Analysis of Polychlorinated Diphenyl Ethers in Fish taken from a Northern Canadian River System

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INTRODUCTION

Polychlorinated diphenyl ethers (PCDPEs) are becoming of great concern because of their structural and toxicological similarity to polychlorinated biphenyls (PCBs).[1] PCDPEs have been reported to be immunotoxic and induce hepatic microsomal enzymes. Toxicity and induction of PCDPEs depends on their structure, but the relationships are not similar to polychlorinated biphenyls (PCBs). The mono-ortho chlorine-substituted congeners of PCDPEs are more potent inducers of AHH and EROD activities and more immunotoxic than their non-ortho analogues.[2-4] The toxic equivalence factor (TEF) proposed by Safe for non- and mono-ortho substituted PCDPEs is 0.001, which is the same as for mono-ortho-coplanar PCBs.[5]

PCDPEs arise as impurities in technical chlorophenol preparations and in chlorinated phenoxyacetic acids. PCDPE residues have been found in several environmental samples including sediments, fish, wildlife, and human tissues. In addition PCDPEs have also been identified in emissions from municipal waste incinerators.[6]

Analyses of burbot fish liver taken from the Peace and Athabasca rivers in Alberta for di- to octa- congeners of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs) revealed the presence of PCDPEs as interferences. These rivers flow towards the North, pass through the Northwest Territories and eventually discharge to the Arctic Ocean. As a result of this finding steps were taken to develop an analytical method and to obtain some preliminary data on levels of PCDPEs in burbot liver samples. Burbot liver has been used as a food source of indigenous people residing in the Northwest Territories.

This work was commissioned by the Northern River Basins Study, a large inter-governmental program commissioned with the task of collecting contaminant data from rivers in Alberta which flow to the North. This study was deemed necessary in order to examine the environmental impact of expanding heavy oil and forestry development in the province of Alberta. Supporters of this study include the province of Alberta, the Northwest Territories and Environment Canada.

EXPERIMENTAL

The standards of PCDPEs were obtained from Cambridge Isotope Laboratories (CIL), Andover, Massachusetts, and include: 2,4,4',5-tetra-, 2,3',4,4'-tetra-, 3,3',4,4'-tetra-, 2,2',4,4',5-penta-, 2,3',4,4,5'-penta-, 3,3',4,4',5-penta-, 2,3,3',4,4',5-hexa-, 2,2',3,3',4,4',5-hepta-, 2,2',3,3',4,4',5,5'-octa- and deca- CDPE. The following surrogates were obtained from CIL: $^{13}C_{12}$-3,3',4,4'-tetra-,$^{13}C_{12}$-2,3,3',4,4'-penta-,$^{13}C_{12}$-2,3,3'4,4',5-hexa-,$^{13}C_{12}$-2,2',3,3',4,4',5-hepta-, and $^{13}C_{12}$-2,2',3,3',4,4',5,5'-octa-CDPE.

The analytical procedure is shown schematically in Figure 1. Briefly, an aliquot of homogenized liver tissue (0.8 - 5.0 g) was ground with sodium sulfate, air-dried for 4 h, and the mixture transferred to Soxhlet extractors. Following the addition of $^{13}C_{12}$-CDPE surrogates (2.0 ng) the samples were extracted with dichloromethane:hexane (1:1) for 16 h. After concentration of the extract, an aliquot (200 μL) was removed for gravimetric lipid determination. The extract (20 mL) was defatted by adding, shaking and removing 5 mL of concentrated sulfuric acid. This process was continued with fresh acid until the acid layer was observed to be clear.

Following defatting with sulfuric acid, further cleanup was performed using a multi-silica column, consisting of: 2 g silica, 4 g 44% (w/w) sulfuric acid on silica, 1 g silica, 2 g 33% (w/w) 1 M sodium hydroxide on silica, 1 g silica, 2 g 10% (w/w) silver nitrate on silica, and 1 g silica. The silica (Silica Gel 60) was obtained from EM Science, Gibbstown, N.J. The defatted extract (20 mL) was applied to the column and eluted with 50 mL of 2% dichloromethane in hexane (v/v). Following concentration, the partially cleaned up extract was

applied to a 12 g Florisil column (U.S. Silica Co., Berkeley Springs, W.Va, 60/100 mesh, 0.8% water deactivated [w/w]). PCDPEs and PCBs were removed by elution with 100 mL of hexane. Following concentration, PCDPEs were separated from PCBs by chromatography using a 5 g basic alumina column (ICN Biomedicals, GmbH, D-3440, Eschwege). PCBs were removed by elution with 100 mL of hexane and PCDPEs were isolated by further elution with 100 mL of DCM. The PCDPE fraction was concentrated just to dryness and reconstituted with 20 μL of toluene containing $^{13}C_{12}$-PCB 169 as an internal standard. Analysis was performed using gas chromatography/high resolution mass spectrometry (10,000 resolution) in the selected ion-monitoring mode.

RESULTS

Results obtained are presented in Table 1. Comparison of these results to those obtained for fish obtained from the Simojoki river, Tenojoki river, and Lake Saimaa in Finland,[7] for congeners 77, 99, 170, and 194 reveal similar data as that shown in Table 2. The one exception was PCDPE#99 which was found in Finland to have a mean concentration in salmon taken from the Simojoki river of 471 pg/g (fresh weight). This value was considerably higher than observed for burbot obtained from Alberta rivers. Other congeners which were detected in salmon exceeding levels of 100 pg/g include PCDPEs # 114, 137, 153, and 154.

At the time of this study these standards were not commercially available, however, a recent paper[8] has reported relative retention times for 54 synthesized PCDPE. Further analyses may allow us to identify and quantitate additional congeners. Comparison of the results in Table 1 to those obtained from carp and pike obtained from the Great Lakes,[8] reveal much lower concentrations for fish obtained from Alberta rivers.

