Characterization and application of a synthetic 4,5-dichloro-chlordene as internal standard for the quantification of toxaphene and chlordane congeners in fish from the Arctic

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1. Introduction

The insecticides toxaphene and chlordane consist of a large number of polychlorinated bornanes or cyclodienes, respectively, which bioaccumulate strongly in the marine food chain. An isomer and/or enantioselective quantitative analysis of these compounds require reference compounds which can be added to the samples before clean-up and which can be used as internal standards for quantification by e.g. mass spectrometry. Usually, both polychlorinated bornanes and cyclodienes are collected in the same fraction during sample clean-up. The ideal solution would have been to synthesize and add the corresponding ^{13}C-isotope-labeled compounds of the congeners to be quantified. However, due to rearrangement reactions which are difficult to control, a synthesis is normally not stereo selective enough, and the obtained isomer mixtures have to be separated and purified further. This makes the production of ^{13}C-labeled toxaphenes and chlordanes rather costly. At present no ^{13}C-labeled toxaphenes and only one mono ^{13}C-labeled cis-chlordane are available. The latter cannot be used for quantification by mass spectrometry due to overlap of the chlorine isotope signals of the labeled and unlabelled compound. Unlabelled substances suitable as internal standards should have a chlordane related structure, a similar chemical and physical behavior and should fulfill the following requirements:

- Not present in the technical chlordane or toxaphene mixture
- No retention time overlap with other congeners present in real samples using high resolution gas chromatography on standard stationary phases and mass spectrometric detection.
- Same polarity and behavior as chlordanes and toxaphenes during sample clean-up and, consequently, collection in the same fraction.
- Resistant against sulfuric acid used during sample clean-up
- About the same response factor and retention time as major chlordane and toxaphene congeners present in biota.
- Easy to synthesize with very high purity.

The chlordane-related compound 4,5-dichloro-chlordene was synthesized. It has all the properties mentioned, and its use as an internal standard for both chlordane and toxaphene quantification will be discussed.

2. Experimental

Dichloro-chlordene synthesis: 9 mg of trans-nonachlor (3-exo,4-endo,5-exo,1,7,8,9,10,10-nonachlorotricyclo[5,2,1,$0^{2,6}$]dec-8-ene) was added under N_2 to a solution of sodium methoxide (6 mg) in methanol and stirred for 24 h. Water was then added, and the mixture was extracted with chloroform. The organic phase was washed with water before drying over magnesium sulfate. High resolution gas chromatography (HRGC) on a fused silica capillary of 25 m x 0,25 mm i.d. coated with 0,25 µm DB-5 showed about 5% of remaining trans-nonachlor and only one more signal at a shorter retention time (relative to trans-nonachlor, 0,847).

Structure elucidation: The electron ionization mass spectrum showed a molecular ion of m/z 404 and the chlorine cluster of an octachloro compound with a fragmentation pattern similar to that of cyclodiene structures. Proton NMR spectroscopy (400 Mhz) of the crude product revealed four signals of the reaction product. The signal at δ 5,84 shows the presence of a vinylic hydrogen indicating that the major product is formed by HCl elimination of trans-nonachlor (see Figure 1). Double quantum filtrated COSY informed that the vinylic hydrogen H_3 coupled moderately strong to H_2 and weakly to H_5. This coupling pattern suggests the structure 1,4,5-exo,7,8,9,10,10-octachlorotricyclo[5,2,1,$0^{2,6}$]dec-3,8-diene where the vinylic hydrogen H_3 will give a strong vicinal coupling to H_2 and a much smaller allylic coupling to H_5. The alternative isomer 1,3,5-exo,7,8,9,10,10-octachlorotricyclo[5,2,1,$0^{2,6}$]dec-3,8-diene is expected to give a strong coupling to H_5 and a weak coupling to H_2. The assignment of the elimination product as 1,4,5-exo,7,8,9,10,10-octachlorotricyclo[5,2,1,$0^{2,6}$]dec-3,8-diene or 4,5-dichloro-chlordene (4,5-DCCD) was further confirmed by two-dimensional NOE spectroscopy. A large NOE was found between H_3 and H_2.

