TIME-DEPENDENT ENANTIOSELECTIVE TRANSFORMATION OF α-HCH, *cis*-CHLORDANE, *trans*-CHLORDANE, AND HEPTACHLOR IN TURBOT LIVERS

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Abstract

Five experimental series were carried out with 25 turbots (*scophthalmus maximus* L.) each that were kept in basins filled with sea water under marine-like conditions. In order to follow the time-dependent enantioselective transformation of pollutants in the turbot liver, four different chiral compounds were injected intraperitoneally, including α-HCH, *cis*-chlordane, *trans*-chlordane, and heptachlor. Increased activity of the microsomal enzymes in the liver was simulated by addition of the planar model compound β-naphthoflavon (β-Nf). The initial addition of β-Nf did not result in strong additional enzymatic transformations in the turbot liver as evidenced by the shift of the enantiomeric ratios of α-HCH. But after having reduced the β-Nf concentration and thus potential toxic effects as well as the α-HCH concentration, an increasing strong shift of the ER values of α-HCH was observed during the experimental period. This observation is in line with the assumption that sea areas with a higher impact of various coplanar pollutants may induce MFO activities in flat fish livers significantly stronger than less polluted sea areas.

Introduction

Enantioselective analyses of flounder liver extracts suggested an induction of stronger microsomal enzymatic activity (mixed functional oxygenases ≡ MFOs) in sea areas with higher pollutant concentrations as compared with lower polluted areas.[1] This conclusion was based upon a comparison of the enantiomeric ratios of α-HCH determined in liver extracts from flounders caught in the Elbe River estuary in January 1991 (mean value of the enantiomeric ratio ER = 0.80 ± 0.05) and flounders caught in the less polluted Eider River estuary (mean ER = 0.89 ± 0.03). However, the authors emphasised that the effects observed based on a consistent, but small data set of eight flounders, and, furthermore, the effect was only slightly beyond the error limits. In order to verify this conjecture, systematic laboratory experiments with flat fish kept in closed water basins had to be carried out. In the present paper, the results will be summarised that were obtained from time-dependent enantioselective analyses of liver extracts from turbots (*scophthalmus maximus* L.) that were kept in basins filled with sea water under marine-like conditions.

Materials and Methods

The turbots were kept in basins of the *Biologische Anstalt Helgoland, Hamburg (BAH), Germany*, filled with sea water (salinity 3.55 to 3.59 %; water temperature 284.2 to 284,8 K). According to the experience of the BAH, turbots can be assumed to be representative of flat fishes of the German Bight, they can be kept in basins with a very low mortality rate, and they can be breeded thus delivering fishes with a very low background contamination. Both the background contamination of the water and of the fishes were determined prior to the start of the systematic experiments. The latter included five parallel series, keeping 25 turbots each in five different basins. In order to follow the enantioselective transformation of the pollutants in the turbot liver, four different chiral compounds were injected intraperitoneally, including α-HCH, *cis*-chlordane, *trans*-chlordane, and heptachlor. Increased activity of the microsomal enzymes in the liver can be simulated by addition of planar model compounds. In the present work, β-naphthoflavon was used which is known to induce the 7-ethoxyresorufin-*O*-deethylase (EROD) activity, which is determined as mmol resorufin/(min × mg microsomal protein)[2].

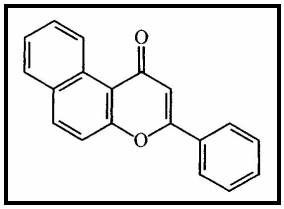


Figure 1:
Structure of β-naphthoflavon, used to induce 7-ethoxyresorufin-*O*-deethylase (EROD) activity[2].

