A Non-Destructive Cleanup Procedure for Chlorobornanes in Water and Sediment

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Introduction

Chlorobornanes are a group of bicyclic chlorinated aromatic hydrocarbons which theoretically consists of more than 32000 individual compounds (1). Analysis of these compounds presents a challenging task due to the large differences in polarity, volatility, and thermal stability that exist among the different congeners. Surprisingly, rather simple cleanup procedures, such as treatment with strong acids or adsorption chromatography, are frequently used prior to instrumental analyses. Such measures will result in decomposition of some of the chlorobornanes, or will not efficiently remove interfering anthropogenic or biogenic substances. In the present study we report on an efficient non-destructive analytical procedure for chlorobornanes in complex matrices, primarily industrial effluent and river sediment.

Materials and methods

Chemicals

All solvents were of distilled-in-glass grade. Anhydrous sodium sulfate, copper granules, Silica gel 60 and Florisil were obtained from Merck (Darmstadt, Germany). Hercules technical toxaphene (X16189-49, Lot No. 8BC25, 68.6% Cl) was provided for the study by the Hercules Corporation (Wilmington, DE, USA), and $^{13}C_{12}$-PCB #180 and $^{13}C_6$-γ-HCH were obtained from Cambridge Isotope Laboratories (Woburn, MA, USA).

Extraction and Cleanup

Prior to extraction all samples were fortified with 100 ng of $^{13}C_6$-γ-HCH. The water samples (ca. 1 L) were filtered through glass fiber filters and the water phase was then extracted with 3 × 100 mL dichloromethane. Sediment samples (ca. 10 g) and the filters from the water samples were extracted in a 50 mL Soxhlet extractor, equipped with a Dean-Stark water trap, using 150 mL toluene. The solvent volume was reduced to 1 mL and subjected to adsorption chromatography on a silica gel column (10 g in a 16 mm i.d. column, topped with 15 mm of copper granules). The column was eluted with three times the bed height with 10% diethyl ether in *n*-hexane, and

5 mL iso-octane was added and the sample volume was reduced to 500 µL. The complete sample was quantitatively injected using a Hitachi AS-4000 autoinjector onto two serially connected 8 × 300 mm high-performance gel permeation chromatography columns (5 µm, 50Å, polystyrene-divinylbenzene copolymer, Polymer Laboratories, Church Stetton, UK). Dichloromethane/ *n*-hexane (35/65) was delivered by a LKB 2150 high-resolution liquid chromatography (HPLC) pump at a flow rate of 1.0 mL/min and the effluent was monitored by an UV detector set at 230 nm. Toluene was used to calibrate the system. The chlorobornane fraction started 0.5 min prior to the onset of the toluene peak, and ended 14.5 min later. In the present study three fractions were collected: 0 to 19, 19 to 34, and 34 to 49 minutes, respectively. The second fraction was gently evaporated to less than 100 µL and was fractionated, using the same system as above, on a chemically activated (2) 4.6 × 250 mm HPLC silica column (Nucleosil NC100, 5 µm particle size, Macherey-Nagel, Düren, Germany). Gradient elution was employed as follows: 0 to 12 min 100% *n*-hexane, 12 to 13 min linear gradient to 5% dichloromethane in *n*-hexane, and 13 to 54 min 5% dichloromethane in *n*-hexane. The flow rate was held at 0.7 mL/min until 13 min, then it was increased to 1.5 mL/min, and the flow direction was reversed one minute after γ-HCH (retention time ca. 30 min) eluted. The system was equilibrated for 6 min between the runs, which resulted in a total analysis time of 1h. In this study, the chlorobornanes were recovered by collecting the effluent between 10.5 and 31 min. Finally, 1 mL of toluene and 10 ng of $^{13}C_{12}$-PCB #180 (recovery standard) was added, and the solvent volume was reduced to a volume appropriate for gas chromatography- mass spectrometry (GC-MS) analysis, *i.e.*, 20- 200 µL.

