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Determination of Enantiomer Ratios of Chlordane Congeners in Cod Tissues by HRGC/NICI-MS. Technical Problems and Influence of Biological Parameters

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Abstract

Enantiomer ratios (ERs) of some chiral chlordane congeners have been determined in liver, gonads and muscle from cod individuals caught in the Barents Sea. ERs were determined by HRGC on heptakis(2,3,6-O-t-butyl-dimethylsilyl)- β -cyclodextrin (TBDMS-CD) which is an ill-defined stationary phase but still the best choice for the enantiomer separation of chlordanes. A test mixture for the enantioselective separation of chiral polychlorinated pesticides has been developed showing substantial differences in the enantioselective properties of different columns based on TBDMS-CD. In cod similar ERs were found in all tissues showing a substantial depletion of one enantiomer for most congeners. The ERs of some congeners such as *trans*-chlordane and MC6 were different between female and male individuals by a factor 3-5 which is opposite to earlier results obtained for herring from the Baltic Sea where no influence of sex was found.

Introduction

Chlordane is a technical pesticide which consists of more than 140 different polychlorinated cyclodienes¹⁾. Recently, it has been reported that chlordane shows a strong synergistic effect on other artificial estrogens and/or endocrine disrupters²⁾. Octa- and nonachloro congeners are most frequently found in marine biota such as fish together with their metabolites such as oxy-chlordane. Some of them are chiral. Enantiomers have usually different toxic and metabolic properties. The racemic enantiomer ratio in the pesticide can be altered by bioaccumulation and degradation in the food chain leading to different enantiomer ratios. Therefore, it is important to determine such compounds not only isomer specific but also enantiomer selective.

High resolution gas chromatography using mixtures of polysiloxanes with modified β -cyclodextrins is the method of choice for an isomer and enantiomer specific analysis. It has been shown that heptakis(2,3,6-O-t-butyl-dimethylsilyl)- β -cyclodextrin (TBDMS-CD) is especially well-suited for a separation of chlordane enantiomers³⁾. However, due to steric interactions this β -cyclodextrin is only randomly derivatised, and a not very well defined mixture is formed⁴⁾. Therefore, the achieved enantiomer separation can vary between different synthesis batches and columns. Consequently, the enantioselective properties of each column should be tested. A recently developed test mixture for the separation of chiral polychlorinated pesticides has been applied to different TBDMS-CD capillaries⁴⁾. Some results are briefly presented and the observed problems discussed.

A recent study found no influence of sex and geographical origin on the enantiomer ratios (ERs) of selected chlordane congeners in individual herring samples from the Baltic Sea⁵⁾. The whole herring was analyzed. In this work the ERs of some octa- and nonachloro congeners have been determined in different tissues from cod individuals caught in the Northern North Atlantic Ocean.

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The aim was to evaluate if the ERs found in muscle, liver and gonads differ significantly due to a different enzyme activity. This information is important for the design of sample collection protocols for future studies of ERs of chiral polychlorinated pesticides in the marine food chain.

Experimental methods

Samples and sample clean up: Cod which was moving from the Barents Sea to the Lofot Islands for spawning, was caught outside Kvaløya, Tromsø (70° N, 17° E). Age, sex, size as well as maturation stage of the gonads were determined. Liver and gonads were weighted and samples were taken including a portion of the filet.

The same sample clean-up was used as described in detail in⁶. It is based on lipid extraction with ethylacetate/cyclohexane 50:50, gel permeation chromatography on Biobeads SX-3 and column chromatography on aluminum oxide. ϵ -hexachlorocyclohexane and ¹³C₁₂-labelled PCB 118 were added as internal standards before extraction. 1,2,3,4-tetrachloro naphthalene was used as recovery standard.

