SIMULTANEOUS QUANTITATION OF SHORT AND MEDIUM CHAIN POLYCHLORINATED *n*-ALKANES IN ENVIRONMENTAL SAMPLES BY HRGC/ECNI-HRMS

Gregg Tomy and Gary Stern

Freshwater Institute, Department of Fisheries and Oceans, Winnipeg MB R3T 2N6 Canada

Introduction

Polychlorinated *n*-alkanes (PCAs), derived by direct chlorination of *n*-alkanes, are a class of chemicals used for a variety of industrial purposes. Common applications include high-temperature lubricants in metal-working machinery and as flame-retardant plasticizers in vinyl plastics.[1] Based on the starting *n*-alkane feedstock used in the chlorination process, PCA mixtures fall into three categories: C_{10}-C_{13} (short), C_{14}-C_{17} (medium) and C_{20}-C_{30} (long); they are further subcategorized into their weight content of chlorine: 40 to 50%, 50 to 60% and 60 to 70%.[2] Subsequent release of these chemicals into the environment can occur during production, storage, transportation, industrial use and carryoff on manufactured products.[3,4]

Only recently has there been a modest increase in our understanding of the fate and behavior of short chain (C_{10}-C_{13}) PCAs (*s*PCAs).[3-7] Much of the earlier difficulties had been associated with their analyses[3,8] and their little known physical-chemical properties.[3,4] Many of these problems have since been addressed, however, by recent physical-chemical property measurements[9,10] and by an interlaboratory study conducted by our laboratory.[11] A survey of the literature, however, showed that even less is known about the behavior and environmental levels of medium chain (C_{14}-C_{17}) PCAs (*m*PCAs). Due to tighter regulations being imposed by environmental agencies on the use of *s*PCAs, it is very likely that the global usage of *m*PCAs could rise; in Europe, for example, the use of *m*PCAs has already surpassed that of *s*PCAs.[12] A recent study has also suggested that compared to *s*PCAs, *m*PCAs may be more bioaccumulative because of reduced biotransformation resulting from the longer carbon chain lengths.[13]

The ability to simultaneously measure *s*- and *m*-PCAs would increase our knowledge of their environmental behavior twofold. In this study, we describe the concurrent quantitation of both *s*- and *m*PCAs based on accelerated solvent extraction (ASE) and HRGC/ECNI-HRMS in the selected ion mode (SIM) at a resolving power (RP) of ~ 11 000. As an example, the method was used to determine levels of *s*- + *m*PCAs in fish from the Detroit River.

Methods and Materials

Two commercial PCA products, used as analytical standards, one of C_{10}-C_{13} chain length and ~60% chlorine by mass (*s*PCA-60) and the other of C_{14}-C_{17} carbon chain length and 52% chlorine by mass (*m*PCA-52) were provided by Dover Chemicals. Extractions were carried out using a Dionex 200 accelerated solvent extractor (ASE) at a temperature of 100°C and a pressure of 136 atm. A mixture of dichloromethane (DCM)/hexane (1:1) was used as the extraction solvent in all cases. Frozen whole fish were ground cryogenically and sub-samples (~10 g, wet weight) were placed into a stainless steel ASE cell (cell size of 33 mL) and spiked with the recovery standard [$^{13}C_1$]chlordane. The dead volume of the extraction cell was then filled with anhydrous Na_2SO_4

(previously baked at 600°C for 6 h). After a 5 min thermal equilibration time, the extraction cell was filled with solvent and extracted under static conditions for 10 mins. Following static extraction, the cell was rinsed with ~30 mL of solvent. This cycle was then repeated. As a final step, the cell was purged with gaseous nitrogen for 100 s. After every sample extraction, there was a rinse cycle in which the system was rinsed with solvent. The length of the extraction was ~30 mins and the volume of extract ~60 mL. Extracts were then solvent reduced (~1 mL) and lipids removed by gel permeation chromatography (GPC).[14] GPC conditions were similar to that described elsewhere.[8] The lipid free extract was then solvent reduced (1 mL) and cleaned on a column (300 mm x 10.5 mm i.d.) of reagent grade Florisil (1.2% deactivated (w/w), 8 g, 60-100 mesh size). Cleanup was achieved with the solvent sequence 38 mL of hexane (F1), 42 mL of 15:85 DCM/hexane (F2), and 60 mL of 1:1 DCM/hexane (F3). Fractions F2 and F3 contained both *m*PCAs and *s*PCAs, along with [$^{13}C_1$]chlordane were combined and the volume reduced to 0.5 mL by a gentle stream of nitrogen prior to GC/MS analysis. A known amount of [$^{13}C_8$]mirex used as an internal standard for SIM, was added to the residual solutions at this stage.

