

## ENANTIOMER SIGNATURES AS EVALUATION TOOL FOR SOURCE ELUCIDATION OF CHIRAL ENVIRONMENTAL POLLUTANTS IN HUMAN TISSUES

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### Introduction

In the early 1990s, enantioselective quantification methods for chiral pollutants were introduced into modern environmental chemistry. During the past decade method and instrumental development in enantioselective trace analysis has developed into a powerful tool for environmental risk assessment and source apportionment. Today, in environmental chemistry, the principles of chirality play an outstanding role in understanding biodegradation and transformation processes. Especially the development of modified  $\alpha$ -,  $\beta$ , and  $\gamma$ -cyclodextrin capillary columns for enantioselective gas chromatographic separation triggered significantly the progress in environmental trace analysis of chiral organic pollutants within the past decade<sup>1</sup>. Today, enantioselective trace analytical methods are widely accepted as versatile tools for risk assessment for a wide range of chiral contaminants. However, Canadian scientists showed that enantiomer signatures (enantiomeric ratios ER or enantiomeric fractions EF) can successfully be used for source elucidation and apportionment for both biota as well as for non-biotic environmental compartments<sup>2</sup>. Thus, not only bioavailability of chiral contaminants but also potential contaminant sources can be investigated through application of enantioselective trace analysis.

A study on the occurrence of POPs in 27 plasma samples of delivering women from the city of Arkhangelsk (Russia) was performed in 2001. High levels for  $\beta$ -HCH (hexachlorocyclohexane) and *p,p'*-DDT (1,1,1-trichloro-2,2-bis-(4-chlorophenyl)-ethane) were determined in these samples (table 1). The generally high levels of *p,p'*-DDT indicate fresh sources presumably technical DDT-mixture<sup>3</sup>. Enantioselective trace analysis was applied for additional information on potential sources for the elevated DDT and HCH values

**Table 1:** Arithmetic mean values for the selected POPs in blood plasma of delivering women from Arkhangelsk (Russia)

Compounds	Arithmetic mean concentration [ $\mu\text{g/L}$ ]	Concentration range [ $\mu\text{g/L}$ ]
<i>o,p'</i> -DDT (Dichloro-diphenyl-trichloroethane)	0.30	0.08 – 0.89
<i>p,p'</i> -DDT	1.16	0.28 – 5.07
<i>cis</i> -chlordane ( <i>cis</i> -CD)	0.07	0.02 – 0.33
<i>trans</i> -chlordane ( <i>trans</i> -CD)	0.16	0.03 – 0.66
$\alpha$ -HCH (hexachlorocyclohexane)	0.13	0.03 – 0.52
$\beta$ -HCH	4.59	1.27 – 11.59
Hexachlorobenzene (HCB)	0.57	0.18 – 1.40
Chlorobornane Parlar #26	0.05	0.04 – 0.10
Chloroborane Parlar #50	0.09	0.02 – 0.70

## Materials and Methods

The sample handling and preparation methods for the here presented study on human blood plasma of delivering women from Arkhangelsk (Russia) has been described earlier in detail<sup>3</sup>. For the enantioselective analysis in the human blood plasma samples, a Fisons Mega II 8560 gas chromatograph (Fisons, Milano, Italy) equipped with an electron capture detector (ECD) was used. *o,p'*-DDT as well as  $\alpha$ -HCH were analysed in the plasma samples and compared with the concentration values derived through gas chromatography –mass spectrometry measurements (GC/MS)<sup>3</sup>. As enantioselective capillary column, a commercially available BGB 172 column was used (BGB-Analytik, Schloßböckelhem, Germany, 30m x 0.25mm ID, 0.25 $\mu$ m film) with 30% *tert*-butyl-dimethyl-silyl- $\beta$ -cyclodextrin as chiral selector in 70%-PS086. An aliquot of 2  $\mu$ l sample eluate was injected at 60°C (2 min isotherm) . The temperature was increased with 10 °C/min to 180 °C. The second ramp was set to 250 (3 °C/min). The temperature was increased to 280 °C (15 °C/min) and held for 10 min before cooling down to 60 °C (initial temperature). The detector base was set to 300 °C and the detector was held at 320 °C throughout the analysis. Enantiomeric ratios were determined for *trans*-chlordane,  $\alpha$ -HCH and *o,p'*-DDT based upon area distribution.

## Results and discussions

From the total of 27 samples, 8 plasma samples were re-analysed for the determination of the enantiomeric signature for *trans*-chlordane,  $\alpha$ -HCH and *o,p'*-DDT (table 2) .

**Table 2:** Enantiomeric ratios for *cis*-chlordane,  $\alpha$ -HCH and *o,p'*-DDT in plasma samples from delivering women in Arkhangelsk (Russia).

