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Determination of PCB and PCT Residues in Fish by Tissue Acid Hydrolysis and Destructive Clean-up of the Extract

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In the analysis of polychlorinated biphenyl (PCB) and polychlorinated terphenyl (PCT) residues in fish tissues, wide variations exist in methods of extraction, clean-up and quantitation from laboratory to laboratory. HATTULA (1974) found variations in extraction efficiencies in PCB residue analysis in fish according to the method used. The disadvantage of clean-up of the extract by liquid-liquid partitioning (hexane-acetonitrile) is that partition coefficients differ for the various components (position and number of chlorine atoms in the phenyl ring) of PCB (STALLING et al. 1972) and PCT, as well as the fact that PCB and PCT are strongly nonpolar and a poor separation from fat obtained. In adsorption chromatography from silica and florisil columns, some authors (MASUMOTO 1972, LIEB and BILLS 1974) observed variations in the relative amounts of the various components in PCB. Some biological material, especially aquatic organisms, synthesized or accumulated various persistent organochloro compounds besides PCB-PCT (LUNDE et al. 1975, LUNDE and STEINNES 1975) and which are coextracted with PCB and PCT. These accompanying compounds interfere in GL chromatograms (because of their electron capture detector response) with PCB-PCT peaks. They could be removed by destructive clean-up. Some investigators used treatment with H_2SO_4 (DOGUCHI et al. 1974, PESTICIDE ANALYTICAL MANUAL 1975, MURPHY 1972), CrO_3 oxidation (COLLINS et al. 1972, MULHERN et al. 1971, TROTTER 1975) or alkali hydrolysis (PESTICIDE ANALYTICAL MANUAL 1975, KRAUSE 1972, TROTTER 1975, YOUNG and BRUKE 1972).

The purpose of the work described in this contribution is (1), to improve the extraction efficiency of PCB-PCT from fish, (2) to note the influence of destructive clean-up on PCB-PCT, and (3), to improve clean-up, with low degradative effects on PCB-PCT but with sufficient elimination of those accompanying non PCB-PCT compounds, which can influence the chromatography pattern. The difference in the GL chromatogram of PCT in fish from that of Aroclor 5460 is due to the effects of the metabolic or environmental pathway (ADDISON et al. 1972, SOSA-LUCERO et al. 1973). This causes difficulties in quantification, and hence we have modified quantification after perchlorination.

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EXPERIMENTAL

Acid hydrolysis. STOLDT-WEIBULL methods (RAUSCHER et al. 1972): The edible portion of fish was homogenized and refluxed for 30 min. with diluted aqueous hydrochloric acid (1+1). After acid hydrolysis, the crude fat was obtained by diethyl ether extraction. The procedure is described in OFFICIAL METHODS of ANALYSIS (1975). The extract was dried on a Na_2SO_4 column and evaporated on a rotovapor to dryness.

Alkali hydrolysis. 0.5 g extracted lipid was refluxed for 30 min. on a steam bath with 30 ml 2 % ethanolic KOH. The hydrolysate was transferred to a separatory funnel with 30 ml water, and the vial and condenser was rinsed triply with 10 ml hexane. The lower phase was separated in a second separatory funnel and shaken with 20 ml hexane. The hexane extract was transferred to the first separatory funnel. The combined hexane extracts were extracted twice with 20 ml aqueous ethanol (1+1).The hexane solution was dried on a Na_2SO_4 column and concentrated to 1 ml at 60°C with a flow of nitrogen.

Oxidation. To the hexane solution (1 ml), 5-10 ml acetic acid and H_2O_2 (30+6) solution was added and refluxed on a steam bath for 1 hr. The solution was neutralized with dil. NaOH, extracted twice with hexane, and the combined hexane extracts washed with water an dried on a Na_2SO_4 column.

H_2SO_4 destructive clean-up. 10 g celite 545 (heated overnight at 130°C) was ground thoroughly with 6 ml 5 % SO_3 H_2SO_4 and transferred to a fritted glass column. The column was prewashed with hexane. The hexane extract from the oxidation step was applied to the column and eluted with 50 ml hexane. The eluate was neutralised with 2 % NaOH solution, dried over Na_2SO_4, concentrated to 2 ml and placed on the miniature 5 cm florisil (activated overnight at 130°C) column (ERNEY 1974). The column was eluated with 70 ml hexane, and the eluate concentrated with a flow of nitrogen at 60°C prior to GLC.

