

Syntheses, Enantioselective Separations of Photocyclodienes and their Prevalence in Ringed Seal Liver Samples

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Introduction

Persistent halogenated xenobiotics such as chlorinated cyclodienes are known to be subject to atmospheric long-range transports into Arctic and Antarctic regions [Arctic Monitoring and Assessment Programme (AMAP) 2004]. As a consequence, chlordane, a complex mixture of various chemically similar components, has been detected in biota of these remote areas. Among the major constituents of this mixture are *cis*- and *trans*-chlordane, *trans*-nonachlor and heptachlor. The chlordane components are metabolized in almost all organisms into two persistent epoxides, oxychlordane and heptachlorepoide. This biotic transformation pathway has been documented in many publications. However, much less effort has been devoted to the photoconversion products of the cyclodiene pesticides, which are being formed during the aerial transport. This may be surprising, because of their higher toxicities and stabilities in the environment in comparison with the parent compounds¹. For example, as compared to heptachlor, its abiotic photoconversion product photoheptachlor was found to be 47 times more toxic to bluegill, 19 times to rat, 4 times to housefly, and 264 times to goldfish. It is also much more toxic to bluegill (4 times) and rat (16 times than heptachlorepoide).

Although it was demonstrated in the laboratory that heptachlor can be transformed into the caged photoisomer photoheptachlor (**Fig. 1**) under ultraviolet irradiation at wavelengths longer than 290 nm, the early 1970s work failed to link photoheptachlor to the presence in the environment. The first possible occurrence in biological samples was proposed in the 1980s by Norstrom et al.³. However, due to lack of standards, the peak could be only tentatively identified as photoheptachlor. In 1993, Buser and Müller reported the presence of photoheptachlor and photo-*cis*-chlordanes in Baltic salmon, Baltic herring, and Antarctic penguin⁴.

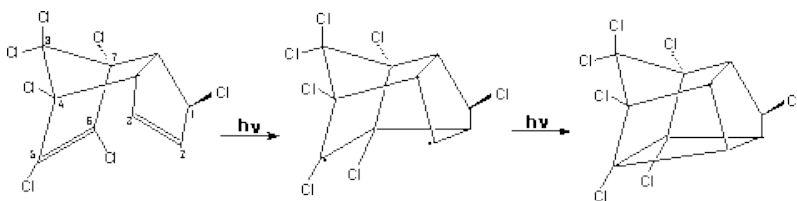


Figure 1: Photoconversion of heptachlor to photoheptachlor

In general, photochemical transformation of chiral xenobiotics is assumed to be largely non-enantioselective. This hypothesis forms the basis for a discrimination between biotic (enantioselective) and abiotic processes like photoconversion. Parlar and co-

workers were the first to report the application of enantioselective GC to the enantiomer separation of the chiral photoconversion products photodieldrin, photoheptachlor, photoheptachlorepoide, and photochlordene¹. In all cases, enantiomeric ratios of 1:1 were verified. Furthermore, Buser and Müller applied enantioselective gas chromatography to a reaction mixture of heptachlor, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, and the technical chlordane which had been exposed to natural sunlight in the presence of air⁴. The photoconversion products from heptachlor and *cis*-chlordane were identified by mass spectrometry as photoheptachlor and two photo-*cis*-chlordanes with caged and halfcaged structures, respectively. While photoheptachlor was formed in enantiomeric ratios of 1:1, in the case of the two photo-*cis*-chlordanes, which are formed in an approximate 2:1 ratio, only two partially resolved peaks were found in the GC chromatogram, which Buser and Müller attributed to the two isomers and not to any pair of enantiomers. As the separation of the enantiomers was unsuccessful in the latter instance, no judgement on the selectivity of this process was possible thus far.

In the present work, we synthesised photoconversion products of heptachlor, *cis*-chlordane, and dieldrin as reference material for their verification and quantification in environmental samples, including enantioselective separation and determination of enantiomeric excesses.

Materials and Methods

The syntheses of the photoconversion products of heptachlor, *cis*-chlordane, and dieldrin (**Figs. 1-3**) were carried out by photosensibilised UV-irradiation (5-6 hrs) of the parent compounds in acetone followed by a proper clean-up. The detailed procedure as well as H-NMR data can be found in ref.⁵. In the case of *cis*-chlordane (**Fig. 2**), the three conversion products (1,5)-, (2,5)- and (2,6)-photo-*cis*-chlordane were formed (relation 2.2 : 1 : 4.2). The formation of the (1,6)-congener is unfavourable because of the presence of a four-membered ring.