CONCLUSION

Analysis of burbot taken from the Peace and Athabasca rivers in Northern Alberta revealed the presence of PCDPEs at levels similar to those reported for lakes and rivers in Finland. Comparison of these results to those obtained for carp and pike obtained from the Great Lakes revealed concentrations several orders of magnitude higher. Synthesis of additional PCDPE standards and determination of relative retention times will allow for more congeners to be reported and quantitated in the future.

Table 1. PCDPEs Detected in Burbot Liver

Congener	CDPE#	Fresh Weight Conc. Range (pg/g)	Lipid Adjusted Conc. Range (pg/g)
2,4,4',5	74	< 0.25 - 6.3	< 0.56 - 16
2,3',4,4'	66	< 0.25 - 23	< 1.7 - 51
3,3',4,4'	77	< 0.25	< 52
2,2',4,4',5	99	3.5 - 286	23 - 640
2,3',4,4',5	118	< 1.0 - 46	< 2.1 - 100
3,3',4,4',5	126	< 1.0	< 2.1
2,3,3',4,4',5	156	< 2.0 - 19	< 4.2 - 49
2,2',3,3',4,4',5	170	11 - 48	73 - 98
2,2',3,3',4,4',5,5'	194	<5.0 - 44	< 10 - 110
2,2',3,3',4,4',5,5',6,6'	209	11 - 101	18 - 260

Table 2. PCDPEs Detected in Fish Obtained from Finland and the Great Lakes

Congener	CDPE#	Finish studies Salmon, Conc Range (pg/g)	Great Lakes Carp, Conc. Range (ng/g)	Great Lakes Pike, Conc. Range (ng/g)
2,4,4',5	74	NA	93 - 1505	91 - 461
3,3',4,4'	77	< 10 - 20	6 - 48	10 - 48
2,2',4,4',5	99	30 - 870	76 - 1289	105 - 524
2,3',4,4',5	118	NA	3 - 52	3 - 17
2,2',3,3',4,4',5	170	< 30	NA	NA
2,2',3,3',4,4'5,5'	194	< 50	NA	NA
2,2',3,3',4,4',5,5'	209	NA	2 - 4	2

REFERENCES

1 Becker, M., T. Phillips and S. Safe (1991). Polychlorinated Diphenyl Ethers - A Review. Toxicological and Environmental Chemistry 33: 189-200.

2. Howie, L., R. Dickerson, D. Davis, and S. Safe (1990). Immunosuppressive and monooxygenase induction activities of polychlorinated diphenyl ether congeners in C57BL/6N mice: quantitative structure-activity relationships. *Toxicol. Appl. Pharmacol.* **105**: 254-263.

3. Chui, Y.C., M.M. Hansell, R.F. Addison, F.C. P. Law (1985). Effects of chlorinated diphenyl ethers on the mixed function oxidases and ultrastructure of rat and trout liver. *Toxicol. Appl. Pharmacol.* **81**: 287-294.

4. Iverson, F., H. Hewsome, L. Hierly (1987). Induction of rat hepatic monooxygenase activity by polychlorinated diphenyl ethers. *Food Cosmet. Toxicol.* **25**: 305-307.

5. Safe, S. (1992). Development, validation and limitations of toxic equivalency factors. *Chemosphere*, **25**: 61-64.

6. Nevalainen, T. and J. Koistinen (1994). Synthesis, structure verification, and chromatographic relative retention times for polychlorinated diphenyl ethers. *Environ. Sci. Technol.* **28**: 1341-1347.

7. Koistinen, J., P.J. Vuorinen and J. Paasivirta (1993). Contents and origin of polychlorinated diphenyl ethers (PCDE) in Salmon from the Baltic Sea, Lake Saimaa and the Tenojoki river in Finland. *Chemosphere*, **27**: 2365-2380.

8. Huestis, S.Y. and D.B. Sergeant (1992). Removal of chlorinated diphenyl ether interferences for analyses of PCDDs and PCDFs in fish. *Chemosphere*, **24**: 537-545.

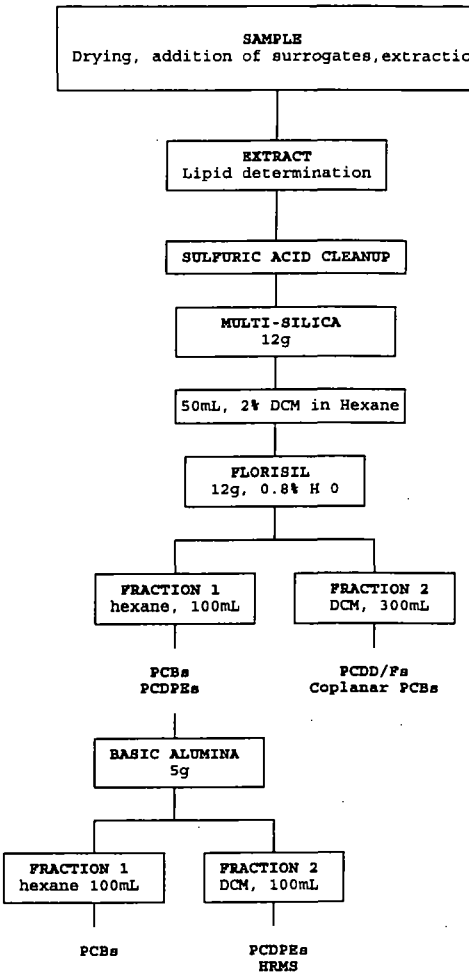

Figure 1: Analytical Procedure for the Analysis of PCDPEs