Quality Control. Precautions used in the analysis of PCAs include use of glass-distilled solvents and high temperature heating of glassware, sodium sulfate and Florisil. Two procedural blanks that were taken through all phases of extraction, isolation and analyses, were included with each sample extraction. For fish, procedural blanks were muscle of Arctic lake trout from Maguse Lake (61°N/95°W), NWT, Canada (previously analyzed and found to have low levels of organochlorine residues). All samples were extracted in triplicate and analyzed in duplicate.

HRGC/ECNI-HRMS. All analyses were performed on a HP 5890 Series II GC, fitted with a HR 30 m x 0.25 mm i.d. (0.25 μm film thickness) DB-5ms fused-silica column, connected through a heated transfer line maintained at 310°C to a Kratos Concept HRMS. All sample injections were made by a CTC A200SE autosampler. The injector port temperature was 280°C, and an electronic pressure program maintained a helium carrier gas flow of 1 mL/min. The column temperature program was as follows: initial 150°C; hold 1 min; ramp to 310°C at 10°C min^{-1}; hold for 15 mins. Details of the MS operating parameters can be found elsewhere.[8]

Results and Discussion

Determining molecular compositions and generating formula group abundance profiles. The first step involves determining the molecular compositions of the standards. As described previously, the average chlorine number, z_{av}, provides a guide for initial selection of ions to be monitored.[8] So that for molecular formulas close to the average formula, the two most abundant isotopic combinations in their respective $[M - Cl]^-$ ion groups were selected. By electronically integrating the responses of the most abundant ion in the $[M - Cl]^-$ ion cluster of a particular formula group, and applying two simple correction factors[8], one can generate formula group abundance profiles (FGAP) of the standards. Parts a and b of Figure 1 show the FGAP of *s*PCA-60 and *m*PCA-52, respectively, determined by injecting each standard separately into the GC. In a similar manner the FGAPs of the fish sample from the Detroit River were generated and are shown in parts c and d of Figure 1.

Simultaneous quantitation of *s*- and *m*PCAs. Once the FGAPs of the environment sample has been generated, one for *s*PCAs and another for *m*PCAs, it now becomes possible to recommend a method for simultaneously quantifying *s* and *m*PCAs. Because *s*PCAs are not discriminated against during the extraction of *m*PCAs, the final cleaned up extract would contain both *s* and *m*PCAs. Using the GC conditions described earlier, and by including specific *m/z* peaks in the final SIM method, *s* and *m*PCAs levels can be estimated. For example, for perch, based on the

FGAP generated here, one can monitor the $[M - Cl]^-$ ions of $C_{14}H_{22}Cl_8$ and $C_{11}H_{16}Cl_8$ in a single GC-injection, and relate their respective responses to that of a standard, injected separately but under similar conditions, containing known amounts of *s* and *m*PCA commercial mixtures. As an illustration, the ECNI selected ion chromatograms of the two species noted above in the perch sample and in the standard are shown in Figure 2. By this method, the total *s*- and *m*PCA levels was estimated to be 1.8 and 0.008 μg/g, respectively.

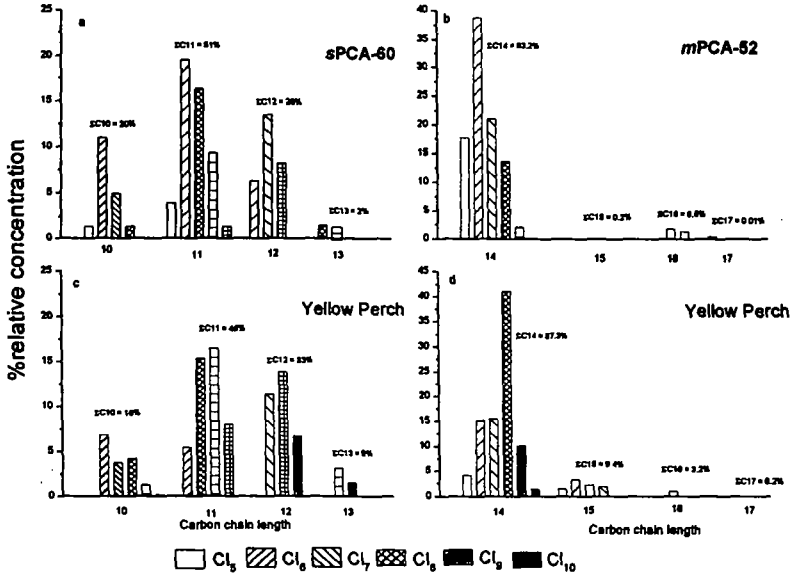

Figure 1. Formula group abundance profiles of *s*- and *m*PCA standards and of the yellow perch.