The five parallel experimental series were carried out with 25 turbots each (mean weight about 130 to 140 g each) as follows:

1. series: intraperitoneal injection of a solution of α-HCH in sun flower oil (30 µg α-HCH/kg fish)
2. series: intraperitoneal injection of a solution of β-naphthoflavon in sun flower oil (25 mg β-naphthoflavon /kg fish)
3. series: intraperitoneal injection of a solution of β-naphthoflavon + α-HCH in sun flower oil (25 mg β-naphthoflavon /kg fish + 30 µg α-HCH/kg fish)
4. series: intraperitoneal injection of a solution of β-naphthoflavon + α-HCH + *cis*-chlordane + *trans*-chlordane + heptachlor in sun flower oil (25 mg β-naphthoflavon/kg fish, other compounds 6 µg/kg fish each)
5. series: control, intraperitoneal injection of pure sun flower oil

The above concentrations were chosen such that no increase in mortality was encountered. The experiments were carried out during a period of 100 days, catching turbots after 12, 21, 28, 42, 54, 77, and 97 days. The respective livers were separated in two aliquots, one of which was extracted and then subject to enantioseletive analysis of the chiral target substances, while the second aliquot was used for the determination of the EROD activity. The analysis of the extracts was performed by enantioselective gas chromatography using a chiral stationary phase. Details of the exact analytical conditions are specified in the Figure captions.

Results and Discussion

Orienting test experiments, carried out for 10 days, confirmed that the concentration of 30 µg α-HCH/kg fish did not exert any toxic effects, i.e., did not lead to any mortalities. The time-dependent development of the α-HCH concentrations in the turbot livers is summarised in **Table 1** for the five experimental series, while the respective results for *cis*-chlordane, *trans*-chlordane, heptachlor as well as the corresponding transformation products oxychlordane and *cis*-heptachloroepoxide are shown in **Table 2**.

Table 1: Time-dependent concentrations of α-HCH (in ng/g wet weight) in turbot livers as determined for the five experimental series; β-Nf ≡ β-naphthoflavon; Mix ≡ mixture of β-naphthoflavon + α-HCH + *cis*-chlordane + *trans*-chlordane + heptachlor; "-" ≡ not determined

Day	**Experimental Series**				
	1st Series α-HCH	**2nd Series β-Nf**	**3rd Series β-Nf + α-HCH**	**4th Series β-Nf + Mix**	**5th Series Control**
0	4.3	4.3	4.3	4.3	4.3
12	49.0	3.3	58.2	16.6	4.1
21	42.2	4.0	45.2	21.3	-
28	14.8	4.6	53.5	-	3.6
42	-	-	-	-	3.8
54	9.2	-	-	-	2.7
77	9.9	-	-	-	4.3
97	5.0	-	-	-	2.1

Table 2: Time-dependent concentrations of a mixture of β-naphthoflavon + α-HCH (see Table 1)+ *cis*-chlordane + *trans*-chlordane + heptachlor in turbot livers; n.d. ≡ below limit of determination

Compound	**Days**		
	0	**11**	**21**
cis-Chlordane	n.d.	24.7	31.0
trans-Chlordane	n.d.	13.7	13.4
Heptachlor	n.d.	14.2	12.0
Oxychlordane	n.d.	3.3	5.3
cis-Heptachloroepoxide	n.d.	2.8	4.3

The development of the α-HCH concentrations shown in **Table 1** suggests that no additional α-HCH input occurs by means of the β-naphthoflavon (series 2) or of the sun flower oil (controls, series 5) injections. Furthermore, the transformation of α-HCH seems to become dominating over the further accumulation in the turbot livers after about 12 days. In the course of the complete experimental period of about 100 days, α-HCH was nearly completely metabolised, finally reaching the original background concentrations between 4 to 5 ng/g wet weight. With regard to *cis*-chlordane, *trans*-chlordane and heptachlor a significant transformation to oxy-chlordane and *cis*-heptachloroepoxide also becomes obvious after about the same time as observed for α-HCH (**Table 2**, 11 days).

The time-dependent development of the EROD activity in turbot livers as determined for the five experimental series is shown in **Table 3**. As to be expected neither α-HCH (1st series), nor pure sun flower oil (5th series) is able to induce mixed functional oxygenases (MFOs) in the turbot livers. By contrast, presence of the coplanar β-naphthoflavon leads to a significant increase in EROD activity (see 2nd, 3rd, and 4th series). The reduction of EROD activity after about 21 days is tentatively interpreted as an increasing toxic effect of β-naphthoflavon. However, this conjecture has to be verified yet.