GC/MS analysis

The chlorobornane fractions were analyzed using a VG Tribrid GC-MS system operated in the electron-capture, negative ion chemical ionization (ECNI) mode with argon as the buffer gas. The ion source temperature was 140 °C. One microliter aliquots of the samples were on-column injected into a 25 m × 0.25 mm SE-54 column via a 1 m × 0.32 mm retention-gap, and the GC oven was temperature programmed as follows: 80 °C for 2 min, then at 20 °C/min to 140 °C, and then at 5 °C/ min to 280 °C, followed by an isothermal hold. The data acquisition started 5 minutes after injection. The two most abundant ions of the M-Cl isotopic distribution cluster were monitored for the penta- through decachlorobornanes and the $^{13}C_6$-γ-HCH internal standard. For the $^{13}C_{12}$-PCB #180 recovery standard the molecular anions at m/z 404 and 406 were monitored. The quantifications were performed using the internal standard technique, and the data reported were thus corrected for $^{13}C_6$-γ-HCH recovery.

Results and Discussion

Preliminary studies

A critical step in the cleanup of chlorobornane samples is the separation of chlorobornanes from other environmental contaminants that might interfere during the instrumental analysis. Especially highly chlorinated PCBs can cause problems during GC- election capture detection or GC-ECNI-MS analyses. Therefore we tested and evaluated three different approaches to separate PCBs and chlorobornanes: (1) Chromatography on Florisil deactivated with 1.2% water; (2) Chromatography on silica gel; and (3) HPLC on Nucleosil NC100. All of these have been claimed to separate the two groups of compounds (3,4,5), although the first two have recently been reported to leak about 15- 25% of the chlorobornanes to the PCB fraction when

using *n*-hexane as the eluent (6). Our preliminary studies support that finding, as 20- 25% of the chlorobornanes in the technical toxaphene were detected in the PCB fraction after Florisil fractionation according to Barrie, *et al.* (3). Our attention was, therefore, focused on the HPLC silica procedure, which due to its higher resolution was supposed to give better separation of PCBs and chlorobornanes. However, this procedure failed to separate these two classes of compounds. The chemically activated Nucleosil NC100 column was tested with several solvents -- *n*-pentane, *n*-hexane, and 1% dichloromethane in *n*-hexane (stored over 3A molecular sieves) -- without any significantly improved separation. Attempts to improve the separation by changing the flow rate or the column temperature did not significantly improve separation. However, the HPLC procedure was still preferred over the low-resolution liquid chromatographic systems because it is easy to calibrate, control, and automate.

Evaluation of the analytical procedure using reference standards

Hercules toxaphene and the PCB formulation Clophen A50 were processed through the whole cleanup procedure, and the PCB and chlorobornane fractions from the HPLC silica were analyzed using full-scan electron impact MS. All of the chlorobornanes were detected in the correct fraction, see Figure 1. As previously noted, the PCBs were not completely removed and a number of tri- through hexaPCBs were detected in the chlorobornane fraction, see Figure 2. However, the majority of the hexa-, and all of the hepta- and octaPCBs were removed, including most of the congeners that interfere with the chlorobornane determinations. PCB #128 was the notable exception since it elutes in the second silica fraction and coelutes with Toxicant Ac on SE-54 type of columns (7). That might cause a problem in GC-ECD analyses, and in GC-ECNI/MS analyses if oxygen is present in the ion source. If that is the case, another GC column should be used.

Applications

We used the described analytical procedure for some fifty river sediment and industrial effluent samples. Figure 3 shows a composite SIM chromatogram from the analysis of a sediment from the Wisconsin River and the corresponding chromatogram from the analysis of an aged toxaphene fortified sewage sludge. Three peaks show up in the sediment chromatogram, of which the first two are the highly persistent Hx-Sed (2-*exo*, 3-*endo*, 5-*exo*, 6-*endo*, 8, 9, 10-heptachlorobornane) and Hp-Sed (2-*exo*, 3-*endo*, 6-*exo*, 8, 9, 10-hexachlorobornane), respectively, recently characterized by Stern, *et al.* (8), while the third corresponds to an unknown chlorinated interference. This is a pattern typical for sediments that primarily receive chlorobornanes via air deposition (9). The concentrations of the Hx-Sed and Hp-Sed were 0.15 and 0.10 ng/g dry weight, respectively.