Separation and quantification: Separation of the cod samples was carried out on a tandem capillary consisting of a 30 m x 0,25 mm id capillary in front coated with 0.1 μ m 90% biscyano-propyl/10% phenyl cyanopropyl polysiloxane (RT_x2330, Restek Corp., Bellefonte, PA, USA) which was coupled to an enantioselective capillary of 23 m length and 0.25 mm id coated with 0.14 μ m of heptakis(2,3,6-O-tert.-butyl-dimethylsilyl)- β -cyclodextrin (TBDMS-CD) in PS086 (1:10). Further details are given in⁶. The separation conditions were: Splitless injection of 1 μ l at 90°C, splitless/isothermal period 2 min, 15°C/min to 180°C, isothermal 44 min, 180-230°C at 2°C/min, isothermal at 230°C for 2 min.

The sample extracts were analyzed by HRGC combined with negative ion chemical ionization (NICI) mass spectrometry on a HP 5989 GC/MS using CH₄ at a pressure of 0.45 torr and an ion source temperature of 200°C. The (M+2)⁻ and (M+4)⁻ ions of the molecular isotope clusters were employed for quantification.

Results and discussion

Enantioselective separation: So far, TBDMS-CD shows the best separation for all chiral chlordane congeners. Such columns are also commercially available. However, due to the bulky t-butyl-dimethylsilyl group not more than 7-10 OH groups of the β -cyclodextrin are derivatised leading to a mixture of different stereo isomers and batch-to-batch variations⁴. Due to the possible inversion of enantiomer elution orders (see Figure 1) it is necessary to test any column before use. A test mixture was developed which contains some polychlorinated pesticides which are especially susceptible to minor alterations in the enantioselective properties of the stationary phase.

As can be seen from Figure 1, substantial changes in the enantioselective separation and the enantiomer elution order of polychlorinated pesticides can be observed for different columns based on TBDMS-CD. Therefore, any TBDMS-CD column should be tested with pure enantiomers before use. Some enantiopure chlordanes are available from Ehrenstorfer (Augsburg, Germany). Furthermore, co-elutions of the enantiomers of *cis*- and *trans*-chlordane might occur. The easiest way to overcome this problem is the use of tandem capillaries⁷ which were also applied here. The improved isomer specific separation minimizes the risk for overlap of enantiomers from different isomers.

Enantiomer ratios in cod: As can be seen from Table 1, the enantiomer ratios (ERs, area of (+)/(-) or first eluting enantiomer divided by the second one) in cod deviate substantially from the racemic ratio. With a few exceptions the ERs found in the all 3 cod tissues are similar within the uncertainty of the ER measurements (3-5 %). This means that any tissue can be used for an ER determination.

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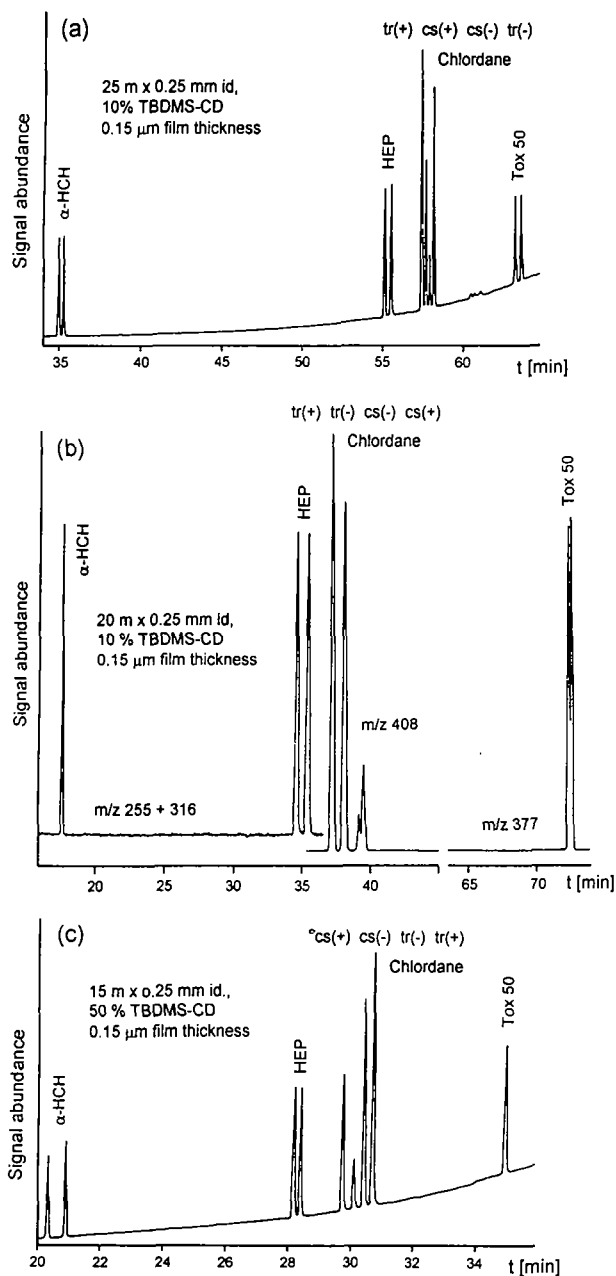


