Chapter 3

Comparison of the Enantiomer Distribution of Chiral Organochlorine Contaminants in Captive West Greenland Sled Dogs and Polar Bears from Baffin Bay

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Enantioselective analysis is a sensitive means of determining contaminant biotransformation, and is essential for proper assessment of the risk posed by chiral pollutants. Similar enantiomer distributions of chiral organohalogen compounds (OHCs) such as hexachlorocyclohexane (HCH) and chlorinated biphenyls (CBs) were previously reported amongst wild *canoidea* species. Therefore, we investigated the comparative enantioselective biotransformation capabilities for chiral OHCs between captive West Greenland (Baffin Bay) sled dogs (*Canis familiaris*) and free-ranging polar bears (*Ursus maritimus*) from Baffin Island, Canada, to understand bioaccumulation dynamics of individual enantiomers within top-predator

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species better, and to examine the feasibility for cross-species comparisons of enantiomer distributions. Enantiomer fractions (EFs) and enantiomer-specific biomagnification factors (BMFs) of OHCs were determined in West Greenland sled dog adipose, liver, thyroid, adrenal, and brain tissues after exposure to OHC-contaminated minke whale (*Balaenoptera acutorostrata*) blubber (exposed cohort) or pork fat (control cohort) for 20 months. Sled dogs biotransformed chiral OHCs enantioselectively. BMFs of (+)-oxychlordane, (-)-α-HCH, and (-)-CB 149 were 1.8-fold, 3.3-fold and 2.5-fold greater than their antipodes, respectively, leading to an enrichment of these enantiomers in sled dog adipose tissue relative to minke whale blubber. Non-racemic distributions of OHCs in two toxicologically sensitive tissues, thyroid and adrenal glands, are also reported for the first time. Except for CB 91, all OHC EFs were similar between Baffin Island polar bears and captive sled dogs. A comparison of enantiomer-specific BMFs revealed similar bioaccumulation dynamics for oxychlordane and heptachlor epoxide between the two species, but contrasting results for α-HCH, despite similar α-HCH EFs. Enantiomer-specific BMFs provide more reliable information for making cross-species comparisons of enantiomer distributions in biota than EFs alone, particularly for related species (e.g., *canoidea*). However, caution should be exercised in making such comparisons without knowledge of enantiomer distributions in underlying foodwebs.

Introduction

Polar bears (*Ursus maritimus*) are apex predators within arctic ecosystems. They biomagnify high concentrations of organohalogen compounds (OHCs), such as certain polychlorinated biphenyls (PCBs, also CBs), organochlorine pesticides, brominated flame retardants, and persistent metabolites of these compounds in their adipose tissue, internal organs, and blood relative to lower trophic level arctic species (*1*). Concentrations of OHCs in polar bears have been correlated with levels of a variety of biomarkers (e.g., endocrine and immunological) (*1*). For instance, a negative correlation was shown to exist between OHC concentrations and plasma testosterone concentrations in free-ranging male polar bears (*2*). While such correlations suggest possible deleterious effects from exposure, uncertainty in factors such as age, health, genetic variation, reproductive status, and other life history variables of wild polar bears leaves the direct cause and effect relationship between contaminant body-burdens and observed toxicological impairments for polar bears unclear (*3*). It is impractical and ethically problematic to conduct experiments on captive polar bears, and thus studies on a model surrogate species are needed to elucidate such relationships.

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Recently, the sled dog (*Canis familiaris*) has been investigated for use as a surrogate species to study the bioaccumulation, fate and effects of OHCs and other environmental contaminants in wild polar bears, as well as other members of the *canoidea* superfamily, including arctic fox (*Alopex lagopus*), arctic wolf (*Canis lupus arctos*), and wolverine (*Gulo gulo*). The captive West Greenland sled dogs that are the subject of part of the present study were fed wild minke whale (*Balaenoptera acutorostrata*) blubber, which was naturally contaminated with OHCs, for nearly two years to simulate natural exposure conditions. Results have shown that dogs from the exposed cohort exhibited similar biomarker endpoint changes as those reported for wild polar bears for a number of health related parameters, such as vitamin status (*4*), sex hormone homeostasis (*5*), and cellular immune response (*6*). A comprehensive review of the comparative health and toxicological findings in wild polar bear and surrogate model species can be found elsewhere (*3*).

Species-specific metabolic differences, due to dissimilarity in cytochrome P-450 (CYP) isozyme substrate specificity, catalytic activity, or expression levels between species, may lead to a differential accumulation or fate of contaminants, or result in varying degrees of formation of toxic metabolites. Such species-specific differences in biotransformation capacity and bioaccumulation of OHCs may thusly affect the toxicological response, and make cross-species extrapolations of toxicity difficult. Investigations into the comparative fate of OHCs between sled dogs and polar bears found both similarities and differences between species (*7*). Although similarities existed in OHC concentrations between the two species, differences in the inferred biotransformation capability and bioaccumulation of contaminant classes and individual compounds led to variation in contaminant and metabolite patterns between polar bears and sled dogs (*7*).

