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Note

Identification of chlorinated phenols as degradation products of chlorinated pesticides in biological materials

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For the identification and determination of residues of degradation products in biological materials chromatographic methods have mainly been used[1–9]. A gas chromatographic (GC) determination of pentachlorophenol (PCP) in human urine and blood after derivatization to alkyl ethers was described by Crammer and Freal[10]. Kawahara[11] prepared pentafluorotolyl ethers of some phenols and showed their excellent chromatographic properties. Langer *et al.*[12] described several methods for the preparation of trimethylsilyl ethers of phenols by reaction with trimethylchlorosilane or hexamethyldisilasane. The preparation of PCP trimethylsilyl ether for quantitative analysis by GC was described by Stark[13]. Rudling[14] developed a method for the determination of a PCP acyl derivative involving reaction with acetic anhydride in the presence of pyridine. Dünges and Bergheim-Irps[15] described a methylation method using methyl iodide for the GC determination of barbituric acids.

We have investigated the identification and determination of chlorinated phenols in meat and poultry liver contaminated with hexachlorocyclohexane (HCH) and hexachlorobenzene (HCB) residues from feeds by GC with an electron-capture detector (ECD), by thin-layer chromatography (TLC) and also by a combination of GC with mass spectrometry (MS) directly or after derivatization with methyl iodide. This technique is suitable for the determination of chlorinated phenols in microgram amounts.

EXPERIMENTAL

Isolation of chlorinated phenols

A 2–10-g amount (depending on the content of the substances to be determined) of poultry liver or meat was cut into small pieces and refluxed for 20 min with 10 ml of 1 *M* potassium hydroxide solution for each gram of the sample. After cooling the mixture, some boiling stones, 50 ml of 5 *M* sulphuric acid and 500 ml of distilled water were added and 400 ml of liquid were distilled off. After adjustment of the pH to 5 the distillate was extracted three times with 15 ml of toluene. The combined toluene extracts were filtered through anhydrous sodium sulphate and evaporated to 1 ml in a rotary vacuum evaporator. This solution was used for the direct determination of chlorinated phenols by GC and a suitable portion was submitted to derivatization with methyl iodide or diazomethane.

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Derivatization of PCP to pentachloroanisole (PCA) by methylation with methyl iodide

Into a 10-ml conical flask was pipetted 1 ml of a 10 $\mu g \cdot ml^{-1}$ solution of PCP in ethanol and the solvent was evaporated. To the residue were added 3–5 mg of potassium carbonate, 0.5 ml of acetone and 1.5 ml of methyl iodide. The mixture was refluxed for 30 min and then evaporated to dryness; the residue was dissolved in 1 ml of ethanol and injected into the gas chromatograph. Standard PCP solutions of concentration 0.5, 1.0, 2.0 and 4.0 $\mu g \cdot ml^{-1}$ were treated in the same way. The results were used to construct a calibration graph.

By using the above procedure, 2,4-dichlorophenol (DCP), 2,4,5- and 2,4,6-trichlorophenol (TCP) and the evaporated toluene extracts of poultry meat and liver samples were methylated.

Derivatization of PCP to PCA by methylation with diazomethane

A solution of diazomethane in diethyl ether[16] was added dropwise to 1 ml of a PCP solution in toluene (20 $\mu g \cdot ml^{-1}$), until a pale yellow colour persisted. After standing for 1 h the solution was evaporated almost to dryness in a current of nitrogen on a sand-bath at 100°C. To the residue 1 ml toluene was added and this solution was injected into the gas chromatograph.