Figure 1: Enantioselective separation of selected chiral polychlorinated pesticides including *cis*- and *trans*-chlordane on different capillaries based on TBDMS-CD in OV-1701. The elution order of *cis*- and *trans*-chlordane enantiomers is marked. (a) recorded by electron capture detection (ECD), (b) NCI-MS detection, (c) ECD detection. The temperature programs were optimized for best separation. α -HCH: α -hexachlorocyclohexane, HEP: heptachlor exo epoxide, Tox 50: 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,9,10,10-nonachlorobornane.

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Compared to the earlier determined ERs in herring (see Table 1 for selected data), the following differences could be found: ER of the octachloro congener U82¹⁸⁾ is below 1 in cod and above 1 in herring. No differences in the ERs between male and female herring were observed. However, in all analyzed cod samples the ERs for *trans*-chlordan and MC6¹⁾ were very different between males and females. Enantiomer degradation was opposite in male and female cod leading to changes of the ERs by a factor of 3-5. A similar but not so unequivocal trend was also seen for *cis*-chlordan. No gender difference could be seen for U81¹⁸⁾ and U82. Figure 2 shows the NICI mass chromatograms of some octa chloro congeners in a male and female cod liver extract.

Table 1: Enantiomer ratios of U81, U82, *trans*-chlordan (tr-CD), *cis*-chlordan (cs-CD) and MC6 in different cod tissues compared to whole herring from the Baltic Sea (viscera excluded, data selected from⁵⁾). F: female, M: male, U: site Utklippan (Rönneby), L: site Landsort (Nykjöping), H: site Harfjärden (Haparanda). For *cis*- and *trans*-chlordan the (+)/(-) ERs are given, for U81, U82 and MC6 the area of the first eluting enantiomer is divided by the second one.

Sample type	Sex (age)	U81	U82	tr-CD	cs-CD	MC6
Cod, muscle	F (6)	0.85	0.73	0.54	0.71	0.29
	F (12)	0.77	0.80	0.73	0.83	0.32
	F(11)	0.83	0.82	0.46	0.91	0.54
	M(9)	0.93	0.97	1.9	1.14	1.6
	M(6)	i	0.92	1.1	1.0	1.8
Cod, gonad	F (6)	1.0	0.76	0.68	0.77	0.23
	F (12)	0.76	0.75	0.77	1.0	0.32
	F (11)	0.85	0.74	0.62	1.1	0.35
	M (9)	0.92	0.76	2.0	1.1	1.0
	M (6)	1.1	0.89	1.1	1.0	1.1
Cod, liver	F (6)	0.93	0.76	0.54	0.71	0.11
	F (12)	0.88	0.76	0.62	0.83	0.26
	F (11)	0.78	0.76	0.41	0.91	0.24
	M (9)	0.95	0.79	2.2	1.2	1.1
	M(6)	0.86	0.89	1.2	1.06	1.0
Herring (U)	F (3)	n.d.	1.1	0.48	1.1	n.d.
	M (3)	n.d.	1.2	0.20	1.2	n.d.
	M (3)	n.d.	1.2	0.27	1.2	n.d.
Herring (L)	M (3)	n.d.	1.1	0.58	1.3	n.d.
	M (3)	n.d.	1.2	0.47	1.5	n.d.
	F (3)	n.d.	1.0	0.36	1.2	n.d.
Herring (H)	F (3)	n.d.	1.0	0.35	1.2	n.d.
	F (3)	n.d.	1.1	0.27	1.6	n.d.
	F (3)	n.d.	1.0	0.32	1.6	n.d.

