Quantification of C_{10}-Chloroparaffines with Purely Synthesized Chloroalkanes as Standards

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Introduction

Chloroparaffines (CPs) are complex mixtures of n-alkane homologues with carbon chain lengths between C_{10} and C_{36} and a chlorination degree between 10 and 72% w/w. These mixtures can be differentiated according to chain length into short chain (C_{10}-C_{13}), medium chain (C_{14}-C_{17}), and long chain (C_n>17) CPs [1]. Because of their convenient physical properties, especially their viscosity, flame resistance, and low vapour pressure, they are used as additives in flame retardants, paints, lubricants, and cutting fluids [2]. Their wide pattern of use and their dispersion tendency has led to the presence of chloroparaffines in very different environmental systems, such as waters in industrial areas, terrestrial organisms, or food [3]. Toxicological investigations revealed that especially short chain chloroparaffines are very toxic to aquatic organisms and are bioaccumulated in different species [4]. Furthermore, the short chain fraction has also been shown to be carcinogenic in mice and rats [5]. Therefore, determination of chloroparaffine residues in the environment has frequently been attempted. It has been very difficult to separate the congeners even with high capacity chromatography columns because of the complexity of these mixtures consisting of numerous homologues, diastereoisomers, and enantiomers. Although many procedures for separation and quantification have been described [6-11], it has not been possible till now correctly to quantify CPs without pure chloroparaffine standards. Recently, single C_{10}-chloroparaffines have been synthesized for the first time [12]. Basing on these standards, a routine analytical procedure is in development.

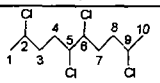
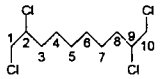
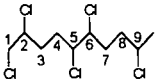
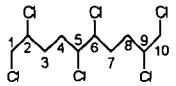
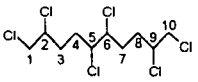
Experimental

Materials:

Single C_{10}-CPs (Table 1) were synthesized and are now available from Fa. Ehrenstorfer, Germany; the method will be published elsewhere [12]. A technical mixture of C_{10}-chloroparaffines was prepared by UV-chlorination of n-decane up to a chlorine content of 49% [12]. Pesticide standards were obtained from Fa. Ehrenstorfer, Germany. Organic solvents (n-hexane, cyclohexane, ethyl acetate, and toluene) were of purity grade for residue analysis. Na_2SO_4 was from Merck, Germany. Biobeads SX3 was from BioRad, Germany.

Extraction and fractionation of a fish oil sample:
Cod liver oil was obtained from Iceland (1996). The sample was kept under −12°C until use. 5g of it was dissolved in 25ml cyclohexane/ethylacetate (1:1). Chloroparaffines were separated from fat by gel permeation chromatography (GPC) [13] (column length 40cm, diameter 2.5cm) with Bio-Beads SX3 as the packing material and cyclohexane/ethyl-acetate (1:1) as eluting solvent. 1g cod liver oil or fish oil, equal to 5ml dissolved sample, was given on the GPC-column. Chloroparaffines were recovered in 115ml (fraction 125-240ml) of the subsequent eluate. The resulting elution speed was ca. 5ml/min. The clean-up with GPC reaches recoveries of 86%.
For the elimination of interfering substances and the rest of the oil (about 5%) a simple mini column chromatography has been executed. For column chromatography, columns were prepared with 1g silica gel 60 (70-230mesh, activated at 140°C for 24h and then desactivated with 1.5% water) and ca.0.5cm anhydrous Na_2SO_4. The chloroparaffine fraction was eluted with 8ml of n-hexane/toluene (65:35) and after that with 8ml of toluene. These two fractions were combined, reduced to 1ml and stored at -12°C. Prior to HRGC-MS-ECNI-SIM analysis, the extracts were reduced to 100µl under a gentle N_2 stream.

Tab.1. Newly synthesized C_{10}-chloroparaffines and their HRGC-MS/ECNI fullscan chromatogram of the chloroparaffin C_{10}-congener mix (GC: HP5890 II, column DB-5 30m × 0.25mm i. d., 0.25µm film thickness, carrier gas He, flow 1.15ml/min, split 1:10, injector 240°C, transfer line 250°C, temperature program 90°C/2min – 10°/min – 160°C/1min – 5°/min – 280°C/10min; MS: Finnigan 8200, reactant gas methane, ion source pressure 2.5×10^{4}, ionization energy 150eV)

No.	Formula	Name	Structure
CP-1	$C_{10}H_{18}Cl_4$	2,5,6,9-Tetrachlorodecane (mixture of 3 diastereoisomers)	
CP-2	$C_{10}H_{18}Cl_4$	1,2,9,10-Tetrachlorodecane (1 diastereoisomer)	
CP-3	$C_{10}H_{17}Cl_5$	1,2,5,6,9-Pentachlorodecane (mixture of 2 diastereoisomers)	
CP-4	$C_{10}H_{16}Cl_6$	1,2,5,6,9,10-Hexachlorodecane (1 diastereoisomer)	
CP-5	$C_{10}H_{16}Cl_6$	1,2,5,6,9,10-Hexachlorodecane (mixture of 2 diastereoisomers)	

HRGC-MS analysis:
Chloroparaffine standards and samples were analyzed on a Hewlett-Packard 5890 Series II gas chromatograph/Finnigan 8200 mass spectrometer. The operating conditions were as follows: column DB-5 30m × 0.25mm i. d., 0.25µm film thickness, carrier gas He,

flow 1.15ml/min, split 1:10, injector 240°C, transfer line 250°C, temperature program 90°C/2min – 10°/min – 160°C/1min – 5°/min – 280°C/5min; reactant gas methane, ion source pressure 2.5×10^{-4}, ion source temperature: 180°C, ionization energy 150eV.

