Absence of Chlorinated Dibenzodioxins and Dibenzofurans from Aquatic Animals

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Residues of chlorinated dibenzodioxins and dibenzofurans were not detectable at detection limits of 0.04, 0.02, 0.02, 0.01 and 0.01 μg/g on tissue wet weight basis, for 2,3,7,8-tetrachlorodibenzodioxin (TCDD), a mixture of di-, tri-, and tetrachlorodibenzofuran (CDF), two isomeric hexachlorodibenzodioxins (HCDD), octachlorodibenzodioxin (OCDD), and octachlorodibenzofuran (OCDF), respectively, in the muscle and liver of white shark (Carcharodon carcharias), eggs of double-crested cormorants (Phalacrocorax auritus) and herring gulls (Larus argentatus), muscle of eel (Anguilla rostrata) and chain pickerel (Esox niger), and in commercial samples of herring oil and groundfish-herring fishmeal.

Chlorinated dibenzodioxins are impurities present in chlorinated phenols and herbicides based on chlorinated phenols (1,2). TCDD and HCDD are the most toxic chlorinated dibenzodioxins (3). According to a preliminary report (4) chlorinated dibenzodioxins do not occur widely in the environment, although they were detected some time ago in certain poultry feeds and found responsible for hydropericardium in chickens (chick edema factor, 5).

Chlorinated dibenzofurans were detected in some commercial polychlorinated biphenyl (PCB) preparations (6). Their presence in environmental samples was not investigated.

A method for the separation of chlorinated dibenzodioxins from PCB was described (7) and the uptake of chlorinated dibenzodioxins was studied in chickens (8).

Experimental

White shark was landed on Deer Island, New Brunswick, Canada, and samples of muscle and liver were provided by Mr. S. N. Tibbo of this Station. Eggs of cormorants and herring gulls, collected on

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Fatpot Island, Bay of Fundy, Canada, were the same as in our previous study (9). Eels and pickerel were taken from the St. John River, New Brunswick. Samples of herring oil and fishmeal were obtained from a reduction plant in New Brunswick. Chlorinated dibenzodioxins and dibenzofuran standards were supplied by Dr. D. Firestone (FDA, Washington, D.C.) and so were preprints (2,8).

The extraction and cleanup were carried out as described (10). Fractions I and II thus obtained were further chromatographed on alumina (7) under slightly modified conditions. Alumina (Fisher Scientific A-540, 2 g), activated at 130°C overnight, was placed in a 45 x 0.5 cm column. Sample, dissolved in 1.5 ml of pesticide-grade hexane (Fisher Scientific H-300) containing 2 % methylene chloride (Fisher Scientific D-37), was applied to the column, washed into the column by an additional 1.5 ml of solvent and the column was percolated with the same solvent to collect 20 ml of effluent. Further elution was carried out with 20 % methylene chloride in pesticide-grade hexane and 10 ml of effluent was collected. Fractions I and II of the original cleanup thus yielded fractions I I, I II, and II I, II II, respectively. The behaviour of PCB in this procedure was tested by using Aroclors 1221, 1232, 1242, 1248, 1254, 1260, and 1268, and Phenoclor DP-6. Chromatography of a commercial preparation of chlorinated naphthalenes was also carried out. If the 20% methylene chloride fraction contained PCB, it was rechromatographed as described.

Conditions of gas chromatography were as described (11,12). CDF and TCDD were determined by chromatography on the 4% SE-30 column. The 3% OV-210 column was used for HCDD, OCDD, and OCDF.

The recovery of chlorinated dibenzodioxins and dibenzofurans was determined in spiked samples of fishmeal.

Refluxing with 3% methanolic potassium hydroxide for 1 h was used in some cases for further cleanup of fractions II II.