The detection limits were 0.2 to 1.4 ng/L and 0.02 to 0.1 ng/g dry matter for the effluents and sediment samples, respectively, and the recovery of the internal standard ($^{13}C_6$-γ-HCH) generally ranged between 45 and 90%. However, the $^{13}C_6$-γ-HCH have somewhat higher vapor pressure than the target analytes and, thus, any evaporation losses during cleanup would result in slightly overestimated chlorobornane levels. One or several ^{13}C-labeled chlorobornanes would have been a better choice, but no such compounds are currently available. Attempts to synthesize such surrogate standards is strongly encouraged.

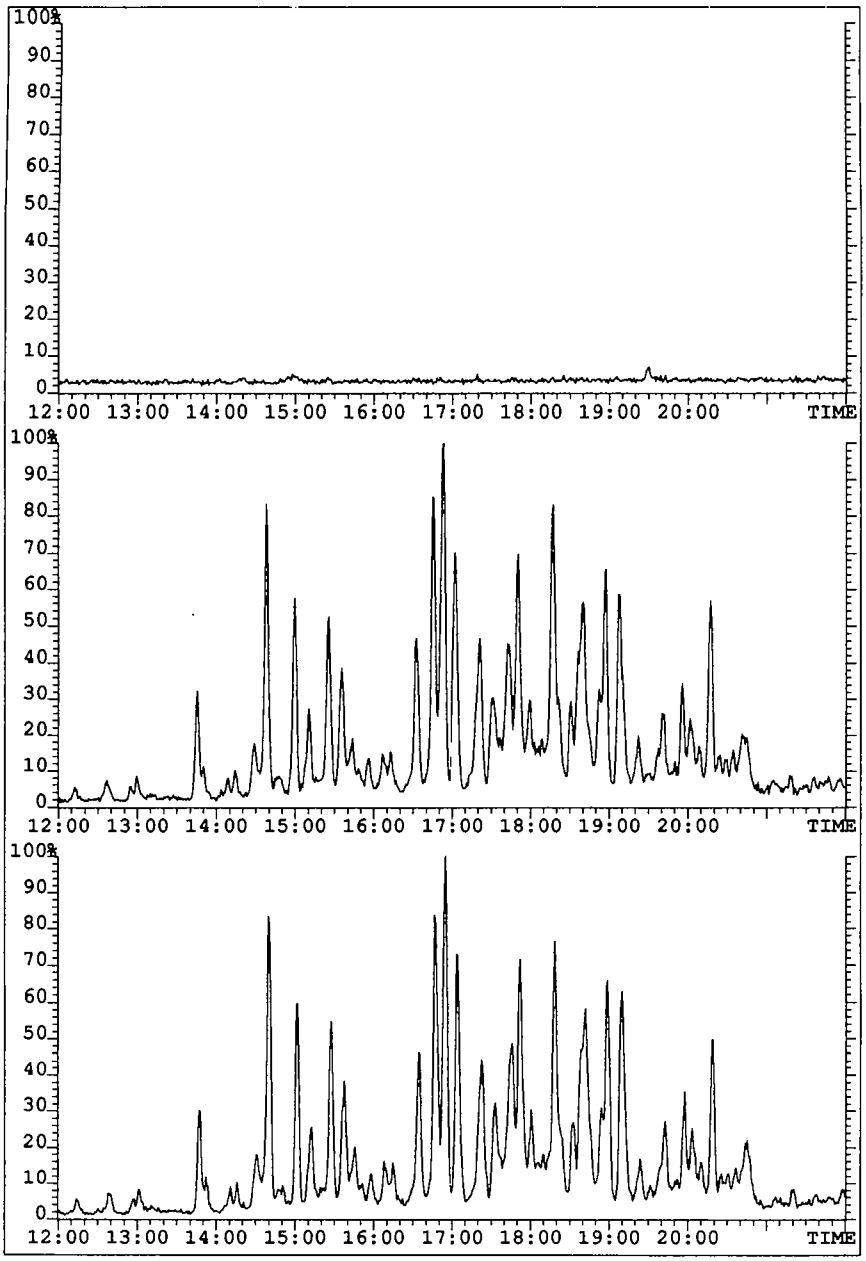