Enantioselective analysis provides a means of quantifying and observing biological processes that are often difficult to quantify using other techniques (*8–10*), and provides an additional method of comparing the bioaccumulation dynamics and biotransformation capabilities and pathways among species. Chiral compounds exist as pairs of non-superimposable mirror images (enantiomers). Environmentally relevant chiral OHCs include 19 atropisomeric PCB congeners (*11*), many components of technical chlordane and their metabolites, and α-hexachlorocyclohexane (α-HCH). These compounds were released into the environment as racemic mixtures (1:1 mixtures of enantiomers), and because enantiomers possess identical physical and chemical properties, only biological processes (e.g., metabolism, protein binding, active uptake/elimination) will alter the relative proportion of enantiomers in the environment. Within biota, differences in the toxicokinetic behavior between enantiomers will lead to a greater accumulation of one enantiomer over the other (*12*). In addition, biological effects may differ between enantiomers. For instance, (*R*)-(-)-*o,p′*-DDT enantiomer is a weak estrogen mimic, while (*S*)-(+)-*o,p′*-DDT has negligible estrogenic effects (*13*). Likewise, individual PCB enantiomers displayed differing potencies towards hepatic enzyme induction (*14*, *15*) and in the enhancement of cellular Ca^{2+} release (*16*). Thus, enantioselective analysis not only provides an

additional means of investigating the bioaccumulation dynamics of organisms, but is essential for the proper assessment of risk posed by chiral compounds.

The comparison of OHC enantiomer distributions among species is complicated. One of the most notable factors influencing inter-species differences is the enantiomeric substrate selectivity of the enzymes that catalyze metabolism. For instance, complete inversions of enantiomer enrichment have been found between closely related species in some laboratory experiments (*9*, *17*, *18*). Similarities were found in the enantioselective biotransformation of CBs 95 and 149 between arctic char and rainbow trout, although opposite enantiomer selectivity was reported for CB 136 between these two members of the *Salmonidae* family (*9*, *17*, *18*). Likewise, bearded seals (*Erignathus barbatus*) from the coast of Alaska were enriched in (-)-oxychlordane, while ringed seals (*Phoca hispida*) from the same location were enriched in the (+)-enantiomer (*19*). The interpretation of enantiomer distributions in a given predator species is also influenced by consumption of non-racemic quantities of chiral OHCs from prey items. Enantiomer distributions of OHCs were shown to vary in eggs and plasma of glaucous gull from three nearby breeding colonies in the Norwegian Arctic, and were likely a result of differences in enantiomer distributions of the selected OHCs in the preferential food sources of each colony (*20*). However, the degree to which the uptake of food-derived enantiomer distributions affects the measured distribution versus biotransformation within the predator has not been investigated.

The objectives of this study were two-fold. The first was to examine the enantiomer distribution of a suite of chiral OHCs in captive West Greenland (Baffin Bay) sled dog adipose, liver, thyroid, adrenal, and brain tissues after 20-month exposure to naturally OHC-contaminated minke whale blubber (exposed cohort) or pork fat (control cohort), in order to understand bioaccumulation dynamics and fate of individual enantiomers within top-predator species in a controlled experiment. Secondly, the enantiomer distributions of chiral OHCs were determined in adipose tissues of free-ranging polar bears from Canadian Baffin Bay subpopulations, and compared to those in West Greenland sled dog adipose tissues. The goal was to understand species-specific biotransformation capabilities for chiral OHC contaminants better, and to examine the feasibility of using surrogate models for cross-species comparisons of enantiomer distributions.

Materials and Methods

Experimental Design

This research was part of a larger study investigating the overall health effects of persistent environmental contaminants on West Greenland sled dogs. Further details on the experimental design are found elsewhere (*6*, *7*, *21–24*). This animal experiment was conducted under a license granted by the Self-Government of Greenland.

Sixteen 2-month old female sled dogs from the community of Aasiaat, Disco Bay, West Greenland, were divided into control (CON; $n = 8$) and exposed (EXP; $n = 8$) groups. Groups were composed of paired sisters, one in each

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group, to minimize age and genetic variation between groups. EXP dogs were exposed (20 month exposure period) to a daily diet of blubber from an individual minke whale collected off the west coast (Baffin Bay) of Greenland as part of a controlled Greenlandic native subsistence hunt. This blubber was naturally contaminated with a suite of OHCs and other environmental contaminants, including mercury and polybrominated diphenyl ethers (*6*, *7*), simulating real-world exposure to multiple contaminants experienced by wild *canoidea* species. The CON group received relatively non-contaminated pork fat, classified for human consumption, for the same feeding duration. Both cohorts were also fed an equivalent amount of standardized Royal Canin Energy 4300/4800 pellets (https://www.royalcanin.com) to fortify the diet with essential vitamins and nutrients not found in either food source. Daily intake of whale blubber or pork fat was 50-200 g/day, leading to an exposure of 10.4-11.7 μg/kg bodyweight of total OHCs in EXP dogs (*25*).

CON and EXP dogs were subjected to a variety of health-related toxicological tests throughout the experiment, including blood sampling and immune system challenges (*6*, *25*). However, such procedures are not expected to alter the relative biotransformation capacity of the two dogs in either treatment groups. Upon termination of the experiment, dogs (mean age 1.5 ± 0.1 years, range 1.5-2 years) were euthanized. Liver, thyroid, brain, adrenal, and subcutaneous adipose tissues were collected and stored at -20 °C until analysis.

Subcutaneous adipose tissue samples were collected from adult and juvenile polar bears (mean age 6.1 ± 3.0 years) from two subpopulations inhabiting Eastern Baffin Island, Canada: Davis Strait (southern Baffin Island; n =11) and Baffin Bay (northern Baffin Island; n =14). All samples were collected between October 2007 and May 2008 as part of Inuit subsistence hunts. Further details on dates and locations of sample collection, as well as sample handling procedures can be found elsewhere (*26*).

