High Resolution Mass Spectrometry Analyses of Toxaphene in the Blubber of Beluga Whales

Terry L. Wade, Laura Chambers, Piero R. Gardinali, José L. Sericano, Thomas J. Jackson and Thomas J. McDonald
Geochemical and Environmental Research Group, 833 Graham Rd, College Station TX 77845
Raymond J. Tarpley
Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77840
Robert Suydam
Department of Wildlife Management, North Slope Borough, Barrow, AK, 99723

1. 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 U.S. as an insecticide on cotton, soybeans and peanuts until the late 1970s when it usage decreased due to heightened environmental concerns. Toxaphene use in the U.S. was limited in 1982 and all uses were canceled in 1987. 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[2]. This widespread distribution of toxaphene, including remote areas, confirms atmospheric transport as an important source.

There is limited information on the chemistry, environmental behavior and toxicology of toxaphene. Therefore, toxaphene was the subject of a recent workshop attended by over 80 environmental chemists and toxicologists. The purpose of the workshop was to evaluate the current state of toxaphene research and recommend future directions[2].

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 or degradation. Currently, analytical methods used for toxaphene analysis have involved gas chromatography with electron capture or negative chemical ionization mass spectrometry 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 (HRSIM-GC-MS) method for determining toxaphene was reported[3]. One objective of this research was to confirm the usefulness of HRSIM-GC-MS for analyses of toxaphene. Since organochlorine concentrations of various marine mammal species have been determined worldwide, the second objective of this study was to apply the HRSIM-GC-MS to the determination of the toxaphene concentration in blubber samples from beluga whales (*Delphinapterus leucas*) that were caught during the Alaskan Native subsistence hunt in 1992.

2. Experimental

Samples of blubber were removed from subsistence-killed beluga whales near the village of Point Lay along Alaska's north coast. All specimens were collected under scientific permits issued by the U.S. National Marine Fisheries Service. Samples were collected on July 6, 1992 within 3 hours postmortem, wrapped in aluminum foil and frozen prior to shipment to Texas A&M University for processing and analyses. Growth layer groups (GLGs) were counted in tooth dentine from each whale in order to estimate their age. It is currently assumed that 2 GLGs are formed per year[4]. Therefore, the GLGs divided by 2 give an estimation of age in years (Table 1). Blubber samples were cut from the internal portion of the original thawed sample with a stainless steel scalpel to avoid surface contamination. Blubber samples include the entire blubber layer from skin to muscle to avoid possible bias between samples[5]. The extraction procedure was previously reported[6]. Sample aliquots (approximately 2 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). After GPC, samples were evaporated to a final volume of 1 mL of hexane.

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 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 employing 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 masses, 158.9769 and 160.9739 corresponding to the dichloro-tropylium ion[7], were monitored and summed for quantitation of the toxaphene isomers[3]. The total area under all the toxaphene peaks in each SIM trace was integrated. PCB103 was used as an internal/quantitation standard and TCMX was used to measure method recovery efficiency.

An initial 6-point calibration was performed by analyzing solutions containing 0.218, 1.09, 2.18, 5.45, 10.9, and 21.8 ng/µL analytical (technical) toxaphene. Response factors relative to internal standard PCB103 were calculated for each standard solution. The 5.45 ng/µL standard was analyzed once during and once after analysis of the blubber samples to demonstrate continued instrumental calibration. The mean relative response factor from the initial calibration was used for quantitation 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 ranged from 0.79 to 7.5 ppm. The mean concentration found by HRSIM analyses of the intercalibration cod liver oil sample in our laboratory was 4.81 ppm, which is within the range for all laboratories.

3. 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, with the toxaphene analyses. Analyses of standards containing toxaphene and technical chlordane, Arochlor 1254, or a pesticide mixture indicated only minor interference with the quantitation 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 beluga whale blubber sample are shown in Figure 1. The differences between technical toxaphene and environmental samples from organisms high in the food web is readily apparent. There are fewer peaks in the cod liver oil than the technical toxaphene and still fewer peaks in the beluga whale blubber, a top predator. 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 beluga whale blubber. These peaks have been previously isolated and identified in beluga whale blubber samples as an octachlorobornane (T2) and a nonachlorobornane (T12)[7].

