ENANTIOSELECTIVE TRANSFORMATION AND ACCUMULATION OF CYCLODIENE PESTICIDES AT DIFFERENT TROPHIC LEVELS OF MARINE AND TERRESTRIAL BIOTA

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1. Introduction

Cyclodiene pesticides and their metabolites, respectively, are still ubiquitously present at all trophic levels in marine and terrestrial ecosystems including biota from remote areas as the Arctic and Antarctic, although their use in Germany has been prohibited since 1971, and by 1988, the application of chlordane was stopped in the United States, Japan and most European Countries. But consumption in other countries continues. Several metabolites are even present in human adipose tissue and milk. Residues found in biological and environmental samples include components from technical chlordane and their metabolites. Technical chlordane is a complex mixture of up to 120 chemically similar components produced by further chlorination of the Diels-Alder adduct of hexachlorocyclopentadiene and cyclopentadiene. However, thus far most authors confined their analysis to at maximum about 10 components and some metabolites. Special emphasis has been placed upon the investigation of *cis*- and *trans*-chlordane, heptachlor and their metabolites. *Exo*-oxychlordane is the principal mammalian metabolite of *cis*- and *trans*-chlordane and of nonachlors, while *cis*-heptachlorepoxide [*exo*], often just referred to as heptachlor epoxide, is the main metabolite of heptachlor. Furthermore, Buser and Müller investigated photoconversion products of some chlordane components in environmental samples[1]. For a review of this earlier work the reader should refer to the paper by Buser et al. and to the literature cited therein[2].

In addition to these "old cyclodiene pesticides", recently evidence was presented that a "new class of cyclodienes" may become a problem for fish: in muscle tissue of rainbow trouts from Danish fish farms and of three different fish species (orfe, bream, and pike) from the river Stör (northern Germany) remarkably high concentrations between 0.09 and 1.23 mg/kg fat of the chiral insecticide bromocyclen were found[3]. The brominated and chlorinated bicycloheptene contact insecticide bromocyclen (trade-names Bromodan, Alugan) is the Diels-Alder adduct of hexachlorocyclopentadiene and allyl bromide. Due to the very low mammalian toxicity, in Europe it is still widely being used against ectoparasites for the treatment of domestic animals (ref.[3], and literature cited therein).

It is important to note that most of the cyclodiene-derivatives and their metabolites mentioned above are chiral. As it is well-known that biological transformation, degradation, accumulation and excretion of chiral compounds can be stereoselective, enzymatic processes can basically be studied by determining enantiomeric excesses at different trophic levels in marine and terrestrial ecosystems[4,5]. König et al. were the first to separate cyclodiene-derivatives including *cis*- and *trans*-chlordane, oxychlordane, heptachlor and heptachlor-epoxide[6], and to determine the order of elution and the optical rotation of these compounds by (semi-)preparative enantiomer separations using packed columns containing modified

cyclodextrins[7,8]. Meanwhile, several research groups have become interested in the investigation of the distribution and fate of chiral xenobiotics by applying enantioselective gas chromatography with cyclodextrin derivatives as chiral stationary phases[2,4,9-11], because the enantiomers often exhibit different activities, toxicities and metabolic pathways.

In the present paper, we shall address the question how the enantioselective degradation and/or accumulation effects of chlordane and heptachlor, as reported for a few individual samples thus far, will show up at different trophic levels of marine and terrestrial biota. Special emphasis will be placed on the question as to whether or not oxychlordane and *cis*-heptachlorepoxide can permeate through the blood-brain-barrier [BBB] enantioselectively as observed earlier for α-HCH.