Perchlorination. The final hexane extract was transferred to a glass vial, and dried on a steam bath (50°C) with a flow of nitrogen. 0.3 ml antimony pentachloride (Merck-Schuchardt) was added and the glass vial sealed. Reaction was carried out for 10 hr at 170°C. The glass vial was cooled, opened and rinsed with a total of 5 ml 6 N HCl, and transferred to a separatory funnel. The reaction vial was rinsed several time with hexane and the rinsings transferred to the separatory funnel. The phases were shaken and allowed to separate; the lower phase was discarded. The hexane extract was washed with 20 ml water, 20 ml 2 % KOH and water. It was then dried

on column of Na_2SO_4 and passed through a miniature florisil column (ERNEY 1974). The florisil column was eluted with 70 ml benzene-hexane (1+1). The eluate was concentrated to a suitable volume for GLC determination.

Side products, which can influence the quantitative determination of PCT after perchlorination - because of contamination of antimony pentachloride with bromide (HUCKINS et al. 1974, TROTTER and YOUNG 1975) was not present in our experimental work ($SbCl_5$ supplied in glass vials from Merck-Schuchardt).

Gas chromatographic equippment. The gas chromatograph was a Varian Mod. 1700 equipped with a 6' x 2 mm (I.D.) pyrex column packed with 2.5 % OV-1 or 2.5 % QF-1 and 2.5 % DC-200 on 100-120 mesh Varaport 30 with an EC (Sc - ^3H) detector. The operating parameters were as follows: nitrogen flow 60 ml/min. Temperature: column 275°C, detector 320°C, injector 290°C for PCT, and 205°C, 220°C and 250°C for PCB respectively.

Quantification. For quantitative determination of PCB we used mixed Aroclor 1254-1260 (1+1) and for PCT Aroclor 5460 standards. PCB and PCT residue was an average value calculated from relating each peak height (for PCB we used 14 and for PCT 13 peaks) in the sample of fish extract to the corresponding peak in the standard. For quantitative confirmation, the PCT was converted by the perchlorination procedure (ARMOUR 1973) to ortho-, meta- and para-perchlorinated terphenyls. As reference standard, pure ortho-, meta- and para-tetradecachloroterphenyls were used. The sum of all three concentrations of tetradecachloroterphenyls was mathematically converted to Aroclor 5460 (with 60 % Cl content). In order to check the method, commercial vegetable oil was spiked with PCB and PCT, as well the blank analysis.

RESULTS AND DISCUSSION

The data in Table 1 show considerable variation in PCB-PCT residue analysis of fish. The extraction of PCB-PCT after complete tissue hydrolysis with HCl is about 1/3 higher than ether tissue extraction in a Soxhlet. The difference is more significant in fish with a low fat level. (The analysis was carried out by steps 1-5 in the experimental part.)

TABLE 1

PCB-PCT residues by different methods of extraction (trout, Soča river, Yugoslavia, non industrial region - ppb/wet fish tissue)

method of extraction	PCB	PCT	PCT perchlor.
ether Soxhlet	20	3	5
ether after HCl hydrolysis	31	5	8

Note on alcoholic KOH hydrolysis. Chemical clean-up with ethanolic KOH apparently causes no significant changes in the PCB-PCT chromatogram pattern. But with some components (Fig.1) especially highly chlorinated isomers, attack through hydrolysis is possible with ethyl ether (HARWEY et al. 1954), or in the case of di-ortho chloro substitution, furan formation (CASE and SCHOCK 1943, VOS et al. 1970).

Note on CrO_3 oxidation. CrO_3 in acetic acid is a frequently used method for destructive clean-up. Our laboratory investigation shows that after CrO_3 oxidation some chromatographic peaks with low retention time appear (Fig. 2). Reaction can be explained by the strongly oxidative nature of CrO_3, which can open one of the phenyl rings (BEILSTEIN 1943).

Experiments with H_2O_2 oxidation in acetic acid did not change the PCB-PCT chromatographic pattern, but eliminated some coextracted accompanying compunds.

The fuming sulfuric acid - celite column treatment in our analysis is a modified FDA method (PESTICIDE ANALYTICAL MANUAL 1975), with a lower concentration of SO_3 and n-hexane used as solvent. Decrease and displacement of some peaks in PCT chromatogram after treatment with a stronger (over 10 %) concentration of SO_3 was noticed.