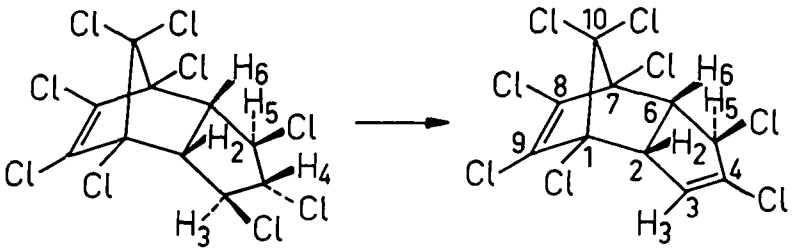

Figure 1: Synthesis of 4,5-dichloro-chlordene from trans-nonachlor. The carbon-atom numbering is according to the polycyclo nomenclature.

Further clean-up by neutral deactivated alumina: The 4,5-DCCD was purified further and the not reacted trans-nonachlor removed by liquid chromatography on a 30 cm x 1,5 cm i.d. column filled with 30 g neutral alumina (0,063-0,2 mm particle size, E. Merck) deactivated with 5 % water (w/w). 4,5-DCCD eluted in the first fraction of 100 ml n-hexane. The obtained solution was controlled by HRGC combined with negative ion chemical ionization (NICI) mass

spectrometry and found free for traces of any polychlorinated compound. The quantity in solution was determined by flame ionization detection assuming the same response factor as for trans-chlordane which has the same carbon skeleton and number of chlorine atoms as well as the same 6 ring structure.

Clean-up of real samples: About 1 g of cod liver or polar cod liver from the Barents Sea was homogenized with a 4-8 times larger amount of sodium sulfate. The homogenate was filled into a 40 cm x 3 cm i.d. glass column, and the internal standard 4,5-DCCD was added. Afterwards, the lipids were extracted by a slow flow of 100 ml cyclohexane (ca. 0,5 ml/min). Further clean-up of the extract was carried out by gelpermeation chromatography (GPC) on a column of 60 cm x 2,5 cm i.d. filled with 50 g Biobeads SX-3 system using cyclohexane/ethylacetate (1+1) as mobile phase. The eluate was concentrated to 500 µl and fractionated further on the deactivated alumina column described before using the following solvents: 0-50 ml, 100% n-hexane; 50-100 ml, n-hexane/methyl-t-butyl ether(MTBE) 50+50; 100-150 ml, n-hexane/MTBE 25+75. Toxaphenes and chlordanes were collected without further separation in a single fraction ranging from 8-130 ml which was reduced to 250 µl. 1,2,3,4-tetrachloronaphtalene was added as recovery standard. The obtained fraction was analyzed by HRGC combined with negative ion chemical ionization (NICI) mass spectrometry on a HP 5987 or 5989 GC/MS using CH_4 at a pressure of 0,45 torr and an ion source temperature of 200°C. The separation conditions were as follows: 30 x 0,2 mm i.d capillary coated with a 0,11 µm film of HP-5, injector temperature, 225°C, splitless injection of 1 µl at 90°C, 2 min splitless time, 90-150°C at 30°C/min, 150-260°C at 4°C/min, 2 min isothermal, transfer line temperature, 260°C.

3. Results and discussion

Characterization of the synthesized 4,5-dichloro-chlordene: The obtained compound was of very high purity (>99,9%). It was resistant against sulfuric acid and eluted in the same sample fraction as chlordanes and toxaphenes when the clean-up procedure described under experimental was used. The NICI mass spectrum did not show the molecular ion. The highest mass fragment was obtained by a double HCl elimination leading to a further aromatisation of the structure and a hexachloro cluster at m/z 332. The $(M\text{-}2Cl\text{-}HCl)^-$ cluster at m/z 298 was the most abundant fragment was and used together with m/z 332 for compound identification. However, the abundance ratio between both clusters changed significantly with the ionization conditions. On a Hewlett-Packard HP 5989 GC/MS system the pattern at m/z 298 was much more abundant than on a HP 5987. Reasons are probably differences in the ion source construction and temperature control as well as the primary electron energy applied for maximum sensitivity. The structure of 4,5-dichloro-chlordene is chiral, and the formed racemate could be separated by HRGC on a fused silica capillary (20 m x 0,25 mm i.d.) coated with 0,15 µm of 10% dimethyl-t-butyl silylated β-cyclodextrin diluted in PS086 (see also[1]).

