Levels and enantiomeric ratios of oxychlordane and α-HCH in harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) from Iceland

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1. Introduction

Oxychlordane and α-HCH are chiral organochlorine compounds. The ubiquitous and persistent xenobiotics accumulate in environmental samples yielding ppt-ppm levels in fatty tissue. Racemic α-HCH is the major part (approx. 65%) of technical HCH which has a global usage of 550.000 metric tonnes[1].
Oxychlordane is the most relevant metabolite of cis- and trans-chlordane, being more persistent and toxic than the parent compounds[2].
In 1989, König et al. separated the enantiomers of α-HCH by application of chiral gas chromatography on modified cyclodextrins[3] and in 1991, Kallenborn et al. published the first paper dealing with enantioselective degradation of α-HCH in biota[4]. Since enantiomers have different chemical properties in an asymetric surrounding[5] the enantioselective determination of chiral organochlorines is important.
In blubber of marine mammal species usually (+)-α-HCH is higher abundant than (-)-α-HCH. However, recently an exception was reported for hooded seals (*Crystophory cristata*) which showed higher (-)-α-HCH levels than (+)-α-HCH levels[6].
Results referring the enantioselective accumulation of oxychlordane in marine mammals are rare[7]. Therefore, we have studied the enantioselective accumulation of oxychlordane in samples of two seal species from Iceland. For this reason we applied a chiral β-TBDM phase (35% heptakis 6-O-*t*-butyl, 2,3 di-O-methyl-β-cyclodextrin in OV-1701) which simultanously separated several chiral organochlorines including α-HCH and oxychlordane with one temperature program.

2. Material and method

Chiral GC conditions

Enantioselective separations were carried out using an HP 5890 gas chromatograph with ^{63}Ni ECD detector. Nitrogen was used as carrier gas (column head pressure 0.6 bar) and as make-up gas.
The β-TBDM phase (35% heptakis 6-O-*t*-butyl, 2,3 di-O-methyl-ß-cyclodextrin in OV-1701) was obtained from M. D. Müller, Eidgenössische Forschungsanstalt Wädenswil, Switzerland. The column parameters were: 20 m length, 0.25 mm internal diameter, 0.15 µm film thickness.
The GC oven was programmed as follows: 60°C, 2 min, 25°C/min to 160°C (10 min); 1°C/min to 200°C; 10°C/min to 220°C (40 min).

Chemicals

Standard solutions of α-HCH, oxychlordane, cis- and trans-chlordane and further organochlorines were from Promochem (Wesel, Germany). The internal standard α-PDHCH (perdeuterated α-HCH), which was synthesized in our laboratory[8], is also available from Promochem (Wesel). The elution order of the enantiomers on β-TBDM was determined by injection of enantiomerically accumulated or pure enantiomers. The (+)-α-HCH enantiomer was obtained after HPLC separation in our laboratory on a

LiChroCart 250-4 column (Merck, Darmstadt, Germany) in accordance to Möller et al.[9]. Enantiomerically accumulated (+)-oxychlordane ($ER_{(+/-)}$ 3) was obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Biological samples

Blubber of harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) were obtained from animals shot on Western Iceland. Organochlorine levels (PCBs, DDT and its metabolites, HCH isomers, compounds related to chlordane) were recently published[10]. The sample clean-up was described in detail before[10][11].

