Determination of Pentachlorophenol and Chlorobiphenylols in Biological Samples

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Pentachlorophenol is a well known, widely used economic poison, and numerous papers on its uses, determination, toxicity, and environmental fate can be found in the literature (for a review see, for example, BEVENUE and BECKMAN 1967). Pentachlorophenol in a concentration of 1-5 and 0.1-0.7 µg/ℓ was recently detected in sewage influent and river water, respectively [BUHLER *et al.* 1973]. This indicates that the distribution of pentachlorophenol in the aquatic environment may be quite widespread and we were prompted to carry out a survey of levels of pentachlorophenol in aquatic fauna in New Brunswick, Canada.

Chlorobiphenylols are metabolites of chlorobiphenyls (for a review see HUTZINGER *et al.* 1974). No data on the possible environmental occurrence of chlorobiphenylols are available. Only a qualitative method for the detection of these compounds, using the commercially obtainable 2,2',6,6'-tetrachloro-2,2'-biphenyldiol as a standard, has been published [BACHE and LISK 1973]. A number of chlorobiphenylols has been prepared recently and their GLC properties have been described [HUTZINGER *et al.*, submitted]. Using some of these as standards this paper describes a method for the detection of chlorobiphenylols in biological samples.

EXPERIMENTAL

Samples. Winter flounder (*Pseudopleuronectes americanus*), cod (*Gadus morhua*), and sea raven (*Hemipterus americanus*) were taken in the St. Croix estuary, silver hake (*Merluccius bilinearis*) and another sample of winter flounder were obtained from the St. John estuary. All samples were collected in the fall of 1972 and kept frozen (-20°C) until analysis. Juvenile Atlantic salmon (*Salmo salar*) were obtained from hatcheries in 1973. White shark (*Carcharodon carcharias*) was landed in Leonardville, New Brunswick, in 1971 and its liver is stored at -20°C. Double-crested cormorant (*Phalacrocorax auritus*) and herring gull (*Larus argentatus*) eggs were collected on White Horse (Bay of Fundy) and Hospital

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Island (Passamaquoddy Bay), respectively, in the spring of 1973. With the exception of white shark liver, subsamples taken from two specimens were combined for analysis. Whole salmon, and muscle tissue of the other fish were analyzed. Fish food was a commercial sample.

Determination of pentachlorophenol was carried out according to RUDLING (1970). Our batches of acetic anhydride (Fisher Scientific A-10) contained a small amount of an impurity with a retention time practically identical with that of pentachlorophenyl acetate, and acetic anhydride was purified by distillation at approximately 134°C, collecting the middle fraction of the distillate. GLC analysis was carried out as described by HUTZINGER *et al.* (1974). In some samples, pentachlorophenol was confirmed by conversion into butyl pentachlorophenyl ether using dimethyl formamide dibutyl acetal (Pierce Chemical Company) and gas chromatography.

Determination of chlorobiphenylols. White shark liver, double-crested cormorant and herring gull eggs, and juvenile Atlantic salmon were analyzed as such and spiked with standard chlorobiphenylols. Sample (1 g) was homogenized in distilled water (3-4 mℓ) in a Sorvall Omni-mixer and the homogenate was transferred to a 15-mℓ centrifuge tube, rinsing the homogenizer with an additional 1-2 mℓ of distilled water. Sulfuric acid (6M, 1 mℓ) was added and, after 10 min, the mixture was extracted with isopropanol-hexane (1:5, 3 x 5 mℓ). The combined organic extract was shaken with sodium hydroxide (0.1M, 2 x 2 mℓ). The combined sodium hydroxide extract was washed with isopropanol-hexane (1:5, 2 mℓ) and the organic layer was examined by gas chromatography. If significant PCB or other unidentified peaks were observed, the washing step was repeated with hexane until a relatively clean tracing was obtained on GLC. The aqueous sodium hydroxide extract was acidified by sulfuric acid (6M, 3 drops) and extracted with hexane (2 x 0.5 mℓ). The hexane extract was treated with acetic anhydride-pyridine (0.4:1) and analyzed by GLC using the same conditions as above. For recovery experiments the samples were spiked in the homogenizer by adding varying volumes of an acetone solution of 2,4'-dichloro-4-biphenylol, 2,5-dichloro-2'-biphenylol, 2,2',5'-trichloro-4-biphenylol (3.2, 0.5, and 2.6 µg/mℓ, respectively).