Extraction

Procedures for the extraction and cleanup of sled dog and polar bear tissue samples for PCBs, HCHs, and chlordane-related compounds have been described in detail previously (*7*, *27*, *28*). Briefly, samples were homogenized with Na_2SO_4 and extracted with either 1:1 acetone/*n*-hexanes (brain tissue) or 1:1 dichloromethane:*n*-hexanes (all other tissues). Lipids were removed with the addition of H_2SO_4 and analytes were fractioned into several chemical classes on a Florisil column. Polar bear adipose tissue was homogenized with sodium sulfate and extracted by pressurized liquid extraction, followed by gel permeation and silica gel chromatography prior to analysis (*26*).

Chemical Analysis

Non-enantioselective separation and quantification was performed on an Agilent 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph-mass spectrometer (GC-MS) with electron impact (EI) ionization, as previously described (*7*, *29*, *30*). Total chlordane (ΣCHL) concentrations

are reported as the sum of six chlordane compounds: Heptachlor epoxide (HEPX), oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, and *cis*-nonachlor. Total PCB (ΣPCB) concentrations in sled dogs and minke whale blubber are reported as the sum of 40 congeners (*7*), while ΣPCB concentrations in Baffin Island polar bears represent the sum of 74 congeners (*26*). A complete list of monitored congeners in both species has been published previously (*7*, *26*).

Enantioselective analysis of OHCs was carried out on a Thermo Trace GC Ultra gas chromatograph coupled to a Thermo DSQII mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA). PCBs were detected with electron impact ionization and selected ion-monitoring (*31*). The separation of PCB 95 and 149 enantiomers was achieved on a Chirasil-Dex column (25 m × 0.25 mm i.d. × 0.25 μm d_f, Varian, Walnut Creek, CA, USA) (*32*). A BGB-172 column (30 m × 0.25 mm i.d. × 0.18 μm d_f, Analytik, Adiswil, Switzerland) was used for the separation of PCB 183 enantiomers (*33*). All columns were calibrated with standard solutions containing all 209 PCB congeners to avoid coelutions with other homologous PCB congeners (*32*). OHC pesticides were detected in electron capture negative chemical ionization mode using previously described methods (*34*). Methane was used as the reagent gas at a flow rate of 2.5 mL/min. A BGB-172 was used for the separation of oxychlordane and HEPX. α-HCH, and *cis*-chlordane were separated on a Betadex-120 column (30 m × 0.25 mm i.d. × 0.25 μm d_f, Supelco, Oakville, ON, Canada).

Data Analysis

Model-fitting software (PeakFit v.4.0, Systat, San Jose, CA, USA) was used for deconvolution and integration of partially co-eluting chromatographic peaks (*35–37*). Enantiomer fractions (EFs) were used to quantify enantiomer distributions (*38*). For compounds with unknown enantiomer elution order (CB 95 on Chirasil-Dex and CB 183 on BGB-172) (*31*), the EF is defined as E1/(E1+E2), where E1 and E2 are the peak areas of the first-eluted enantiomer and second-eluted enantiomer, respectively. For all other analytes the EF was determined as the peak area of the (+)-enantiomer divided by the sum of the peak areas of the (+) and (-) enantiomers (*34*, *39*).

Enantiomer fractions and concentration data are presented as mean ± 1 standard deviation unless otherwise noted. Mean measured EFs of all racemic standards ranged from 0.493 to 0.499, depending on the analyte. Non-racemic EFs were determined by statistical comparison to racemic standards. Comparisons between and amongst groups were done using a Student's *t* test or one-way analysis of variance (ANOVA) with Tukey Honestly Significant Difference post-hoc test, respectively, with $\alpha = 0.05$.

Results and Discussion

Organochlorine Contaminant Concentrations

Elevated concentrations of chlorinated OHCs were detected in naturally contaminated minke whale blubber used for the exposed cohort diet, which

had ΣPCB concentrations and ΣCHL concentrations (mean ± standard error) of 1150±60 and 196±5 ng/g wet weight, respectively (*24*). None of the analytes were detected in the control cohort diet or dietary supplements (limit of quantification range: 0.01-1.5 ng/g wet wt., depending on the analyte). Exposure to contaminated minke whale blubber led to increased concentrations of all investigated OHCs in EXP cohort dog tissues. A detailed description of the tissue concentrations and accumulation patterns of OHCs and metabolites in sled dogs arising from exposure to contaminated minke whale blubber was recently published (*7*), and will not be reiterated here. It is important to point out, however, that after two years of exposure, adipose tissue concentrations (mean ± standard error) of ΣPCB and ΣCHL in EXP sled dogs were 2710 ± 500 ng/g wet wt. and 1480 ± 140 ng/g wet wt., respectively (*7*). These concentrations were 56-fold higher in ΣPCB and 43-fold higher in ΣCHL than CON dogs (*7*). Adipose tissue concentrations (mean ± standard error) of ΣPCB and ΣCHL in CON dogs were 48 ± 12 ng/g wet wt. and 34 ± 8 ng/g wet wt., respectively.

No differences in ΣPCB and ΣCHL concentrations were found between the Davis Strait and Baffin Bay polar bear subpopulations of Baffin Island (mean concentrations of 3080 ± 2770 ng/g wet wt. and 1150 ± 690 ng/g wet wt., respectively (*26*). In polar bears and sled dogs, ΣPCB contributed the most to the overall chlorinated OHC body burden. Additionally, oxychlordane was the predominant chlordane compound found in both species. A more detailed description of the congener and compound distributions in sled dogs and Baffin Island polar bears can be found elsewhere (*7*, *26*).

