

ENANTIOMERIC FRACTIONS OF CHIRAL POLYCHLORINATED BIPHENYLS
PROVIDE INSIGHTS ON BIOTRANSFORMATION CAPACITY OF ARCTIC BIOTANICHOLAS A. WARNER,[†] ROSS J. NORSTROM,[‡] CHARLES S. WONG,^{*†} and AARON T. FISK[§][†]Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada[‡]Department of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6, Canada[§]Warnell School of Forestry Resources, University of Georgia, Athens, Georgia 30602–2152, USA

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Abstract—Chiral polychlorinated biphenyls (PCBs) were measured in an arctic marine food web from the Northwater Polynya (NOW) in the Canadian Arctic to examine potential biotransformation of chiral PCB atropisomers. Organisms under investigation included pelagic zooplankton, Arctic cod (*Boreogadus saida*), seabirds, and ringed seals (*Phoca hispida*). Previous studies using achiral methods (e.g., biomagnification factors) have shown that chiral PCB congeners biomagnified in the NOW food web, but provided little information about biotransformation of PCBs except in extreme cases (i.e., high biotransformation). In this study, highly nonracemic enantiomeric fractions (EFs) were observed in several seabird species and ringed seals, but racemic EFs were found in prey (zooplankton and fish). This suggests stereoselective and species-specific biotransformation of individual PCB stereoisomers by birds and mammals. These results are consistent with previously reported biotransformation activity of chiral organochlorine pesticides, α -hexachlorocyclohexane, and chlordane within these organisms. This study demonstrates the utility of using chiral analysis of PCBs to investigate biotransformation within biota of arctic food webs.

Keywords—Chiral polychlorinated biphenyls Biotransformation Enantiomer fractions Northwater Polynya
Food web

INTRODUCTION

Due to the hydrophobic nature of polychlorinated biphenyls (PCBs), organisms accumulate these chemicals from the surrounding environment, which can lead to biomagnification in organisms from lower to higher trophic levels [1,2]. The use of PCBs over the years has decreased since the manufacturing of these chemicals in North America ceased in 1977. However, these chemicals still are of concern due to their stability within the environment. Long-range transport and numerous volatilization and deposition steps have led to the global distribution of PCBs [3].

Chiral analysis is an area of study offering enhanced insight into biological processes affecting chemical pollutants. A chiral compound exists as pairs of nonsuperimposable mirror images called enantiomers. Chiral PCBs and most other chiral organohalogen chemicals are introduced into the environment as racemates (equal concentrations of enantiomers) because their synthesis is an achiral process. Nineteen of the 209 PCB congeners are substituted asymmetrically and are axially chiral (atropisomerism) due to restricted rotation around the C-C bond [4]. Enantiomers have the same physical and chemical properties, such as hydrophobicity, vapor pressure, and polarity, that are important for determining their behavior in the environment, but they may exhibit different biological [5,6] and toxicological properties [7,8]. Selective biotransformation of one enantiomer over the other can result in enantiomeric enrichment in an organism. Thus, chiral analysis may be useful for detecting and elucidating biological fate processes, particularly biotransformation, within organisms [6,9–16].

The Northwater Polynya (NOW) is a highly productive

open-water region in the northern Baffin Bay of the Canadian Arctic. The NOW food web consists of a variety of zooplankton and invertebrate communities, Arctic cod consuming invertebrates, seabirds (dovekie, thick-billed murre, black guillemot, northern fulmar, ivory gull, black-legged kittiwake, glaucous gull) feeding mainly upon invertebrates and forage fish, and marine mammals (ringed seals) eating mostly Arctic cod [17]. This region, and the Arctic in general, are ideal for studying trophodynamics of persistent and bioaccumulative organochlorine pollutants [2,11,16], including PCBs [2,15,18], in food webs due to the reduced diversity of species, long food webs that are similar across the region, and limited impact of pollution point sources [19]. Previous studies have reported trophic magnification and biomagnification factors for PCBs in the NOW [2]. However, little information on PCB biotransformation exists except in extreme cases.

The objective of this study was to investigate the fate of chiral PCBs within the NOW and to provide insight on biotransformation activity of PCBs among organisms occupying the different trophic levels. This is of particular significance, because the enantiomer composition of chiral PCBs at lower trophic levels within arctic marine food webs has received little attention. Enantiomer composition of chiral PCBs in higher trophic-level organisms, such as seals and birds, is of particular significance. These organisms have greater metabolic capabilities compared to zooplankton and fish and can provide insight into possible detoxification mechanisms in warm-blooded organisms. To date, chiral analysis of PCBs in seals is limited to a few studies [20,21] and in birds is limited to eggs [22]. To the best of our knowledge, this is the first report of direct chiral analysis of enantiomer composition of PCBs in zooplankton, fish, and seabirds in an ecosystem.