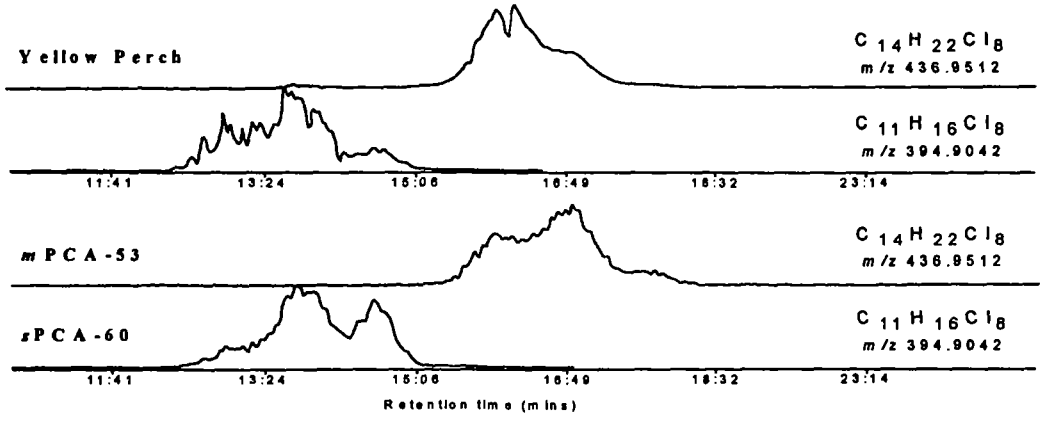

Figure 2. ECNI selected ion chromatograms of $C_{14}H_{22}Cl_8$ and $C_{11}H_{16}Cl_8$ in the perch sample and in the standard containing known amounts of *s*- and *m*PCAs.

Before a thorough assessment of the exposure and risk *s*- and *m*PCAs poses to aquatic or terrestrial life much work needs to be done in determining their concentrations in environmental *compartments*; a greater knowledge of their spatial and temporal variations is also needed. The work presented here represents the first step in addressing some of these issues.

Acknowledgements

The authors thank the Government of Canada and the Natural Sciences and Engineering Research Council of Canada (NSERC) for the Visiting Fellowship to G.T. We are also grateful to Doug Haffner (Great Lakes Institute) for providing the environmental sample.

Reference

1. Zitko, V.; Arsenault, E. (1974) Chlorinated Paraffins: Properties, uses and pollution potential. Tech. Rep. No. 491, Canadian Dept. Environ., Fisheries and Marine Services, Research and Development Directorate.
2. Serrone, D.M.; Birtley, R.D.N.; Wiegand, W.; Millischer, R. (1987) Food Chem. Toxicol. *25*, 553.
3. Tomy, G.T.; Fisk, A.T.; Westmore, J.B.; Muir, D.C.G. (1998) Rev. Environ. Toxicol. Chem. *158*, 53.
4. Muir, D.C.G.; Stern, G.A.; Tomy, G.T. (1999) in: Handbook of Environmental Chemistry, (Paasivirta, J., Ed.) ISBN 3540658386
5. Tomy, G.T.; Stern, G.A.; Lockhart, W.L.; Muir, D.C.G. (1999), Environ. Sci. Technol. 33, 2858
6. Fisk, A.T.; Tomy, G.T.; Muir, D.C.G. (1999) Environ. Toxicol. Chem. 18, 2894.
7. Fisk, A.T.; Cymbalisty, C.D.; Tomy, G.T.; Muir, D.C.M. (1998) Aquatic Toxicol. 43, 209.
8. Tomy, G.T.; Stern, G.A.; Muir, D.C.G.; Fisk, A.T.; Cymbalisty, C.D.; Westmore, J.B. Anal. Chem. 1997, 69, 2762-2771.
9. Drouillard, K.G.; Tomy, G.T.; Muir, D.C.G.; Friesen, K.J. (1998) Environ. Toxicol. Chem. 17, 1252.
10. Drouillard, K.G.; Heibert, T.; Tran, P.; Tomy, G.T.; Muir, D.C.G.; Friesen, K.J. (1998) Environ. Toxicol. Chem. 17, 1261.
11. Tomy, G.T.; Westmore, J.B.; Stern, G.A.; Muir, D.C.G.; Fisk, A.T. (1999) Anal. Chem. 71, 446-451.
12. Willis, B.; Crookes, M.J.; Diment, J.; Dobson, S.D. (1994) Environment Hazard Assessment: Chlorinated Paraffins. Toxic Substances Division. Department of the Environment. London UK.
13. Fisk, A.T.; Tomy, G.T.; Cymbalisty, C.D.; Muir, D.C.G. (1999) Environ. Toxicol. Chem. in press
14. Stalling, D.L.; Tindle, R.C.; Johnson, J.L. (1972) J. Assoc. Off. Anal. Chem. 55, 32.