ENANTIOSELECTIVE DETERMINATION OF CHIRAL CHLOROBORNANES IN SEDIMENTS FROM THE BALTIC SEA

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Abstract

Two sediment samples from the Baltic Sea were analyzed for chlorobornanes. Identification of the chlorobornanes was achieved by various high-resolution gas chromatographic/mass spectrometric (HRGC/MS) techniques, including a chiral stationary phase resulting in the enantiomeric resolution of chiral compounds. The samples were collected close to the coast and in the open sea approximately 150 km from the coast. The coastal sampling point was located 4 km from a pulp mill and all sampling was done at a time (1985-86) when free chlorine was used for bleaching. An earlier study showed a dramatic decrease between the two stations for various analytes correlated to pulp bleaching (extractable organic compounds, PCDDs, PCDFs, alkyl-PCDFs, 3,4,5-trichloroguaiacol). In both sediment samples a series of hexa- to nonachlorobornanes was detected. There is a higher concentration of the hexa- and heptachlorobornanes in the sample nearer the coast, and there are differences in the isomer patterns of these compounds. For the octa- and nonachlorobornanes, however, no such differences were observed. There are small changes in the enantiomeric composition of these chlorobornanes from that of technical toxaphene.

Introduction

Toxaphene was a widely used insecticide manufactured by the chlorination of camphene. It consisted of a complex mixture of chlorinated derivatives, mostly chlorobornanes with six to ten chlorine atoms. More than two hundred chlorobornanes or related compounds have been identified in technical toxaphene mixtures. A relatively smaller number of these components have been detected in biota, indicating extensive alteration of the original mixture (1). Most of the chlorobornanes are chiral and exist in two enantiomeric forms. The drastic changes in congener and isomer composition of chlorobornanes in biota may be due to biotic and/or abiotic processes. However, changes in the enantiomeric composition of chiral compounds would only be due to enantioselective biotic processes.

Chlorobornanes have also been detected in sediment samples. Recently, Stern *et al.* identified two major chlorobornanes in sediment from a toxaphene treated lake, a hexa- and a heptachloro congener designated as Hx-Sed and Hp-Sed (2). Hx-Sed was previously identified as a reductive dechlorination product of toxicant B, a component of technical toxaphene (3). Hx-Sed also was found to be the major

degradation product of a series of other chlorobornanes and of technical toxaphene under anaerobic conditions in soil by Fingerling *et al.* (4). They reported the loss of a chlorine from the *geminal-* (*gem-*) Cl_2 group at C-2 in the 6-membered carbon ring from these chlorobornanes. Stern *et al.* (2) determined that Hp-Sed is most likely TC1, characterized by MS and MS/MS techniques in a technical toxaphene mixture (1). In this report we present results from enantioselective and non-enantioselective analyses of two Baltic Sea sediment samples. We also observed a reduced isomer pattern of chlorobornanes, and show that there are some changes in enantiomeric composition of some chiral components, including Hx-Sed and Hp-Sed.

Experimental Methods

Sample Description. In an earlier study, a series of sediment samples was collected in 1985-1986 from the northern part of the Baltic Sea (see ref 5). The samples were taken at varying distances from a pulp mill on the coast that bleached with free chlorine. Various pulp bleaching related parameters and compounds, including 2,3,7,8-TCDD and -TCDF, alkyl-PCDFs, 3,4,5-trichloroguaiacol, and extractable organic chlorine decreased as distance from the pulp mill increased. At 4 km from the mill the concentrations of some key components were 20-100 times higher than those found at a distance 150 km away. In this study, the sample 4 km from the mill ("coastal" sample) and the sample 150 km from the mill ("open-sea" sample) were analyzed for chlorobornanes.

Sample Preparation. The sediment samples were air-dried and then Soxhlet-extracted with toluene. The crude extract was treated with copper to remove sulfur, percolated through silica gel and then fractionated on Florisil (6). Fractions 1 and 2 were pooled and further cleaned-up by gel-permeation chromatography (7) for removal of lipids. Final clean-up was effected by normal phase silica HPLC (8). The purified extract was concentrated, exchanged for toluene and a small aliquot injected for HRGC/MS analysis.