Gas chromatography

A Carlo Erba GI 452 gas chromatograph was used under the following conditions: glass column, 180 × 0.3 cm I.D.; ^{63}Ni ECD detector; detector voltage, 10 V; column temperature, 150 and 200°C; temperature of injection block, 210°C; carrier gas (nitrogen) flow-rate, 60 $ml \cdot min^{-1}$; chart paper speed 0.5 $cm \cdot min^{-1}$; injection volume, 1 μl. The following columns were used: (1) 4% SF-96 + 8% QF-1 on silanized Gas-Chrom P (80–100 mesh)[13]; (2) 2% QF-1 + 1.5% OV-17 on Chromosorb W AW (80–100 mesh)[17]; (3) 4% SE-30 + 6% SP-2401 on Supelcon AW DMCS (100–120 mesh)[18]; (4) 1% Igepal CO 800 + 1% orthophosphoric acid + 2% Apiezon L on Chromosorb W (80–100 mesh).

The identification was completed by mass spectrometry using a quadrupole mass spectrometer combined with a Hewlett-Packard 5985 gas chromatograph. The separation of chlorinated phenols has been performed in the column 4 and in an SE-52 capillary column with direct entrance into the ion source. The carrier gas (helium) from the packed columns was separated in a jet separator. The vapours of the substances leaving the column were ionized at 70 eV and the ions produced, after separation in a quadrupole analyser according to their *m/e* values, were detected by using an electron multiplier with a maximum amplification of 10^6.

RESULTS AND DISCUSSION

The results were evaluated according to peak heights with the aid of a calibration graph constructed from three points corresponding to concentrations of individual standards in the range 3.0–0.25 $\mu g \cdot ml^{-1}$. The most suitable column for the separation of a mixture of 2,4-DCP, 2,4,6-TCP, HCB and PCP is column 4 (Fig. 1). On this column 2,3,4- and 2,4,5-TCP produce a common peak. For the separation of corresponding anisoles, column 2 is the most suitable.

After the derivatization of TCP and PCP with methyl iodide we analysed the

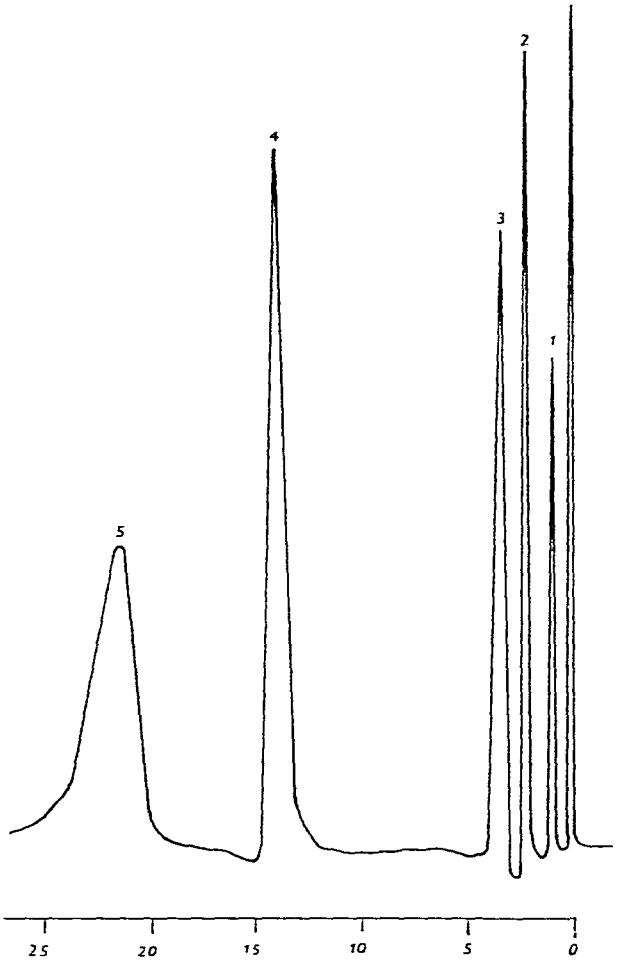

Fig. 1. Separation of a standard mixture of chlorinated phenols and benzenes on a column packed with 1% Igepal CO 880 + 1% H_3PO_4 + 2% Apiezon L on Chromosorb W (60–60 mesh). Peaks: 1 = 2,4-DCP; 2 = 2,4,6-TCP; 3 = 2,3,4- + 2,4,5-TCP; 4 = HCB; 5 = PCP.

reaction mixture by GC and GC–MS, and obtained peaks with retention times differing from those of the original chlorinated phenols. The mass spectra indicated that they were the expected anisoles, trichloroanisole and pentachloroanisole.

