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LEVELS AND ENANTIOMERIC RATIOS OF α -HCH, OXYCHLORDANE, AND PCB 149 IN BLUBBER OF HARBOUR SEALS (*Phoca vitulina*) AND GREY SEALS (*Halichoerus grypus*) FROM ICELAND AND FURTHER SPECIES

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Abstract

The chiral stationary phase β -TBDM (35% heptakis(6-*O*-*tert*-butyldimethylsilyl)-2,3-di-*O*-methyl)- β -cyclodextrin in OV-1701) was applied to study the enantioenrichment of α -HCH, oxychlorthane, and PCB 149 in blubber of two seal species from Iceland by gas chromatography with electron capture detection. The examined harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) showed α -HCH enantiomeric ratios (ER) > 1. The ER of PCB 149 was comparable in the two species from Iceland but for oxychlorthane ER < 1 was observed in harbour seals while the oxychlorthane ER in grey seals was > 1. In blubber of Weddell seals (*Leptonychotes Weddelli*) from the Antarctic we determined an α -HCH ER < 1 which is in contrast to the Icelandic seal species and a sample from Lake Baikal.

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Introduction

Enantioselective gas chromatography for the determination of enantiomeric ratios (ER) of chiral compounds in biological samples is a new and growing research topic in analytical chemistry. In 1989, the first successful enantiomer separation of the chiral organochlorine pesticide aaeeee-1,2,3,4,5,6-hexachloro-cyclohexane (α -HCH) was achieved on modified cyclodextrins by König et al. [1]. The authors stated that the enantiomer separation of α -HCH may be used to study the enantioselective biodegradation of this

compound in the environment [1]. Racemic α -HCH is the major part (approx. 65%) of technical HCH which had a global usage of 550.000 metric tonnes [2]. In 1991, Kallenborn et al. published the first paper dealing with the enantioselective degradation of α -HCH in biota [3]. In blubber of several marine mammal species such as harbour seals (*Phoca vitulina*) [4-6], northern fur seals (*Callorhinus ursinus*) [7], grey seals (*Halichoerus grypus*) [5,8], harp seals (*Phoca groenlandica*) [5], and different cetaceans [5,9] (+)- α -HCH was more abundant than (-)- α -HCH. However, exceptions of this "rule" were hooded seals (*Cystophora cristata*) and a Weddell seal (*Leptonychotes weddelli*) which showed higher (-)- α -HCH than (+)- α -HCH levels in their blubber [5,10-11].

Oxychlordane (1-exo,2-exo,4,5,6,7,8-octachloro-2,3-exo-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane) is the common metabolite of cis- and trans-chlordane and other chlordane-related compounds [12]. It is more persistent and toxic than the parent compounds [13]. In 1992, Buser and Müller enantiomer separated oxychlordane in biological samples on *tert*-butyldimethylsilylated β -cyclodextrin [14]. However, studies on the enantioselective accumulation of oxychlordane in biological samples are still scarce [15].

The third chiral compound studied here was the atropisomeric PCB 149 (2,3,6,2',4',5'-hexachlorobiphenyl). Technical PCB products are mixtures of 80-100 congeners which have been used for diverse industrial applications. 19 of the 209 PCB congeners exist as stable atropisomers due to the hindered rotation about the phenyl-phenyl bond [16]. A requirement for stable PCB atropisomers is a nonsymmetrical substitution pattern on both phenyl rings together with at least three chlorine atoms in ortho-position. In 1993, Schurig and Glausch and König et al. reported the enantiomer separation of PCB atropisomers [17-18]. Up to now, only a few studies of the enantioselective determination of atropisomeric PCBs have been published [15]. In this work we focussed on the enantiomer separation of PCB 149 since this tri-ortho substituted PCB congener was present at measurable levels in seal blubber [19].

In this study we used a chiral stationary phase (CSP) consisting of 35% heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin diluted in OV-1701 (β -TBDM) which enantiomer separated α -HCH, oxychlordane, cis- and trans-chlordane, heptachlorepoxide, PCB 149 [11,20], some polychlorinated bornanes (toxaphene) [21] and other chiral organochlorines [11,22].

