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Note

Method for the determination of higher chlorinated diphenyl ethers in chicken tissue

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Polychlorinated diphenyl ethers occur as impurities in chlorophenol preparations[1–3] and have been estimated at 100–1000 ppm in some formulations[1]. In studies with lower chlorinated congeners, a relatively high persistence in fish was found[4,5]. Trichloro- and tetrachlorodiphenyl ethers have been identified in mussels in Narragansett Bay, in association with a trichlorodibenzofuran[6]. Little is known of the toxicity of the chlorinated diphenyl ethers, although the induction of liver enzymes has been studied in rats[7]. The ethers have been shown to be converted to chlorodibenzofurans by irradiation with UV light[8,9] and to chlorinated dibenzofurans and dibenzo-*p*-dioxins by pyrolysis[10].

Chickens raised on wood chip litter have been shown to assimilate chlorophenol impurities such as dioxins and furans[11] or chloroanisoles[12] resulting from prior treatment of lumber with wood preservative. Thus, it is possible that chicken tissues could be contaminated with chlorodiphenyl ethers which could serve as precursors to dioxins and furans. Accordingly, a method was developed for the analysis of these ethers, particularly the hepta-, octa-, and nonachloro congeners which appear to predominate in commercial pentachlorophenol.

EXPERIMENTAL

Materials

Polychlorinated diphenyl ethers (compounds 1–11, Fig. 1) were synthesized from the respective chlorinated diphenyliodonium salt and chlorophenol by a procedure analogous to that of Nilsson *et al.*[13], using 3-μmole quantities of reactants. The products were purified by chromatography on a 6 × 1.5 cm column (10 g) of activity I neutral alumina (BioRad Labs, Richmond, VA, U.S.A.) packed in hexane. The column was eluted first with hexane (50 ml) and then with dichloromethane–hexane (2:98) (50 ml) to recover the diphenyl ether fraction. The identity of the product was ascertained by electron impact mass spectrometry. The purity of each isomer was established by gas–liquid chromatography (GLC) on a 30-m capillary column and was found to exceed 99%.

For fortification of tissues, a mixture containing approximately 50 ng ml^{-1} of each diphenyl ether was prepared in acetone and was added to the samples in volumes of 50 or 100 μl. For GLC, the same mixture was diluted in hexane to a nominal concentration of 2 ng ml^{-1} in each component.

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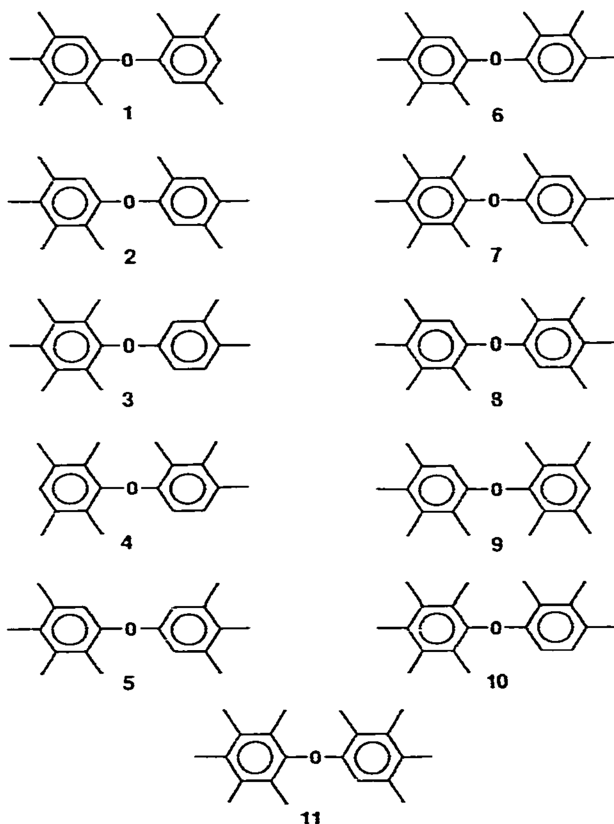

Fig. 1. Structures of polychlorinated diphenyl ethers used to fortify chicken tissue.

Florisil for column chromatography (Floridin Co., Berkley Springs, WV, U.S.A.) was washed with methanol and then dichloromethane to remove impurities. After removal of the dichloromethane by heating overnight at 100°C, it was activated by heating to 320°C for 8 h. Before use, Florisil was deactivated by bringing the water content to 2% with distilled water and mixed by shaking for 2 h.