The methylation of chlorinated phenols with methyl iodide was also suitable for quantitative purposes and can replace the carcinogenic diazomethane. In GC the excess of methyl iodide does not interfere as its elution time corresponds with that of the solvent. The degree of conversion in the methylation with methyl iodide is 76–80%.

The elution peak of PCA in the samples of poultry liver (Fig. 2) is partly covered by that of HCB, the content of which is about 100 times greater, as a result of its cumulation from the contaminated feed.

We also confirmed the identity of the substances examined by TLC on Silufol plates[19]. The separation of mixed chlorinated phenols was achieved by using *n*-hexane–benzene–ethyl acetate (6:4:1) as the mobile phase in the order 2,4-DCP (R_F = 0.37), 2,4,6-TCP (R_F = 0.45), 2,3,4-TCP (R_F = 0.51), 2,4,5-TCP (R_F = 0.59) and PCP (R_F = 0.65).

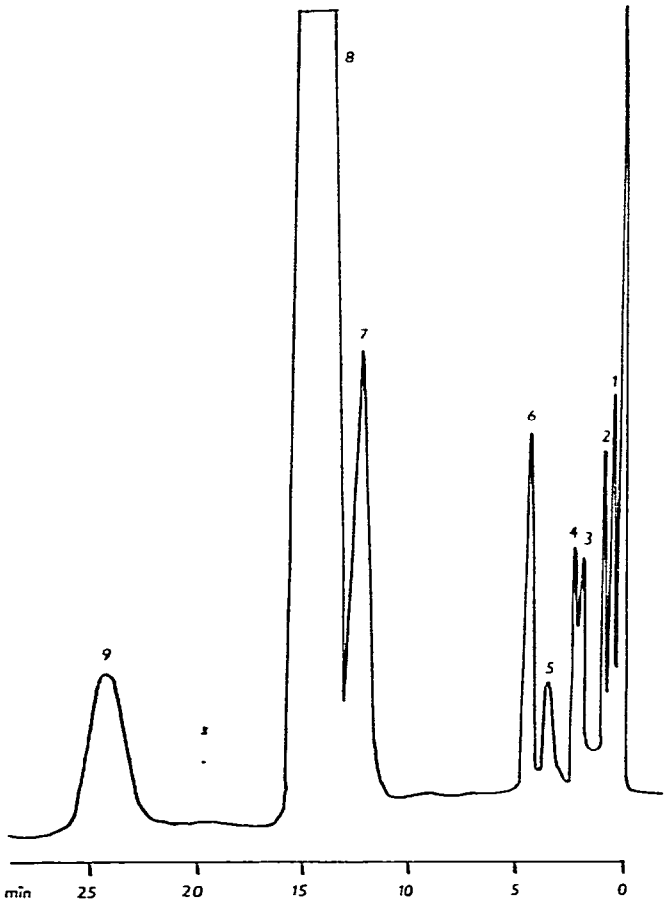

Fig. 2. GC of a sample of poultry liver from chicken fed with a feed contaminated with HCB after methylation with methyl iodide. Peaks: 2 = 2,4-DCP; 3 = 1,2,4,5-TeCB; 4 = 2,4,6-TCP; 5 = 2,3,4- + 2,4,5-TCP; 7 = PCA; 8 = HCB; 1,6,9 = not identified.

The yield of the chlorinated phenols was in the range 92–98%. The sensitivity of the GC method is $1 \cdot 10^{-2}$ $\mu g \cdot g^{-1}$ and of TLC 0.5–5 $\mu g \cdot g^{-1}$.

The method was tested on samples of poultry liver and meat from laying hens in a feeding experiment with HCB in which chlorinated phenols as degradation products after a thermal treatment were determined. The presence of 2,4-DCP, 2,4,6-TCP and PCP was established[20].

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