The enantiomeric ratios were studied in blubber of two seal species from Iceland. Organochlorine concentrations as well as the ER of α -HCH determined on a CP-Cyclodex phase (β -PMCD) have been published before [5,8]. However, the levels of PCB 149 were not measured. Furthermore, the earlier published α -HCH levels and enantiomeric ratios were reconsidered due to slightly differences from the results obtained on new stationary phases which allowed an improved separation in this work. In addition, the α -HCH ERs in blubber of Weddell seals (*Leptonychotes Weddelli*) and a Baikal seal (*Phoca sibirica*) were determined by GC/ECNI-MS to confirm our recent findings of higher (-)- α -HCH levels in some marine mammals [11].

MATERIALS AND METHOD

Biological samples and clean-up procedure

Blubber of eight harbour seals (*Phoca vitulina*) and eight grey seals (*Halichoerus grypus*) was obtained from animals shot on Faxaflói (Western Iceland). Blubber of Antarctic Weddell seals (*Leptonychotes weddelli*) was from animals shot for biological studies in January and February 1990 at the Drescher Inlet (72° 52' S, 19° 25' W), Vestkapp, Riiser Larsen Ice Shelf on the east coast of the Weddell Sea. The biological data of these animals are given in Table 1 and 2, respectively (see Results and Discussion). The sample clean-up has been described in detail before [8,19]. The blubber of the Baikal seal (*Phoca sibirica*) was from an animal caught in 1995 on the east coast of Lake Baikal.

Chemicals

Standard solutions of α -HCH (10 ng/ μ L toluene), oxychlorane (10 ng/ μ L methanol), PCB 149 (10 ng/ μ L cyclohexane), cis- and trans-chlordane (10 ng/ μ L cyclohexane) and further organochlorines were from Dr. Ehrenstorfer (Augsburg, Germany). The organochlorines were combined and diluted with isooctane to final concentrations of 100 pg/ μ L. A solution of 2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane (B8-1413 [23] or Parlar #26 [24]) (10 ng/ μ L cyclohexane) was from Dr. Ehrenstorfer (Augsburg, Germany). Enantioenriched (+)- α -HCH was obtained after HPLC enantiomer separation on a LiChroCart 250-4 column (Merck, Darmstadt, Germany) following the procedure of Möller et al. [11,25]. The optical rotation and elution order was determined with HPLC and a chiral detector [26]. Enantioenriched (+)-oxychlorane (1 ng/ μ L cyclohexane) [27] was from Dr. Ehrenstorfer.

Gas chromatography with electron capture detection (GC/ECD)

Enantiomer separations were carried out using an HP 5890 gas chromatograph equipped with a ^{63}Ni ECD detector. Nitrogen was used as both carrier (column head pressure 0.6 bar) and make-up gas. The parameters of the β -TBDM phase (M. D. Müller, Eidgenössische Forschungsanstalt Wädenswil, Switzerland) were: 20 m length, 0.25 mm internal diameter, 0.15 μm film thickness. The enantiomers of oxychlorane and α -HCH were separated with the following temperature program: 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). For PCB 149 the GC conditions were: 60°C (2 min), 10°C/min to 150°C (180 min), 0.5°C/min to 170°C, 10°C/min to 220°C (40 min) [11].

Achiral separations were performed with a CP-Sil 2 column of 50 m length, 0.25 mm internal diameter, and 0.12 μm film thickness (Chrompack, Middelburg, The Netherlands) which was installed in an HP 5890 gas chromatograph equipped with a ^{63}Ni ECD detector. Nitrogen was used as carrier- and make-up gas. For α -HCH and oxychlorane the following GC oven program was used: 70°C (1.5 min), 40°C/min to

200°C (2 min), 1.5°C/min to 250°C (2 min), 6°C/min to 300°C (15 min). For PCB 149 the following settings were applied: 75°C (1.5 min), 25°C/min to 200°C (2 min), 2°C/min to 250°C (1 min), 15°C/min to 280°C (35 min).

