Toxaphene Analyses of Fish Livers by High Resolution Mass Spectrometry

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Abstract

Fish may bioaccumulate toxaphene compounds from their environment and store them in their fatty tissues. Liver samples of burbot from a Russian lake contain polychlorinated camphenes. The concentrations ranged from 144 to 2,122 ng/g wet weight. These concentrations are similar to concentrations reported for burbot from other locations and the toxaphene pattern is similar to that seen for cod liver oil. The source of toxaphene to this remote area is likely global distillation from lower latitudes. Toxaphene was not detected in livers of trout samples from Alaskan Arctic lakes using these analytical procedures, possible due to the method detection limit (125 ng/g) for small sample size (0.5 g wet weight). High resolution gas chromatography/high resolution mass spectrometry in selected ion monitoring mode (HRGC/HRMS-SIM) is a useful tool for analyzing toxaphene in samples in the presence of other organochlorine contaminants.

Introduction

Toxaphene (Polychlorinated camphene; PCC) is produced by the chlorination of camphene to a chlorine content of 67 to 69%[1]. This process produces a broad spectrum pesticide comprised of over 650 polychlorinated bornane/bornene congeners. Toxaphene was widely used in the southern United States as an insecticide on cotton, soybeans and peanuts until the late 1970s when toxaphene usage decreased due to heightened environmental concerns. Toxaphene use was limited in 1982 and all uses were canceled in 1987 in the United States. Toxaphene may still be used in the former Soviet Union. Current global usage distribution indicates the United States and the former Soviet Union were the major users from 1970 to the present, with a usage of over 100,000 tones[2]. Surprisingly, more than 10 years after its

major usage, toxaphene was found to be the most abundant pesticide detected in Arctic aquatic organisms and one of the major contaminants of fish from small and large lakes around the world[3]. This widespread distribution of toxaphene indicates that atmospheric transport is an important source to remote areas.

Toxaphene is difficult to quantify because the composition of the commercially used product, "Technical Toxaphene", is different from the toxaphene found in environmental samples. These differences are likely due to preferential metabolism and/or degradation. Analytical methods currently used for toxaphene analysis involve gas chromatography with electron capture detectors or GC/MS with negative chemical ionization detectors. The analytical methods for toxaphene are at a developmental stage, similar to the situation with PCBs before individual congeners were available. Recently an alternative high resolution selective ion monitoring GC/MS (HRGC/HRMS-SIM) method for determining toxaphene was reported[4]. One objective of this research was to confirm the usefulness of HRGC/HRMS-SIM for analyses of toxaphene. Since organochlorine concentrations of various fish species have been determined worldwide, the second objective of this study was to apply the HRGC/HRMS-SIM method to the determination of the toxaphene concentration in fish livers from Arctic lakes.

Experimental

Samples of liver were removed from burbot from a lake in Russia. Fish were collected, the livers removed and frozen prior to shipment to Texas A&M University for processing and analyses. The extraction procedure was previously reported[5]. Sample aliquots (approximately 0.5 g wet weight) were macerated with 40 g anhydrous Na_2SO_4 and 100 mL of methylene chloride for 3 minutes using a Tekmar Tissumizer®. The solvent was then decanted, and the process was repeated two more times with an additional 100 mL of methylene chloride each time. Percent lipid content was determined gravimetrically using a 20 mL aliquot of the extract. Samples were evaporated to 10 mL. Hexane (5 mL) was added and the sample evaporated to 1 mL. Lipids that might interfere with the analyses were removed by size exclusion gel permeation chromatography (GPC).

A HP5890 gas chromatograph fitted with a 60m DB-5MS fused silica capillary column was used for the analyses. The injector was operated at 280°C in the splitless mode with a helium carrier gas head pressure of 30 psi. A fast temperature ramp was employed to facilitate short analysis times and a compressed retention time window for the toxaphene isomers.

All analyses were performed on a VG AutoSpec Ultima high resolution mass spectrometer operated in the selected ion monitoring (SIM) mode at a resolving power of 10,000 or better. Electron impact (EI) ionization at 35eV was used. Two microliters of the concentrated extract were injected and

data were acquired at a rate of one scan per second. Perfluorokerosene (PFK) was used for mass calibration. Two mass/charge ratios, 158.9769 and 160.9739 corresponding to the dichlorotropylium ion[5], were monitored and summed for quantification of the toxaphene isomers[4]. The total area under all the toxaphene peaks in each SIM trace was integrated. PCB103 was used as an internal/quantification standard and tetrachlorometaxylene (TCMX) was used to measure method recovery efficiency.