Distributions of OHC Enantiomers in Minke Whale Blubber

Enantiomer distributions of α-HCH, *cis*-chlordane, HEPX, and CB 91 were non-racemic in minke whale blubber (Figures 1 and 2). α-HCH was enriched in the (+)-enantiomer, while both HEPX and *cis*-chlordane were enriched in the (-)-enantiomer (Figure 1). A slight, although not significant, enrichment of (+)-oxychlordane (EF = 0.565 ± 0.022; $p > 0.05$) was also observed (Figure 1). Amongst PCB congeners, only the second-eluting enantiomer of CB 91 was enriched, while CBs 95, 149, 174, and 183 were racemic (Figure 2). This appears to be the first report of enantiomer distributions of chiral OHCs in minke whale blubber. Similar EFs for chlordanes, α-HCH and CB 149, both in the magnitude and the direction of enrichment were reported in bowhead whale from Barrow, AK (*19*). HEPX, on the other hand, was enriched in the (+)-enantiomer in bowhead whales (EF = 0.64) (*19*), in contrast to the enrichment of the antipode in this study.

Accumulation and Disposition of Enantiomers in Sled Dogs

Non-racemic distributions of all analytes except CB 174 were found in the tissues of EXP sled dogs (Figures 1 and 2). CB 95 was below the limits of detection, and therefore EFs could not be quantified. To increase the understanding of the sled dog enantioselective biotransformation and bioaccumulation capacity, biomagnification factors (BMF: concentration of each enantiomer in predator

divided by the concentration in the prey) from minke whale blubber to EXP sled dog adipose tissue were calculated on an enantiomer-specific basis. An inverse relationship exists between the BMF and the elimination rate constant of a compound, based on the equation BMF = $\alpha F/k_{el}$, where α is the assimilation efficiency (%), F is the feeding rate ($g_{food}\ g_{body\ weight}^{-1}\ day^{-1}$), and k_{el} is the elimination rate constant (day^{-1}) (*40*). Feeding rates should be identical for enantiomers, and assimilation efficiencies should also be identical due to the identical physical properties of enantiomers and the passive absorption and uptake of hydrophobic compounds (*41*). The latter assumption is supported by the observation that enantiomer-specific assimilation efficiencies were similar for α-HCH, *trans*-chlordane, CB 95, and CB 136 enantiomers in rainbow trout (*9*). Therefore, the ratio of the enantiomer-specific BMFs provides an approximation of the biotransformation rate constant of the more highly accumulated enantiomer relative to its antipode.

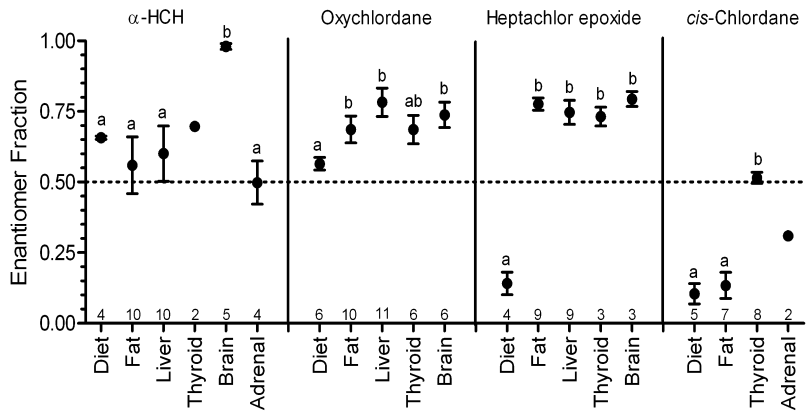

Figure 1. Enantiomer fractions (EFs) of chlorinated organohalogen compounds in exposed cohort sled dog tissues and diet (minke whale blubber). Points indicate mean value, while error bars indicate standard deviation. EF distributions sharing a letter designation are not statistically different. Dotted line represents theoretical racemic EF (EF = 0.5). Numbers above x-axis represent number of samples.

With the exception of (+)-α-HCH, both CB 149 enantiomers, and the first-eluting enantiomer of CB 183, all enantiomers had BMFs greater than 1 in EXP sled dogs (Table I), indicating a propensity for dogs to accumulate these enantiomers from the diet. Conversely, those enantiomers for which the BMF was less than 1 were not being accumulated, and thus were being biotransformed and/or eliminated more rapidly than they were being absorbed from the diet. Moreover, clear differences in enantiomer-specific bioaccumulation and/or biotransformation were evident. The BMF of (+)-oxychlordane was 1.8-fold