HRGC/MS Analyses. The analyses were carried out using an achiral 25-m SE54 HRGC column and a chiral 20-m OV1701-BSCD HRGC column (BSCD= *tert*-butyldimethylsilyl-β-cyclodextrin) using primarily electron-capture, negative ionization mass spectrometry (ECNI-MS) (1). Full-scan ECNI mass spectra were recorded for analyte identification; selected-ion-monitoring (SIM) was used for congener group analysis using the $(M\text{-}Cl)^-$ (or satellite) ions at m/z 307, 341, 375, 409 and 443 for hexa- to decachlorobornanes. The conditions and the identification of the various chlorobornanes were described previously (1). Several additional chlorobornanes were available as reference compounds and used for further assignments of toxaphene components. The technical toxaphene used as reference was Camphechlor (Ehrenstorfer, Augsburg, Germany).

Results and Discussion

Chlorobornanes in the Sediment Samples. Hexa- to nonachlorobornanes were detected in both the "coastal" and the "open-sea" samples at total concentrations estimated to be in the low ng/g range (dry-weight). The isomer profiles for both samples had fewer chlorobornanes peaks compared to the technical toxaphene mixture and showed some resemblence to those observed in biota (1).

In the "open-sea" sample a single major hexachlorobornane was detected. This compound is also present in technical toxaphene. Based on retention time, this compound was identified as Hx-Sed (2). Two major heptachlorobornane congeners, TC1 and TC2, and several minor hepachlorobornanes were identified. TC1 is Hp-Sed, as pointed out by Stern *et al.* (2). The "open-sea" sample's isomer profile is similar to technical toxaphene except that a higher proportion of TC1 is present and another component, identified as toxicant B, is lower. Three major octachlorobornane congeners were present in this sample (TC5, TC6 and TC7), fewer than in technical toxaphene. Also, the octachlorobornanes TC8 and TOX8 were lower than in technical toxaphene; as pointed out previously (1), TC8 very likely is toxicant A. There was a single major nonachlorobornanes isomer (TOX9) present in the "open-sea" sample whereas there are up to nine medium to large peaks for the nonachlorobornanes in technical toxaphene. The "open-sea" sediment sample thus shows a much simpler isomer pattern compared to the technical toxaphene, particularily for the hepta-, octa- and nonachlorobornanes. Two of the missing components in the sediment are toxicant A and B, both of which contain a *gem*-Cl_2 substitution for which enhanced anaerobic degradation was previously reported (4).

The "coastal" sample is both similar and dissimilar to the "open-sea" sample depending on the congener group considered. The "coastal" sample shows additional halogenated components in the analyzed fraction, despite the fact that an elaborate and sophisticated clean-up for chlorobornanes was used. Consequently, there is the potential for misidentifications. These additional components yielded different mass spectra and different SIR response ratios, or they eluted outside the retention time windows for chlorobornanes. In case of the octa- and nonachlorobornanes, the same major congeners as in the "open-sea" sample were present at similar concentrations. The same heptachlorobornanes congeners were present but Hp-Sed (TC1) was higher in the "coastal" sample. In case of the hexachlorobornanes in the "coastal" sample, there is a more complex isomer pattern and the chromatogram showed additional peaks due to compounds which are not hexachlorobornanes (wrong relative response ratios). Hx-Sed is present in the sediments from both locations. Another component, tentatively identified as a hexachlorobornane in the "coastal" sample, was present, if at all, only at low concentration in technical toxaphene.

Degradation Pathways. The isomer patterns of these two sediment samples indicate significant alteration compared to the technical toxaphene mixture. The most drastic changes were in the nonachlorobornanes where only 1 out of 5-9 major peaks was observed, followed by the octachlorbornanes (3 out of 7 majors peaks). Smaller changes were observed for the heptachlorobornanes. For the hexachlorobornanes the pattern for the "coastal" sample was more complex than either the "open-sea" sediment and the technical toxaphene. These findings are consistent with a more extensive degradation of the higher chlorinated congeners, resulting in the formation of the lower chlorinated ones, as reported by Fingerling *et al.* (4) for the anaerobic soil degradation. The changes observed in the hexachlorobornanes may well be caused by reductive dechlorination of the higher chlorinated congeners, in particular those containing *gem*-Cl_2 substitution. A relatively higher proportion of hexa- over heptachloro congeners also was reported by Howdeshell and Hites (9) in sediments from Lake Ontario.

