DETERMINATION OF TOXAPHENE CONGENERS AND TOTAL TOXAPHENE IN ENVIRONMENTAL MATRICES BY ACCELERATED SOLVENT EXTRACTION – HRGC/ECNI-HRMS

Gregg Tomy, Gary Stern and Brian Billeck

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

Introduction

Toxaphene is a complex mixture consisting of several hundred chlorinated bicyclic hydrocarbons of which chlorinated bornanes ($C_{10}H_{18-x}Cl_x$, x=5-10) are most predominant. Introduced in the USA in 1945 by Hercules Co. as a new insecticide to control a variety of insect pests, two-thirds of toxaphene production was used for insect control on cotton. It was also used on vegetables, small grains, soya beans and for control of external insects on livestock.[1,2] In Canada and the USA, toxaphene was also used extensively in fish eradication programs. This practice, however, was discontinued when it was found that toxaphene was extremely persistent and that lakes could not be restocked for years after treatment.[3-5]

Prior to its ban in 1982 by the US EPA, toxaphene was the most extensively used pesticide in the USA and many parts of the world.[6,7] Maximum usage of ~27 x 10^6 kg/yr[8] occurred between 1972 and 1974. Global production has been estimated to be 1.33 megatonnes[9], with production in the United states (1946-1982) accounting for about 0.45 megatonnes.[6,9]

The most common method for toxaphene analysis today is based on GC-MS operated under ECNI conditions.[11,12] Because of the unavailability of individual toxaphene congeners, early GC-ECNI-MS methods were based solely on technical toxaphene as the analytical standard. For example, Swackhammer *et al.* described a SIM method in which four retention time windows were used to monitor the two most abundant peaks in the $M^{-\bullet}$ (Cl_6) and the $[M - Cl]^-$ (Cl_7-Cl_{10}) ion clusters; environmental concentrations were determined by comparing the response of technical mixture, which was used as the analytical standard, to the response of toxaphene in the samples[13]. Because low resolution (LR) MS was employed, additional ions had to be monitored to correct for coeluting interferences. Recently, Glassmeyer *et al.* developed an automated protocol by which interfering ions could be corrected from the ions of interest.[14]

Today, concerted efforts have been made to quantify toxaphene based on individually synthesized standards, which only recently have become available commercially.[11,15] Because not all congeners are yet available, technical toxaphene is still needed to *estimate* total toxaphene concentrations in environmental matrices.

Operating the MS at high-resolution (HR) can circumvent the specificity and interference limitations encountered using GC-ECD and under LR-MS conditions. In this study, we report on the use of accelerated solvent extraction (ASE) and HRGC/ECNI-HRMS in the SIM mode for the extraction and quantatition of toxaphene in fish and sediment samples from the Detroit River, near its entry to Lake Erie. Congener specific concentrations were determined using an individual congener standard and total toxaphene estimated using a technical toxaphene mixture.

Methods and Materials

Technical toxaphene was purchased from Radian Chemical Inc. A toxaphene standard (Tox 482) consisting of 25 individual toxaphene congeners was graciously provided by Promochem (Wesel, Germany). Isotopically labeled [$^{13}C_1$]chlordane (99% ^{13}C) and [$^{13}C_8$]mirex (99% ^{13}C) were purchased from Cambridge Isotope Laboratories Inc.

Extraction and Isolation. Extractions were carried out using a Dionex 200 ASE at a temperature of 100°C and a pressure of 136 atm. A mixture of DCM/hexane (1:1) was used as the extraction solvent in all cases. Sub-samples (~10 g, wet weight) of frozen fish and freeze dried sediment were placed into separate stainless steel ASE cells (cell size of 33 mL) and spiked with the recovery standard [$^{13}C_1$]chlordane. The dead volume of the extraction cells 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 cells were filled with solvent and extracted under static conditions for 10 mins. Following static extraction, the cells were rinsed with ~30 mL of solvent. This cycle was then repeated. As a final step, the cells were 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. Fish extracts were then solvent reduced (~1 mL) and lipids removed by gel permeation chromatography (GPC).[16] GPC columns (29.5 mm i.d. x 400 mm) were packed with 60 g (dry weight) of 200-400 mesh SX-3 BioBeads that had been soaked in DCM/hexane (1:1) overnight. The column was eluted with 325 mL of DCM/hexane; the first 150 mL contained lipids and was discarded. Sediment extracts were treated with copper powder (nitric acid washed) for 15 mins at room temperature to remove sulfur-containing compounds. Extracts were 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). Fraction F1 contained the less polar components of toxaphene, all the PCBs, chlorinated benzenes, DDT and its metabolites, and other chlorinated organics. F2 contained the more polar components of toxaphene, along with [$^{13}C_1$]chlordane, while F3 contained other more polar organochlorines such as heptachlor epoxides and dieldrin. For toxaphene analysis, F1 and F2 were combined and solvent exchanged to isooctane, 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.