* To whom correspondence may be addressed
(charles.wong@ualberta.ca).

Table 1. Mean polychlorinated biphenyls (PCBs) enantiomer fractions \pm standard deviation data for Northwater Polynya (Canada) biota. Sample sizes for analysis are given in parentheses for each organism. Asterisks (*) represent enantiomer fraction values based on a single measurement. ND = not detected (correlates to concentrations less than 0.1 ng/g wet wt per analyte)

Organism	Enantiomeric fractions					
	PCB 91	PCB 95	PCB 149	PCB 174	PCB 176	PCB 183
<i>Calanus hyperboreus</i>	0.507 \pm 0.012 (n = 3)	0.490 \pm 0.001 (n = 3)	0.493 \pm 0.006 (n = 3)	ND	ND	ND
Arctic cod	0.524 \pm 0.001 (n = 2)	0.463 \pm 0.001 (n = 3)	0.509 \pm 0.036 (n = 3)	ND	ND	ND
Dovekie	ND	0.503 \pm 0.007 (n = 2)	0.111*	ND	ND	ND
Thick-billed murre	0.676*	0.742 \pm 0.078 (n = 5)	0.151 \pm 0.071 (n = 5)	ND	ND	ND
Black guillemot	0.191*	0.586 \pm 0.037 (n = 2)	0.200 \pm 0.032 (n = 5)	ND	ND	ND
Northern fulmar	0.623 \pm 0.009 (n = 3)	0.762 \pm 0.041 (n = 3)	0.418 \pm 0.045 (n = 5)	ND	ND	0.294*
Ivory gull	0.497*	0.840 \pm 0.015 (n = 2)	0.262 \pm 0.089 (n = 5)	ND	ND	ND
Black-legged kittiwake	0.542 \pm 0.162 (n = 4)	0.694 \pm 0.179 (n = 4)	0.403 \pm 0.008 (n = 5)	ND	ND	ND
Glaucous gull	0.137 \pm 0.024 (n = 5)	0.609 \pm 0.177 (n = 5)	0.277 \pm 0.052 (n = 5)	0.828*	ND	0.443*
Ringed seal	0.063 \pm 0.034 (n = 12)	0.849 \pm 0.072 (n = 12)	0.478 \pm 0.045 (n = 13)	0.590 \pm 0.055 (n = 12)	0.435 \pm 0.049 (n = 3)	0.461 \pm 0.042 (n = 12)

MATERIALS AND METHODS

Sample collection was carried out during the 1998 April to July voyage of the *CCGV Pierre Radisson*, and consisted of zooplankton (*Calanus hyperboreus*), Arctic cod (*Boreogadus saida*), ringed seal (*Phoca hispida*), and seabirds including dovekie (*Alle alle*), thick-billed murre (*Uria lomvia*), black guillemot (*Cephus grille*), northern fulmar (*Fulmaris glacialis*), ivory gull (*Pagophila eburnea*), black-legged kittiwake (*Rissa tridactyla*), and glaucous gull (*Larus hyperboreus*). Details of sample collection have been published previously [2,11].

Sample extraction, cleanup, and analysis are described elsewhere [15,18,23]. Briefly, sample tissues (whole organism for zooplankton and fish, liver tissues for birds, and blubber from ringed seals) were ground with anhydrous sodium sulfate and extracted by Soxhlet extraction using dichloromethane/hexane (1:1). Chiral analysis of PCBs 91, 95, 149, 174, 176, and 183 on a subset of the collected samples [2,11] of the species mentioned above were carried out by gas chromatography-mass spectrometry using a combination of columns: Cyclosil-B (J&W Scientific, Folsom, CA, USA), Chirasil-Dex (Varian, Palo Alto, CA, USA), and BGB-172 (Analytik, Adiswil, Switzerland). Details of analysis have been described elsewhere [24]. Chiral PCB compositions were expressed as enantiomer fractions (EFs) [25]

$$EF = \frac{E_1}{E_1 + E_2} \quad (1)$$

where E_1 and E_2 represent the concentrations of the first and second eluting enantiomers, respectively, for a given column when the enantiomer elution order was unknown (i.e., PCBs 91 and 95 on Chirasil-Dex, and PCB 183 on BGB-172 [24]). When the elution order was known (for PCB 149, the [−] enantiomer elutes first and, for PCBs 174 and 176, the [+] enantiomer elutes first on Chirasil-Dex [24]), enantiomer fractions were calculated as

$$EF = \frac{(+)}{(+)+(-)} \quad (2)$$

Racemic standards had EF values that ranged from 0.496 \pm 0.004 to 0.505 \pm 0.008 [26]. The 95% confidence interval (± 0.032) was used as a conservative measure of EF precision for the racemic standards (EF = 0.5) [26]. Enantiomer fraction distributions among different species were compared using a one-way analysis of variance with Tukey honestly significant difference tests run on all significantly different groups (overall $p < 0.05$).