Table 3: Time-dependent development of the EROD activity (in mmol resorufin/(min × mg microsomal protein)) in turbot livers as determined for the five experimental series; β-Nf ≡ β-naphthoflavon; Mix ≡ mixture of β-naphthoflavon + α-HCH + *cis*-chlordane + *trans*-chlordane + heptachlor

Day	**Experimental Series**				
	1st Series α-HCH	**2nd Series β-Nf**	**3rd Series β-Nf + α-HCH**	**4th Series β-Nf + Mix**	**5th Series Control**
0	0.037	0.037	0.037	0.037	0.037
12	0.016	0.179	0.240	0.152	0.025
21	0.028	0.026	0.078	0.138	-
28	0.022	0.049	-	0.033	0.013
42	-	0.021	-	-	0.043
97	0.018	-	-	-	0.014

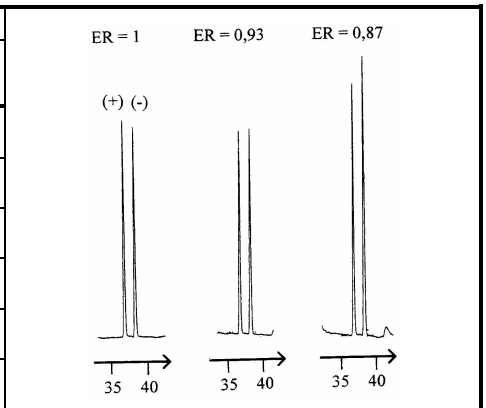
The time-dependent development of the enantiomeric ratios of α-HCH [(+)-/(-)-α-HCH] for the 1st, 3rd, and 4th experimental series is shown in **Table 4**. In all cases, the (+)-α-HCH enantiomer was preferentially transformed, where the addition of β-naphthoflavon did not give rise to an increased metabolisation during the first 12 days of the experimental period. However, in the 4th series the presence of β-naphthoflavon plus additional cyclodienes leads to a faster transformation of the (+)-α-HCH. This result is in line with the assumption that sea areas with a higher impact of various coplanar pollutants may induce MFO activities in flat fish livers significantly stronger.

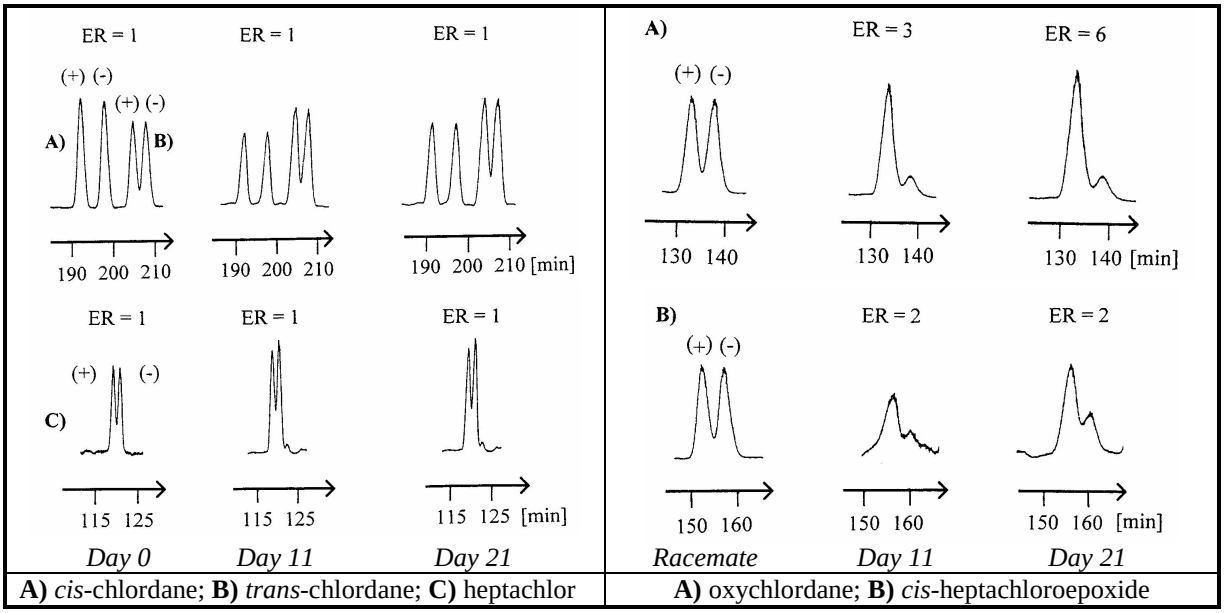
The time-dependent development of the enantiomeric ratios of the cyclodiene parent compounds and their transformation products as determined in turbot liver extracts is displayed in **Figure 2**. During a period of 21 days the ER values of the parent compounds remain 1.00 which indicates that their transformation in the liver is overcompensated by the transport of the injected racemate into the liver (**Figure 2, left hand side**). However, evidence about the actually occurring metabolisation process can be inferred from the results of the transformation products oxychlordane (ER = 6 after 21 days) and *cis*-heptachloroepoxide (ER = 2; **Figure 2, right hand side**).