HRGC/ECNI-HRMS. GC and MS conditions are described elsewhere.[17]

Quality Control. Two procedural blanks, taken through all phases of extraction, isolation and analyses, were included with each sample extraction. For sediment, procedural blanks consisted of sodium sulfate and for fish were muscle of trout that were reared in holding tanks at the Freshwater Institute. All samples were extracted in triplicate and analyzed in duplicate.

Results and Discussion

Selection of ions to be used for SIM. The behavior of toxaphene congeners in the ion source of the MS under ECNI conditions has been well documented.[12] Based on these studies, and some confirmatory studies of our own, we found that at an ion source temperature of 120°C, the [M – Cl]$^-$ ion for each Cl-homologue group, *i.e.*, Cl_6-Cl_7, to be suitable for monitoring purposes. For quantitation of the B9-1025 (P62) congener, however, the [M – HCl – Cl]$^-$ fragment ion was used,

as it is known that this ion is more abundant than the $[M - Cl]^-$ ion.[12] For each Cl-homologue group, therefore, we selected the two most abundant isotopic combinations in their respective $[M - Cl]^-$ and $[M - HCl - Cl]^-$ (for B9-1025) ion clusters. The most abundant *m/z* value was used as a quantitation ion for SIM and the next most abundant as a confirmation ion.

Elution Profiles and Retention Time Windows. The retention time windows were established using the technical toxaphene standard, as congeners in the individual congener standard would elute in a similar manner to that in the technical standard. Parts a and b of Figure 1 show the elution profile of technical toxaphene and that of the individual congener standard, injected separately, but under the same conditions, respectively. Four windows were found to be convenient and practical for determining these profiles. Because the concentration of each congener in the congener standard solution were equal, Figure 1b illustrates clearly the wide range of response factors observed for congeners within the same homologue class.

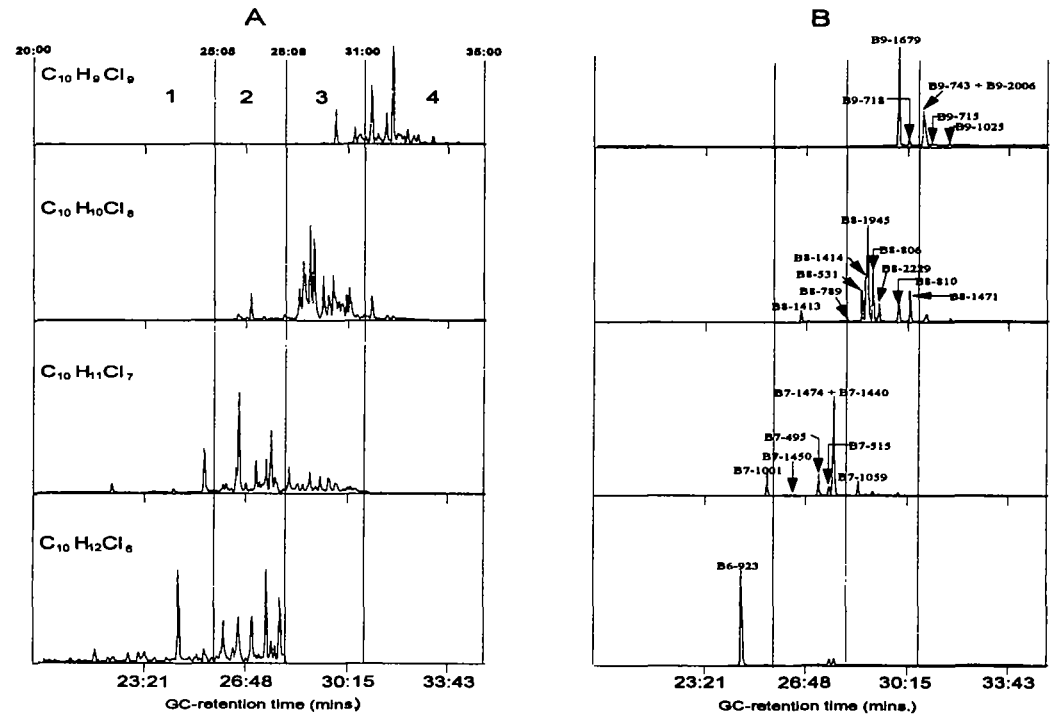

Figure 1. HRGC/ECNI-HRMS elution profiles of monitored ions in (A) technical toxaphene and (B) individual congener standard aligned to show the four-retention time windows used.

Quantitative Measurements. Congener specific concentrations were done by comparing the integrated response of each congener in the sample to that of the individual congener standard, injected separately, but under identical conditions. QA criteria for peak detection included retention time window and correct isotope ratio (± 15%) for the two mass fragments monitored for each Cl-homologue class. Homologue concentrations were estimated by comparing the total integrated response of all the congeners in a particular homologue group in the sample to that in the technical standard, injected separately, but under identical conditions. Total toxaphene (ΣCHB) was calculated by summing the hexa- to nona- homologue concentrations.

