Enantioselective Analysis of Organochlorines in the Arctic Marine Food Chain: Chiral Biomagnification Factors and Relationships of Enantiomeric Ratios, Chemical Residues and Biological Data

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Introduction

Persistent lipophilic organochlorines (OCs) tend to biomagnify, and are thus found at high concentrations at the top of the food chains. The polar bear (*Ursus maritimus*) food chain is simple due to the limited biodiversity in the arctic marine environment. The main food for polar bears is the blubber of ringed seal (*Phoca hispida*), which in turn consumes largely arctic cod (*Boreogadus saida*) and amphipods (1). Several OC pesticides, as well as their metabolites, are chiral and exist as two enantiomers, which may elicit quite different toxicological properties. The enantiomeric ratios (ERs) are racemic in technical products. However, ER changes may occur when chiral compounds are subjected to biotic processes. The ER changes seem to be highly species specific (2,3), and differences between organs and sexes have also been demonstrated (3,4). Chlordane and its metabolites (CHLs) are a group of chemicals, in which many chiral and highly biomagnifying compounds are found. For the hexachlorocyclohexanes (HCHs), α-HCH is the only chiral OC. The objective of the present study was to examine the ERs and the biomagnification of enantiomers of CHLs and α-HCH in the polar bear food chain. Relationships of ERs, OC residues (CHLs, HCHs, DDTs, chlorinated benzenes, sPCB, $sMeSO_2$-PCB, atropisomeric $MeSO_2$-PCBs etc) and biological (enzyme induction and cytochrom P450 content) data were examined. Differences between species, organs and sexes were studied.

Material and Methods

Whole body pools of arctic cod (n=2), blubber (n=11) and liver (n=7) of male and female ringed seals (RS) and fat (n=7) and liver (n=13) of male polar bear (PB) samples from Resolute Bay animals (Canadian high Arctic) were obtained for the study. The tissue sampling, the extraction, and clean up procedure are described in detail elsewhere (1,5). Two chiral columns were used for enantioselective analysis viz. Betadex-120 (Supelco) and BGB-172 (BGB Analytik) and for the quantitative OC analysis, a DB-5 column was used. Detection was by LRMS, and the analysis has been described in (6) and (7). The ER is defined as the peak area of (+)/(-)-enantiomers in the case where the optical rotations are known. Otherwise, the ERs are expressed as peak area of the first eluting enantiomer (E_1) / the second eluting enantiomer (E_2).

Results and Discussion

The cod showed near-racemic ERs for most of the compounds, indicating that bioconcentration take place without or with minor selective metabolism. Ringed seal and polar bear exhibited large ER changes. As (+)-α-HCH was transferred up the food chain it became more abundant relative to (−)-α-HCH (Figure 1a). The same trend was observed for E_2 relative to E_1 of the octachlordane MC5 (Figure 1b). Different food chain trends were also observed, e.g. for the octa- and nonachlordanes MC4 and MC6 (Figure 1b), heptachlor-*exo*-epoxide (HEPX) and oxychlordane OXY. The (+)-HEPX enantiomer was in excess in cod and polar bear, whereas the (−)-enantiomer dominated in ringed seal. The mean ER for OXY was similar for all species in the food chain. Preferential enantioselective metabolism and differential rates of metabolism are the most likely explanations for the ER differences within the food chain and between the fat and liver tissues. Apparent chiral and achiral BMFs were calculated (Table 1). Chiral BMFs provide new insight into OC biotransformation in food chains. For example, in the polar bear food chain the (+)-α-HCH biomagnify from seal to bear in contrast to (-)-α-HCH. Further, the difference in BMFs between enantiomers may be significant. The BMF of MC4-E_1 was 5-fold greater than MC4-E_2 from cod to seal.

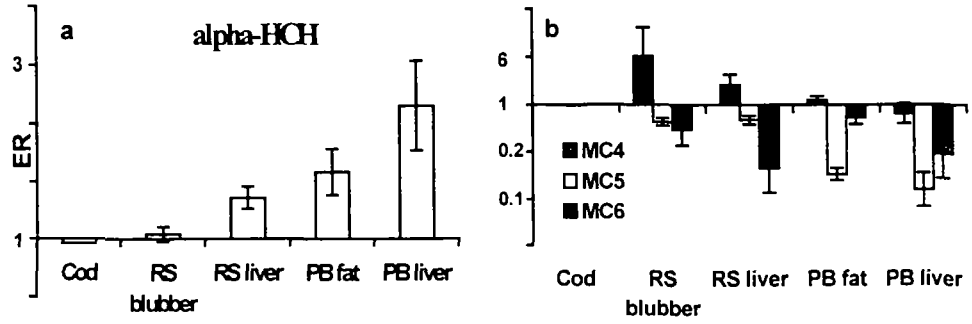

Figure 1. Average ER ± standard deviation of a) α-HCH and b) CHLs in the polar bear food chain.