Sample	ER (+/-) $\alpha$ -HCH	ER-(I/II) <i>o,p</i> -DDT	ER (I/II) <i>cis</i> -CD
1	2.1	1.1	5.0
2	7311	1.0	n.d.
3	2.5	1.0	4.5
4	10.8	1.1	1.8
5	2.4	1.0	n.d.
6	n.d.	0.9	n.d.
7	50.6	1.0	n.d.
8	167	0.3	n.d.

n.d. = not detected

The obtained concentration values (table 1) revealed clearly, that the distribution of the selected persistent organic pollutants (POPs) was clearly dominated by *p,p'*-DDT and  $\beta$ -HCH. However, neither *p,p'*-DDT and  $\beta$ -HCH are chiral substances but are important representatives of technical mixtures where chiral isomers are important constituents. Within the technical DDT mixture, earlier applied as insecticide but now banned for agricultural use in western countries, the *o,p*-DDT and *o,p'*-DDD are chiral compounds. Also the technical mixture of hexachlorocyclohexane-isomers (HCH) was earlier applied as insecticide mixture although the insecticidal effect was only attributed to  $\gamma$ -HCH (Lindane<sup>®</sup>) with about 15% of the technical mixture. 60% of the technical mixture is attributed to  $\alpha$ -HCH, the only chiral HCH isomer. The application of the technical HCH mixture as insecticide in agricultural applications is not allowed anymore in western countries, whereas the usage of  $\gamma$ -HCH is restricted. All chemicals have been released into the environment as racemic mixtures (enantiomeric ratio +/- = 1). Only enzymatic biodegradation processes or enantioselective transport through biomembranes can alter the enantiomeric ratio. Therefore it is

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assumed, that a racemic distribution of the enantiomers indicates a fresh, nearby source. Whereas  $ER \neq 1$  indicate sources where the enantiomeric ratio is altered by enzymatic biodegradation and/or enantioselective up-take.

For  $\alpha$ -HCH and *cis*-chlordane (*trans*-CD) significant enantiomeric excess of the first eluting enantiomer ((+)- $\alpha$ -HCH and (I)-*cis*-CD) was found. This is a clear indication that the  $\alpha$ -HCH and *cis*-CD contamination is due to "old" sources or long-range transport. The enantiomeric ratio has obviously been altered during the passage in the food-web before the chiral contaminants were taken up in the human organism through food consumption. However, additional changes in the enantiomeric ratio through biochemical transformation in the human organism cannot be excluded. Therefore, also for  $\beta$ -HCH as major source for the elevated levels, long-range transport and subsequent up-take in the food web can be assumed which lead to elevated levels in food products (e.g., marine fish and cattle). However, we cannot exclude, that imported food items may have significantly contributed to the elevated  $\beta$ -HCH values.

Except for sample number 8, all plasma samples revealed a racemic distribution ( $ER = 1,0 \pm 0,1$ ) for *o,p'*-DDT. Thus, the enantiomeric distribution is not yet altered neither by biochemical processes nor selective up-take through bio membranes. The racemic distribution of *o,p'*-DDT indicates a fresh source where the technical mixture of DDT was applied to agricultural land. The up-take of the racemic *o,p'*-DDT is obviously caused by the consumption of agricultural products (local or imported) where technical DDT was applied. Therefore, the elevated levels of *p,p'*-DDT, earlier reported, is obviously caused by the direct application of technical DDT to food products consumed by the delivering women who participated in the study.

### Conclusion

Sandanger et al. (2003) concluded, that the evidence of elevated levels for *p,p'*-DDT and  $\alpha$ -HCH in plasma samples from delivering mothers from Archangelsk (Russia) indicate fresh contamination from source nearby the living areas of this populations. However this conclusions must be modified based on the outcome of the here presented study. In general, the ratio of *p,p'*-DDT and *p,p'*-DDE (major transformation product) is usually used as indication for exposure to fresh or already environmentally degraded DDT mixtures. Predominant levels of *p,p'*-DDT always indicates long-term exposure to unchanged DDT and points thus towards newly application of DDT or intake to DDT-treated food items.

For hexachlorocyclohexane isomers the ratio of the major constituents  $\alpha$ - and  $\gamma$ -HCH is considered as good indicator for source elucidation. Predominant  $\alpha$ -HCH in biota (incl. humans) indicates the exposure to an unchanged technical mixture whereas elevated levels of  $\gamma$ -HCH indicate direct up-take through the respective food webs.

Thus, the application of enantioselective trace analytical methods can add additional significance to this type of interpretation. Based upon enantioselective analysis indication for different origins of the elevated levels of HCH and DDT in the plasma samples were found. Elevated levels of HCH were probably caused by the consumption of food products which was contaminated through direct up-take and biochemical ER change in the food web (e.g. fish and game) and reflect, thus, the normal exposure to this type of chemicals. However, the elevated DDT levels are obviously caused by the consumption of food products which were directly treated with technical DDT mixture as insecticide (agricultural application). This is indicated by the elevated levels of *p,p'*-DDT in combination with the overall racemic distribution of the *o,p'*-DDT enantiomers. However, based upon the here applied method, we cannot discriminate between the influences of local and imported food items on the elevated DDT and HCH levels. In addition, tissue specific enantioselective degradation processes may also contribute to the enantiomeric excess of the (+)- $\alpha$ -HCH in the plasma samples analysed.

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