n.d.: not determined; i: interference

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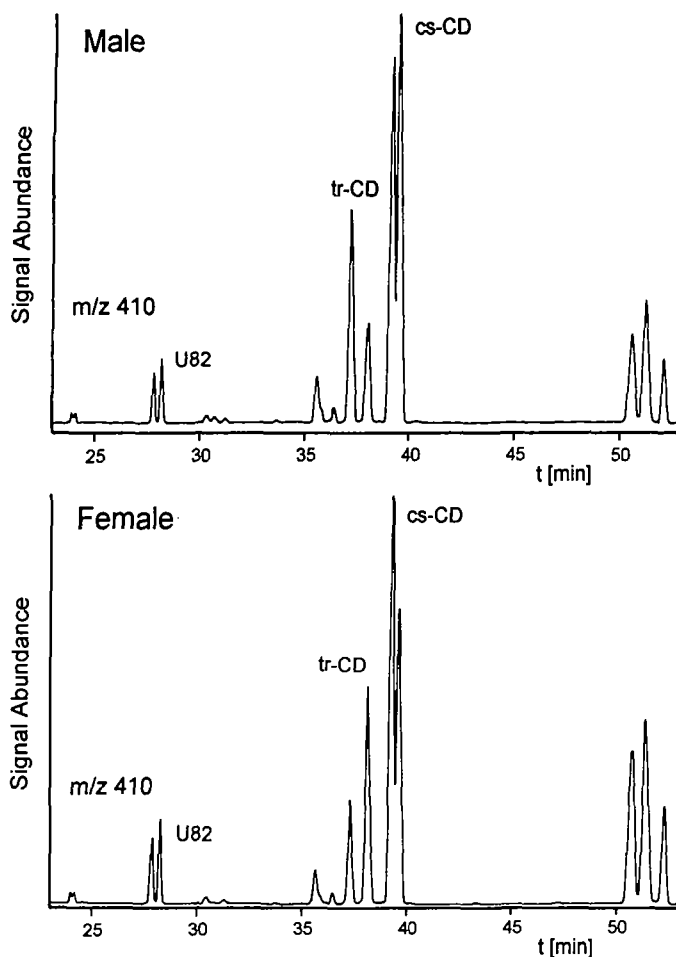


Figure 2: NICI mass chromatograms of the enantioselective separation of the octachloro congeners U82, *cis*- and *trans*-chlordane in cod liver of male and female cods.

The differences in influence of sex on the ERs of the congeners cannot be explained yet. One reason might be differences in their structures. It is assumed that U81 and U82 have a 5+3 chlorine distribution between ring 2 and 1, while all other congeners are of the 6+2 (octachloro congeners) or 6+3 type (nonachloro congeners)¹⁾. The technical pesticide chlordane has shown a significant synergism concerning artificial estrogenic effects together with other pesticides such as dieldrin²⁾. This might be an explanation for the different ERs in male and female cod. However, this does not explain the missing gender influence at herring. Other factors such as the position in the food chain and unlike enzyme systems can also be of importance. Interestingly, the isomer specific quantitative results (not given here) did not show any gender related change. The results indicates that accumu-

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lation/degradation processes of enantiomers might be more susceptible to biological factors. This underlines once more the importance of an enantiomer specific quantification of chiral polychlorinated pesticides.

Acknowledgment

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