Control of possible retention time interferences: *4,5-dichloro-chlordene elutes on the* applied stationary phase HP-5 just in front of all chlordanes and toxaphenes present in real samples at a retention time where no other compound is present with chlorine fragments at m/z 298-304 and 332-340 (see Figure 2).

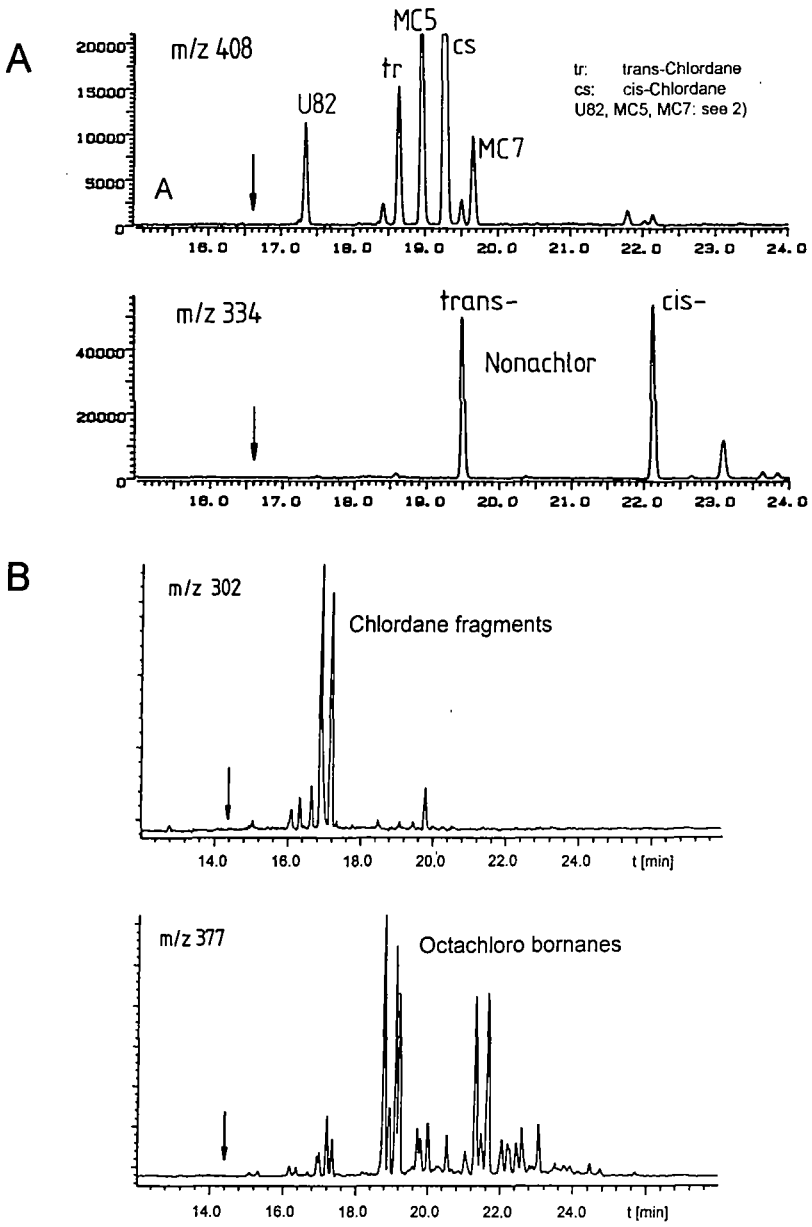

Figure 2: Mass fragmentograms showing the absence of 4,5-DCCD (represented by m/z 334 and 302, respectively, and retention time indicated by arrows) in herring oil (A) quantified for chlordanes (m/z 408) and in cod liver from the Barents Sea (B) quantified for toxaphene. Only the fragmentogram for octachloro bornanes (m/z 377) is shown. Compounds giving additional signals at m/z 302 and 334 are marked

