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Chlorinated Benzene Residues in Fish in Slovenia (Yugoslavia)

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Chlorinated benzenes (CB) can enter the environment as a waste product of the electro-industry and as a result of its use as a solvent, pesticide, heat transfer fluid, flame retardant and chemical intermediate (FISHBEIN 1979) as well as from side reactions in the production of chlorinated compounds, waste water chlorination (YOUNG & HEESEN 1978) and from lindane and HCB transformation (ENGST et al. 1977, 1979). Because of their lipophylic character and slow degradation in the environment they may accumulate in the food chain. Little data on fish contamination with CB are reported (YOUNG & HEESEN 1978, BAUMANN OFSTAD et al. 1978, MORITA 1977, KÖLLE et al. 1972). In the present contribution, capillary GLC was used for the determination of CB residues in fish. This method is convenient as better separation is obtained from interfering substances.

EXPERIMENTAL

Fish and mussels were taken from rivers in Slovenia and the Gulf of Triest (Yugoslavia) in the autumn of 1978. Freshwater fish were 4-7 years of age. The edible tissue (50-100 g) of 1-3 fish and 12 mussels was ground and extracted twice with a mixture of n-hexane and 2-propanol (1+1) in a shaking apparatus at room temperature for 3 h. The solvent to sample ratio was 1 : 1. Water was added to the combined extracts to separate 2-propanol from the hexane layer. The hexane was washed with water and dried with Na_2SO_4 and evaporated in a rotovapor (35°C). The oil residue remaining (0.5 - 1 g) was dissolved in hexane (5 mL) and the solution was mixed twice with a double volume (10 mL) of concentrated sulfuric acid. The hexane extract was passed through a Florisil column (1 cm I.D.; 7 cm Florisil + 0.5 cm Na_2SO_4 on the top) and eluted with 20 mL of 6 % diethyl ether in hexane. The eluate was concentrated to 2 mL at 50°C in the nitrogen flow and chromatographed. To avoid losses during evaporation, especially of lower CB isomers, 50 µL of 1,3,5-trimethylbenzene was added. Futher clean-up was done with hydrolysis. To the hexane eluate evaporated to 1 mL, 1 mL of 2 % ethanolic KOH was added, sealed in a glass vial and hydrolysed 1 h at 95°C. After cooling, water was added to the reaction mixture, extra-

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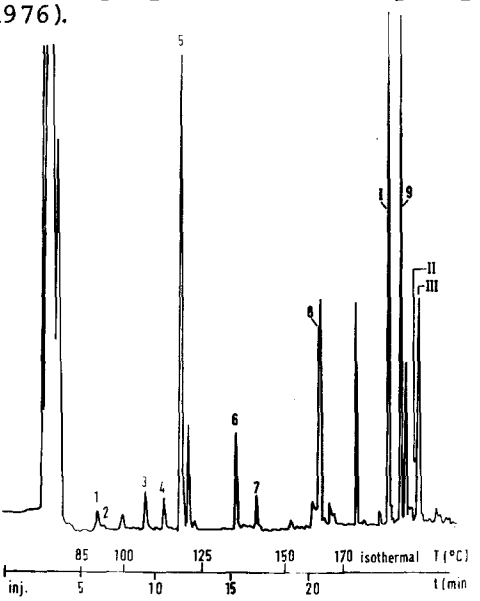
cted with hexane and cleaned-up through concentrated sulfuric acid and Florisil as above and analysed by GLC.

Gas chromatography system. The samples were injected into a GC equipped with an electron capture detector (^{63}Ni). The 200 cm x 2 mm I.D. glass columns were packed with 1.5 % OV-17 + 1.95 % QF-1 and 3 % OV-1 separately coated on 100/120 mesh Varaport 30. The chromatographic conditions - temperatures: injector 210°C; column isothermal at 110° and 150°C; detector 290°C; nitrogen flow 30 and 40 mL/min, respectively.
The conditions for the capillary GLC: glass column lenght 20 m, I.D. 0.22 mm impregnated with SE-30; temperatures: injection port 210°C; detector 300°C; column temperature programmed from 85°C to 170°C, 5°C/min with an initial hold of 5 min; splitless sampling; sample size 2-4 µL; purge activation time 40 sec; nitrogen flow:column 0.8 mL/min, injector purge 120 mL/min.