RESULTS AND DISCUSSION

Pentachlorophenol in aquatic fauna. Low, but easily detectable levels of pentachlorophenol were found in most of the samples (Table 1). In comparison with literature

data, the concentrations found are most likely insignificant toxicologically. Guppies (*Lebistes reticulatus*), killed by pentachlorophenol within 18 h, contained approximately 100 µg/g wet weight of pentachlorophenol [STARK 1969], and the level of pentachlorophenol in fish from a lake receiving pulpmill waste was 0.15-3.0 µg/g. Eel (*Anguilla anguilla*), exposed to pentachlorophenol in sea water (0.1 mg/ℓ, 8 days), contained 9.4 µg/g of pentachlorophenol in the muscle and this level decreased to 3.6 µg/g after 8 days in clean water [HOLMBERG *et al.* 1972]. WEBB and BRETT (1973) found measurable effects of pentachlorophenol at 1.8 µg/ℓ on growth rate and conversion efficiency of juvenile sockeye salmon (*Oncorhynchus nerka*), but did not report the associated tissue levels of pentachlorophenol.

TABLE 1.

Concentration of pentachlorophenol in aquatic fauna and commercial fish food.

Sample	Weight (g)	Pentachlorophenol (ng/g wet weight)
Cod	486.8	0.82
Winter flounder	157.8	1.77
	128.2*	3.99
Sea raven	875.9	<0.5
Silver hake	214.0	1.75
Atlantic salmon	50.3	1.26
	8.5	0.54
White shark liver	-	10.83
Double-crested cormorant egg	46.2	0.36
Herring gull egg	96.1	0.51
Fish food	-	2.23

*St. John estuary

Determination of chlorobiphenylols. The chlorobiphenylol fraction from white shark liver gave four peaks on gas chromatography, those from double-crested cormorant and herring gull eggs gave two and three peaks, respectively. The chlorobiphenylol fraction from juvenile Atlantic salmon yielded one peak, eluted very closely to that of 2,5-dichloro-2'-biphenylol. This peak was also present in

all the other samples and made an accurate quantitation of this compound in the recovery experiments impossible. The identity of the above peaks is not known. They are most likely phenols since they are not extractable with isopropanol-hexane from aqueous sodium hydroxide and can be acetylated.

Care must be taken in the cleanup process to remove completely neutral organochlorine compounds such as PCB and p,p'-DDE before the extraction of phenols. Three washings, one with the isopropanol-hexane and two with hexane were sufficient for white shark liver, which contains PCB, p,p'-DDE, p,p'-DDD, and p,p'-DDT at 218, 335, 43, and 63 µg/g wet weight, respectively [ZITKO *et al.* 1972].

The recovery of 2,4'-dichloro-4-biphenylol and 2,2',5'-trichloro-4-biphenylol from the spiked samples decreased with decreasing level of spiking (Table 2).

TABLE 2.

Recovery of chlorobiphenylols from spiked samples.

2,4'-dichloro-4-biphenylol		2,2',5'-trichloro-4-biphenylol	
Spiked (µg/g)	Recovery (%)	Spiked (µg/g)	Recovery (%)
2.29	104	1.60	89.4
1.63	68.6	1.33	57.7
0.60	43.8	0.48	43.2

CONCLUSION

Pentachlorophenol is detectable in samples of aquatic fauna from relatively clean areas. The levels are probably not significant toxicologically.

The described procedure for the determination of chlorobiphenylols may be used to detect these compounds in the µg/g range in biological samples. All samples contained some unidentified compounds in the chlorobiphenylol fraction.

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