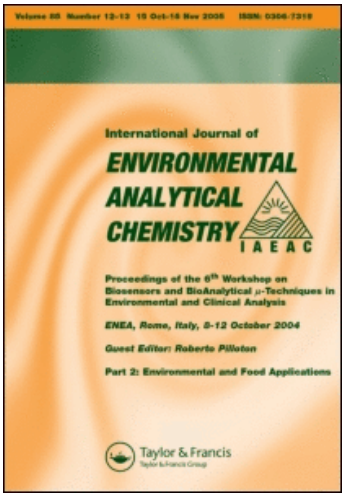

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A Method for the Determination of Octa- and Nonachloro-2-phenoxyphenols in Chicken Tissue

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A gas–liquid chromatographic method was developed capable of determining octa- and nonachloro-2-phenoxyphenols in chicken liver or muscle at 0.25 ppb and fat at 2.5 ppb. The method involves extraction with acidified acetone:hexane, cleanup with concentrated H_2SO_4 and Florisil column chromatography, methylation with diazomethane, and quantitation by capillary column gas–liquid chromatography with electron capture detection. Fortification of liver and muscle at 0.25 or 0.5 ppb and fat at 2.5 or 5.0 ppb and subsequent analysis yielded recoveries averaging 91% for octa- and 97% for nonachlorophenoxyphenol.

KEY WORDS: Chlorinated 2-phenoxyphenol, analysis, chicken tissue.

INTRODUCTION

Chlorinated 2-phenoxyphenols have been identified as major impurities in chlorophenol preparations where they have been estimated at levels of 1–5%.[1–3] In Canada, pentachlorophenol appears to be used to the greatest extent[4] and the predominant impurity is nonachloro-2-phenoxyphenol with smaller amounts of octachloro-2-phenoxyphenols being present.[2,3,5,6] Chloro-2-phenoxyphenols have been shown to convert to chlorodibenzodioxins when heated[2,3] or subjected to ultraviolet irradiation[7] and thus are a potential source of chlorodibenzodioxins.

Chlorophenols are used primarily for the preservation of wood and as such are known to enter the food supply via shavings or sawdust used as bedding for livestock. Ryan and Pilon[8] have reviewed examples of contamination of animals and reported finding hexa-, hepta-, and octachlorodibenzodioxins in the livers of chickens maintained on bedding containing pentachlorophenol. It may be assumed that dioxin precursors could also migrate to the tissues. Methods for the determination of chloro-2-phenoxyphenols in foods are unavailable in the literature although they have been determined in wood dust with low (30–70%) recovery. The following method was developed to determine the higher chlorinated 2-phenoxyphenols which might be expected in chicken tissues as a result of the use of wood-based bedding.

MATERIALS AND METHODS

Reference standards

Nonachloro-2-phenoxyphenol was synthesized from pentachlorophenol by the route shown in Figure 1 as described by Deinzer *et al.*[10] The product of reaction was dissolved in dichloromethane (50 ml) and extracted with four aliquots (50 ml) of 0.1 *N* $NaHCO_3$. The organic layer was taken to dryness, redissolved in a minimum volume of iso-octane: dichloromethane: methanol (925:50:25) and chromatographed on a 20 g column of silica (Woelm, activity I, 63–100 μm) using the same solvent system as eluant. The 250–350 ml fraction containing nonachloro-2-phenoxyphenol was evaporated to dryness and further purified by preparative high pressure liquid chromatography on a 1.0×25 cm column of 5 μm Lichrosorb Si 60 using iso-octane: dichloromethane: methanol (925:50:25) as mobile phase. Mass spectral examination of the product showed a fragmentation pattern essentially the same as that published for nonachloro-2-phenoxyphenol.[6] Treatment with ethereal diazomethane and subsequent gas–liquid chromatography on a 30 M SE-54 capillary column showed a single peak indicating >99% purity.

Octachloro-2-phenoxyphenol was synthesized by reacting 2,2',3,3',4,4',5,5' octachlorodiphenyl iodonium bisulfate with tetrachloroguaiacol in aqueous KOH (Figure 1). The