higher than that of (-)-oxychlordane, while BMFs of (-)-α-HCH and (-)-CB 149 were 3.3-fold and 2.5-fold greater than their antipodes, respectively. Clear accumulation of (+)-HEPX was also apparent, as the BMFs of (+)- and (-)-HEPX were 19.5 ± 5.9 and 1.1 ± 0.3, respectively. Although minke whale blubber was enriched in (-)-HEPX, the greater accumulation of (+)-HEPX by sled dogs resulted in an inversion in the enantiomer enrichment of HEPX between minke whale blubber and sled dog tissues (Figure 1). Similarly, CB 183 was racemic in minke whale blubber, but was highly enriched in the second-eluting enantiomer in sled dog adipose tissue (EF = 0.139 ± 0.067, Figure 2). The differences in biotransformation between individual OHC enantiomers led to a significant enrichment of the (+)-enantiomer of oxychlordane and HEPX, the (-)-enantiomer of α-HCH and CB 149, and the second eluting enantiomer of CBs 91 and 183 relative to the food. No change in the enantiomer distribution of *cis*-chlordane or CB 174 was found between the food and adipose tissue, resulting in similar BMFs between enantiomers for these two compounds. These changes in OHC EFs between minke whale blubber and sled dog adipose tissues provides strong evidence that sled dogs enantioselectively biotransformed chiral OHCs (Figures 1 and 2). The mechanism responsible for enantiomer enrichment in biota is unknown at the present time, although it has been suggested that CYP-mediated metabolism may be responsible based on the enantioselective metabolism of PCB congeners by isolated CYP isozymes *in vitro* (*42*, *43*). Time-dependent enrichments in the enantiomer distribution of OHCs occur in fish, invertebrates, and mice, further suggesting that metabolic processes may be responsible (*8*, *9*, *12*).

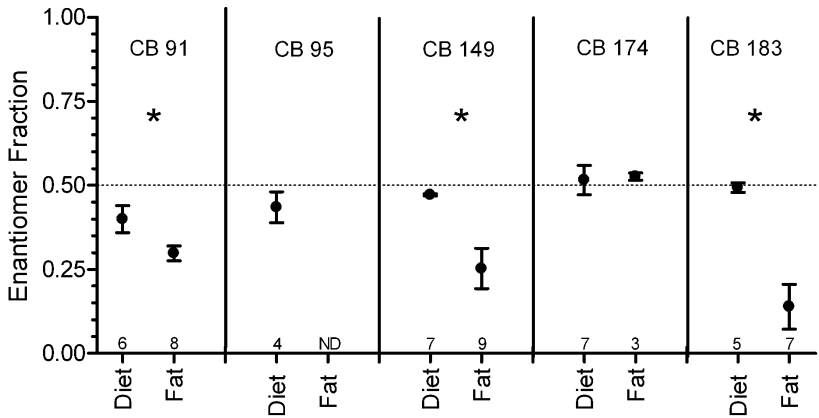

Figure 2. Enantiomer fractions (EFs) of polychlorinated biphenyls in diet (minke whale blubber) and sled dog adipose tissue. Points indicate mean value, while error bars indicate standard deviation. Asterisk indicates significant differences between diet and fat. Dotted line represents theoretical racemic EF (EF = 0.5). Numbers above x-axis represent number of samples. ND = non-detectable.

Table I. Biomagnification Factors (BMFs) and their ratios of individual organohalogen compound enantiomers from minke whales to West Greenland sled dog exposed (EXP) cohort and from ringed seal to polar bears from Resolute Bay, Canada

	Biomagnification Factors[a]			
	EXP Sled Dog		*Resolute Bay (Canada) Polar Bears*[b]	
	BMF	*Ratio*	*BMF*	*Ratio*
(+)-α-HCH	0.60 ± 0.2	3.3	1.4	2.0
(-)-α-HCH	2.0 ± 0.7		0.71	
(+)-HEPX	19.5 ± 5.9	17.7	7.4	3.2
(-)-HEPX	1.1 ± 0.3		2.3	
(+)-Oxychlordane	6.6 ± 4.1	1.8	7.1	1.2
(-)-Oxychlordane	3.6 ± 1.7		5.8	
(+)-*cis*-Chlordane	30.2 ± 13.5	1.2	0.0043	1.1
(-)-*cis*-Chlordane	24.3 ± 9.2		0.0047	
E1-CB 91	na		na	
E2-CB 91	na		na	
(+)-CB 149	0.010 ± 0.005	2.5	na	
(-)-CB 149	0.025 ± 0.008		na	
E1-CB 174	na		na	
E2-CB 174	na		na	
E1-CB 183	0.28 ± 0.15	6.7	na	
E2-CB 183	1.9 ± 0.10		na	

[a] Biomagnification factors (BMF) = concentration of each enantiomer in predator divided by the concentration in the prey. [b] Data from (*54*) E1 = first eluting enantiomer E2 = second eluting enantiomer na = data not available.

The second-eluting enantiomer of CB 183 was eliminated nearly 7 times slower than the first-eluting enantiomer, leading to a significant enrichment of the second eluting enantiomer of CB 183 in EXP sled dog adipose tissue. CB 183 is a metabolic precursor to 4-hydroxylated CB 187 (4-OH-CB187), a major OH-PCB congener in the blood of wildlife species, including polar bears (*27*, *44–46*). Concentrations of 4-OH-CB 187 in the present EXP sled dog plasma were reported to be nearly three-fold higher than the second most abundant OH-PCB congener, and represented approximately 25% of the total burden of OH-PCBs in sled dogs (*7*). Similarly, high concentrations of 4-OH-CB 187 have also been found in polar bears and other wildlife species (*27*, *44–46*). The large ratio of CB 183 enantiomer-specific BMFs in EXP sled dog adipose tissue and the

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enrichment of the second eluting CB 183 enantiomer compared to minke whale blubber suggest substantial biotransformation occurred, which may partially explain the high concentrations of 4-OH-CB 187 in EXP sled dog blood plasma.