Gas chromatography with electron capture negative ionization mass spectrometry (GC/ECNI-MS)

GC/ECNI-MS experiments were carried out on an HP 5989B MS Engine connected to an HP 5890 II plus gas chromatograph (Hewlett-Packard, Waldbronn, Germany). Helium was used as carrier gas and methane as reagent gas. The ion source was kept at 200°C and the quadrupole at 100°C. The system was optimized by manual tuning using the perfluorotributylamine (PFTBA) masses m/z 312, m/z 414, and m/z 464. In the SIM mode m/z 252.9 and m/z 254.9 were monitored for α -HCH after a solvent delay of 8 min. The dwell time was 75 ms per ion. 1 μ L of the samples was splitless injected (1.5 min) at 225°C.

The chiral stationary phase (CSP) in the GC oven consisted of 10% heptakis(2,3,6-tri-*O*-methyl)- β -cyclodextrin chemically bonded to CP-Sil 5 (25 m length, 0.25 mm internal diameter, and 0.2 μ m film thickness), hereafter abbreviated as β -PMCD. The β -PMCD column was from Chrompack (Middelburg, The Netherlands). The following temperature program was applied: 80°C (1 min), 15°C/min to 140°C, 2°C/min to 170°C, 20°C/min, 232°C (18.5 min); the total run time was 40 min.

RESULTS AND DISCUSSION

Characterization of enantiomer separations on the β -TBDM phase

A detailed description of achievable enantiomer separations on β -TBDM and other chiral stationary phases (CSPs) has been published recently [11,22]. Therefore, only some special aspects will be discussed. On β -TBDM, an enantiomer separation of α -HCH, oxychlordan, *cis*- and *trans*-chlordan, *cis*- and *trans*-heptachlorepoxid can be obtained in one run [11]. Due to possible interferences when using the non-selective GC/ECD, a confirmation of the ER on a second column with different polarity is desirable. However, this is difficult to fulfill with CSPs since the enantiomer separation of a chiral organochlorine is sometimes only attainable on one CSP. Therefore, we have recorded the retention times of many organochlorine standards (e.g. PCB congeners, compounds of technical toxaphene (CTTs), HCH isomers, DDT and its metabolites, nonachlor and others) with the GC conditions optimized for the enantioselective determination of α -HCH, oxychlordan, and PCB 149 on β -TBDM. In this way, interferences due to coelutions with these compounds would be detectable. For example, B8-1413 (Parlar #26), a major recalcitrant CTT in many samples [28,29], coeluted with the first eluted peak of *trans*-chlordan and *trans*-nonachlor interfered the second eluted peak of *cis*-chlordan (see **Figure 1a**). Although B8-1413 (Parlar #26) is chiral, our attempt to separate the enantiomers of B8-1413 (Parlar #26) failed on β -TBDM [11].

Due to the coelutions mentioned above, an enantioselective analysis of *cis*- and *trans*-chlordane on β -TBDM was not possible with GC/ECD although the four enantiomeric peaks of *cis*- and *trans*-chlordane were baseline separated (see Figure 1b).