Methods

Samples of chicken muscle (10 g), liver (10 g), or fat (1 g) were homogenized for 1 min in acetone–hexane (2:1) (90 ml) using a Silverson homogenizer. The extract was filtered, using vacuum, on a 4.25-cm Büchner funnel containing Whatman No. 1 paper. The filtrate was added to a 125 ml separatory funnel and partitioned with water (30 ml). The hexane layer was removed by Pasteur pipette and taken to dryness on a rotary evaporator with a 25°C bath.

A 50 × 2 cm glass chromatography tube was dry packed with 2% deactivated Florsil (12 g) and topped with a 0.5 cm layer of anhydrous sodium sulfate. The extract was dissolved in hexane (2 ml) and added to the Florisil column. After penetration of the sample, further aliquots (2 ml) of hexane were added until the entire column was wetted with solvent. The diphenyl ether fraction was then eluted with hexane (85 ml).

For high-performance liquid chromatographic (HPLC) cleanup, the hexane eluate was reduced to 2–3 ml on a rotary evaporator, transferred to a 15-ml conical centrifuge tube and taken to dryness. The residue was dissolved in acetonitrile (200 μl) and injected into the 100-μl sample loop of the HPLC. The appropriate fraction, as determined by calibration with a standard solution of diphenyl ethers in acetonitrile was collected and diluted to 9 ml with water. The ethers were extracted with hexane (2 $\times$ 3 ml) and the extract taken to dryness in a conical tube. The samples were then dissolved in hexane (500 μl) and analyzed by capillary GLC.

Instrumentation

HPLC was carried out on a 25 $\times$ 0.46 cm column packed with 5-μm Spherisorb ODS (Cosyns & Spain, Mount Royal, Canada) and supplied with a mobile phase of water–acetonitrile (10:90) by an Altex Model 110 A pump at a flow-rate of 1 ml min^{-1}. The effluent was monitored with a Waters Model 440 UV detector fitted with a 254 nm filter and connected to a 1-mV Honeywell recorder.

GLC was performed on a Hewlett-Packard 5700 fitted with an SGE splitless injection system and a 30 m 0.25 mm I.D. J & W capillary column coated with SE-54. A helium carrier gas flow with a linear velocity of 33 cm sec^{-1} was maintained and the ^{63}Ni electron-capture detector was purged with argon–methane (95:5) at a flow-rate of 30 ml min^{-1}. Injector and detector temperatures were maintained at 300°C and the column oven was operated isothermally at 240°C. Sampies were injected using an SGE solids injector and applying 5 μl of the hexane solution to the spiral needle. Components were quantitated by comparison of the peak heights to those of a standard.

Mass spectra were obtained on a Varian MAT 311 A at a resolution of 1000 using a 70 eV ionizing voltage. Samples were introduced on a probe.

RESULTS AND DISCUSSION

During development of the method attempts were made to separate the various polychlorinated biphenyl (PCB) congeners and isomers of *e.g.* Aroclor 1260 from the diphenyl ethers. Using adsorbents such as silicic acid, alumina, Florsil, 10% silver nitrate on silicic acid[14], or sulfuric acid on silicic acid[15] many of the PCBs were separated from each other, but it was not possible to remove them as a class from the diphenyl ethers. Further, reversed-phase HPLC or normal-phase HPLC on silica did not provide the required isolation. Thus, in the event of the presence of PCBs, mass spectrometry must be used to avoid interference.

For preliminary cleanup, concentrated sulfuric acid was examined as a reagent for lipid removal but was found to result in the generation of non-polar material which interfered with the subsequent HPLC cleanup step. A 12-g column of 2% deactivated Florisil was found effective in removing essentially all of the lipid from the non-polar fraction while permitting elution of all 11 diphenyl ethers with 85 ml of hexane. Care had to be exercised to avoid overloading the Florsil column since the presence of lipid material resulted in low recoveries from the HPLC step. In the absence of HPLC cleanup numerous negative peaks were produced on the GLC which, interfered with determination of the diphenyl ethers. The separation of hepta-, octa-, and nona-chlorinated congeners by HPLC is shown in Fig. 2.