Results and Discussion

To detect TCDD, HCDD, and CDF, fractions I and II of the cleanup-chromatography must be further chromatographed on alumina. The HCDD standard contained

two isomers with retention times of 0.87 and 1.00 relative to decachlorobiphenyl, respectively. The former is recovered mainly in fraction I II, the latter in fraction II II. TCDD (retention time of 1.86 relative to p,p'-DDE) is equally divided between fractions I II and II II, while CDF (retention times of 0.42, 0.85, and 1.72, relative to p,p'-DDE, respectively) is eluted in fraction II II. OCDD and OCDF are eluted in fraction I and because of the long retention times (2.68 and 2.53 relative to decachlorobiphenyl, respectively) may be quantified directly in this fraction. After chromatography on alumina, OCDD and OCDF appear in fraction I II.

In samples containing a large amount of p,p'-DDT, some of it may be eluted in fraction II and occur finally in fraction II II, where it interferes with the quantification of CDF. This interference is eliminated by refluxing fraction II II with methanolic potassium hydroxide. CDF is not affected by this treatment while p,p'-DDT yielded p,p'-DDE.

Analyses of spiked samples of commercial fishmeal are summarized in Table 1.

TABLE 1

Analysis of spiked fishmeal samples, μg/g wet weight

Sample	TCDD		HCDD		OCDD	
	Added	Found	Added	Found	Added	Found
1	1.18	0.96	1.08	1.15	1.30	1.29
2	0.59	0.64	1.08	1.04	0.65	0.57
3	0.29	0.33	0.26	0.31	0.32	0.50

	CDF		OCDF	
	Added	Found	Added	Found
4	1.21	1.41	0.91	0.96
5	0.60	0.62	0.45	0.38
6	0.30	0.28	0.23	0.14

Levels of PCB (as Aroclor 1254) and chlorinated hydrocarbon pesticides in samples analysed for chlorinated dibenzodioxins and dibenzofurans are presented in Table 2.

With the exception of the white shark liver extract, the removal of PCB from fraction I in fraction I I caused no difficulties. Neither PCB nor any other

peaks were detected in the fraction I II. In the shark liver extract, containing PCB in very high concentration, some PCB were carried over into fraction I II and this fraction was rechromatographed. No peaks were detectable after rechromatography.

TABLE 2

PCB and chlorinated hydrocarbon pesticides in analysed samples

			μg/g wet weight		
Sample	Tissue	PCB	p,p'-DDE	p,p'-DDD	p,p'-DDT
Herring oil		3.55	2.27	-	0.37
Fishmeal		0.54	0.19	0.11	0.10
Chain pickerel	muscle	0.35	0.15	0.03	0.08
Eel	muscle	0.46	0.48	0.13	0.15
Double-crested cormorant	eggs	45.6	32.1	-	0.10
Herring gull	eggs	7.45	2.70	-	0.10
White shark	muscle	0.77	0.44	-	0.12
" "	liver	218	335	43	63

No peaks corresponding to the available CDF standards, TCDD, and HCDD were detected in fractions II II after reflux with methanolic potassium hydroxide. Fraction II II of the shark liver extract had to be rechromatographed.

An unidentified peak with a retention time of 0.89 relative to p,p'-DDE, yielding a peak with retention time of 0.58 relative to p,p'-DDE, after the potassium hydroxide treatment, was present in most II II fractions. Another small peak with a retention time of 0.76 relative to p,p'-DDE, unchanged by the potassium hydroxide treatment was present in several II II fractions.

The chromatography on alumina (7) as modified in this work is very efficient in removing PCB in the 2% methylene chloride fraction. In model experiments 700 ng of Aroclors 1242, 1254, 1260, and 1268, Phenoclor DP-6, and chlorinated naphthalenes was completely eluted in this fraction. One rechromatography of the 20% methylene chloride fraction was necessary to remove 500 μg of Aroclor 1254, 1260, and Phenoclor DP-6 and no peaks were detected in the chromatograms of the purified fractions. Peaks with

retention times of 0.25, 0.53, and 1.07 relative to p,p'-DDE, respectively, were found in the rechromatographed 20% methylene chloride fractions of Aroclors 1248 and 1242, when 800 μg was originally applied to the column. Aroclors 1232 and 1221 yielded under the same conditions one additional peak with a retention time of 0.09 relative to p,p'-DDE. The peak with retention time of 1.07 was absent from Aroclor 1221. According to a preliminary investigation by mass spectrometry, the rechromatographed 20% methylene chloride fractions of Aroclors 1248, 1242, 1232, and 1221 contain mono-, di-, and trichlorobiphenyls, mono-, and dichloroterphenyls, and some other unidentified compounds (O. Hutzinger, personal communication).

