Characterization of Two Major Toxaphene Components in Lake Sediment.

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1. Introduction

Toxaphene, a complex mixture consisting primarily of chlorinated bornanes (CHB's), was once widely used as a insecticide and briefly used as a fish toxin during the late 1950's and 1960's in many North American Lakes. The technical material is thought to consist of between two and three hundred penta- to undecachlorobornanes and camphene/diene isomers[1,2]. It is well known, however, that toxaphene residues extracted from biotic and abiotic samples do not resemble the technical material as analysed by HRGC, but instead consists of a more limited number of hexa-, hepta-, octa- and nonachlorobornanes[3]. In previous work[4,5], we structurally identified the two major CHB congeners, T2 (2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,10,10-Octachlorobornane) and T12 its nonachloro- analog (2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,9,10,10-Nonachlorobornane) found in beluga blubber. We report here the structures, as determined by mass spectrometry and ^{1}H-NMR, of two more environmentally significant toxaphene congeners, a hexa- and heptachlorobornane (Hx-Sed and Hp-Sed, respectively), from the sediment of two lakes located in Alberta Canada[6]. Both lakes were treated in the early 1960's by addition of toxaphene to the water column at low ug/L concentrations. Highest toxaphene concentrations (~500-1600 ng/g dry wt.) were found in sediments of both lakes in slices dated to the early 1960's. In these slices the chromatographic pattern resembled that of the toxaphene standard while in more recent slices, the number of CHB peaks was greatly reduced with the two most prominant peaks corresponding to Hx-Sed and Hp-Sed. Surface sediments of an untreated basin of Peanut Lake was also analysed for toxaphene and was found to contain levels < 0.1 ng/g. This suggests that external sources of toxaphene to the treated lakes are negligible and that the observed chromatographic pattern in the surface sediment is the sole result of the introduction of the toxaphene during treatment. Sediment cores from three Yukon lakes (Laberge (61°11'N, 135°12'W), Kusawa (60°20'N, 136°22'W) and Fox (61°14'N, 135°28'W)) were also analysed for toxaphene and their chromatographic profiles compaired to those of the two treated Alberta lakes.

2. Experimental

Toxaphene extraction and isolation of Hx- and Hp-Sed.

Approximately 10 kg (wet wt.) of Chatwin lake surface sediment (0 to 10 cm deep) was collected using a modified Ekman dredge and was stored in 3, 4 L glass bottles. This corresponded to 735 g (dry wt.) of sediment after freeze drying. The freeze dried sediment was refluxed, 100 g at a time, in 800 ml DCM for 5 hours. Extracts were treated with 1:1 fumming nitric:sulfuric acid to remove organic pigments and chlorinated aromatics[7] and with copper to remove sulfur. To isolate Hx- and Hp-Sed from other organochlorines, we used high-performance liquid chromatography (HPLC) on a Nova-Pak HR C18 preparative column (Waters Scientific), employing an isocratic solvent system consisting of a acetonitrile-water (65:35, v/v) mixture (4.0 ml min^{-1}). GC-ECNIMS was used to analyse the various HPLC fractions for Hx- and Hp-Sed. Cores were collected from the deep zone of each lake using a 15-cm KB corer and sliced at 1 cm intervals. Sediment were placed in plastic bags and stored (-20°C) until analysis. Sediments (10 g dry wt.) were Soxlet extracted with DCM as described by Muir *et al*[6].

Mass Spectrometry

GC-EIMS, GC-ECNIMS and linked field scanning were performed on a Kratos Concept high resolution mass spectrometer (EBE geometry) controlled using a Mach 3X data system. EI mass spectra were scanned from 35 to 450 daltons at a scan rate of 1 sec per decade. The ion source was maintained at a temperature of 220°C, the trap current was 500 uA, the ion accelerating voltage was 8 kV and the electron energy was adjusted for maximum sensitivity (~ 50 eV) . Decompositions of selected ions in the first field region were identified by a series of linked-field scans (B/E, B^2/E and CNL(daughter and parent)). Ion decompositions were enhanced by collisional activation by introducing argon into the collision cell at a pressure to give approximately 50 % attenuation of the m/z 231 ion of PFK. Selected ion ECNIMS was performed at a spectrometer resolution of $M/\Delta M$ ~14000. Methane was used as the moderating gas and PFK as the mass calibrant. Optimum sensitivity was obtained at a gas pressure of ~2 x 10^{-4} torr as measured by the source ion guage. The electron energy was adjusted for maximum sensitivity (~180 eV), the accelerating voltage was 5.3 kV and the ion source temperature was 120°C. The following characteristic ions were monitored from the $(M-Cl)^-$ isotopic cluster of the hexa- to nonachlorobornane homolog groups; Cl_6 308.9352, 310.9323; Cl_7 342.8962, 344.8933; Cl_8 376.8573, 378.8543; Cl_9 410.8183, 412.8154.