2. Experimental

The samples were stored in a refrigerator at about 248 K prior to sample preparation. Then, the samples were defrosted and homogenised with anhydrous Na_2SO_4 (1:3) supplying a powder that was extracted in a Soxhlet apparatus for eight hours with 200 ml *n*-hexane of analytical-reagent grade. The extract was concentrated to 1 mL at 360 mbar and 313 K followed by column chromatography over a partially deactivated Al_2O_3-column (5 % water; for details see ref.[12]). After elution with 80 ml *n*-hexane and subsequent concentration of the eluate to 1 mL, higher-molecular lipids were separated by means of conc. H_2SO_4 of analytical-reagent grade ("H_2SO_4 clean-up"). The remaining *n*-hexane solution was concentrated by a weak nitrogen stream to 200 µL and then fractionated over a LiChrosorb 100 Si-column (200 x 8 x 4 mm; partical size 5 µm; Merck) by high-performance liquid chromatography (HPLC) using a L-6200 pump of Merck-Hitachi and *n*-hexane as mobile phase (flow rate 1 mL/min).

The cGC analysis was performed on a HP 5890 gas chromatograph (Hewlett-Packard) equipped with a DB 5 and DB 1701 fused-silica capillary column (both 60 m, 0.25 mm i.d., film thickness 0.25 µm). Temperature program: 393 K, 2 min → 20 K/min → 443 K → 3 K/min → 483 K → 1.5 K/min → 543 K, 15 min; carrier gas helium (120 kPa); split/splitless injection (1 µL, 60 s, 543 K). The chlordane compounds were analysed by a Mega 5300 gas chromatograph (Carlo Erba) equipped with a DB 608 fused-silica capillary column (25 m, 0.32 mm i.d., film thickness 0.25 µm). Temperature program: 393 K → 2 K/min → 523 K. Detection was carried out in all cases with a ^{63}Ni-electron-capture detector (ECD; make-up gas nitrogen).

For the enantiomer separation of *cis*-heptachlorepoxide and oxychlordane a 25 m fused-silica capillary column coated with heptakis(2-*O*-methyl-3,6-di-*O*-pentyl)-β-cyclodextrin (0.25 mm i.d., film thickness 0.125 µm) was used. Column temperature program: 323 K → 11 K/min → 388 K, 170 min isothermal; carrier gas hydrogen (60 kPa); on-column injection. The column was installed in a VEGA gas chromatograph (Carlo Erba) equipped with a ^{63}Ni-electron capture detector (ECD; make-up gas nitrogen).

The standards *cis*-heptachlorepoxide [66429-35-4] and oxychlordane [26880-48-8] were purchased from Promochem, Wesel, Germany. The solvents, *n*-hexane, acetone, toluene and petroleum ether of analytical reagent grade, were bought from Merck, Darmstadt, Germany.

3. Results and Discussion

Terrestrial Ecosystem

The concentration values of α-HCH as well as those of the cyclodiene metabolites oxychlordane and *cis*-heptachlorepoxide are summarized in Table 1. In general, the concentrations of all three compounds found in the livers of hare and roe-deer turned out to be relatively high, where, in the case of hare livers, the concentrations of the two cyclodiene metabolites were higher by about a factor of 4 and 8, respectively, as compared with the concentrations of α-HCH, while the values of the three compounds determined in roe-deer livers appear to be comparable. Comparison between the concentrations found in hare and

roe-deer, respectively, reveals that in roe-deer livers a higher enrichment of α-HCH takes place (factor 2 to 14), while the accumulation of the cyclodiene metabolites shows the same order of magnitude at these different trophic levels. As a consequence, no significantly different degradation processes of the cyclodiene derivatives can be inferred from a comparison of concentration values between hare livers and roe-deer livers.

However, a clear difference of the enzymatic degradation of the three pollutants investigated herein in hare and roe-deer livers is revealed by the enantiomeric ratios [ER] summarized in Table 1: in the case of the hare livers, the enantiomeric ratios of α-HCH are close to one and in part even inverted, i.e. some ER-values are higher and some values are slightly lower than one (ER ≡ 0.8 - 1.5). As for oxychlordane, the ER-values are also close to one (ER ≡ 1.0 - 1.5), though in most cases significantly larger than one. This implies a preferential accumulation of (+)-oxychlordane. The most pronounced degradation in hare livers can