RESULTS AND DISCUSSION

Zooplankton and Arctic cod

Chiral analysis of the omnivorous copepod *C. hyperboreus* showed racemic EF values of 0.507, 0.490, and 0.493 for PCBs 91, 95, and 149 respectively, but other chiral PCBs investigated were not detected (Table 1). Enantiomer compositions at the lower trophic levels (except PCB 95 in Arctic cod) were in agreement with other studies on zooplankton and fish for chiral organochlorine pesticides within the NOW and chiral PCBs within other Arctic regions [9–11]. Unfortunately, other invertebrate species could not be investigated in this study. Non-racemic EFs have been observed in mysids and amphipods in freshwater food webs for chiral PCBs [26]. It is unclear whether stereoselective degradation had occurred or whether the nonracemic EFs observed were from uptake from primary producers. Borga et al. recently reported nonracemic EFs for organochlorine pesticides in Arctic marine ice fauna, zooplankton, and benthos [27]. Although the authors could not rule out biotransformation, the nonracemic EFs observed most likely were due to accumulation from the water column and sediments.

Enantiomer fractions in Arctic cod for PCBs 91 and 149 were 0.524 and 0.509, respectively, which suggests no biotransformation and/or selective elimination of the enantiomers. However, a nonracemic average EF value of 0.463 was ob-