Marine mammals, including toothed whales, have been employed as biomonitors of the presence of PCBs and chlorinated pesticides[6,9], primarily because they are mobile, long-lived, and are top predators in the food web, making them repositories for organochlorine contaminants. Marine mammals 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. Some marine mammals accumulate high body burdens of organochlorine contaminants in their blubber and other tissues through ingestion of contaminated prey. 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 beluga whale samples, consisting of two males, four females, and one fetus, was chosen for analyses. The toxaphene concentrations ranged from 1.49 to 8.94 µg/g dry weight (Table 1). The two males had estimated ages of 6 and 13 years, respectively. The males had higher concentrations of toxaphene, when compared to the females or the fetus that ranged in estimated age from near term fetal to 19 years. The older male had toxaphene concentrations four times higher than the younger male (8.94 µg/g vs. 2.01 µg/g). The younger male had slightly higher concentrations (1.2 times) than the females, all of which had very similar concentrations regardless of age.

Female marine mammals transfer pesticides/PCBs to their offspring during gestation and through lactation. It is therefore not surprising that the females have lower concentrations than the males. The female/fetus pair have nearly identical concentrations of toxaphene (range 1.49 to. 1.66 µg/g). These differences are within the uncertainty of the analytical method. The female beluga whales have lower toxaphene concentrations as compared to the males probably due to reproductive processes of birth and lactation that transfer contaminants to the offspring. Within the females there is no consistent trend of higher contaminant concentrations with increased age. It is of interest to look at the total contaminant concentrations of the beluga whales in terms of potential human health effects. The legal limit for toxaphene in fish and fishery products is 0.1 µg/g wet weight in Canada and 5 µg/g wet weight in the United States[10]. All the samples exceed the Canadian limit, while only the 13 year old male exceeds the U.S. limit.

4. Conclusions

HRSIM-GC-MS 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. Beluga whales bioaccumulate toxaphene from their environment. The source is likely global distillation from lower latitudes. Based on this limited set of seven samples, consumption of older male whales may pose a human health concern.

5. References

1) Melnikov N.M. (1971) Chemistry of Pesticides. Res. Review. 36. 51
2) Bidleman T.F. and D.C.G.Muir (1993) Analytical and Environmental Chemistry of Toxaphene. Chemosphere. 27. 1825-1826
3) Andrews P., W.H. Newsome,M. Boyle and P. Collins (1993) High Resolution Selective Ion Monitoring GC-MS Determination of Toxaphene in Great Lakes Fish. Chemosphere. 27, 1865-1872
4) Goren A.D., P.F. Brodie, S. Spotte, G.C. Ray, W.W. Kaufman, A.J. Gwinnett,J.J. Sciubba and J.D. Buck (1987) Growth Layer Groups (GLGs) in teeth of an Adult Beluga Whale (*Delphinapterus leucas*) of Known Age: Evidence for TwoAnnual Layers. Marine Mammal Sci. 3 14-21
5) Aguilar A. and A. Borrell (1991) Heterogeneous Distribution of Organochlorines Contaminants in the Blubber of Baleen Whales:Implications for Sampling Procedures. Mar. Environ. Res. 31 275-86
6) Salata G., T.L. Wade, J. Davis, J.L. Sericano, and J.M. Brooks: (1995) Organochlorine Contamination of Marine Mammals in the Gulf of Mexico, Environ. Poll. 88: 167-175.
7) Saleh M. A. (1983) Capillary Gas Chromatography-Electron Impact and Chemical Ionization Mass Spectrometry of Toxaphene. J. Agric. Food Chem. 31 748-751
8) Stern G.D., D.C.G. Muir, C.A. Ford, N.P. Grift, E. Dewailly. T.F. Bidleman and M.D. Walla (1992) Isolation and Identification of Two Major Recalcitrant Toxaphene Congeners in Aquatic Biota. Environ. Sci. Technol. 26 1838-1840
9) Muir D.C.G., C.A. Ford, R.E.A. Stewart, T.G. Smith, R.F. Addison, M.E. Zinck and P. Beland (1990) Organochlorine Contaminants in Beluga, *Delphinapterus leucas*, from Canadian Waters. Can. Bull. Fish. Aqua. Sci. 224 165-190
10) Nauen, C.E. (1983) Compilation of legal Limits for Hazardous Substances in Fish and Fishery Products. FAO Fisheries Circular No. 764, United Nations, Rome Italy. 102pp.