3. Results and Discussion

3.1. Separation of organochlorine standards on β-TBDM

α-PDHCH was used as internal standard to monitor the accuracy of the enantiomeric separation of α-HCH.
On β-TBDM simultanous separation of the enantiomers of α-HCH and α-PDHCH, oxychlordane, cis- and trans-chlordane, cis- and trans-heptachlorepoxid was obtained by application of the oven program mentioned above[12]. To even out slight differences in peak heights of the enantiomers of racemic α-HCH which also varied slightly from injection to injection α-PDHCH was used as internal standard[12]. This was possible since α-PDHCH and α-HCH were separated in four peaks and both standards were subject to the same variations on β-TBDM. Therefore, the $ER_{(+/-)}$ of α-HCH in the samples were corrected by the factor determined for α-PDHCH[12].
Furthermore, the elution order of enantiomers was checked by pure or enantiomerically enriched standards. On β-TBDM, (+)-α-HCH and (-)-oxychlordane were the first eluting enantiomers. The detailed description of the chiral separation on this and other chiral columns was recently presented[12].
To guarantee the unambiguous elution of the chiral compounds we determined the retention times of many organochlorine standards (e.g. PCB congeners, toxaphene congeners, HCH isomers, DDT and its metabolites, nonachlor and others) on β-TBDM. Unfortunately, B8-1413 (2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane) and trans-nonachlor interferred cis- and trans-chlordane under the conditions mentioned above. Thus, quantitation of cis- and trans-chlordane by GC/ECD was not possible though the chlordane standards were resolved in four peaks on the β-TBDM column. The enantiomers of heptachlor and heptachlorepoxide were also separated on β-TBDM. However, recoveries of these compounds using our clean-up procedure were not tested so far. Work is ongoing to estab-lish heptachlorepoxide levels and ER ratios in biological samples. In this study we have focussed on the determination of $ER_{(+/-)}$ of α-HCH and oxychlordane in biota.

3.2. Enantioselective determination of α-HCH and oxychlordane in seal blubber

High levels of compounds related to chlordane were determined in blubber of all harbour and grey seals from Western Iceland. This confirmed that chlordane is a major contaminant in this region[10]. Table 1 lists levels of enantiomers and enantiomeric ratios of α-HCH and oxychlordane in blubber of harbour and grey seals.
Both seal species revealed α-HCH $ER_{(+/-)} > 1$ (see Table 1) which is the common result for seal species. Up to now, hooded seals are the only marine mammal species which accumulated α-HCH $ER_{(+/-)} < 1$ in blubber[6].
Enantiomeric ratios of oxychlordane in blubber of marine mammals were only published for two harbour seal samples. Both samples had lower levels of (+)-oxychlordane than (-)-oxychlordane[7]. Oxychlordane $ER_{(+/-)} < 1$ were also determined in all of our harbour seal samples (see Table 1). Interestingly, all blubber samples of grey seals showed (+)-oxychlordane as the higher abundant oxychlordane enantiomer (see Table 1). Figure 1 shows a part of the GC/ECD chromatogram of blubber of both harbour seal and grey seal. The levels determined on the chiral phase were confirmed on the non-chiral phase.

Table 1: Biological data, levels (ng/g) and enantiomeric ratios $ER_{(+/-)}$ of oxychlordane (Oxy) and α-HCH in blubber of harbour seals and grey seals from Faxaflói (Western Iceland)

Harbour seals	H1	H2	H3	H4	H5	H6	H7	H8	Mean
Sex	F	F	F	F	F	M	M	M	
Age	ad	23y	3y	2y	5m	8y	3y	5m	
(+)-α-HCH	3.0	3.4	2.8	2.8	7.1	3.8	4.5	5.4	4.1
(-)-α-HCH	3.0	2.6	2.2	2.2	3.9	3.2	3.5	3.6	3.0
$ER_{(+/-)}$ α-HCH	1.0	1.3	1.3	1.3	1.8	1.2	1.3	1.5	1.4
(+)-Oxy	47	27	21	42	115	148	56	130	73
(-)-Oxy	66	38	27	71	165	212	93	144	102
$ER_{(+/-)}$ Oxy	0.7	0.7	0.8	0.6	0.7	0.7	0.6	0.9	0.7