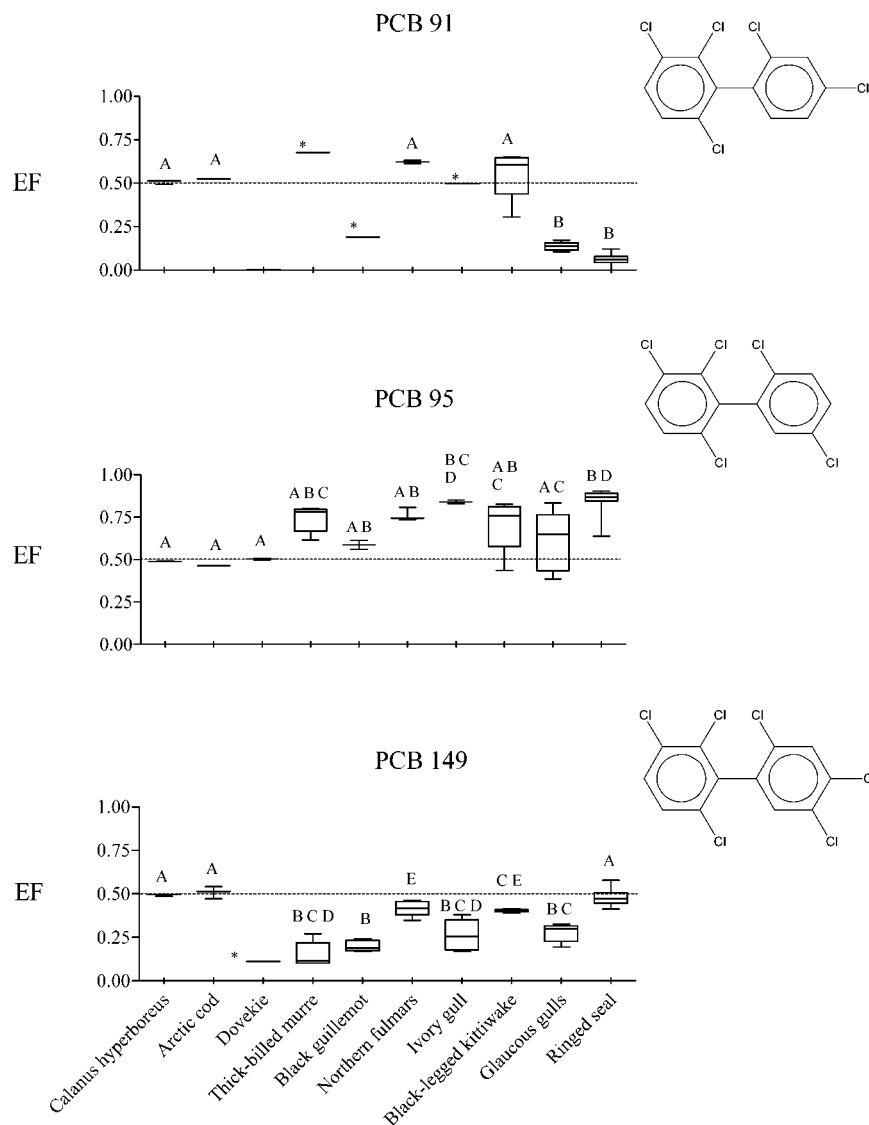


Fig. 1. Enantiomer fraction (EF) distributions for polychlorinated biphenyls (PCB) 91, 95, and 149 within Northwater Polynya (Canada) biota. Boxplots are defined as follows: Center line, median; boxplot edges, 25th and 75th percentile; whiskers, smallest, and largest value within the distribution. Dotted line represents racemic EFs of 0.5. Distributions that were not significantly different from one another share the same letter designation. Asterisks (*) represent EF value based on a single measurement.

served for PCB 95. One possible explanation for this nonracemic EF value is stereoselective biotransformation, which has been observed in forage fish in freshwater food webs. Wong et al. [26] found a mean PCB 95 EF of 0.286 for sculpin in Lake Superior, USA, a value that was significantly different from the EFs observed in their prey. It is unclear why PCB 95 was susceptible to enantioselective processing in Arctic cod compared to PCBs 91 and 149. All three congeners have vicinal hydrogen atoms in meta-para positions, making them susceptible to cytochrome P450 2B (CYP2B) metabolism [28,29]. Wong et al. [30] observed differences for EF distributions between PCBs 95 and 136 in rainbow trout. Both congeners have similar chlorine substitution patterns (PCB 95: 2,3,6–2,5 on the two phenyl rings; PCB 136: 2,3,6–2,3,6) and have vicinal hydrogen atoms in both of their meta-para positions. However, PCB 95 remained racemic in trout throughout uptake/deuration experiments, and PCB 136 was degraded enantioselectively. This suggests that enantioselective degradation of chiral PCBs is regioselective and species-depen-

dent, because PCB 95 remained racemic in trout but not in Arctic cod.

Seabirds

Comparing EF distributions of predator-prey relationships within the NOW suggests stereoselective biotransformation of chiral PCBs within higher trophic-level organisms (i.e., seabirds and ringed seals). The two primary food sources for NOW seabirds are Arctic cod and zooplankton [31], both of which had racemic EF values for PCBs 91, 95 (except Arctic cod), and 149 (Table 1, Fig. 1). Most seabirds exhibited nonracemic EFs for PCBs 91, 95, and 149 (Fig. 1). Glaucous gulls had an extreme mean EF value of 0.137 for PCB 91. All seabirds, with exception of dovekie, exhibited mean EF values of 0.586 or greater for PCB 95. Ivory gulls had the extreme nonracemic mean EF of 0.840. For PCB 149, all seabirds exhibited EFs of 0.403 and lower, with the alcids (dovekie, thick-billed murre, black guillemot) having the lowest EFs. Nonracemic EF values also were found for PCBs 176 and 183

in northern fulmars and in glaucous gulls (Table 1); however, these EF values are based on only a single measurement.

Seabirds may feed on a variety of different zooplankton species throughout the year [31]. Although only one species was examined in this study, previous reports have found that EFs of other organochlorine pollutants in zooplankton were similar to those in the water column within the NOW and other Arctic regions [11,16,27]. Thus, the EFs observed in *C. hyperboreus* likely would be representative of EFs present in the water column and other plankton. The importance of invertebrates cannot be overlooked for their role in contributing to nonracemic distributions of chiral PCBs observed in higher trophic-level organisms and is the focus of future research. However, it is unlikely that the seabird EFs solely were due to accumulation from invertebrates. Nonracemic EFs present in invertebrates would need to be on the magnitude of those observed in seabirds, which is unlikely due to the low biotransformation ability of marine invertebrates [11,16,27]. Also, the seabird diet does not consist of invertebrates alone, but also Arctic cod, which had racemic EFs for PCBs 91 and 149. Although Arctic cod had nonracemic PCB 95 EFs, the average EFs observed in seabirds for PCB 95 were higher than the EFs in the prey species, indicating that stereoselective biotransformation had occurred.