Table 1:
Concentrations [μg/kg extractable organic matrix (EOM)] and enantiomeric ratios [ER] of α-HCH, oxychlordane, and *cis*-heptachlorepoxide in sea-gull (eggs), hare (liver), roe-deer (liver), and seal (liver, blubber, brain), respectively (n.d. ≡ not determined).

sample		α-HCH concentr. [μg/kg]	α-HCH ER [+/-]	oxychlordane concentr. [μg/kg]	oxychlordane ER [+/-]	*cis*-heptachlorepoxide concentr. [μg/kg]	*cis*-heptachlorepoxide ER [+/-]
TERRESTRIAL ECOSYSTEM							
hare (liver)	1	10	0.8	40	1.1	80	3.3
	2	10	0.8	20	1.0	80	2.5
	3	10	1.5	40	1.3	70	3.7
	4	10	1.2	40	1.3	100	2.6
	5	10	0.8	40	1.5	90	3.2
roe-deer (liver)	1	60	0.15	40	9	10	1
	2	140	0.06	60	12	40	2
	3	80	0.06	50	14	100	6
	4	100	0.03	30	11	40	2
	5	100	0.04	60	17	90	7
	6	50	0.07	40	12	70	9
	7	40	0.40	30	11	30	5
	8	20	0.35	10	7	20	5
MARINE ECOSYSTEM							
sea-gull (eggs)	1	1	0.7	48	2.3	n.d.	n.d.
	2	1	0.1	99	2.1	n.d.	n.d.
	3	1	0.2	15	1.8	n.d.	n.d.
	4	1	1.9	6	1.5	24	2.7
	5	1	9.3	3	1.5	9	1.6
seal 1: liver		20	1.47	200	0.57	60	0.06
blubber		20	1.50	430	0.66	70	0.14
brain		100	11.5	40	0.57	10	0.11
seal 2: liver		10	1.27	140	0.45	50	0.05
blubber		20	1.49	350	0.54	60	0.18
brain		30	23.8	10	0.48	10	n.d.

be inferred from the ER-values of *cis*-heptachlorepoxide (ER≡ 2.5 - 3.7), which also indicates a clear enantiomeric excess of the (+)-enantiomer. In contrast to hare livers, the ER found in roe-deer livers reflect a strong enzymatic degradation of all three pollutants: the ER-values of α-HCH range between 0.03 and 0.40 and show a weak negative dependence on the concentration of this pollutant (SPEARMAN-Rank-Coefficient: $r_S = -0.35$[13]). In the case of oxychlordane (ER ≡ 7 - 17) and *cis*-heptachlorepoxide (ER ≡ 1 - 9) a very clear correlation between the concentration of the respective pollutant and the enantiomeric ratios was found (SPEARMAN-Rank-Coefficients: oxychlordane $r_S = 0.92$; *cis*-heptachlorepoxide $r_S = 0.76$[13]). This result implies that enzymatic degradation both of chlordane and of heptachlor, or of their metabolites may be induced and/or enhanced by increasing the concentrations of these compounds. At this stage it cannot be decided as to whether or not the ER-values reflect a preferential degradation of the repective cyclodiene-enantiomer that forms the (+)-enantiomer of the metabolites. Alternatively, a faster degradation of (-)-oxychlordane and (-)-*cis*-heptachlorepoxide would also give rise to the enantiomeric excesses of the metabolites as observed in hare and roe-deer livers.

Marine Ecosystem

α-HCH and oxychlordane were found in all five sea-gull eggs investigated herein, while the concentrations of *cis*-heptachlorepoxide were below the detection limit in three eggs. The presence of *cis*-heptachlorepoxide shows that the transformation of both *cis*- and *trans*-heptachlor to *cis*-heptachlorepoxide is not only confined to mammalian biota, but it is also the exclusive transformation pathway in sea birds. While the concentrations of α-HCH and *cis*-heptachlorepoxide are significantly lower than those of hare and roe-deer livers, oxychlordane shows comparable concentration values.