HRGC-ECD analysis:
Standards and samples were analyzed on a Dani 86.10 system. The operating conditions were as follows: column: DB-5 (30m × 0.25mm, film thickness 0.25µm; carrier gas: N_2; injector temperature: 260°C; detector temperature: 300°C; column temperature program: initial temperature 90°C/2min – 10°C/min – 160°C/1min – 5°C/min – 280°C/5min).

Results and Discussion

HRGC-ECD-quantification
Generally, the concentration of single C_{10}-CPs measured with ECD are similar to those obtained with ECNI-SIM-MS. This indicates that with this method interferences can nearly be excluded. However, the different CP-classes could not be completely separated because highly chlorinated short chain CPs interfere with long chain CPs of low chlorination degree. Furthermore, overlapping with other chlorinated hydrocarbons during the determination of C_{10}-CPs residues in environmental samples is possible. Neither gel permeation nor mini silica gel column chromatography is sufficient totally to eliminate cyclodienes and toxaphenes because of their behavior similar to the C_{10}-chloroalkanes under these conditions. Therefore, residue data obtained only with HRGC-ECD should be considered with caution.

HRGC-MS/ECNI-SIM-quantification
The ions 242 and 243 (tetrachlorodecanes), 278 and 279 (pentachlorodecanes), and 312 and 313 (hexachlorodecanes) have been selected for quantification while the masses 244, 245, 276, 277, 314, and 315 were additionally registered for identification. For low level quantification only the masses 243 (M–Cl) for $C_{10}H_{18}Cl_4$, 279 (M–Cl) for $C_{10}H_{17}Cl_5$, and 313 (M–Cl) for $C_{10}H_{16}Cl_6$ were used. When monitoring selected fragments, many chlorinated contaminants cannot interfere. There are only some substance groups which may lead to problems because they form ions with similar masses to C_{10}-CP fragments. They include chlorostyrenes, PCBs, the DDT group, chlorocamphenes, and the cyclodiene insecticides, such as dieldrin, cis- and trans-chlordane, heptachlor, and their photoproducts. However, these compounds give rather small molecular ion clusters and almost no $(M–Cl)^-$ ion clusters during ECNI measurements. The resulting ions differ sufficiently from those produced from chloroparaffin standards to avoid interferences or mistakes.

HRGC-MS/ECNI-SIM-quantification in technical products
A technical mixture of C_{10}-chloroparaffines with a chlorination degree of 49% w/w has been used as a model mixture for quantification with single substances in technical products (Table 1). The amounts obtained of tetrachlorodecane, (quantified with CP-2), of pentachlorodecanes (quantified with CP-3), and of hexachlorodecane (quantified with CP-4) measured with HRGC-MS/ECNI full scan are not comparable to those obtained with HRGC-MS/ECNI-SIM. The results are slightly higher than the theoretical amount

with the last method. In this case, the low determination level of the ECNI-SIM method evidently gives false results. Further study of quantification of C_{10}-chloroparaffines with different methods with regard to their sensitivity and reliability is needed.

HRGC-MS/ECNI-SIM-quantification in cod liver oil samples

Possible peaks of tetra-, penta-, and tetrachlorodecanes could be identified with the help of retention time windows and selection of typical fragments (243, 245, 277, 279, 313, and 315). Assuming that all peaks registered actually belong to C_{10}-CPs, a residue level of 4.06mg/kg has been determined. As this value appears to be rather high, interferences cannot be excluded. This was confirmed by the determination in the fullscan mode where the peaks at 20.05min, 21.11min, 21.30min, and 22.22min could be assigned to chlorostyrenes, whereas the peak at 23.18 was caused by dieldrin. A possible way to reduce these interferences may be the irradiation of the samples.

Literature

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Tab. 1. Results of the HRGC-MS/ECNI-SIM quantification of the technical C_{10}-CP mixture with 49% Cl (533.4 ng/µl) and of the fish sample extract (12.00-18.00min with m/z 243 and 279; 18.00-25.00min with m/z 279 and 313) with the single substances

	Quantification masses M-Cl	Technical C_{10}-CP mixture		Fish oil sample extract		
		Peak area $\times 10^5$	ng/µl	Peak area $\times 10^5$	ng/µl	mg/kg
ΣCP-1 + CP-2	243	11.7	253.4	–	–	–
ΣCP-3	279	152.65	214.2	2.598	1.82	0.91
ΣCP-4 + CP-5	313	180.95	90.51	27.796	6.95	3.48
Total			558.11		8.11	4.06