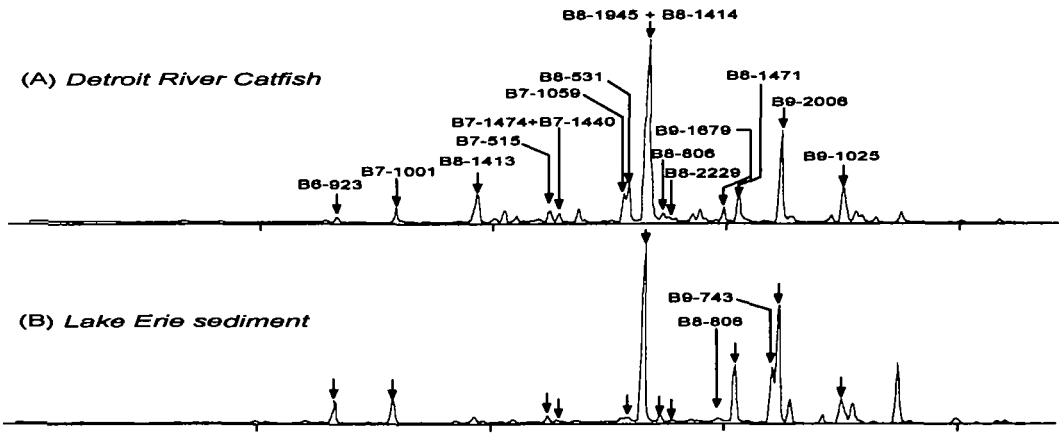

Figure 2. Total ion chromatogram of catfish from the Detroit River and sediment from Lake Erie.

Environmental levels. The SIM program was then applied to the quantitation of toxaphene in fish and sediment samples from the Detroit River and Lake Erie, respectively. Figure 2 shows the TIC (ΣCl_6-Cl_9) of (a) catfish and (b) sediment. Individual congener concentrations in sediment range from 7.3 pg/g (B7-1474 + B7-1440) to 3.4 ng/g (B9-743); for catfish, the range is 15.1 pg/g (B7-1474 + B7-1440) to 9.2 ng/g (B9-2006). ΣCHB concentrations in fish and sediment were determined to be 11.5 and 19.1 ng/g, respectively. In summary, this study reports on a method for measuring environmental concentrations of individual toxaphene congeners and ΣCHB based on ECNI-HRMS.

References

1. Cairns, T.; Siegmund, EG.; Foberg, JE. (1981) Biomed. Mass Spectrom. 8, 568.
2. Casida, JE.; Holmstead, RL.; Khalifa, S.; Knox, JR.; Ohsawa, T.; Palmer, KJ.; Wong, RY. (1974) Science 183, 520.
3. Nash, RG.; Woolson, EA. (1967) Science 157, 924.
4. Hermanson, HP; Gunther, FA; Anderson, LD, Garbe, MJ. (1971) J. Agric. Food Chem. 19, 722.
5. Nash, RG; Harris, WG; Ensor, PD.; Woolson, EA. (1973) J. Assoc. Off. Anal. Chem. 56, 728.
6. Saleh, MA. (1991) Rev. Environ. Contam. Toxicol. 118, 1.
7. *Fed. Regist.* (1982), 47, 53784.
8. Bidleman, TF.; Zaranski, MT.; Walla, MD. (1988) in: Toxic Contamination in Large Lakes Vol 1 (Schmidtke, N.W., Ed). pp 257.
9. Voldner, EC.; Li, YF. (1993) Chemosphere 27, 2073.
10. Barrie, LA.; Gregor, D.; Hargrave, B.; Lake, R.; Muir, DCG.; Shearer, R.; Tracey, B.; Bidleman, T.F. (1992) Sci. Total Environ. 122, 1.
11. Vetter, W; Oehme, M. (1999) in: *New Types of Persistent Halogenated Compounds* (Paasivirta, J., Ed.) ISBN 3540658386
12. Lau, B.; Weber, D.; Andrews, P. (1996) Chemosphere 32, 1021.
13. Swackhamer, DL.; Charles, MJ.; Hites, RA. (1987) Anal. Chem. 59, 913.
14. Glassmeyer, ST.; Shanks, KE.; Hites, RA. (1999) Environ. Sci. Technol. 71, 1448.
15. Gill, US.; Schwartz, HM.; Wheatley, B.; Parlar, H. (1996) Chemosphere 33, 1021.
16. Stalling, DL.; Tindle, RC.; Johnson, JL. (1972) J. Assoc. Off. Anal. Chem. 55, 32.
17. Stern, GA. and Tomy, GT. (2000). To be Submitted to Anal. Chem.