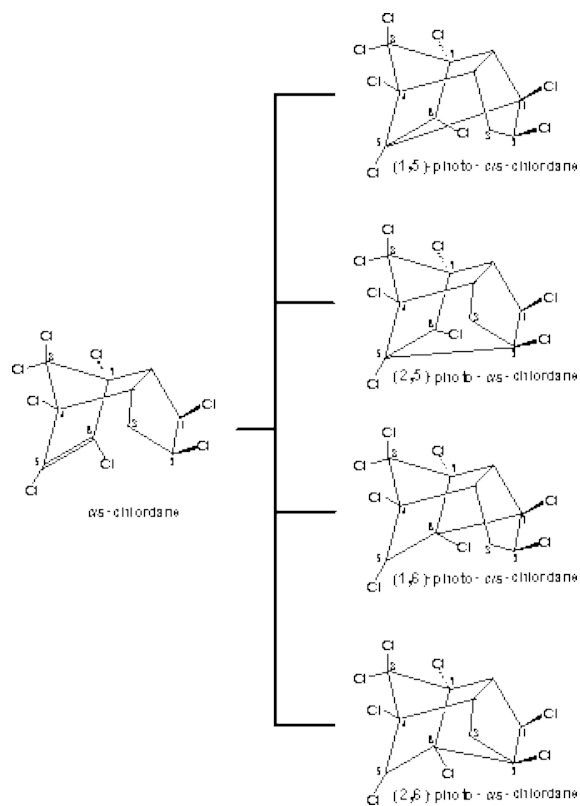


Figure 2: Photoconversion of *cis*-chlordane by photosensibilised UV-irradiation (300 nm).

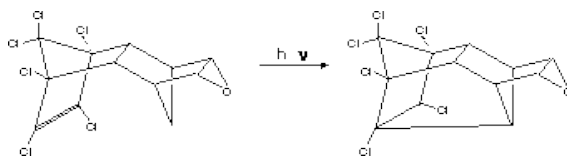


Figure 3: Photoconversion of dieldrin by photosensibilised UV-irradiation (300 nm).

The enantioselective gas chromatographic separations were carried out on a capillary column containing heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin/ OV 17 (50 % w/w) as stationary phase (length: 30 m; diameter: 0.32 mm; film thickness: 0.25 μ m).

Results and Discussions

In five liver extracts of Arctic ringed seals (*Phoca hispida*) caught east of Greenland in 1995, contents of photoheptachlor, (1,5)-photo-*cis*-chlordane, (2,5)-photo-*cis*-chlordane and photodieldrin in the lower mg/kg EOM (extractable organic matrix) range were detected (**Table 1**). The fact that these substances were generally identified in all sample extracts, in combination with their high toxicity and persistence, proved the environmental relevance of these photoconversion products and suggested their inclusion in further studies.

Table 1: Concentrations of photo-conversion products [mg/kg EOM] in liver extracts of five ringed seals (*Phoca hispida*) RS 1 – RS 5.

Photo-conversion Product	RS 1	RS 2	RS 3	RS 4	RS 5
	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]	[mg/kg]
Photoheptachlor	0.5	0.7	0.4	1.7	1.7
(1,5)-Photo- <i>cis</i> -chlordane	1.1	1.0	1.2	1.8	1.6
(2,5)-Photo- <i>cis</i> -chlordane	2.4	2.2	2.6	3.7	3.1
Photodieldrin	0.5	0.8	0.4	1.1	1.6

The enantioselective gas chromatographic separation of the photoconversion products investigated herein was carried out on a capillary column containing heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin/OV 17 (50 % w/w) as stationary phase. It is worth noting that in nearly all five cases a baseline-separation of the peaks was achieved (**Fig. 4**). Furthermore, the isomers of the three photo-*cis*-chlordanes were completely separated from each other on the chiral column, while the main products (1,5)- and (2,5)-photo-*cis*-chlordane were only partly separable on achiral columns (SE-54; DB 5 MS).