The ER-values of oxychlordane and *cis*-heptachlorepoxide in sea-gull eggs are of particular interest: in both cases, the values are larger than one, indicating a preferential accumulation of the respective (+)-enantiomer. In contrast, the corresponding values determined in different tissues of seals were smaller than one (as will be discussed below). This may possibly suggest that sea-gulls are reflecting rather terrestrial characteristics than marine ones. This holds at least for the enzymatic processes that give rise to enantioselective accumulation of oxychlordane and/or *cis*-heptachlorepoxide.

The investigation of three different tissues of two seals aimed at the question whether or not oxychlordane and *cis*-heptachlorepoxide can permeate through the blood-brain-barrier [BBB] enantioselectively as observed earlier for α-HCH (see refs.[14,15], and literature cited therein). During a previous study of brain tissue from the harbour seal (*Phoca vitulina*) we encountered a surprising phenomenon: in brain samples from eight Icelandic seals we detected almost exclusively (+)-α-HCH (the values for the enantiomeric ratio were 55.6, 66.2 and six values of 100:0). In contrast, blubber tissue from the same animals yielded enantiomeric ratios of only 1.2 to 1.4. This result supported the widespread hypothesis that the BBB is also partially effective towards certain fat-soluble organic pollutants. The "blood-brain barrier" is normally understood as mechanisms which greatly inhibit the transport of *non*-lipid-soluble substances such as proteins from blood vessels into the surrounding interstitial nervous system tissue (glia) and the capillary endothelium. These mechanisms thus ensure a constant environment for the neurons. The respiratory gases carbon dioxide and oxygen, however, can cross the capillary walls with ease. The exact mechanisms involved, especially for the selective inhibition of entry for certain lipid-soluble pollutants into the brain tissue, still require more detailed study. Whether the barrier effect is enhanced by enzymes in the endothelial cells (enzyme barrier) also still appears controversial.

In order to link this earlier study with the present work about cyclodiene metabolites, in Table 1 the concentrations and enantiomeric ratios are given for both α-HCH and oxychlordane as well as *cis*-heptachlorepoxide as determined in liver, blubber and brain of the two seals investigated herein. A comparison between the ER-values is shown in Figure 1. The concentrations of α-HCH in brain of the two seals are clearly higher than those found