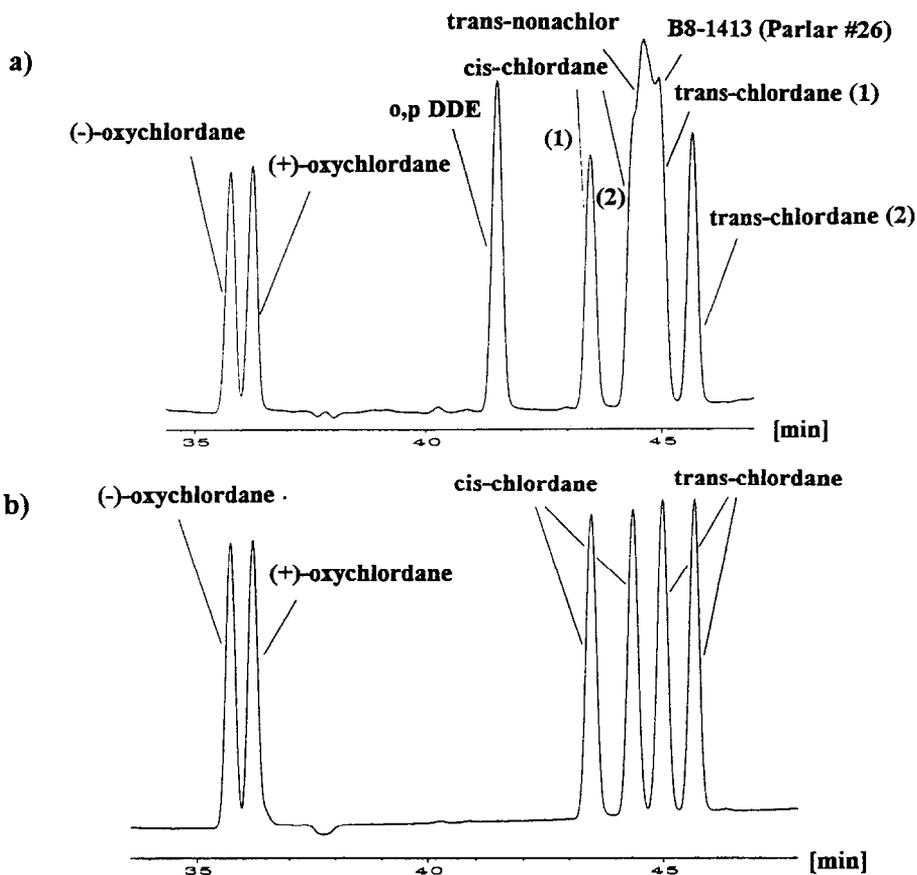


Figure 1: a) Separation of oxychlordane, *cis*- and *trans*-chlordane, B8-1413 (Parlar #26) and *trans*-nonachlor on β -TBDM

b) Enantiomer separation of oxychlordane, *cis*- and *trans*-chlordane on β -TBDM

No interference was observed for α -HCH, oxychlordane and PCB 149, but the separation of enantiomers of PCB 149 was more difficult to achieve compared to α -HCH and oxychlordane. On the applied β -TBDM column, a run time > 3 h was necessary to separate the atropisomers of PCB 149. As an

additional quality control measure the level of the sum of both enantiomers ($c_{(+)} + c_{(-)}$) was compared with that determined on an achiral column ($c_{(\pm)}$).

This allows to exclude a substantial interference of one or both enantiomers when the ER is determined by GC/ECD only. The elution order of enantiomers was determined by injection of enantiopure or enantioenriched standards. On β -TBDM, (+)- α -HCH and (-)-oxychlorane were the first eluted enantiomers, respectively. Recently, Haglund isolated some enantiopure PCB atropisomers with HPLC and a CSP [30], and Haglund and Wiberg determined the elution order of several PCB atropisomers on β -PMCD [31]. Unfortunately, enantiopure PCB 149 could not be obtained by enantioselective HPLC and the elution order of PCB 149 atropisomers is still unknown. Therefore, we defined the ER of PCB 149 as ratio of the first to the second eluted enantiomer ($ER_{(1/2)}$) on our column.

Levels and enantiomeric ratios of α -HCH

Without exception, harbour and grey seals revealed α -HCH $ER_{(+/-)} > 1$ (see Table 1). Note that the levels and enantiomeric ratios were corrected compared with our earlier presentations [8,20]. The levels determined on the chiral β -TBDM (i. e. the sum of the levels of the enantiomers) and the achiral CP-Sil 2 agreed very well (see Table 1). The average enantioenrichment of (+)- α -HCH was comparable for both seal species ($ER_{(+/-)} = 1.4$). This confirms the earlier results reported for harbour seals and grey seals [4-8].