Tissue-specific differences in the accumulation of individual enantiomers were also evident. The direction of enantiomer enrichment of chlordane compounds and α-HCH was similar amongst all tissues of the EXP cohort dogs, although some differences existed in the magnitude of enrichment (Figure 1). Extremely non-racemic EFs (EF = 0.960 ± 0.011) of α-HCH were found in the brain tissue, consistent with the highly enriched EFs of α-HCH found in brain tissues of other species, including seals (*47*), rats (*36*), mice and quail (*48*). Enantiomer-specific differences in α-HCH bioaccumulation have been attributed to selective uptake of the (+)-enantiomer across the blood-brain barrier (*36*). An enrichment of (+)-α-HCH was found in all tissues relative to the adrenal gland, which was racemic. Similarly, (+)-oxychlordane in the liver was enriched relative to the fat and thyroid, while the (-)-enantiomer of *cis*-chlordane in fat was enriched relative to the other tissues.

Differences in the accumulation of individual OHC enantiomers among tissues has been reported previously, both in wildlife and laboratory animals. Enantiomer fractions of *trans*-chlordane were different in rats between abdominal fat and the liver (*49*), while differences in the tissue distribution of oxychlordane enantiomers occurred in rats (*49*) and bowhead whales (*19*). In bowhead whales, the liver contained racemic proportions of *cis*-chlordane, while adipose tissue was significantly enriched in the (+)-enantiomer (*19*). Variation in the enantiomer distribution of chiral OHCs between liver and adipose tissue was attributed to metabolism or selective protein binding within the liver (*19*), although the exact mechanism has yet to be elucidated. However, this is the first time that enantiomer distributions of chiral OHCs have been determined in adrenal or thyroid tissue, and therefore the toxicological significance of such findings is unknown. Although no histological changes in either adrenal or thyroid tissue were found in exposed sled dogs (*3*), rats dosed orally with technical chlordane or *trans*-nonachlor (which is metabolized to *trans*-chlordane) developed hypothyroidism and pathological alterations of the thyroid gland (*50*, *51*). Therefore, evaluation of the enantiomer-specific toxicological effects of chiral OHCs and further investigation of the distribution of OHC enantiomers in sensitive tissues are warranted.

Enantiomer distributions of chiral OHCs were similar between CON and EXP cohort dogs for most analytes. This may be expected, due to the age and genetic similarity between CON and EXP groups. However, adipose tissue EFs of oxychlordane in CON dogs were significantly more enriched in the (+)-enantiomer than EXP dogs (Table II). This difference likely resulted from the uptake of more racemic enantiomer distributions of oxychlordane from minke whale blubber by EXP cohort dogs. Oxychlordane was not detectable in pork fat, and the EF of oxychlordane in minke whale blubber (Figure 1) deviated less from racemic than the distributions measured in the tissues of either EXP or CON dogs (Figure 1 and Table II). Enantiomer distributions in sled dogs and wildlife are likely at a steady-state balance between uptake of non-racemic proportions from the food and enantioselective biotransformation (*52*). Changes in the

distribution of OHC enantiomers in prey items or in the magnitude of exposure to non-racemic quantities will thus alter the enantiomer distribution within the consuming organism.

Table II. Enantiomer Fractions of chlorinated organohalogen contaminants in tissues of *canoidea* species

	CON Sled Dogs[a]	*EXP Sled Dogs*[a]	*Baffin Island Polar Bear*[a]	*Resolute Bay Polar Bear*[b]	*Wolverine*[c]	*Arctic Fox*[c]
α-HCH	0.700 ± 0.196	0.559 ± 0.100	0.639 ± 0.079	0.59	0.423 ± 0.020	0.414 ± 0.036
HEPX	nq	0.776 ± 0.022	0.725 ± 0.089	0.69	0.554 ± 0.019	0.732 ± 0.014
Oxychlor-dane	0.778 ± 0.040	0.686 ± 0.047	0.560 ± 0.067	0.62	0.712 ± 0.020	0.676 ± 0.019
cis-Chlordane	0.131 (n=1)	0.134 ± 0.046	0.299 ± 0.159	0.78	nq	0.607 ± 0.035
CB 91	0.321 (n=1)	0.300 ± 0.022	0.666 ± 0.150	nq	0.497 ± 0.022	0.546 ± 0.060
CB 149	0.315 (n=1)	0.258 ± 0.060	0.403 ± 0.104	nq	0.461 ± 0.030	0.535 ± 0.007
CB 174	0.540 (n=1)	0.526 ± 0.010	nq	nq	nq	nq
CB 183	0.072 (n=1)	0.139 ± 0.067	0.122 ± 0.028	nq	nq	nq

nq = not quantified. [a] Adipose tissue. [b] Adipose tissue data from (*54*). [c] Liver data (mean ± SD) from (*55*).

Comparative Enantiospecific Bioaccumulation

Except for oxychlordane, no differences were found in enantiomer distributions between Baffin Island polar bear sub-populations (data not shown). For oxychlordane, the difference in EF between the Davis Strait subpopulation (EF = 0.512 ± 0.049) and the Baffin Bay subpopulation (EF = 0.597 ± 0.063) may be attributable to differences in prey consumption. Davis Strait and Baffin Bay polar bear subpopulations were shown to feed at different trophic levels and employ different foraging strategies, based on stable isotope and dietary fatty acid analysis (*53*). For the purposes of comparing to sled dogs and other *canoidea* species, both Baffin Island subpopulations were combined into a single data set.