Table 1. Apparent chiral and achiral BMFs in the polar bear food chain for α-HCH and chlordane metabolites (lipid weight basis).

	S-α-HCH	(+)-α-HCH	(–)-α-HCH	S-OXY	(+)-OXY	(—)-OXY	S-HEPX	(+)-HEPX	(–)-HEPX
Cod to seal	1.9	2.0	1.7	148	152	124	6.8	4.8	9.3
Seal to bear	1.1	1.4	0.7	6.6	7.1	6.7	4.3	7.4	2.3
Cod to bear	2.0	2.8	1.2	970	1075	834	29	35	21

Cod: Whole body arctic cod; Seal: Ringed seal blubber; Bear: Polar bear fat

The data was comprised of 40 objects (samples) and x 59 variables (ERs, OC residues and biological data for the cod, seal blubber, seal liver, bear fat, and bear liver respectively). Enzyme induction and cytochrom P450 content were measured for the polar bear livers exclusively, and $MeSO_2$-PCBs were the only chemical residues determined for the seal livers. In total, the percentage of missing values of chemical residues and ERs was 25%.

Multivariate projection methods, such as Principal Component Analysis (PCA), provide a means by which patterns and trends in complex data may be analyzed and interpreted. Thus, in order to get an overview of the data and find outliers, PCA was used. In the model, the chemical residues and the ERs and all objects were included. The first and second principal components (PCs) of the model explained 48% and 17% of the variance in the data respectively. In the score plot of PC1 and PC2 (Figure 2), the clusters of objects clearly reflects the sample categories, i.e. the cod, seal blubber, seal liver, bear fat and bear liver, with one outlier only (bear liver D). Important variables for the PC1 were the ERs of MC4, MC5 and MC6 (negative correlation) and a group of mainly polar compounds viz. $MeSO_2$-PCBs, OXY and HEPX (positive correlation). For the PC2, DDT and DDE (negative correlation), PCBs, 3-$MeSO_2$CB91 and the ER of trans-CHL (positive correlation) contributed most to the separation.

Partial Least Squares – Discriminant Analysis (PLS-DA) was used to investigate differences between organs and between sexes, and also to find out whether the variation in ERs contributed to the separation of the sample categories. In PLS-DA an indicator matrix (1/2) reflecting class structure is generated and the existing differences between classes can be revealed. Three cases, with two classes in each, were studied viz. 1) seal blubber and liver 2) bear fat and liver and 3) female and male seals. In all three cases significant PLS models were found either by using the ER variables only, or by using both ERs and OC residue data.

The initial step for many non-planar OCs (including the chiral α-HCH and CHLs) metabolism is probably a CYP2B-type enzyme-mediated process. Enantiomers may induce enzyme formation differently, and/or be metabolized by the same enzyme differently. It was therefore of interest to examine the dependence of the CYP2B-content variable derived from the bear liver samples (y) to the chiral and chemical residue data (x). A PLS model was created with the CYP2B-content as dependent variable, and the OC residue and ERs as predictor variables. It was found that for some of the chiral compounds (e.g. MC6 and MC4), the concentration of one enantiomer was more important for the model than the level of the other enantiomer.

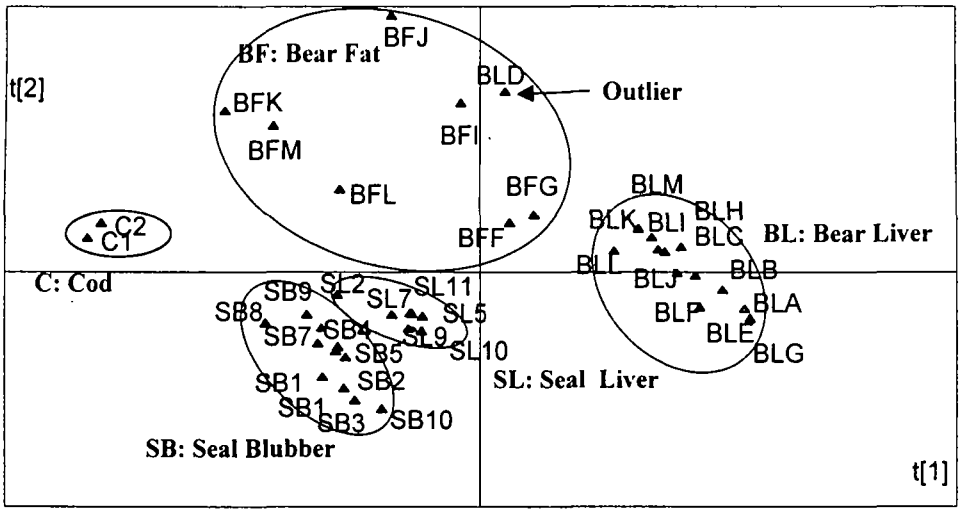

Figure 2. The score plot of PC1 and PC2 for the objects in the polar bear food chain.

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