An $ER_{(+/-)} > 1$ was also found in blubber of northern fur seals (*Callorhinus ursinus*) [7] and harp seals (*Phoca groenlandica*) [9], as well as for several cetaceans [5,9]. However, (-)- α -HCH was more abundant in blubber of three hooded seals (*Cystophora cristata*) [5,10] and a Weddell seal [11]. To confirm these findings, the $ER_{(+/-)}$ of α -HCH was determined in blubber samples of this and further seven Weddell seals together with a seal blubber sample from Lake Baikal (see Table 2).

The determination was carried out with GC/ECNI-MS using the two most intensive fragment ions m/z 252.9 and m/z 254.9 of the M-Cl⁻ ion cluster. As quality control, we checked if the ER was the same for both fragment ions and if the ratio of m/z 252.9 : 254.9 was identical with the theoretical value of 0.6352. The maximal deviation from the theoretical value of both the isotopic ratios and ERs was 2.6 % in all samples (see Table 2). This result confirms the accuracy of the GC/ECNI-MS method for enantiomer separation of chiral organochlorines. In agreement with our preliminary results [11], all blubber samples of Weddell seals exhibited an $ER_{(+/-)} < 1$ (see Figure 2). Two samples showed a particularly low ER. At the moment, we have neither explanations for the variations in the ER nor for the very low ER in sample W1 and W8 (see Table 2). A systematic error of the GC-MS method presented above can be excluded. Finally, we determined the ER of α -HCH in a sample of blubber from Lake Baikal. The Baikal seal (*Phoca sibirica*) showed an ER of 1.1 which agrees well with the ER determined in the two seal species from Iceland.

Table 1: Levels [$\mu\text{g}/\text{kg}$] and enantiomeric ratios of α -HCH, oxychlordane, and PCB 149 in blubber samples of harbour seals and grey seals from Faxaflói (Western Iceland)

Harbour seals	α -HCH			Oxychlordane			PCB 149		
	achiral ¹⁾	chiral ²⁾	ER _(+/-)	achiral ¹⁾	chiral ²⁾	ER _(+/-)	achiral ¹⁾	chiral ²⁾	ER _(1/2)
51 (F, ad)	11	10	1.4	34	34	0.7	27	35	1.4 ³⁾
52 (M, 8y)	16	13	1.4	187	210	0.7 ³⁾	47	45	2.3
53 (F, 5m)	23	20	1.8	166	172	0.7	51	50	1.9
54 (M, 3y)	18	15	1.5	123	117	0.6	40	40	2.1
55 (F, 23y)	14	12	1.3	107	73	0.7 ³⁾	17	15	2.5
56 (M, 5m)	22	18	1.4	162	166	0.9	174	160	1.7
57 (F, 2y)	9	11	1.1	24	24	0.6	-	-	-
58 (F, 3y)	14	10	1.5 ³⁾	59	53	0.8	28	25	1.7

Grey seals	α -HCH			Oxychlordane			PCB 149		
	achiral ¹⁾	chiral ²⁾	ER _(+/-)	achiral ¹⁾	chiral ²⁾	ER _(+/-)	achiral ¹⁾	chiral ²⁾	ER _(1/2)
59 (F, 15y)	18	15	1.4	89	83	1.4	28	30	1.9
60 (F, 18y)	23	21	1.6	113	123	1.5	49	45	1.6
61 (F, 19y)	15	14	1.4	94	90	1.6	-	-	-
62 (M, 26y)	13	10	1.4 ³⁾	336	418	1.1 ³⁾	61	60	1.9
63 (F, 8y)	19	19	1.5	130	133	1.2	33	30	1.5
65 (M, juv)	22	19	1.5	96	92	1.4	-	-	-
66 (F, juv)	18	15	1.5	171	183	1.1	37	40	2.3
67 (M, juv)	20	19	1.3	61	57	1.5	15	15	1.8

Age classes: ad = adult; juv = juvenile; y = years; m = month; sex: M = male; F = female