Grey seals	G1	G2	G3	G4	G5	G6	G7	G8	Mean
Sex	F	F	F	F	F	M	M	M	
Age	19y	18y	15y	8y	juv	26y	juv	juv	
(+)-α-HCH	4.0	6.2	4.4	4.9	4.2	3.0	5.4	6.0	4.8
(-)-α-HCH	4.0	4.8	3.6	4.1	2.8	2.0	3.6	5.0	3.7
$ER_{(+/-)}$ α-HCH	1.0	1.3	1.2	1.2	1.5	1.5	1.5	1.2	1.3
(+)-Oxy	86	107	63	95	105	245	71	32	100
(-)-Oxy	53	71	45	79	96	222	50	22	80
$ER_{(+/-)}$ Oxy	1.6	1.5	1.4	1.2	1.1	1.1	1.4	1.5	1.3

Ages are given as ad, adult; juv, juvenile; y, years; m, months. Sex: M, male; F, female.

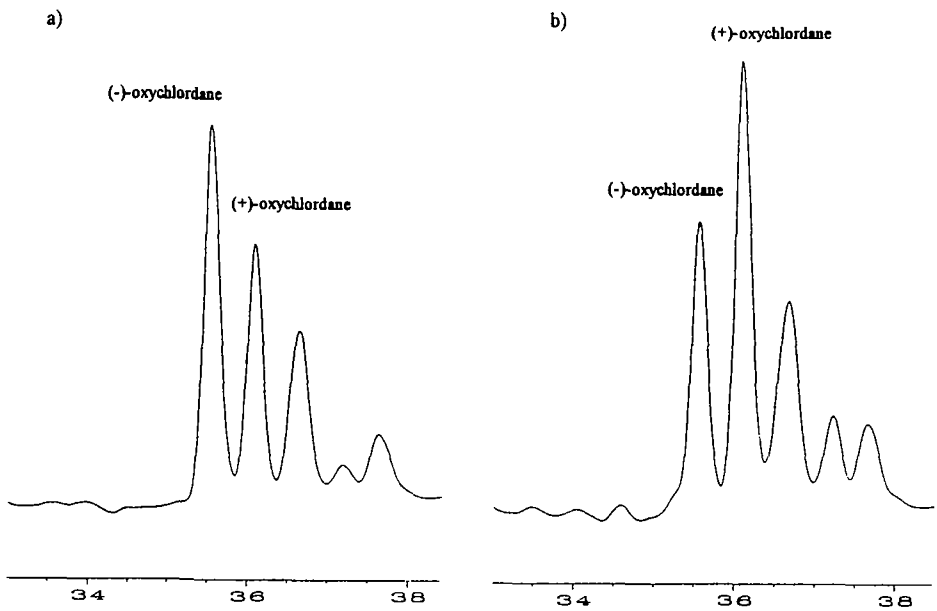

Figure 1: Typical GC-ECD chromatogram (part) of the chiral separation of oxychlordane in blubber of a) harbour seal and b) grey seal on β-TBDM

$ER_{(+/-)} > 1$ for α-HCH in both seal species but higher (-)-oxychlordane levels in harbour seals and lower (-)-oxychlordane level in grey seals were very astonishing to us. Thus, we have studied if there are connections between α-HCH and oxychlordane levels and enantiomeric ratios.
The highest oxychlordane level was determined in the blubber sample of an adult male grey seal (G6, see Table 1). However, the α-HCH level in this sample (G6) was the lowest of all grey seal samples and also the extend of enantioselective enrichment of α-HCH and oxychlordane was different. Generally, neither levels nor enantiomeric ratios of α-HCH and oxychlordane did correlate in harbour seals or grey seals. Also referring age and sex no trend in ER of α-HCH or oxychlordane was observed in the two seal species.

4. Conclusions

We have no explanations for the inverse enantiomeric ratio of oxychlordane ($ER_{(+/-)} < 1$ in harbour seals and $ER_{(+/-)} > 1$ in grey seals) in blubber of marine mammals. More data are necessary to explain this phenomenon.
However, the results obtained from the two seal species clearly confirm that species specific factors play an important role during the enantioselective biodegradation of organochlorine compounds. Therefore, it should be avoided to conclude from results in one marine mammal on results in another species.

5. References

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