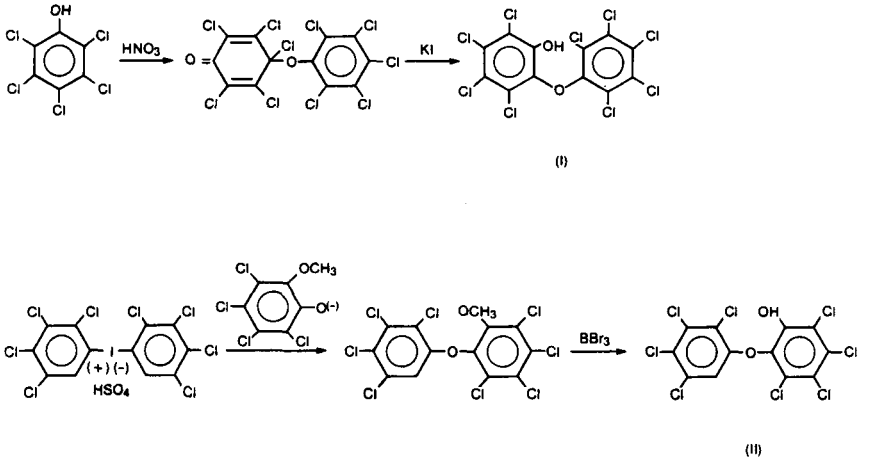

FIGURE 1. Reaction schemes for the synthesis of octachloro-2-phenoxyphenol(II) and nonachloro-2-phenoxyphenol(I).

octachlorodiphenyl iodonium bisulfate was prepared from 2,3,4,5 tetrachlorobenzene as described by Nilsson *et al.*[11] Tetrachloroguaiacol, prepared by the chlorination of guaiacol[4] was provided by Dr. I. Chu, Environmental Toxicology Division, Environmental Health Directorate, Health and Welfare Canada. Typically, tetrachloroguaiacol (1.31 g; 5 mmole) was stirred in 40 ml of H_2O with KOH (280 mg; 5 mmole) and octachlorodiphenyl iodonium bisulfate (3.27 g; 5 mmole) added. The reaction mixture was refluxed for 1 h, cooled, and extracted with dichloromethane (50 ml). The extract was washed twice with 1 *N* NaOH (50 ml), once with water (50 ml), then dried over sodium sulfate and the solvent removed on a rotary evaporator. The orange coloured product was chromatographed on a 20 g column of neutral alumina (activity I, Bio Rad Laboratories, Richmond, CA, U.S.A.) with the first 100 ml of hexane eluate yielding 2.7 g of colourless octachloro-2-phenoxyanisole. The methyl ether was cleaved by dissolving it in dichloromethane (25 ml), cooling to −78°C and adding dropwise a cold solution consisting of 2 ml of BBr_3 in 5 ml of dichloromethane. The solution was stirred 1 h at −78° then allowed to warm to room temperature and react for 3 days. After demethylation, excess BBr_3

was destroyed by the dropwise addition of water (50 ml), the organic layer dried over sodium sulfate, and the solvent removed on a rotary evaporator. The resulting octachloro-2-phenoxyphenol was purified by chromatography on a 12 g column of 2% deactivated Florisil. The column was eluted with hexane (100 ml), then 10% dichloromethane in hexane (100 ml) and the product finally removed with dichloromethane (100 ml). White crystals, mp 169–171° were obtained after crystallization from dichloromethane/hexane. The electron impact mass spectrum was consistent with that of octachloro-2-phenoxyphenol, while capillary gas–liquid chromatography of the methyl ether indicated a purity >99%.

Standards for gas–liquid chromatography were prepared by dissolving the octa- and nonachloro-2-phenoxyphenol in methanol and diluting serially to give a concentration of 100 ng ml^{-1} of each compound. Fifty μl aliquots (5 ng) were treated with ethereal diazomethane along with the samples and analyzed after bringing to a volume of 1.0 ml. For fortification of tissues, 100 or 200 μl aliquots of a 25 ng ml^{-1} solution in methanol were added to the samples prior to extraction.

Adsorbents

Florisil for column chromatography was obtained from Floridin Co., Berkeley Springs, W.VA. U.S.A. and was purified before use by washing with methanol, then dichloromethane and drying overnight at 110°. After activation by heating 8 h at 350°, the Florisil was cooled and deactivated by the addition of 2% water.

Instrumentation

Gas–liquid chromatography was carried out on a Varian 1400 fitted with an S.G.E. (Scientific Glass Engineering, Victoria, Australia) splitless injection system and a 30 m × 0.25 mm id. fused silica capillary column coated with SE-54 (J & W Scientific, Inc. Rancho Cordova, CA. U.S.A.). Helium carrier gas was supplied to the column to give a linear velocity of 33 cm sec^{-1} while the ^{63}Ni electron capture detector was purged with nitrogen at a flow rate of 30 ml min^{-1}. The column oven was maintained at 260° while the injector block and injector were 310°. Under these conditions, the

injection of 25 pg of either octa- or nonachloro-2-phenoxyanisole produced a peak with half scale deflection on a 1 mV recorder at an attenuation of 4×10^{-12} amp/mV.