Gas Chromatography

GC separations were performed on a Hewlett Packard model 5890 Series II gas chromatograph using a a 60m x 0.25mm i.d. DB-5ms fused silica column (Chromatographic Specialities) which was connected directly to the ion source of the mass spectrometer. He was used as the carrier gas. Samples were run using splitless injection (2 min.) with the injector at 260°C. The initial column temperature was 80°C; at 2 minutes the oven was ramped at 20°Cmin^{-1} to 200°C, then at 2°Cmin^{-1} to 230°C then at 10°Cmin^{-1} to a final temperature of 300°C and held for 8 minutes. Electronic pressure programming was used increase the pressure during the injection cycle and then to maintain a constant flow of 1 ml min^{-1} during the remainder of the run. All injections were made by a CTC A200SE autosampler under data system control.

3. Results and discussion

The EI positive ion mass spectra of Hx-Sed and Hp-Sed are shown in Figure 1. In the mass spectra of both compounds, a 110 Da neutral species is eliminated from $[M\text{-}HCl\text{-}Cl]^+$ (m/z 271 for Hx-Sed, 305 for Hp-Sed) to yield the m/z 161 ion in the mass spectrum of Hx-Sed and the m/z 195 ion in that of Hp-Sed. The observation of these decompositions, verified for both compounds by linked field scanning, provides us with important structural information because the neutral species lost are comprised of the bridging carbon (C7) and its two substituents *i.e.* $CH_2ClC{:}CH_2Cl$. Observed decompositions involving the loss of $\cdot CH_2Cl$ in the mass spectra of both compounds and the absence of corresponding decompositions involving the loss of $\cdot CHCl_2$ further support the positioning of two monochloromethyl moieties on C7. The group of peaks starting at m/z 306 and 307 in the mass spectrum of Hx-Sed and at m/z 340 and 341 in the mass spectrum of Hp-Sed correspond to the ions $[M\text{-}Cl]^+$ and $[M\text{-}HCl]^+$, respectively. Verified by linked-field scanning, the neutral fragment $C_2H_2Cl_2$ is eliminated from the latter ion giving rise to the even mass odd-electron ion $[M\text{-}HCl\text{-}C_2H_2Cl_2]^{+\cdot}$ (m/z 210 for Hx-Sed, 244 for Hp-Sed). In the mass spectrum of Hx-Sed loss of C_2H_3Cl is also observed and gives rise to the even mass odd-electron ion $[M\text{-}HCl\text{-}C_2HCl_3]^{+\cdot}$ (m/z 244). Losses of both C_2H_3Cl and $C_2H_2Cl_2$ in the mass spectrum of Hx-Sed suggest that there are three chlorines on the ring, two on one side and one on the other. In the mass spectrum of Hp-Sed, loss of only $C_2H_2Cl_2$ and not C_2H_3Cl, C_2HCl_3, C_2H_4 or C_2Cl_4 suggest a total of four ring chlorines, two on each side. In the mass spectra of T2 and T12, competitive losses of $Cl\cdot$ and HCl from the molecular ion, were observed while those of toxicants A and B (2,2,5-*endo*,6-*exo*,8,8,9,10/8,9,9,10-Octachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane,respectively), showed an enhance elimination of a chlorine from the molecular ion[5]. It was suggested that the enhanced HCl elimination from the molecular ions in T2 and T12 with respect to that of toxicants A and B was to be expected from the greater acidity of the 5-*endo* and 6-*exo* hydrogen of T2 and T12 relative to the 5-*exo* and *endo* hydrogens of toxicant A and B, due to the electron-withdrawing properties of the 5-*exo* and 6-*endo* chlorines. This competitive loss of HCl and $Cl\cdot$ from the molecular ion is also observed in the mass spectrum of Hp-Sed. This result along with conclusions drawn by Hainzl[2] with regard to relevant structures of CHB's suggest that Hp-Sed, like T2 and T12, has a 2-*exo*,3-*endo*,5-*exo*,6-*endo* ring conformation. The postulated structure for Hp-Sed is thus, 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,9,10-heptachlorobornane and was varified using ^{1}H-NMR. Positioning of the ring chlorines on Hx-Sed required the use of ^{1}H-NMR. Based on these results and the mass spectral results discussed above, the structure of Hx-Sed was determined to be 2-*exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane. The enhanced elimination of $Cl\cdot$ from the molecular ion relative to that of HCl loss may result from the reduced acidity of the 5-*exo* proton in absence of the electron-withdrawing properties of a 5-*endo* chlorine.

Toxaphene concentrations in the Yukon sediment core slices (covering the past three decades) were highest in Fox Lake, comparable to levels found in other Arctic cores[8] and considerably lower (1000 fold) than the levels observed in Chatwin and Peanut Lake. These result would seem to suggest that the source of toxaphene to these lakes is atmospheric rather than from elevated inputs of toxaphene from a point source. However, the chromatographic profile of toxaphene in Fox Lake sediment was found to be very similar to that which was observed for Chatwin and Peanut Lake sediment (Figures 2 and 3.). Approximately 60% of the total ion intensity is due to the presence of Hx- and Hp-Sed. If elevated levels of these two CHB's is a signature by which lakes exposed to an *in situ* source of toxaphene can be

identified, it would imply that Fox lake may have in the past been contaminated by a direct source. The lower levels would be more consistent with a small spill(s) than with direct application as a piscicide. Laberge sediment was also found to contain elevated levels of Hx- and Hp-Sed, however, in this case they were responsible for a much smaller portion of the total toxaphene concentration. This could, howerver, be due to the much larger relative size of Laberge compared to Fox and the resulting dilution effect. Kusawa sediment had a toxaphene profile consistent with contamination by an external source only.

