 14.8 minutes for the fat residue and a peak at 14.9 minutes for PPCP, as shown in Figure 2.

Mass spectrometric studies identified this HPLC peak as PPCP. EI did not give the molecular ions, but both PPCP and the isolated material gave characteristic fragment ions of m/z 263, 265, 267 ($C_6Cl_5O^+$) and m/z 239 ($C_{15}H_{31}CO^+$) as shown in Figure 3. CI_{CH4} mass spectrometry of the PPCP standard gave the pseudomolecular ion at m/z 503, 505 and 507 (m + 1). Selective ion monitoring of the CI_{CH4} mass spectrum of the isolated material at masses 503, 505 and 507 (shown Figure 4), confirmed the presence of PPCP in the fat sample. Approximately 0.1 µg of PCPP was isolated, corresponding to 0.24 µg/g fat or 240 ppb.

This study shows that PCP is present in human adipose tissue as an ester of palmitic acid. To the best of our knowledge this is the first demonstration of an esterified xenobiotic in human fat. This finding is another example of the relationship between lipids and toxin metabolism, which has been found to involve a wide variety of compounds containing hydroxyl groups such as codeine (Leighty and Fentiman, 1983a), DDOH [2,2-bis (p-chlorophenyl)ethanol] (Leighty et al., 1980), trichloroethanol (Leighty and Fentiman, 1981), 11-hydroxy-Δ^8-tetrahydrocannabirol and 11-hydroxy-Δ^9-tetrahydrocannabirol (Leighty et al., 1976), 2-napthylmethyl cyclopropane carboxyl palmitate (Pryde and Hanni, 1983), the amine cyclamate (Leighty and Fentiman, 1983b), as well as others. These studies are significant because they have shown a mechanism by which xenobiotics may become more lipophilic, which would favor deposition in fat and other tissues rather than renal excretion. Fat and tissue reservoirs are not fixed, and may be released during lipid mobilization, and possibly liberating sequestered reservoirs as well.

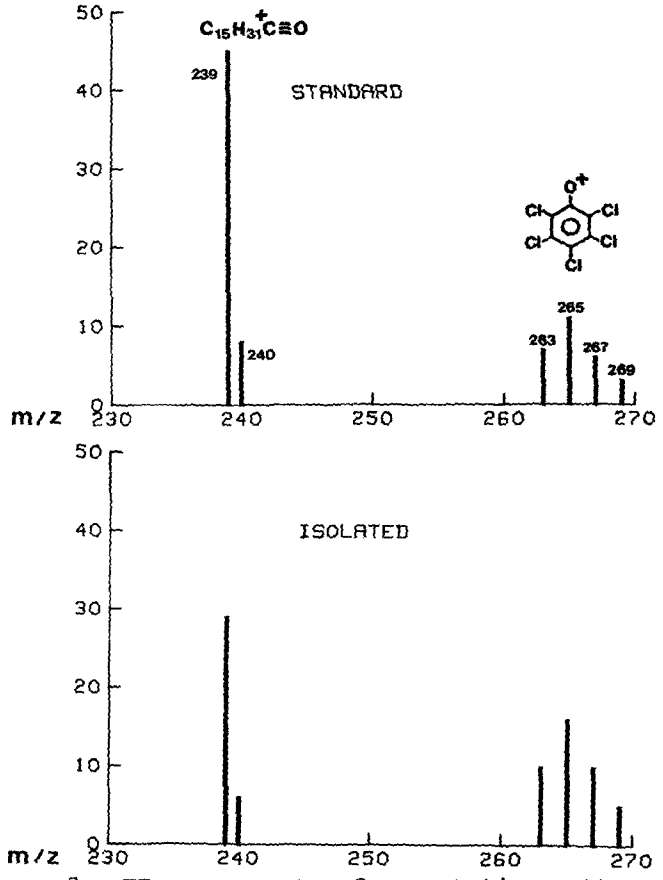


Figure 3. EI mass spectra fragmentation pattern of PPCP standard and isolated material.

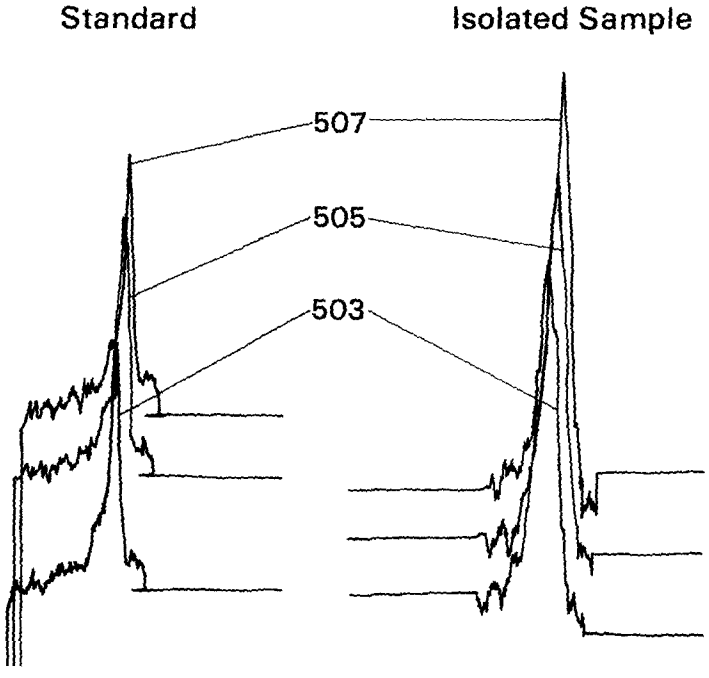


Figure 4. Selective ion monitoring of CI mass spectra of PPCP standard and isolated material.

The mechanism for the formation of PPCP in humans is not known. Whether the formation of PCP ester is enzymic (Leighty and Fentiman, 1982) or formed nonenzymically during transport as an albumin complex being in appropriate proximity to fatty acids needs to be answered. Another possibility for consideration could be that PPCP may be formed in PCP contaminated food and then consumed, especially in the light of 2-chloroethyl palmitate and 2-chloroethyl linoleate found in French dressing (Heikes and Griffitt, 1979), as well as oleanilide (Diachenko et al., 1982) and C_{16} and C_{18} esters of chloropropanediol (Gardner et al., 1983) found in adulterated Spanish cooking oils.

PCP has been shown to be present in human adipose tissues at levels from 4 to 250 ppb (Shafik, 1973; Ohe, 1979; Reynolds et al., unpublished results). The extraction procedures used in the above studies would not hydrolyze an ester bond, therefore PCP present in ester form would remain unaccounted, and PCP quantities present in human fat should be much higher than reported in the literature. A new procedure for extraction and simultaneous monitoring of PCP and PPCP or a procedure for hydrolysis of PPCP before extraction of PCP should be designed for accurate assessment of PCP exposure.

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