Analysis of PCB-PCT residues in fish by comparison to a standard is not satisfactory because of the untypical pattern of chromatograms (Fig. 3), which may be due to metabolic effects or environmental exposure, and probably also some accompanying non PCB-PCT residue compounds. The elimination of these influences and the increase in sensitivity with an EC detector in GLC are the principal advantages obtained after perchlorination. PCT is a mixture of components with different persistence in the environment. The components differ in structure (degree of chlorination, the position of chlorine atoms and existence of the o-, m-, and p-isomers). The relative amounts

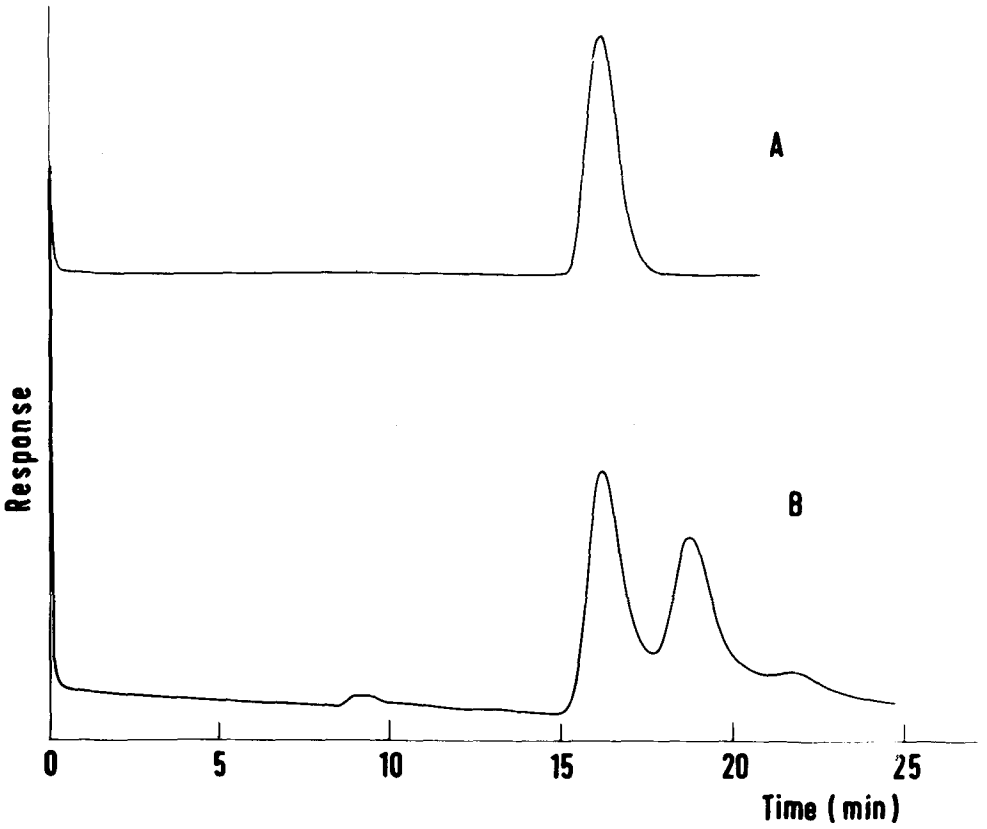

Fig. 1. GL chromatograms (2.5 % OV-1)
A - decachlorobiphenyl
B - decachlorobiphenyl after alkaline hydrolysis (30 min. on reflux with 2 % ethanolic KOH)

of the o-, m-, and p-isomers after perchlorination differ between the Aroclor 5460 standard and the samples from the environment.

Quantification of PCT by formation of o-, m- and p-tetradecachloroterphenyls has to be calculated from the sum of the individual isomers. The electron capture detector response is susceptible to structural differences. Thus the relative electron capture detector response of the ortho isomer of tetradecachloroterphenyl is 2.0 times higher than the corresponding para isomer.

This investigation shows that preliminary acid hydrolysis of fish tissue increases the efficiency of subsequent extraction of PCB-PCT residues. The experimental data indicates that the analyst has to be aware that the destructive clean-up can influence some PCB-PCT isomers. On the other hand, the destructive method