Chromatographic steps involved in the purification of standards were monitored by reverse phase high pressure liquid chromatography on a 150 × 4.1 mm PRP-1 column (Hamilton Co., Reno, N.V. U.S.A.) using a mobile phase consisting of 8 ml of 25% tetramethylammonium hydroxide and 125 ml water/l of methanol. Solvent was delivered to the column at 1 ml min^{-1} by an Altex model 110 A pump and the effluent monitored by absorption at 254 nm with a Waters model 440 ultraviolet detector.

Mass spectra were obtained on a ZAB-2F spectrometer using 70 eV ionizing voltage and a resolution of 2000. Samples were introduced on a probe.

Analytical procedure

Samples of liver (10 g), muscle (10 g) or fat (1 g) were homogenized for 1 min at high speed in a Silverson Homogenizer with 90 ml of acetone: hexane (2:1) containing 0.5 ml of conc. HCl. The homogenate was filtered through a 4.25 cm dia. disc of Whatman No. 1 paper on a Buchner funnel and the filtrate transferred to a 250 ml separatory funnel where it was partitioned with water (30 ml). The aqueous phase was discarded and hexane (70 ml) added to the upper layer. Lipid in the hexane extract was removed by shaking with successive aliquots (10 ml) of conc. sulfuric acid until the sulfuric acid remained colourless. After treatment with sulfuric acid, the hexane was washed by shaking twice with 50 ml portions of water, then dried over sodium sulfate, filtered, and the solvent removed on a rotary evaporator.

The residue was taken up in 10% dichloromethane in hexane (1.0 ml) and applied to a 2 g column of 2% deactivated Florisil dry packed in an 8 × 200 mm glass chromatographic tube. The flask which had contained the residue was rinsed with small portions of hexane and these added to the column until the entire bed of Florisil was wetted with hexane. The column was then eluted with 15 ml of hexane followed by 16 ml of ethyl acetate. The phenoxyphenols were recovered by elution with 20% methanol in ethyl acetate (25 ml). After removal of the solvent on a rotary evaporator, the samples

were transferred with methanol to 15 ml graduated centrifuge tubes and taken to dryness on a rotary evaporator.

The residue was dissolved in methanol (50 μl) and, along with standards consisting of 5 ng each of octa- and nonachloro-2-phenoxyphenol, methylated overnight with a solution (0.5 ml) of diazomethane in methanol: diethyl ether (10:90). The solvent was removed with a stream of nitrogen and the residues taken up in hexane (1.0 ml). Aliquots (10 μl) were spotted onto an S.G.E. solid injector (Scientific Glass Engineering, Victoria, Australia) and analyzed by capillary gas–liquid chromatography.

RESULTS AND DISCUSSION

The extraction procedure was similar to that of Van Renterghem and Devlaminck[12] for PCBs, but incorporated HCl which enhanced the recoveries by approximately 10%, particularly from liver. Lipid was removed from the extracts by repeated shaking with H_2SO_4; a reagent in which the phenoxyphenols were stable. The elution sequence used with Florisil column cleanup removes chlorinated diphenyl ethers and PCBs in the first fraction and chlorodibenzodioxins and chlorodibenzofurans in the second while the relatively polar phenoxyphenols are not removed until elution is carried out with 20% methanol in ethyl acetate. Deviation from the

TABLE I

Recoveries of octa- and nonachloro-2-phenoxyphenols from fortified chicken tissue

Tissue	Level added (ppb)	Recovery[a] (%) 8-Cl	9-Cl
Liver	0.25	83	91
	0.50	81	94
Muscle	0.25	96	102
	0.50	89	91
Fat	2.5	109	103
	5.0	89	100

[a]Values are the means of duplicate determinations.

stated elution solvents, for example, by replacing ethyl acetate in the second fraction with dichloromethane resulted in incomplete recovery of phenoxyphenol in the third fraction even if the percentage of methanol or volume of eluate were increased.