Enantiomer distributions of chiral OHCs have previously been determined in polar bears from Resolute Bay, Canada (*54*), as well in arctic fox and wolverines from the Canadian Arctic (*55*). The overall enantiomer profiles of chiral OHCs in the current study were comparable among Baffin Island polar bears, captive EXP

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sled dogs, and other arctic *canoidea* species (Table II). Similarities in chiral OHC enantiomer distributions amongst *canoidea* species have been noted previously by Hoekstra et al. (*55*) for arctic fox, wolverine, and Resolute Bay polar bears. The results from our study agree well with these previous observations based on visual inspection of the enantiomer distributions. Amongst the OHCs determined in all 4 species, the (+)-enantiomer of both oxychlordane and HEPX was enriched (Table II) in EXP sled dogs, arctic fox (*55*), wolverines (*55*), and polar bears (*54*). Despite similarities in the direction of enrichment, the magnitude of enrichment tended to vary amongst species. For example, the magnitude of enantiomer enrichment of oxychlordane ranged from an EF of 0.560 in Baffin Island polar bears to 0.712 in wolverines, while EFs of HEPX ranged from 0.554 in wolverines to 0.776 in EXP sled dogs (Table II).

The above comparisons, however, are based solely on measured EFs, with no knowledge of the enantiomer distributions within the underlying food web. To account for the dietary uptake of non-racemic distributions of OHCs, the comparative bioaccumulation dynamics and relative rate constants of individual enantiomers were investigated between sled dogs and polar bears from Resolute Bay (*54*) (which is to our knowledge the only data set in which prey species EFs were determined) using enantiomer-specific BMFs (Table II). The average BMF of (+)-oxychlordane between ringed seals and polar bears was 1.2-fold greater than the BMF of (-)-oxychlordane, similar to the 1.8-fold greater BMF of (+)-oxychlordane than (-)-oxychlordane between minke whale blubber and EXP sled dogs (Table I). BMF ratios of greater than unity in both species indicate a preferential elimination of (-)-oxychlordane, although at a rate that is only marginally slower than (+)-oxychlordane. In a similar vein, the BMF of (+)-HEPX in EXP sled dogs was 18 times greater than (-)-HEPX, while in polar bears this ratio was only 3.2 (Table I). In both species, the greater accumulation of (+)-HEPX led to a reversal in the EF between predator and prey, and ultimately to EFs of similar magnitude. Both HEPX and oxychlordane are persistent metabolites, and non-racemic distributions can arise due to the enantioselective metabolism of the parent compounds, or due to the enantioselective biotransformation/elimination of HEPX or oxychlordane itself. The predominant pathway leading to the non-racemic EFs is unclear, but given the comparability in BMF ratios of oxychlordane enantiomers between both species, it is apparent that similarities exist in the enantioselective bioaccumulation dynamics of oxychlordane between polar bears and sled dogs. Likewise, the similarity in enrichment of (+)-HEPX suggests similar mechanisms of enrichment (e.g., enzyme mediated degradation or elimination of HEPX), but differences in rate determining factors, such as catalytic enzyme activity.

Enantiomer distributions of several analytes varied among species. The enrichment of (-)-*cis*-chlordane found in sled dogs and Baffin Island polar bears contrasts the enrichment of the (+)-*cis*-chlordane observed previously in arctic fox (*55*) and Resolute Bay polar bears (*54*) (Table II). However, no alteration in *cis*-chlordane EF was observed between minke whale blubber and EXP sled dog adipose tissues, suggesting that sled dogs were not biotransforming *cis*-chlordane in an enantioselective manner. A similar lack of enantiomer enrichment of *cis*-chlordane was found between ringed seals and polar bears from Resolute

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Bay, Canada (*54*). If the other *canoidea* species similarly lack the ability to biotransform *cis*-chlordane enantioselectively, it is possible that the variation amongst species is a result of differences in the enantiomer distribution of *cis*-chlordane within prey items. Thus the observed EF may be a reflection of the enantiomer distribution within prey items rather than *in vivo* metabolism, as observed elsewhere (e.g., for glaucous gulls (*20*)).

Enantiomer fractions of α-HCH in EXP sled dogs were similar in magnitude and direction to those in Baffin Island polar bears and to α-HCH EFs previously reported for polar bears from Resolute Bay, Canada (*54*), although the enrichment of (+)-α-HCH in polar bears and EXP sled dogs contrasts the enrichment of (-)-α-HCH in wolverines and arctic fox (*55*) (Table II). Arctic fox are phylogenetically closer to polar bears than to sled dogs, consistent with our EF observations. The enrichment of (+)-α-HCH in EXP sled dogs and polar bears from both Resolute Bay and Baffin Island suggests similarities in the enantioselective biotransformation capabilities between species. However, as already discussed, (+)-α-HCH was enriched in the tissues of EXP sled dogs due to the consumption of minke whale blubber enriched in the (+)-enantiomer, as the greater BMF of (-)-α-HCH than (+)-α-HCH indicates EXP sled dogs were preferentially eliminating (+)-α-HCH. In contrast, a greater BMF of (+)-α-HCH relative to (-)-α-HCH in Resolute Bay polar bears clearly demonstrates differences in the enantioselective biotransformation and bioaccumulation dynamics between these two species. In addition, the contrasting enantiomer-specific BMFs between the two species, despite similar EFs, further highlights the influences of dietary uptake on the interpretation of EFs in biota.