The species analysed in this work are in high trophic levels of the aquatic food web and may serve as good indicators of environmental contamination by cumulative compounds. The results indicate no detectable contamination of the food chains by chlorinated dibenzodioxins and dibenzofurans.

The leakage of TCDD into the environment may occur mainly from the applications of 2,4,5-T. This may be insignificant, since, in the past, the levels of TCDD in 2,4,5-T were 20 μg/g or less (4) and preliminary reports indicate that TCDD is not very mobile in the environment (13). However, the extreme toxicity of TCDD warrants a thorough investigation of the problem.

Tetra- and pentachlorophenol are probably the main source of HCDD and OCDD, containing these compounds in the μg/g range (2). Similarly to TCDD, HCDD, and OCDD originate in the production of these phenols and are not generated in the environment, where other types of condensation reactions take place (14,15). The amount of HCDD and OCDD released into the environment may be very small.

Commercial PCB preparations are a potential source of chlorinated dibenzofurans. No detailed data on the levels of chlorinated dibenzofurans in PCB preparations are available. It is possible that the leakage of chlorinated dibenzofurans into the environment is negligible.

Additional spotchecks in different geographic areas are required to assess the contamination of the environment by chlorinated dibenzodioxins and dibenzofurans.

References

1. WOOLSON, E. A. and THOMAS, R. F., ACS, 161st National Meeting, Div. Pesticide Chem. (1971).
2. FIRESTONE, D., RESS, J., BROWN, N. L., BARRON, R. P., and DAMICO, J., J. Assoc. Offic. Anal. Chemists, in press (1971).
3. ROWE, V. K., NORRIS, J. M., SPARSCHU, G. L., SCHWETZ, B. A., and GEHRING, P. J., ACS, 162nd National Meeting, Div. Pesticide Chem. (1971).
4. WOOLSON, E. A., ACS, 162nd National Meeting, Div. Pesticide Chem. (1971).
5. RESS, J., HIGGINBOTHAM, G. R., and FIRESTONE, D., J. Assoc. Offic. Anal. Chemists 53, 628 (1970).
6. VOS, J. G., KOEMAN, J. H., VAN DER MAAS, H. L., TEN NOEVER DE BRAUW, M. C., and DE VOS, R. H., Fd. Cosmetics Toxicol. 8, 625 (1970).
7. PORTER, M. L. and BURKE, J. A., J. Assoc. Offic. Anal. Chemists, in press (1971).
8. FIRESTONE, D., FLICK, D. F., RESS, J., and HIGGINBOTHAM, G., J. Assoc. Offic. Anal. Chemists, in press (1971).
9. ZITKO, V., and CHOI, P. M. K., Bull. Environ. Contam. Toxicol. 7(1),(1972).
10. ZITKO, V., Bull. Environ. Contam. Toxicol., 6, 464 (1971).
11. ZITKO, V., J. Chromatogr. 59, 444 (1971).
12. ZITKO, V., HUTZINGER, O., JAMIESON, W. D., and CHOI, P. M. K., Bull. Environ. Contam. Toxicol., in press (1972).
13. KEARNEY, P. C., ISENSEE, A., HELLING, C. S., WOOLSON, E. A., and PLIMMER, J. R., ACS, 162nd National Meeting, Div. Pesticide Chem. (1971).
14. MUNAKATA, K. and KUWAHARA, M., Residue Reviews 25, 13 (1969).
15. STEHL, R. H., PAPENFUSS, R. R., BREDEWEG, R. A., and ROBERTS, R. W., ACS, 162nd National Meeting, Div. Pesticide Chem. (1971).