Gas Chromatographic Method for the Analysis of Chlorinated Paraffins in Biological Samples.

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Introduction

Chlorinated paraffins (CP) are complex chemical products consisting of mainly poly-chlorinated n-alkanes. Depending on chain length and chlorination degree these products are often divided into six groups: short (C_{10-13}), medium (C_{14-17}) or long (C_{18-30}) chain length with high (>50%) or low (<50%) chlorination degree (1).

The number of theoretically possible congeners within these limits is huge, which explains the complexity of the products. CP is used as a flame retardant and plasticiser in polymers and textiles and as a lubricant in metal working fluids. The world-wide annual production 1993 was estimated to be 300 000 tonnes (2), but over the period 1993 to 1996 the use in Sweden has decreased from about 1400 to 750 tonnes (3) due to voluntary agreements.

The complex nature of CP products makes it difficult to analyse these compounds, especially in biological matrices. Thin layer chromatography has been used with an argentation method for the detection (4) and electron capture mass spectrometry with the sample fraction directly introduced into the ion source (5). A photochemical technique with high intensity ultraviolet light has been suggested to eliminate chlorinated aromatic interferences in the analysis (6). Most methods used today utilise gas chromatography for the final separation. Since the CP congeners completely cover a wide retention window all compounds with overlapping retention will interfere in the determination. Very specific clean-up or detection techniques has to be used to avoid this problem. Low resolution mass spectrometers have been used (7, 8, 9, 10 and 11) and a high resolution technique has also been applied (12). The available data base on environmental levels of CP is very limited and the aim of the present investigation was consequently to develop a method suitable for monitoring of CP in biological samples.
A combination of photolysis, HPLC and GC-ECD was chosen in this study.

Material and Methods

Chemicals

The solvents n-hexane (LiChrosolve), dichloromethane, acetone and 2,2,4-trimethylpentane were all, except n-hexane, of p.a. grade and purchased from Merck. Sulphuric acid (95-97% w/w), 1-iodododecane and 1-bromododecane (both >97-98%, for synthesis) were also purchased from Merck. Technical mixtures of chlorinated paraffins are presented in the table below.

Product	Producer	Chain length	Chlorine content (%)
Cereclor 50LV	ICI	C_{10-13}	49
Cereclor 63L	ICI	C_{10-13}	63
Cereclor S45	ICI	C_{14-17}	45
Cereclor 42	ICI	C_{22-26}	42
Chloroparaffin 60C	Hüls	C_{10-13}	60
Chloroparaffin 70C	Hüls	C_{10-13}	70
Chloroparaffin 40G	Hüls	C_{14-17}	40

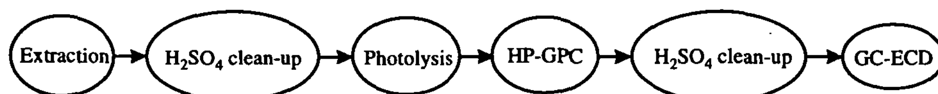
Instruments

The high performance liquid chromatography (HPLC) system was composed of a Varian 9001 pump (with SSI LP-21 lo pulse), a Rheodyne 7125 injector with a 200 µl loop, a Jones Chromatography 7955 column chiller and a Merck-Hitachi L-5200 fraction collector. The system was equipped with four serially coupled HPLC gel permeation columns (PL-gel, 5 µm, 50 Å, 300 mm × 7.5 mm from Polymer Laboratories).

Gas Chromatography (GC) was performed on a Fisons Instruments GC 8160 equipped with an electron capture detector, ECD 800, an AS 800 autosampler and a split/splitless injector operated in splitless mode (injection volume 1 µl). The column used was a fused silica capillary column (DB1, 10 m × 0.18 mm ID, 0.18 µm film thickness from J&W Scientific Inc.) and the temperature program was: 90°C; 12°/min to 220°C; 8°/min to 245°C; 4°/min to 290°C (5 min isothermal). Helium was used as carrier gas (flow rate 0.7 ml/min) and argon/methane (10%) as make-up gas. The injector and detector temperatures were set to 250° C and 320°C, respectively.

Homogenisation of tissues was performed with an Ultra-turrax Ika homogeniser and the photolysis was performed with a high-energy mercury lamp.

Analytical procedure

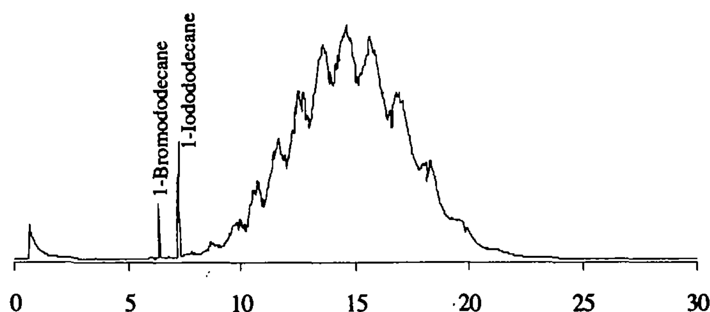


Extraction and removal of fat by treatment with sulphuric acid was performed as described by Jansson *et al.* (9) except that no internal standards were added until after photolysis.

The samples, in approximately 4 ml n-hexane, were transferred into a quartz test tube and irradiated for 15 minutes at a distance of 30 mm from the lamp. The sample volumes were then reduced to 200 μl under a gentle stream of nitrogen and internal standards 1-iodododecane and 1-bromododecane were added.

The samples were further cleaned-up by HP-GPC on four PL-gel columns coupled in series and placed in a column chiller set at 20°C. Dichloromethane/ hexane (1:1) was used as mobile phase at a flow rate of 0.75 ml/min. Each sample was injected onto the column and a CP-fraction including the internal standards were collected between 38-46 min. The sample volumes were reduced to 3 ml and further treated with sulphuric acid before the analysis by GC-ECD.

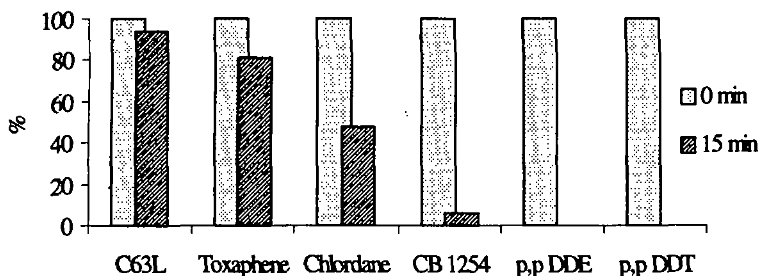
Results and Discussion



The figure above shows a chromatogram of Cereclor 63L, 1-iodododecane and 1-bromododecane detected with GC-ECD. The short capillary column enables to use relatively low temperatures to avoid thermal degradation of CP and reduces the analysis times. The temperatures used in the injector was found not to change the CP composition.

Photolysis of CP for 15 minutes gives a recovery of 94%. The effect on some other organohalogens that may interfere was also investigated and the results can be seen below. 1-Iodododecane and 1-bromododecane are also partly destroyed. Therefore the internal standards are not added until after photolysis.

Separation of chlordane and toxaphene from CP is then further performed with HP-GPC. At present four columns have to be used to isolate CP from remaining chlordane, but the performance of these columns differs and sometimes two columns in series have been enough.



This method will now be applied to determine CP in moose samples from Norway, Finland and two areas in Sweden, Dalarna and Västergötland. Dalarna is used as a reference area to Västergötland, where moose is threatened by a serious disease.

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