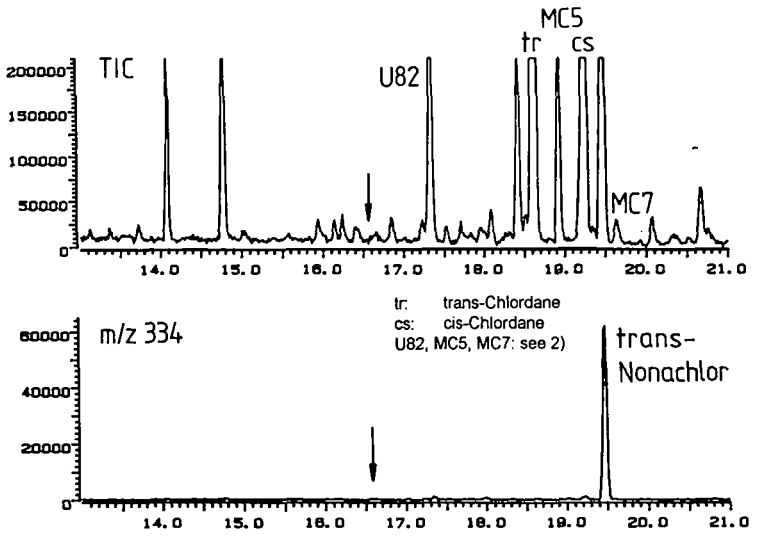

Figure 3: Absence of the synthesized 4,5-DCCD in technical chlordane. The total ion current chromatogram (TIC) and mass fragmentogram (single ion monitoring) for the m/z 334 fragment of 4,5,-DCCD is shown. The retention time of 4,5-DCCD is marked by an arrow.

Presence of 4,5-DCCD in technical chlordane: At the retention time of 4,5-DCCD no signal was present in the fullscan NICI chromatogram of technical chlordane (see Figure 3). Furthermore, no compound with fragment clusters at m/z 298-304 and 332-340 were found in the selected ion mode which confirms that this compound is not present in technical chlordane even in trace amounts. Also Dearth and Hites[2] could not find compounds with a molecular ion m/z 404 in the technical product which confirms that dichloro-chlordenes are not formed during synthesis of chlordane.

Usefulness of 4,5-DCCD for quantification by electron capture detection (ECD): Buchert et al.[3] used alkali treatment of cod liver oil extracts to transfer partly trans-nonachlor to 4,5-dichloro-chlordene. The reaction was used as an additional verification criterion for the presence of chlordane compounds in cod liver oil when electron capture detection was applied. However, the HRGC chromatograms of the untreated sample extracts showed already a minor interfering signal with the same retention time which complicated a confirmation in this way (see Figure 5 in Buchert et al.[3]). To enable detection by ECD, further work has to be done to separate chlordanes and toxaphenes better from the compounds interfering with 4,5-DCCD.

Quantification with 4,5-DCCD: So far, the experiences are promising in using 4,5-DCCD as internal standard for chlordane and toxaphene quantification by NICI mass spectrometry. The response factor and retention time is comparable to those of the major chlordane and toxaphene congeners. However, the chemical and thermal stability is much better than for the higher chlorinated toxaphenes. Table 1 shows the levels of some major toxaphene congener in polar cod and cod liver samples from the Barents Sea which were quantified with 4,5-DCCD as

internal standard. Deviations compared to a quantification with ε-hexachlorocyclohexane were within 15-20% which corresponds to the present precision of the method.

Table 1: Levels of selected toxaphene congeners in polar cod liver and cod liver from the Barents Sea

Sample description	Concentration in ng/g wet weight			
	Tox 26 [a] (297-603)	Tox 32 (195-241)	Tox 50 (297-643)	Tox 62 (099-643)
Cod liver 1	39	<0,15	77	33
Polar cod liver 2	23	<0,3	50	26
Cod liver 3	19	i	37	11
Cod liver 4	27	<1,6	44	17
Cod liver 5	70	<0,9	122	51

[a] Numbering after Burhenne et al.[4]. The structure related numerical code according to Oehme and Kallenborn[5] is given in parenthesis. i: Interference.

4. References

1) Oehme, M., R. Kallenborn, K. Wiberg, and C. Rappe (1994): Simultaneous enantioselective separation of chlordanes, a nonachlor compound, and o,p'-DDT in environmental samples using tandem capillary columns. J. High Resoln Chromatogr. 17, 583-588.
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