An initial 6-point calibration was performed by analyzing solutions containing 0.545, 1.09, 2.18, 5.45, 10.9, and 21.8 ng/µL technical toxaphene. Response factors relative to internal standard PCB103 were calculated for each standard solution. The 5.45 ng/µL standard was analyzed before and after analysis of the liver samples to demonstrate continued instrumental calibration. The mean relative response factor from the initial calibration was used for quantification of toxaphene in the analytical samples.

The concentration of toxaphene in NIST SRM 1588, Cod Liver Oil, was measured using the same instrumental and analytical procedures. Fourteen laboratories from five countries participated in the first phase of an interlaboratory round-robin conducted by the Bureau of Chemical Safety, Health Canada (P. Andrews, personal communication). Concentrations of toxaphene reported in the cod liver oil reference material ranged from 0.79 to 7.5 ppm. The mean concentration found by HRGC/HRMS-SIM analyses of the NIST SRM 1588 cod liver oil sample analysed in the same analytical batch with the burbot livers was 2.68 and 2.38 ppm, which is within the range of concentrations reported for all laboratories and close to the 2.3 ppm reported for HRGC/HRMS-SIM method.

Results and Discussion

A set of mixed standards were analyzed in order to establish that there would not be any interferences from other chlorinated hydrocarbons that might be present in the samples. Analyses of standards containing toxaphene and technical chlordane, Aroclor 1254, or a mixture of organochlorine pesticide indicated only minor interference with the quantification ions (158.9769 and 160.9739) selected. This allows the method to be used without the necessity of separating toxaphene from these other chlorinated hydrocarbons and allows PCB 103 to be used as an internal standard.

The mass chromatograms of technical toxaphene, NIST SRM 1588 Cod Liver Oil and a typical burbot liver sample are shown in Figure 1A, B and C, respectively. The differences between technical toxaphene (Figure 1A) and environmental samples from organisms high in the food web (Figure 1B and C) is readily apparent. There are fewer peaks in the cod liver oil (Figure 1B) and the burbot liver (Figure 1C) than the technical toxaphene (Figure 1A). To illustrate these changes two peaks have been labeled as T2 and T12 (Figure 1). These peaks are minor constituents in the technical toxaphene, but are

the major peaks in both the cod liver oil and the burbot liver samples. These peaks have been previously isolated and identified in beluga whale blubber samples as an octachlorobornane (T2) and a nonachlorobornane (T12)[6].The cod liver oil chromatogram has peaks in the 28 to 29 min. range that are not seen in the burbot liver, while, the burbot liver contains two peaks in the 26 to 27 min. retention range that are not seen in the cod liver oil. These differences may be the result of different metabolism between the cod and burbot or differences in the toxaphene in the food that they consume.

Fish have been employed as biomonitors of the presence of PCBs and chlorinated pesticides[7,8]. Fish are also used to assess organochlorine bioavailability and bioaccumulation in the marine environment over long time spans (years) and geographic areas, and may provide a model for human exposure to organochlorines from seafood consumption. When exposed, fish accumulate high body burdens of organochlorine contaminants in their fatty tissues, including their livers, through food consumption. The bioaccumulation may be dependent on many factors, including species, age, gender, lipid content, environmental conditions, and chemical and biological characteristics of the organism.

A selected suite of burbot liver samples was chosen for analyses. The toxaphene concentrations ranged from 0.14 to 2.12 µg/g wet weight (Table 1).The legal limit for toxaphene in fish and fishery products is 0.1 µg/g wet weight in Canada and 7 µg/g wet weight in the United States. All the samples exceed the Canadian limit, while none exceeded the U.S. limit.

Table 1. Burbot liver Toxaphene Concentrations (µg/g wet weight)

Burbot Liver Sample	Concentration (µg/g)
A	0.14
B	0.27
C	0.45
D	0.83
E	2.12

The concentration of toxaphene in burbot liver samples from lakes in the Yukon ranged from 0.54 to 2.81 µg/g wet weight with significant variations between lakes[7]. The range of toxaphene concentrations reported for fish from the North Sea and North-East Atlantic was <.001 to 1.3 µg/g wet weight[8]. The GC/ECD method used for toxaphene analyses of fish samples[7,8] may be more prone to interference from chlordanes than the HRGC/HRMS-SIM method described here. The toxaphene concentrations reported for burbot livers from Russia were within these ranges.

