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Enantiomer-Specific Accumulation of PCB Atropisomers in the Bowhead Whale (*Balaena mysticetus*)

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Blubber ($n = 40$) and liver ($n = 20$) samples from the bowhead whale (*Balaena mysticetus*) were collected during the 1997−1998 Native (Inuit) subsistence harvests in Barrow, AK. Bowhead tissues and zooplankton were analyzed for polychlorinated biphenyl (PCB) concentrations and the enantiomeric fractions (EFs) of eight chiral PCB congeners (PCB-91, 95, 135, 136, 149, 174, 176, and 183) to quantify the enantiomer-specific accumulation of PCBs in this cetacean. PCB concentrations in bowhead blubber were low (mean ± 1 SE: 610 ± 54 ng g^{-1} lipid) relative to other cetaceans. The accumulation of several chiral PCBs (PCB-91, 135, 149, 174, 176, and 183) in bowhead blubber was enantiomer-specific relative to bowhead liver and zooplankton, suggesting that biotransformation processes within the bowhead whale are enantioselective. The EFs for PCB-95 and 149 were significantly correlated with body length in male and female whales, while EFs for PCB-91 correlated with length in males only. Despite evidence for enantioselective biotransformation, all three congeners bioaccumulated in the bowhead relative to PCB-153. Results suggest that enantioselective accumulation of PCB-91, 95, and 149 is influenced by PCB concentrations, age, and/or the modification of an uncharacterized stereoselective process (or processes) during sexual maturity.

Introduction

The distribution of the enantiomers of chiral organochlorine contaminants (OCs) can provide insights into environmental fate processes. Enantiomers of chiral compounds have the same physical and chemical properties but may exhibit different biological and toxicological characteristics (*1*−*4*). Thus, the quantification of enantiomer fractions (EFs) (*5*) of chiral compounds can be used to assess potential biotransformation pathways (*6*) and enantiomer-specific biological activity (*7*). Axially symmetrical PCBs (called PCB atropisomers) are chiral as a result of restricted rotation at the central biphenyl bond (*2, 8*). While chiral PCBs are released into the environment as a racemic mixture, previous studies have found them in nonracemic proportions in biota, indicating that enantioselective biotransformation had occurred (*9*−*14*).

The bowhead whale (*Balaena mysticetus*) is a large mysticete found in the Arctic waters of the Bering, Chukchi, and Beaufort Seas that feeds on marine invertebrates (*15*). Native subsistence whalers hunt this species under careful regulation by the International Whaling Commission and the Alaskan Eskimo Whaling Commission. This harvest provides a valuable opportunity to study the accumulation of organochlorine contaminants in a large number of potentially healthy cetaceans.

Few studies have measured chiral PCBs in cetaceans, and these have consisted of limited sample sizes collected from strandings or epizootic events (*11, 12*). The limited number of samples has meant that the influence of biological parameters (i.e. age, gender) on the accumulation of PCB atropisomers in cetaceans is not known. In addition, the possible modification of chiral signatures by lower trophic organisms in cetacean diets has not been addressed. While previous investigations have documented PCB concentrations in bowhead whale tissue (*16*−*18*), the EFs of chiral PCBs or potential metabolism of PCB congeners by this species from lower trophic prey items have not been studied. In this study, we determined the enantiomeric distribution and accumulation of eight PCB atropisomers and other achiral PCB congeners in *Calanus* spp. (a representative prey zooplankton) and bowhead whale blubber and liver to examine mechanisms of PCB bioprocessing in this cetacean.

Materials and Methods

Sampling. Methodology for the collection of calanoid zooplankton samples (*Calanus* spp.) and bowhead whale has been previously described (*18*). Complete blubber cores ($n = 40$) and liver ($n = 20$) samples were obtained from Native subsistence hunters in Barrow, AK (Figure 1) through the North Slope Borough Department of Wildlife Management (DWM). Samples were collected during three consecutive seasonal hunts of the bowhead whale from the Bering-Chukchi-Beaufort Sea stock in 1997−1998. DWM personnel recorded the specimen information (i.e. body length, sex, etc.) for each whale harvested. Body length classification was based on lengths at sexual maturation and known age characteristics (juveniles: 6 m − 8.9 m; subadults: 9 m − 12.9 m; adults: >13 m) (*19*).

Extraction and Cleanup. Zooplankton and bowhead tissue extraction and cleanup procedures followed an established protocol (*20, 21*). Briefly, zooplankton and bowhead liver samples were homogenized with sodium sulfate and Soxhlet extracted using dichloromethane (DCM). Blubber samples were extracted with DCM using a Polytron homogenizer. PCB surrogate recovery standards, PCB-30 and PCB-204, were added to all samples at the extraction step. Lipids were removed using gel permeation chromatography and lipid content was determined gravimetrically. Each sample was concentrated and separated on 100%-activated silica gel into two fractions: 65 mL of 100% hexane (F1) and 90 mL of 50% hexane:50% dichloromethane (by volume, F2). Fraction 1 contained PCB congeners and was analyzed in this study. Samples were transferred to 2,2′,4-trimethylpen-