6. Acknowledgments

We are grateful to the hunters and villagers of Point Lay, Alaska for their help and cooperation in collection of the beluga whale tissues for analyses. This study was funded by the Alaskan Beluga Whale Committee (NOAA GRANT NA27FX0258-01) and the North Slope Borough, Department of Wildlife Managment. Toxaphene method development was partially funded by US EPA Arctic Contaminants Research Program.

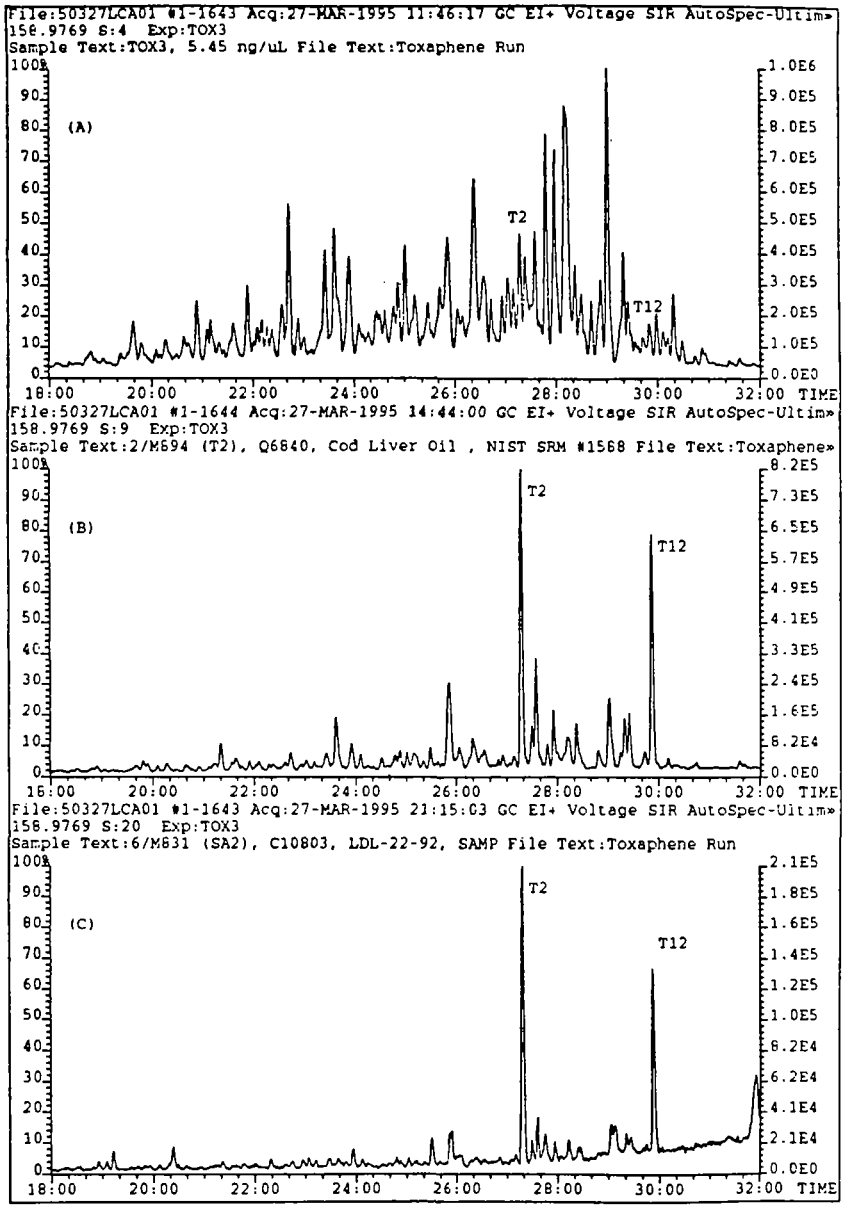

Figure 1. Mass chromatograms of Technical Toxaphene (A), NIST SRM 1588 Cod Liver Oil (B), and belgua whale blubber (C). (See text for explanation of peaks T2 and T12).

Table 1. Beluga Whale Estimated Age, Sex and Toxaphene Concentrations

Estimated Age Years	Sex	Dry Weight µg/g	Wet Weight µg/g	Lipid Weight µg/g
0	Fetus	1.64	1.35	3.71
16	Mother	1.55	1.35	3.20
11	Female	1.62	1.39	3.54
16	Female	1.66	1.58	3.44
19	Female	1.49	1.40	2.38
6	Male	2.01	1.79	4.06
13	Male	8.94	8.21	15.94