1) levels in [$\mu\text{g}/\text{kg}$] determined on the achiral column CP-Sil 2

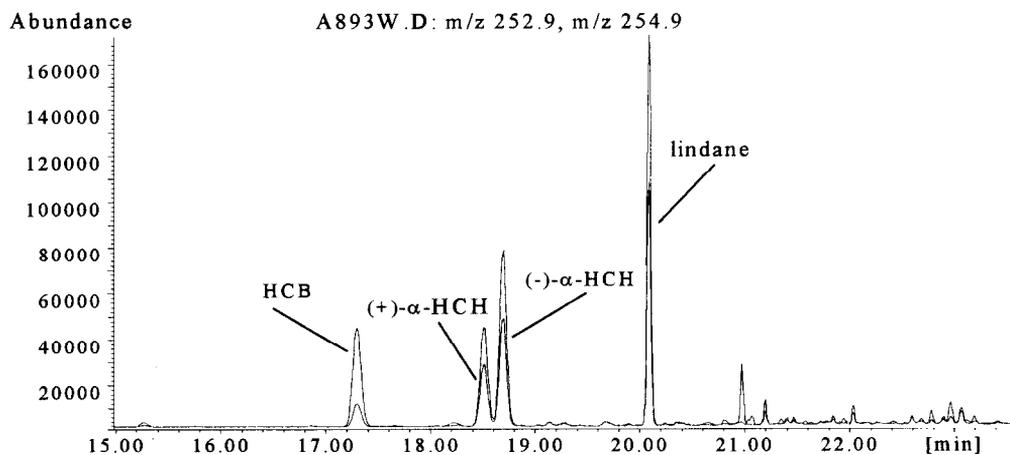
2) levels in [$\mu\text{g}/\text{kg}$] determined on the chiral column β -TBDM

3) ER insecure (levels on chiral and achiral columns deviated significantly)

Table 2: ER of α -HCH in blubber from Antarctic Weddell seals and a Baikal seal

Seal species	ER _(+/-)	Ion ratio m/z 252.9/254.9	
		(+)- α -HCH	(-)- α -HCH
W1 (male, adult)	0.12 ¹ (0.119; 0.121) ²	0.6119 ³ (0.979) ⁴	0.6217 (0.994)
W2 (male, adult)	0.97 (0.966; 0.967)	0.6310 (1.009)	0.6320 (1.011)
W3 (female, subadult)	0.87 (0.874; 0.875)	0.6203 (0.992)	0.6212 (0.993)
W4 (male, adult)	0.80 (0.794; 0.801)	0.6208 (0.993)	0.6267 (1.002)
W5 (male, adult)	0.73 (0.732; 0.732)	0.6225 (0.996)	0.6225 (0.996)
W6 (female, juvenile)	0.63 (0.620; 0.628)	0.6216 (0.994)	0.6292 (1.006)
W7 (male, juvenile)	0.87 (0.874; 0.875)	0.6192 (0.990)	0.6196 (0.991)
W8 (female, juvenile)	0.10 (0.101; 0.100)	0.6129 (0.980)	0.6090 (0.974)
Baikal seal	1.10 (1.093; 1.097)	0.6208 (0.993)	0.6231 (0.996)

- 1 ER (mean value of the ER of m/z 252.9 and m/z 254.9)
- 2 (ER of m/z 252.9; ER of m/z 254.9)
- 3 measured ion ratio of m/z 252.9/254.9 (theoretical value: 0.6253)
- 4 deviation of the measured ion ratio from the theoretical value of 0.6253

**Figure 2: GC/ECNI-MS chromatogram (m/z 254.9 and m/z 254.9) of the enantiomer separation of α -HCH in blubber of an Antarctic Weddell Seal (*Leptonychotes Weddelli*)**

Levels and ER of oxychlordanes

An interesting observation was made during the analysis of oxychlordanes in blubber of harbour and grey seals. All harbour seal samples showed an enantioenrichment of (-)-oxychlordanes, while in the grey seal samples the amounts of (+)-oxychlordanes were higher (see Figure 3). With one exception, the level determined on the chiral β -TBDM phase agreed well with those determined on the achiral CP-Sil 2 phase (see Table 1).