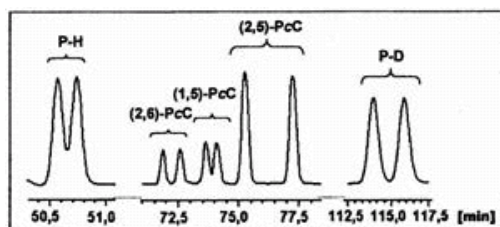


Figure 4: Enantioselective separation of standards of photoheptachlor (P-H), (2,6)-photo-*cis*-chlordane [(2,6)-PcC], (1,5)-photo-*cis*-chlordane [(1,5)-PcC], (2,5)-photo-*cis*-chlordane [(2,5)-PcC], and photodieldrin [P-D] by a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin/OV 17 column (50 % w/w).

The results obtained by enantioselective separation of photoheptachlor, (1,5)-photo-*cis*-chlordane, (2,5)-photo-*cis*-chlordane, and photodieldrin in ringed seal liver extracts are summarised in **Fig. 5** and **Table 2**. It is evident that these photo-conversion products that were formed as racemic mixtures in the atmosphere undergo further enzymatic transformation in ringed seals, despite their persistence. The enantiomeric ratios (ER) determined for photoheptachlor ranged between 1.20 and 1.31, while those for the other photo-cyclodienes investigated herein lay under 1.00.

Moreover, a correlation between the concentrations of the photo-cyclodienes and the corresponding enantiomeric shifts (ES), defined as $(ES) = |1 - (ER)|$, can be inferred from **Table 2**, which may give rise to different conjectures:

- The enantiomeric shift increases with increasing metabolisation of the photo-cyclodienes, because the activity of the enzyme responsible for the enantioselective transformation is being induced by higher concentrations.

· Different stereoselective transport processes occur in the organism, one of which is substance specific, while the other one is assumed to act along a concentration gradient.

It should be noted that the enantioselective separation of the parent compounds and their enzymatic transformation products in the present ringed seal extracts (not shown herein) revealed even stronger enantioselective processes, e.g., for *cis*-chlordane (ER) up to > 30. Moreover, the correlation of their enantiomeric shifts with their concentrations showed characteristics similar to those observed for the photo-cyclodienes. This may suggest that both the parent compounds, their enzymatic transformation products and the photoconversion products are being transformed by the same enzymatic systems within the ringed seal liver.

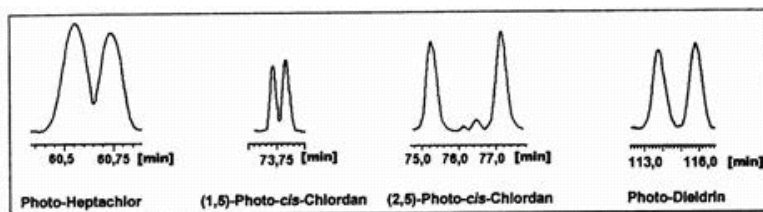


Figure 5: Enantioselective separation of photoheptachlor, (1,5)-photo-*cis*-chlordane, (2,5)-photo-*cis*-chlordane, and photodieldrin in ringed seal liver extracts by a heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)- β -cyclodextrin/OV 17 column (50% w/w).

Table 2: Concentrations [mg/kg fresh weight] and enantiomeric ratios (ER) of photoheptachlor, (1,5)-photo-*cis*-chlordane, (2,5)-photo-*cis*-chlordane, and photodieldrin in five ringed seal liver extracts RS 1 –RS 5.

Sample	photo-heptachlor		(1,5)-photo- <i>cis</i> -chlordane		(2,5)-photo- <i>cis</i> -chlordane		photo-dieldrin	
	Conc.	(ER)	Conc.	(ER)	Conc.	(ER)	Conc.	(ER)
	RS 1	0.019	1.20	0.041	0.79	0.089	0.82	0.019
RS 2	0.047	1.23	0.067	0.82	0.147	0.78	0.054	0.84
RS 3	0.021	1.21	0.064	0.81	0.338	0.72	0.021	0.81
RS 4	0.104	1.31	0.110	0.87	0.226	0.75	0.067	0.85
RS 5	0.066	1.25	0.062	0.81	0.121	0.80	0.062	0.96

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