Diazomethane produced complete methylation within 1 h, but gave more consistent results and permitted decomposition of the

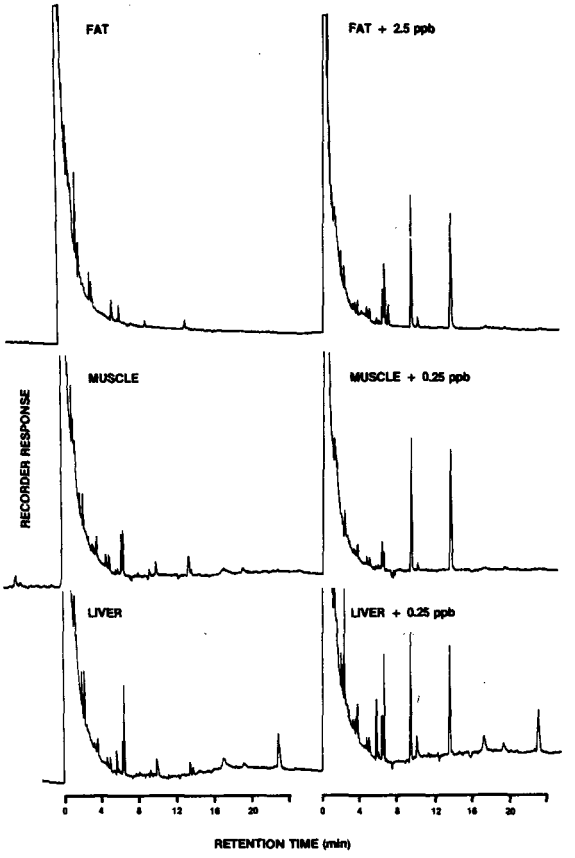

FIGURE 2. Gas–liquid chromatograms of purified extracts from fortified and unfortified chicken tissue. Fortification level was 0.25 ppb for liver and muscle, and 2.5 ppb for fat. Chromatography was performed on a 30 m × 0.25 mm fused silica column coated with Se-54 and run at 260°C using 33 cm sec^{-1} helium carrier. Octachloro-2-phenoxyanisole has a retention time of 9.25 min and nonachloro-2-phenoxyanisole elutes at 13.5 min.

residual reagent if the reaction was carried out overnight. Attempted alkylation with methyl iodidetetramethylammonium hydroxide[13] resulted in an approximately 50% yield of derivative while dimethyl sulfate-NaOH gave only traces of the methyl ether.

As shown by the data in Table I, acceptable recoveries were obtained at levels of 0.25 or 0.50 ppb in liver and muscle, and 2.5 or 5.0 ppb in fat. When 6 samples of liver were fortified before extraction at 0.25 ppb and analyzed simultaneously a coefficient of variation of 14.7 and 13.0% was found for octa- and nonachloro-2-phenoxyphenol respectively. Corresponding values for muscle fortified at 0.25 ppb were 15.0 and 16.1%.

Figure 2 depicts chromatograms obtained from the various blank and fortified tissues. It is estimated that a lower limit of detection of 0.1 ppb in liver or muscle could be achieved, accepting a 2:1 signal:background ratio.

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References

1. C.-A. Nilsson and L. Renberg, *J. Chromatogr.* **89**, 325 (1974).
2. S. Jensen and L. Renberg, *Ambio.* **1**, 1 (1972).
3. C. Rappe and C.-A. Nilsson, *J. Chromatogr.* **67**, 247 (1972).
4. Chlorinated Phenols: Criteria for Environmental Quality. Publication NRCC No. 18578. National Research Council of Canada. NRC Associate Committee on Scientific Criteria for Environmental Quality. (1982).
5. M. Deinzer, D. Griffin, T. Miller and R. Skinner, *Biomed. Mass. Spectrom.* **6**, 301 (1979).
6. M. Deinzer, J. Lamberton, D. Griffin and T. Miller, *Biomed. Mass. Spectrom.* **5**, 566 (1978).
7. C.-A. Nilsson, K. Andersson, C. Rappe and S.-O. Westermark, *J. Chromatogr.* **96**, 137 (1974).
8. J. J. Ryan and J. C. Pilon, In: Chlorinated Dioxins and Related Compounds. O. Hutziner *et al.* eds. Pergamon Press, New York, pp. 183–189, 1982.
9. J.-O. Levin and C.-A. Nilsson, *Chemosphere* **1**, 443 (1977).
10. M. Deinzer, T. Miller, B. Arbogast and J. Lamberton, *J. Agric. Food Chem.* **29**, 679 (1981).
11. C.-A. Nilsson, A. Norstrom, M. Hansson and K. Andersson, *Chemosphere* **9**, 599 (1977).
12. R. Van Renterghem and L. Devlaminck, *Z. Lebensm. Unters. Forsch.* **170**, 346 (1980).
13. I. M. Kapetanovic, J. S. Dutcher and J. M. Strong, *Anal. Chem.* **49**, 1843 (1977).