In conclusion, the simulated induction of MFO activities by means of β-naphthoflavon (β-Nf) did not result in strong additional enzymatic transformation in the turbot liver as evidenced by the shift of the enantiomeric ratios. But this result may have been influenced by the choice of the concentration of β-Nf as suggested by its toxic effect after about 21 days and by the concentrations of the target compounds. The latter aspect is supported by the stronger shift of the ER values of α-HCH after having reduced the β-naphthoflavon concentration and thus potential toxic effects as well as the α-HCH concentration. In the latter case, the results are in line with the assumption that sea areas with a higher impact of various coplanar pollutants may induce MFO activities in flat fish livers significantly stronger.

Table 4, left: Time-dependent development of the enantiomeric ratios of α-HCH [(+)-/(-)-α-HCH] for the 1st, 3rd, and 4th experimental series; **right hand side:** enantioselective GC separation of the α-HCH enantiomers of turbot liver extracts after 0, 12, and 28 days for the 3rd series (β-Nf + α-HCH); 25 m fused silica capillary column; stationary phase 50 % w/w octakis(2-*O*-butyryl-2,6-di-*O*-*n*-pentyl)-γ-cyclodextrin in 50 % OV 1701 (LIPODEX E®), on-column injection, ECD-detection; carrier gas He 60 kPa; temperature program 333 K → 11 K/min → 423 K, 50 min

Day	Experimental Series		
	1st Series α-HCH	3rd Series β-Nf + α-HCH	4th Series β-Nf + Mix
0	1.00	1.00	1.00
12	0.93	0.93	0.81
21	-	0.89	0.79
28	0.80	0.87	-
54	0.61	-	-
77	0.57	-	-
97	0.55	-	-





A) *cis*-chlordane; **B)** *trans*-chlordane; **C)** heptachlor

A) oxychlordane; **B)** *cis*-heptachloroepoxide

Figure 2: Enantioselective GC separation of cyclodiene parent compounds (**left hand side**), and their transformation products (**right hand side**) of turbot liver extracts; 25 m fused silica capillary column; stationary phase heptakis(2-*O*-methyl-3,6-di-*O*-*n*-pentyl)-β-cyclodextrin, on-column injection, ECD-detection; carrier gas H_2 60 kPa; temperature program 323 K → 11 K/min → 388 K, 210 min (metabolites 170 min, resp.)

References

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2. Pfaffenberger B, Untersuchungen zur enantioselektiven Anreicherung von chiralen organischen Schadstoffen im marinen und terrestrischen Ökosystem, Ph.D. thesis, University of Hamburg, Germany, 1992, 182 pp.

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