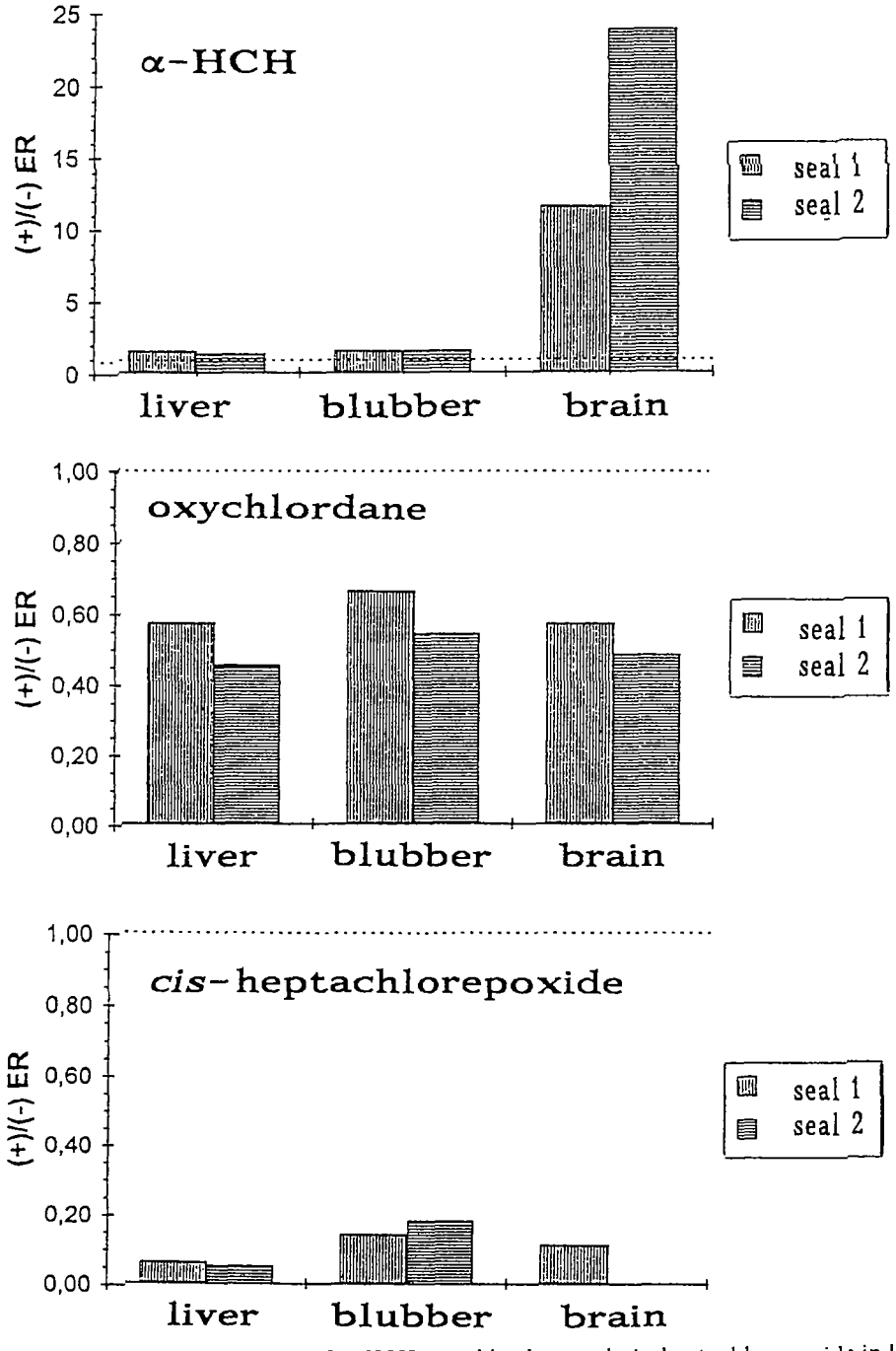

Figure 1: Enantiomeric ratios (ER) of α-HCH, oxychlordane and *cis*-heptachlorepoxide in liver blubber and brain tissue of two harbour seals (*Phoca vitulina*). The ER-values indicate that (+)-α-HCH can permeate through the blood-brain-barrier [BBB] enantioselectively, while the BBB is effective towards both enantiomers of oxychlordane and *cis*-heptachlorepoxide.

in liver and blubber of the same animals. In both cases, the ER-values in brain are significantly higher than those in the other two tissues. This result is in line with our earlier results summarized above, i.e., it reflects the enantioselective permeation of (+)-α-HCH through the BBB.

In contrast, the concentrations of oxychlordane in the brain tissues of the two seals are lower by about one to two orders of magnitude compared to liver and blubber tissues, while the concentrations of *cis*-heptachlorepoxide in brain tissue are lower by a factor of about five to seven. This implies that the BBB is effective towards these two metabolites by inhibiting their permeation through the cell walls.

Furthermore, both for oxychlordane and for *cis*-heptachlorepoxide the ER-values are largely the same in the three tissues, i.e., the "blood-brain barrier" inhibits the transport of both enantiomers from blood vessels into the surrounding interstitial nervous system tissue (glia) and the capillary endothelium equally effectively, or vice versa, no enantioselective permeation of oxychlordane and *cis*-heptachlorepoxide through the cell walls is encountered.

In conclusion, the permeation of the chiral environmental pollutants α-HCH, *cis*- and *trans*-chlordane, as well as *cis*- and *trans*-heptachlor and their metabolites, respectively, through the blood-brain-barrier shows extremely different characteristics. Therefore, the potential impact of these compounds on the central nervous system is expected to be of quite different relevance.

4. Literature

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