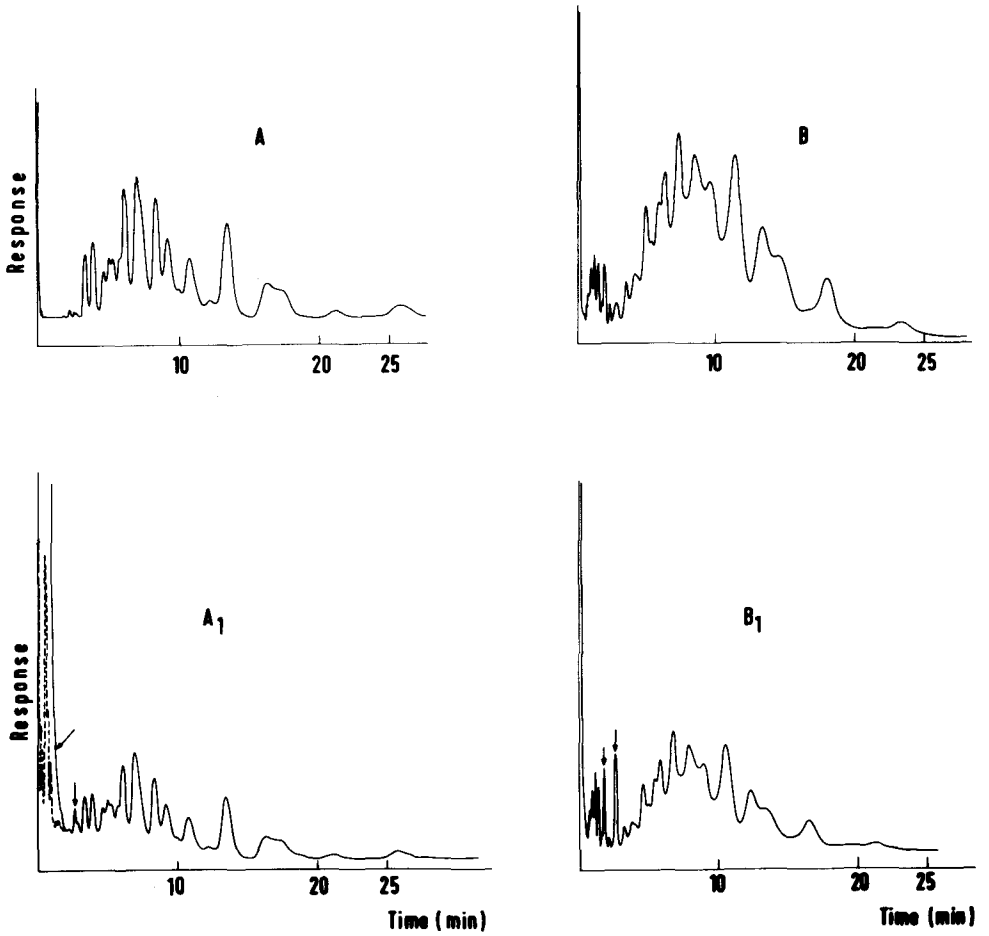

Fig. 2. GL chromatograms of : A - polychlorobiphenyls (Aroclor 1254-1260, 1:1) on 2.5 % QF-1 and 2.5 % DC-200; B - polychloroterphenyls Aroclor 5460 on 2.5 % OV-1;

A_1 and B_1 after oxidation of corresponding compounds (1.5 g CrO_3 in 1 ml water and 59 ml Ac. Ac under reflux on steam bath for 30 min., neutralised with dil. NaOH, hexane extract).

has proved to be practicable for the clean-up of fish extracts; especially because they eliminate some coextracting compounds from biological material. The usefulness of the perchlorination procedure is accepted because it eliminates some other residues which could interfere in PCB-PCT total peak area quantification.

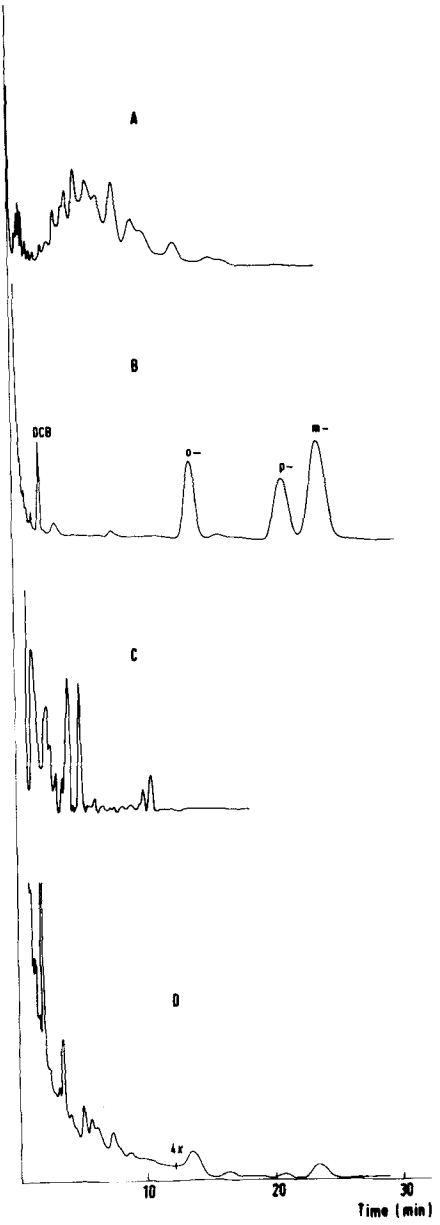

Fig. 3. GL chromatograms (2.5 % OV-1): A - Aroclor 5460; B - perchlorinated Aroclor 5460 (DCB-decachlorobiphenyl, o-, m-, p-tetradecachloroterphenyl isomers, respectively; C - PCT residue in fish extract using the described clean-up before perchlorination, and D - after perchlorination.

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