Figure 1: Composite ECNI SIM-chromatograms m/z 307, 343, 377, 413, and 445 from the analysis of chlorobornanes in a river sediment sample from Wisconsin River (top); the same sample fortified with 900 ppt of Hercules toxaphene (middle); and, technical Hercules toxaphene (bottom).

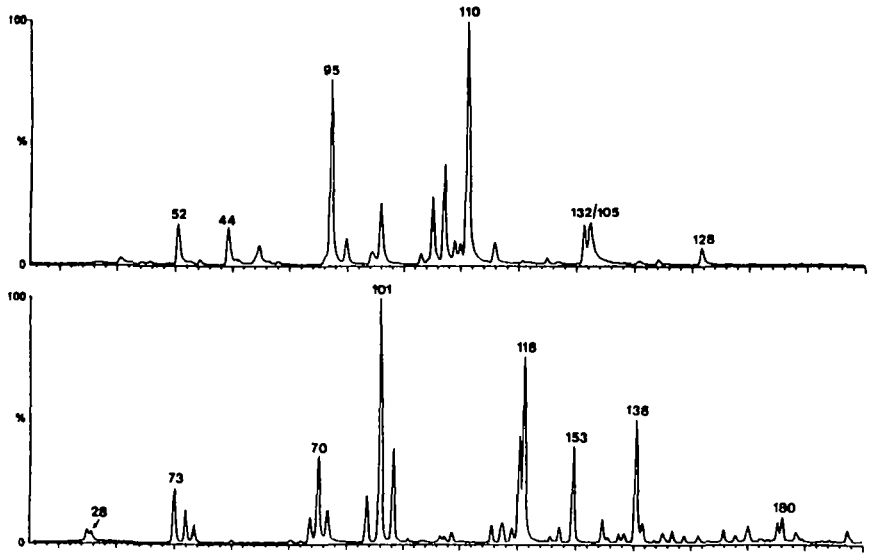

Figure 2: Composite electron impact (70 eV) SIM-chromatograms m/z 256, 292, 326, 360, 394, and 430 from the analysis of PCBs, from Clophen A50, in the first (lower) and second (upper) HPLC silica fractions.

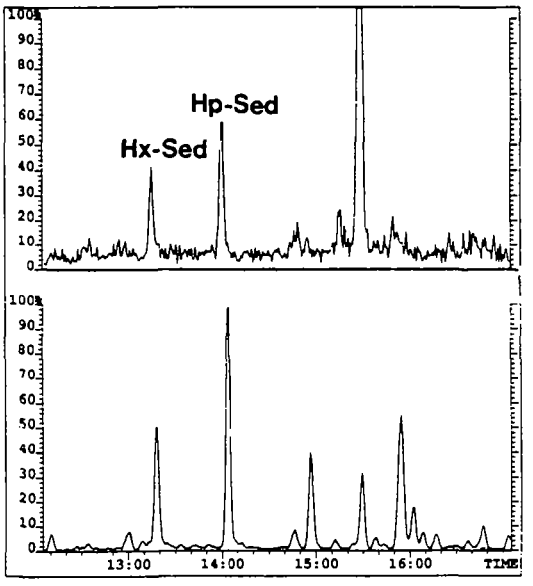

Figure 3: Composite ECNI SIM-chromatograms m/z 307, 343, 377, 413, and 445 from the chlorobornane analysis of a river sediment sample from Wisconsin River (upper) and an aged toxaphene fortified sewage sludge sample (lower).

Acknowledgments

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