Conclusions

HRGC/HRMS-SIM is a valuable tool for analyzing toxaphene in samples in the presence of other organochlorine contaminants. Further advancements in analytical techniques for toxaphene must wait for standards of the major environmental toxaphene metabolites and toxicants to be available. Burbot bioaccumulate toxaphene from their environment. The source is likely global distillation from lower latitudes as toxaphene is not used in the Arctic.

Acknowledgments

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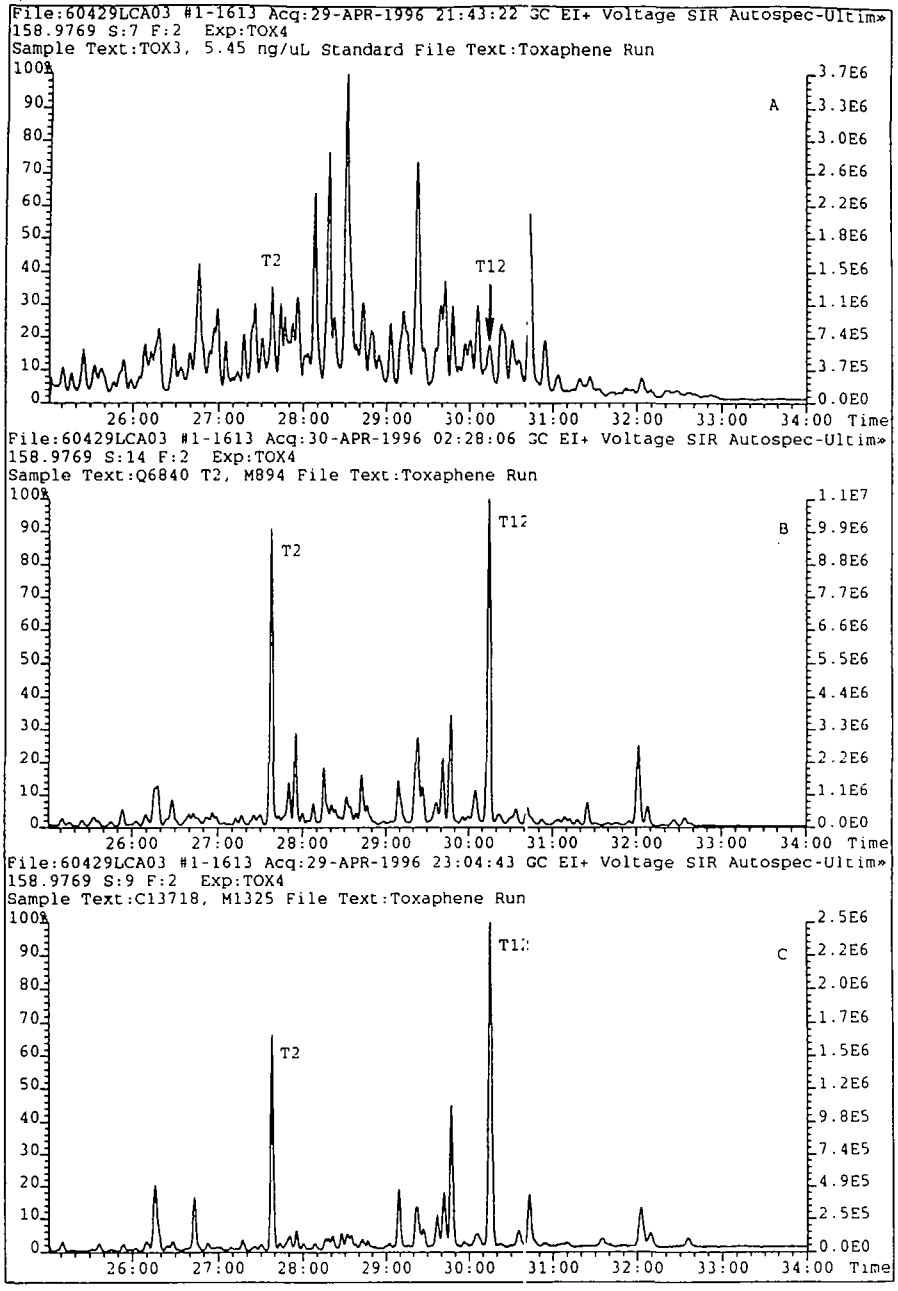

Figure 1. Mass chromatograms of Technical Toxaphene (A), NIST SRM 1588 Cod Liver Oil (B), and burbot liver (C). (See text for explanation of peaks T2 and T12).