Enantiomer distributions of CBs 91 and 149 have been measured in arctic fox (*55*), wolverine (*55*), polar bears, and sled dogs, while EFs of CB 183 have only been determined in EXP sled dogs and Baffin Island polar bears. CB 149 was enriched in the (-)-enantiomer in sled dogs, polar bear, and wolverine, but was enriched in the (+)-enantiomer in arctic fox. Similarly, biotransformation of CB 91 in EXP sled dogs resulted in an enrichment of the second eluting enantiomer of CB 91, whereas the antipode was enriched in polar bears and arctic fox (Table II). Enantiomer distributions of PCBs have not been determined in both wild *canoidea* species and their prey, precluding the comparison of enantiomer-specific BMFs between EXP sled dogs and other *canoidea* species. However, racemic distributions of CB 149 and a considerable enrichment of the second eluting enantiomer of CB 91 (EF = 0.063) were found in ringed seals from the Northwater Polynya (NOW) (*56*), a nearby area located on the northern tip of Baffin Bay. Assuming similar enantiomer distributions in the ringed seals from Baffin Island, the enrichment of antipodes of CB 91 between the polar bears and sled dogs suggests differences in the enzyme systems involved in the biotransformation of CB 91. However, without knowledge of the true enantiomer distributions in ringed seals from Baffin Island, this conclusion remains speculative.

No differences were found in the enantiomer distribution of CB 183 between EXP sled dogs and Baffin Island polar bears (Table II). As discussed earlier, metabolism of CB 183 may lead to formation of 4-OH-CB 187, although 4-OH-CB 187 may also be formed metabolically from CB 187. A greater ratio of CB 187 + CB 183 to 4-OH-CB 187, a metric of inferred biotransformation

capacity, was found in East Greenland polar bears compared to EXP sled dogs (*7*). It was postulated that different species-specific biotransformation capacities might be involved in the metabolism of CB 183 between the two species (*7*). While the similarities in both direction and magnitude of the CB 183 enantiomer enrichment between the two species suggests similarities in enantioselective biotransformation of CB 183, other factors (e.g., 4-OH-CB 187 retention or greater metabolic specificity or activity towards CB 187) not investigated here may also play a role in the differences between species in 4-OH-CB 187 accumulation.

Conclusions, Perspectives, and Recommendations

Previous investigations into comparative non-enantioselective bioaccumulation dynamics between EXP sled dogs and polar bears from East Greenland found similarities in the bioaccumulation dynamics of compound classes (i.e. PCBs, chlordanes, etc.) between species, although the accumulation of individual compounds/congeners tended to vary (*7*). Differences were also found between species in OH-PCB metabolite retention and/or formation, with OH-PCB congener patterns in sled dogs being composed primarily of penta- and hexa-chlorinated congeners. Polar bears accumulated greater concentrations of OH-PCBs and the congener profile was dominated by hepta-and octa-chlorinated congeners (*7*). The species-specific congener/compound patterns and differences in metabolite formation were attributed to species-specific differences in biotransformation capacity (CYP enzyme content, activity and specificity), selective retention/excretion of congeners and compounds, or differences in dietary influence between species. Similarly, in this study, both similarities and differences in the enantioselective biotransformation and enantiomer accumulation were found between species. While the biochemical processes mediating the enantioselective biotransformation of OHCs are not well understood, similarities in the direction of enrichment between Resolute Bay polar bears and EXP cohort sled dogs suggests similarity of the substrate specificity responsible for the biotransformation of OHC contaminants between polar bears and sled dogs. However, differences in the relative rates of the biotransformation of individual enantiomers suggest dissimilarity in the rate determining processes (e.g., catalytic enzyme content and/or activity) between species. Due to the lack of data on the enantiomer distributions of chiral OHCs in many Baffin Island prey items of polar bears, arctic fox, and wolverines, it is unclear whether such a conclusion may be extrapolated to these species as well, although the similarities in direction of enrichment suggest that it may. However, differences in the enantiomer preferences in biotransformation of OHCs were also found, most notably in the reversal of enantiomer preference of α-HCH between sled dogs and polar bears. This observation indicates that sled dogs are not a completely accurate surrogate of polar bears, at least for α-HCH.

At present, the toxicological implications of enriched enantiomer distributions are unclear. *In vitro*, exposure of rat hepatocytes to (+)-α-HCH resulted in both higher cell death and greater induction of mitosis than (-)-α-HCH (*57*).

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The almost exclusive presence of (+)-α-HCH in sled dog brain tissues, and the enrichment of (+)-α-HCH in both polar bear and sled dog tissues, suggests the possibility of increased deleterious effects. Enantiomers of several chiral PCB congeners have been shown to induce drug metabolizing enzymes to different extents (*14*, *15*). *In vitro,* individual CB 84 enantiomers differed in potency for increasing translocation of protein kinase C from the cytosol to the cell membrane in rat cerebral granular cells (*58*), and the (-)-enantiomer of CB 136 increased the sensitivity of ryanodine receptors (*16*), which are broadly expressed Ca^{2+} release channels necessary in cellular signaling and muscle contractions. However, none of these PCB congeners were investigated in the current study, and aside from α-HCH, enantiomer-specific toxicological investigations of other analytes is lacking.

In conclusion, this study highlights several challenges in understanding and interpreting enantiomer distributions in biota. Changes in EF between CON and EXP dogs illustrate the role that dietary uptake of non-racemic proportions of chiral contaminants has on resulting EFs in biota, and/or exposure-induced differences in metabolic capacity. Moreover, the reversal in the relative enantiomer-specific biotransformation rate constants between polar bears and sled dogs, despite the similar observed EFs, demonstrates the danger in interpreting EFs in biota or making cross-species comparisons without knowledge of the enantiomer distribution in the underlying foodweb.

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