Enantioselective Determinations of Chlorobornanes. Chlorobornanes are among the halogenated compounds that are chiral. A number of chlorobornanes in technical toxaphene and in biota have in fact been enantiomerically resolved (1, 10). In Figure 1 we show SIM chromatograms from enantioselective analyses of the hexa- and heptachlorobornanes in the technical toxaphene and in the "open-sea" sediment. While Hx-Sed is only marginally resolved, as indicated by an increased peak width, the two major heptachloro congeners TC1 (Hp-Sed) and TC2 are clearly resolved. The data suggest that all three components are chiral, which for the Hx-Sed and Hp-Sed is in agreement with their structural assignments (2, 4); the exact structure of TC2 is unknown. The chromatograms indicate enantiomeric ratios for the heptachloro congeners in the sediment somewhat differing from an exact 1:1 ratio.

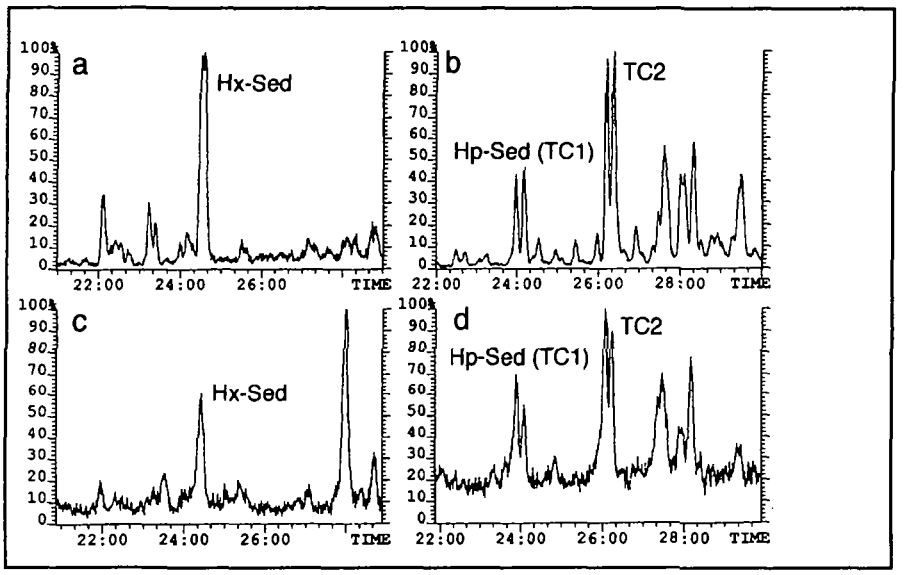

Figure 1. ECNI SIM chromatograms from enantioselective analyses showing elution of hexachlorobornanes (m/z 309, left-side panels a and c) and heptachlorobornanes (m(z 345, right-side panels b and d in technical toxaphene (top panels a and b) and in the "open-sea" sediment sample (bottom panels c and d).

Sources of Chlorobornanes. The major chlorobornanes (TC1, TC2, TC6, TC7, TOX9 and others) detected in the "open-sea" and the "coastal" sediment sample have been detected in various biota from widely distant locations (Arctica, Antarctica, and others)(1). They are all major constituents in technical toxaphene, and some are present in biota in similar relative proportions as in the technical mixture. It is, therefore, probable that aerial deposition of the technical mixture followed by degradation in the sediment is the source for a majority of the chlorobornanes found in these samples. However, there appear to be additional chlorobornanes, in particular hexachloro congeners, which are specific to the "coastal" sample. The trend observed between the "coastal" and the "open-sea" sediment samples appears to be different from that observed for the above mentioned pulp mill analytes. It remains to be investigated whether these hexachlorobornanes are the

result of a selective secondary degradation or a primary formation by another source.

Conclusions

The analysis of environmental samples such as the sediments in this study for chlorobornanes and similar compounds is complex due to the large number of halogenated compounds present in these samples. There is the possibility of misidentification. In order to minimize this possibility we suggest the following:

1) an elaborated, sophisticated multi-step selective clean-up procedure, in order to remove as many of the interfering compounds as possible;
2) HRGC separation in order to resolve as many of the chlorobornane congeners as possible;
3) use of various MS detection techniques for the specific, selective and sensitive detection of these compounds, including MS/MS and high-resolution MS for the selective detection of specific isomers, particularily those present in environmental samples;
4) careful inspection of SIM chromatograms for isotopic ratios as defined by the chlorine clusters of the chlorobornanes;
5) recording of full-scan mass spectra for confirmation of the target compounds; and
6) enantioselective determination of chiral chlorobornanes to confirm resolution into enantiomers.

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