RESULTS AND DISCUSSION

Chlorinated benzene residues in fish and mussels are listed in Table 1. The chromatogram using a glass capillary column is shown in Fig.1. The total content of CB residues in fish (sum of di-; tri-; tetra-; penta-; and hexa-CB) is of the same order of magnitude as organochlorine pesticide nad polychlorinated biphenyl residues (JAN et al. 1976).

FIGURE 1



Capillary GL chromatogram of chlorinated benzenes in marine fish (pilchard). The CB peaks are numbered 1 to 9:1, 1,4-diCB; 2, 1,2-diCB; 3, 1,3,5-triCB; 4, 1,2,4-triCB; 5,1,2,3-triCB; 6, 1,2,3,5- and/or 1,2,4,5-tetraCB; 7, 1,2,3,4-tetraCB; 8, pentaCB; 9, hexaCB; and HCH isomers I to III: I, alpha-; II, beta-; and III, gamma-HCH, respectively.

TABLE 1

Chlorinated benzene residues in fish and mussels (μg/g on a fat basis)

Species and location	No.of species	1,4-dichlorobenzene	1,2-dichlorobenzene	1,3,5-trichlorobenzene	1,2,4-trichlorobenzene	1,2,3-trichlorobenzene	1,2,4,5- and/or 1,2,3,5-tetrachlorobenzene	1,2,3,4-tetrachlorobenzene	penta-chlorobenzene	hexa-chlorobenzene
trout (Salmo trutta) Kokra r. agricult./wood-land	1	tr.	0.12	0.001	0.001	0.012	0.002	tr.	0.006	0.050
nase (Chondrostoma nasus) Drava r. after Maribor	3	0.45	1.14	0.005	0.005	0.048	0.012	0.002	0.075	0.160
nase Mura r. basin Slovenia	3	0.35	1.20	0.001	0.002	0.008	0.055	0.005	0.010	0.080
nase Sava r. after Ljubljana	2	0.22	1.10	0.005	0.003	0.015	0.022	0.003	0.020	0.080
whiting (Leuciscus cephalus) Sava r. after Ljubljana	1	tr.	tr.	0.003	0.015	0.010	0.015	0.002	0.090	0.220
mullet (Mugillidae sp.) Gulf of Triest	2	0.15	0.05	0.021	0.010	0.080	0.015	0.004	0.015	0.030
pilchard (Sardina pilchardus) Gulf of Triest	3	0.22	0.03	0.015	0.007	0.130	0.018	0.003	0.009	0.026
date shell(Lithophaga lithophaga) Piran	12	tr.	tr.	0.120	0.910	0.330	0.081	tr.	0.125	0.015

tr. - trace; r. - river

In freshwater fish caught in highly populated and industrialized regions the level of total CB residues (1.8 µg/g fat basis) was appreciably higher with respect to fish from river-basins of lightly polluted agricultural- and wood-land (0.2 µg/g) and marine fish (0.4 µg/g). In comparison with other data the level of CB residues in fish should be recognized as rather low; it is in the same order of magnitude as in California (YOUNG & HEESEN 1977) and Japan (MORITA 1977) and lower than in polluted regions of Norway (BAUMANN OFSTAD 1978). We could speculate that the different contribution of CB isomers in water organisms depends on the contamination in different regions, different species, and on the physical properties of CB as partition coefficient lipid/water and water/air (ERNST 1977, SATO & NAKAJIMA 1979). However, metabolism also influences the level of some CB isomers. The metabolism of lower chlorinated isomers is fast (KOHLI et al. 1976) and also depends on the position of chlorine atom: less stable ortho-diCB compared to para-diCB (MORITA 1977) could explain lower ortho: para proportion in marine fish with regard to freshwater fish.

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