The oxychlordanes levels were comparable in blubber of grey and harbour seals. A correlation of the ER with age or sex was not observed. ER data of oxychlordanes in blubber of marine mammals are scarce in the literature [15]. König et al. found lower levels of (+)-oxychlordanes than (-)-oxychlordanes in two blubber samples of harbour seals from the German North Sea coast [27] and Buser and Müller published an $ER_{(+/-)} > 1$ in blubber of a Baltic grey seal [32] which confirms the results obtained in our samples (see Table 1).

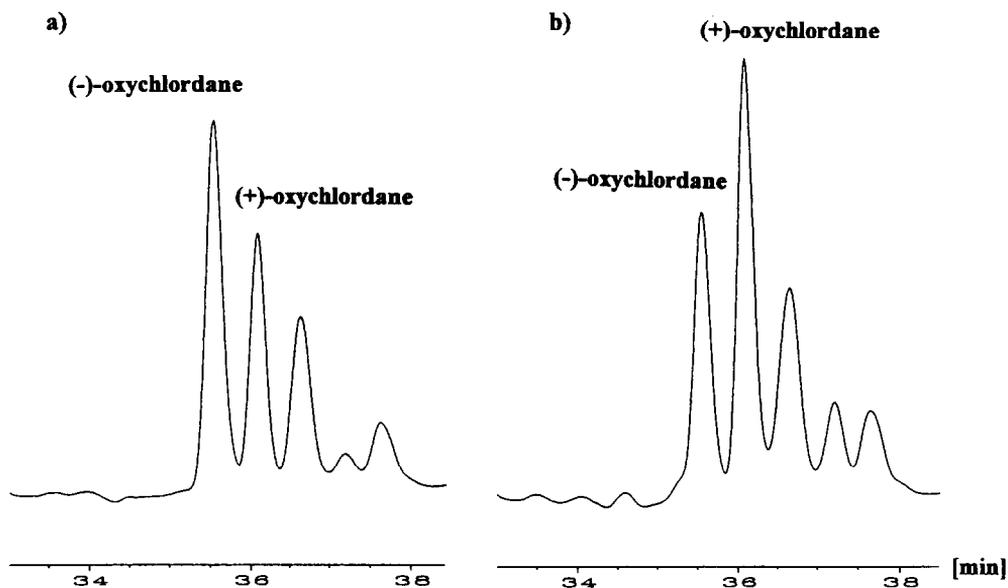


Figure 3 GC/ECD chromatogram (part) of the enantiomer separation of oxychlordanes on β -TBDM
 a) blubber of harbour seal (*Phoca vitulina*)
 b) blubber of grey seal (*Halichoerus grypus*)

Levels and ER of PCB 149

Levels of PCB 149 in the blubber samples ranged from 15 - 174 $\mu\text{g}/\text{kg}$. In all blubber samples of harbour and grey seals, the first eluted enantiomer was more abundant which resulted in $\text{ER}_{(1/2)} > 1$ (see Table 1). However, the ER varied also from species to species. Blubber of a caspian seal (*Phoca caspica*) had a PCB 149 $\text{ER}_{(1/2)} = 1$, in blubber of the Baikal seal (*Phoca sibirica*) the $\text{ER}_{(1/2)}$ was < 1 , and the $\text{ER}_{(1/2)}$ in four harbour seals from the North Sea ranged from 0.9 to 1.1. Again, we observed no correlation between enantiomeric ratio and biological parameters of the samples.

Conclusions

While the $\text{ER}_{(+/-)}$ of α -HCH and the $\text{ER}_{(1/2)}$ of PCB 149 were > 1 in both harbour and grey seals, the $\text{ER}_{(+/-)}$ of oxychlordan was reversed in blubber of the two seal species. Results for α -HCH in blubber of Antarctic Weddell seals also showed that in 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.

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