4. References

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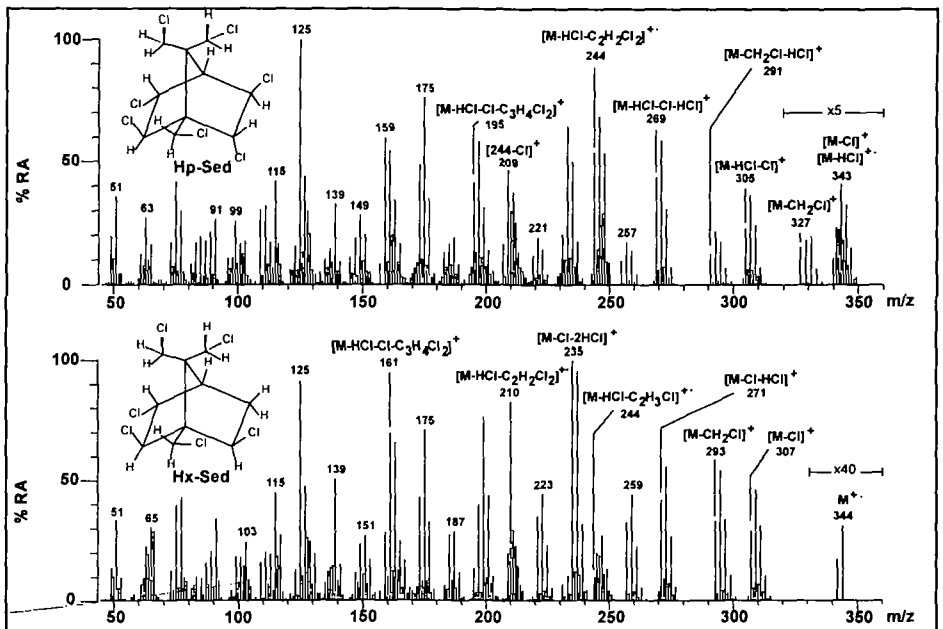

Figure 1. EI positive ion mass spectra of Hp-Sed (top) and Hx-Sed (bottom).

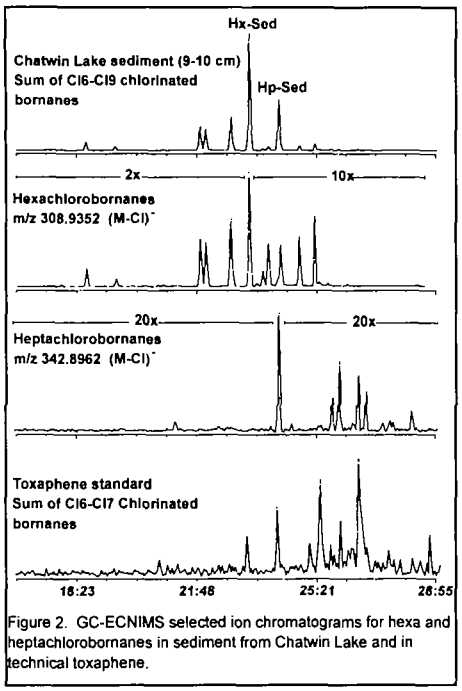

Figure 2. GC-ECNIMS selected ion chromatograms for hexa and heptachlorobornanes in sediment from Chatwin Lake and in technical toxaphene.

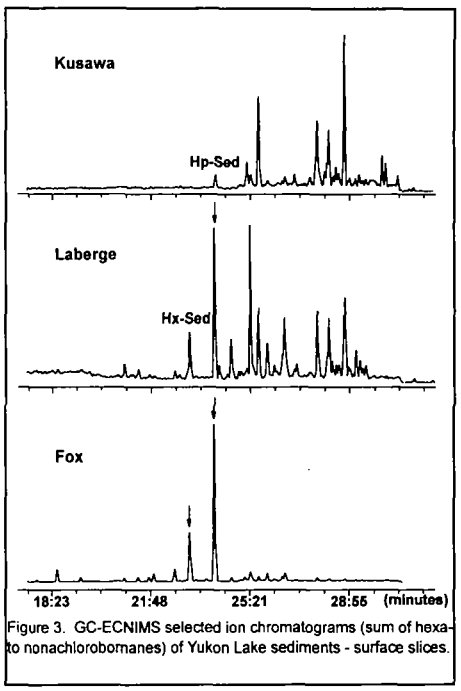

Figure 3. GC-ECNIMS selected ion chromatograms (sum of hexa- to nonachlorobornanes) of Yukon Lake sediments - surface slices.

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