Enantiomer fractions for PCBs 95 and 149 were nonracemic in the same direction for all seabird species (except dovekie for PCB 95; Fig. 1) with the first eluting enantiomer of PCB 95 and (–)-PCB 149 being enriched. Vicinal hydrogen atoms present in the meta-para positions make these particular congeners more susceptible to CYP2B-like enzyme attack [28,29] compared to recalcitrant congeners (no vicinal hydrogen atoms). Drouillard et al. [32] found that PCB congeners having vicinal hydrogen atoms in one or both meta-para positions had lower biomagnification factors (relative to recalcitrant PCB 180) and higher elimination rates in American kestrels (*Falco sparverius*) compared to congeners with no vicinal meta-para hydrogen atoms. The most important mechanism contributing to high elimination rates of these readily cleared congeners in birds is biotransformation [33,34]. Fisk et al. [12] saw similar results in regards to chiral chlordane components within the same seabird species of the NOW food web, with enrichment of (+)-oxychlordane and (+)-heptachlor epoxide in all seabirds. These results suggest that seabirds may possess similar stereoselective biotransformation processes for chiral PCBs 95 and 149 and chlordane compounds. A species-dependent EF profile for PCB 91 (2,3,6–2,4 substitution) among seabirds was observed compared to PCBs 95 (2,3,6–2,5) and 149 (2,3,6–2,4,5; Fig. 1), with EFs that ranged from 0.137 to 0.676. Thus, PCB 95 has two unsubstituted vicinal hydrogen atoms in its meta-para sites, whereas PCB 91 has only one of its meta-para sites occupied with vicinal hydrogen atoms. As with the case for PCB 95 in Arctic cod, it is likely that regioselective metabolic processes were affecting the EF distribution of PCB 91 among seabird species.

The EF distributions for PCB 95 in glaucous gulls and PCBs 91 and 95 in black-legged kittiwakes were substantially larger compared to distributions observed in other seabirds. Glaucous gulls and black-legged kittiwakes showed substantial differences in enantiomer enrichment within individuals of these species (Fig. 1), with distributions crossing the racemic value. It is assumed that, within a given species, individuals will possess the same biological pathways. These wide distributions may be attributed to the wide range of organisms consumed

in their diets. For example, glaucous gulls are opportunistic feeders. They consume Arctic cod and seabird chicks and scavenge marine mammal carcasses [31]. Scavenging behavior also has been observed in ivory gulls and northern fulmars [31], which could be another source for the observed nonracemic EF distributions. The distributions of EFs for scavenging seabirds (i.e., ivory gulls and northern fulmars) and ringed seals (Fig. 1) were not statistically different ($p > 0.05$) for PCB 95, supporting this hypothesis.

Ringed seals

Nonracemic EF values were observed in ringed seals for PCBs 91 and 95 (Fig. 1), but racemic EF (except PCB 95) values were observed in the primary prey species (Arctic cod). Although seals have limited CYP2B-type biotransformation compared to other mammals [19,28], results from our study suggest that stereoselective biotransformation of chiral PCBs nevertheless may be occurring in ringed seals. This is supported further by work done by Wolkers et al. [35], where the presence of a CYP2B-like enzyme was found in ringed seals, although its functional significance still is unknown. Nonracemic EFs also were detected for PCBs 174, 176, and 183 (Table 1) in ringed seals for this study.

Enantiomer fraction distributions among PCBs 91, 95, and 149 were different in ringed seals. Though all have vicinal hydrogen atoms present in one or both meta-para positions, nonracemic EFs were observed for PCB 91 (second eluting enantiomer-enriched) and PCB 95 (first eluting enantiomer-enriched), but not for PCB 149. Ringed seals have been found with 3- and 4-methylsulfonyl PCB 149 metabolites [36], showing that they are capable of biotransforming PCB 149. Thus, our results indicate that enantiomers of PCB 149 are metabolized at equal rates (nonstereoselective). However, Wiberg et al. [36] found nonracemic distributions of 3-methylsulfonyl PCB 149 in ringed seals, suggesting that the production or elimination of this metabolite is stereoselective. In addition, the second eluting enantiomer for PCB 91 and the first eluting enantiomer for PCB 95 were enriched in ringed seals. As mentioned earlier for birds, PCB 95 has two sets of vicinal meta-para hydrogen atoms, though PCB 91 only has one (Fig. 1), and both are susceptible to CYP2B-like metabolism. Why different enantiomers are enriched between the two congeners if they are being degraded by the same mechanism is unclear. Stereoselective biological processes that alter EFs in ringed seals and other Arctic species also appear to be regioselective. Though exact pathways of PCB accumulation in these biota are unknown, it is clear that they are stereoselective.

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