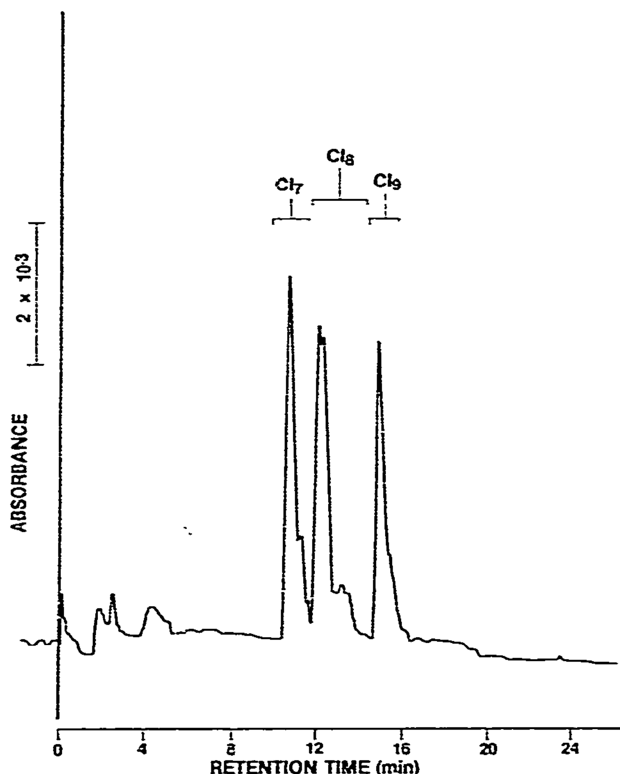

Fig. 2. High-performance liquid chromatogram of hectachloro- (Cl_7; 60 ng), octachloro-(Cl_8; 40 ng), and nonachloro-(Cl_9; 24 ng) diphenyl ethers. Mobile phase is water–acetonitrile (10:90) at 1 ml min^{-1} on a 25 × 0.46 cm column of Spherisorb ODS.

TABLE I

RECOVERIES OF 11 POLYCHLORINATED DIPHENYL ETHERS FROM CHICKEN TISSUE FORTIFIED AT TWO LEVELS

Values are the means of duplicate determinations; for structures, see Fig. 1.

Diphenyl ether added	*Diphenyl ether recovery (%)*					
	Fat		*Liver*		*Muscle*	
	2.5 ppb	*5.0 ppb*	*250 ppt*	*500 ppt*	*250 ppt*	*500 ppt*
1	97	100	91	87	86	103
2	95	103	86	81	77	104
3	84	90	83	78	85	102
4	86	97	81	79	78	102
5	69	90	81	81	91	100
6	79	95	72	64	71	98
7	88	101	79	82	84	102
8	101	100	81	80	91	100
9	82	99	89	88	86	103
10	87	102	86	97	101	101
11	89	104	82	81	85	99

As shown by the data in Table I, the method provides acceptable recoveries of the 11 ethers at levels down to 250 ppt (10^{12}) in muscle or liver and 2.5 ppb (10^9) in fat. When 5 samples of muscle were fortified at 500 pt and analyzed, a mean recovery for the 11 ethers of 103% was obtained with a coefficient of variation of 5.8%.

The separation of congeners and isomers obtained from a fortified fat sample is shown in Fig. 3. Compared to the fortified sample, the fat blank contains no peaks which interfere with the diphenyl ethers at the concentrations tested. Similar chromatograms were obtained in the case of muscle or liver tissue.

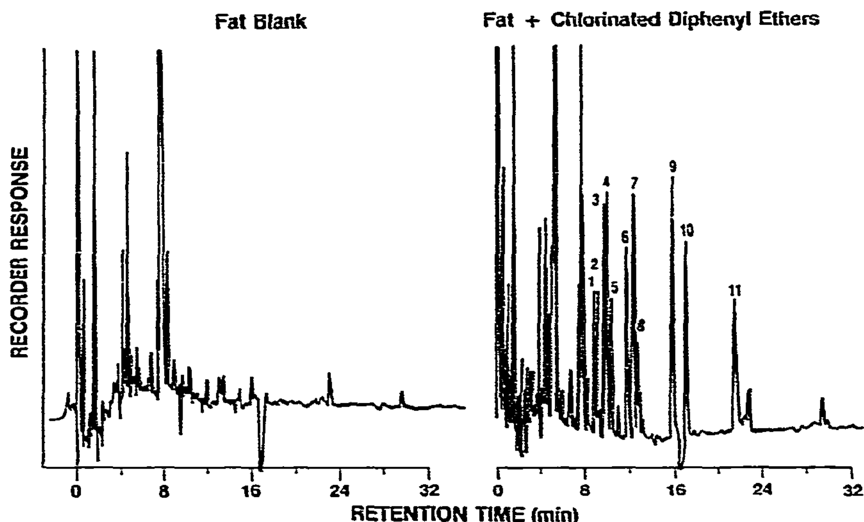

Fig. 3. Gas–liquid chromatogram of extracts of fortified and unfortified chicken fat after cleanup on Florisil and HPLC. Diphenyl ethers were added at a nominal concentration of 2.5 ppb and are identified as shown in Fig. 1.

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