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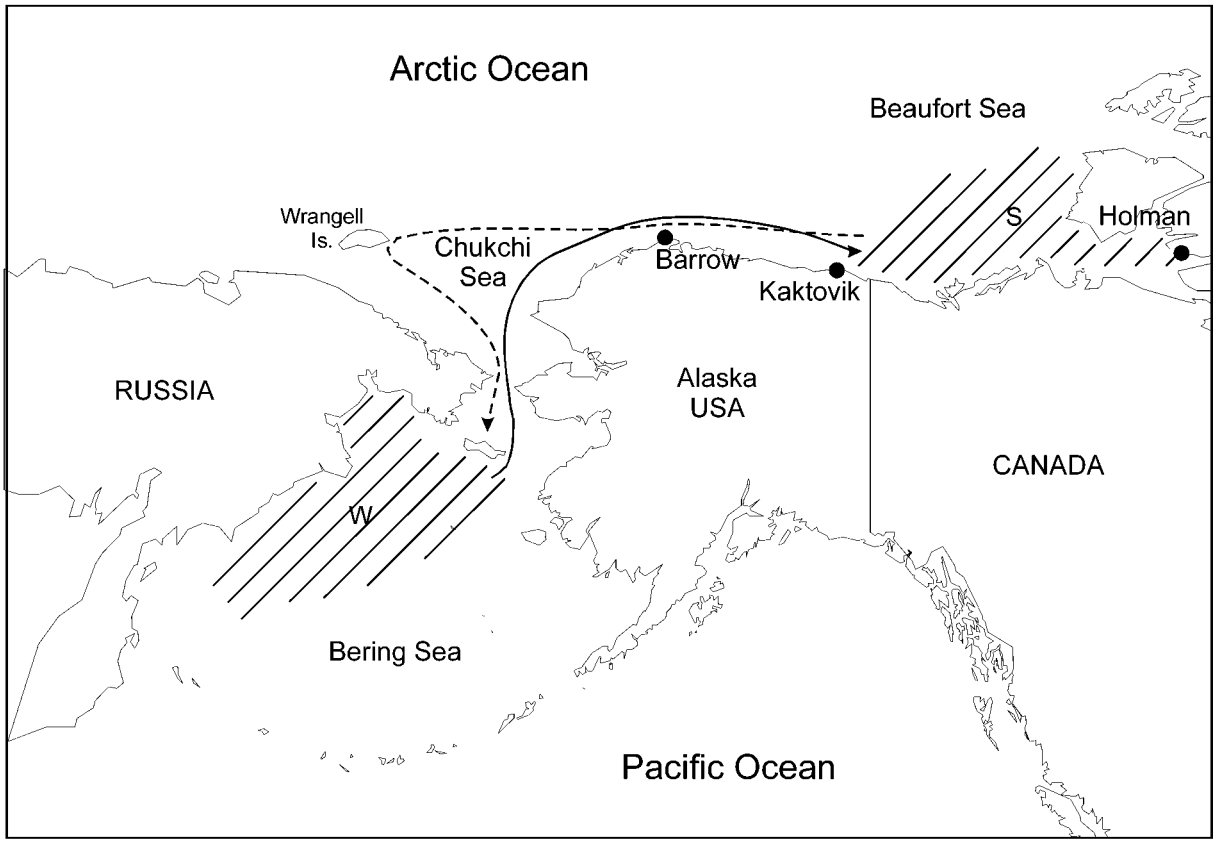

FIGURE 1. Annual migration route of the bowhead whale (Bering-Chukchi-Beaufort Sea stock) between summering (S) and winter (W) grounds. Solid and dashed lines indicate spring and fall migrations, respectively. Biota sampling locations are indicated (●).

tane and concentrated to approximately 100 μL. PCB-166 was added as a volume corrector.

PCB Analysis. PCB concentrations in zooplankton and bowhead whale tissue samples were determined using a Hewlett-Packard (HP) 5890 gas chromatograph (GC) with a ^{63}Ni-electron capture detector (ECD) (*20*). Compound separation was completed using a DB-5 column (60 m × 0.25 mm i.d. × 0.25 μm internal film thickness; J&W Scientific, Folsom, CA) with H_2 carrier gas. Confirmation was accomplished using a HP6890 GC-5973 mass-selective detector (GC-MSD) with election impact (EI) ionization (*20*).

Enantiomers of eight chiral PCBs (PCBs 91, 95, 135, 136, 149, 174, 176, and 183) were quantified by using GC-MSD on either a HP6890/5973 or a HP 5890/5971 operating in EI mode, using a suite of chiral columns: PCBs 91, 95, and 136 on Cyclosil-B (30 m × 0.25 mm i.d. × 0.25 μm internal film thickness, J&W Scientific); PCBs 91, 136, 149, 174, and 176 on Chirasil-Dex (25 m × 0.25 mm i.d. × 0.25 μm internal film thickness, Chrompack, Raritan, NJ); and PCBs 135 and 183 on BGB-172 (30 m × 0.25 mm i.d. × 0.25 μm internal film thickness; BGB, Adiswil, Switzerland). Individual columns were calibrated to quantify chiral PCBs with no coelution with other PCB congeners that had the same target ions (*22*). The three most abundant ions of each molecular cluster were scanned: *m*/*z* 324, 326, and 328 (PCB-91 and 95), *m*/*z* 358, 360, and 362 (PCB-135, 136 and 149), and *m*/*z* 392, 394, and 396 (PCB-174, 176, and 183).

Enantiomeric fractions (EFs) for the chiral PCB atropisomers were calculated as follows (*5*):

$$\mathrm{EF} = \frac{\mathrm{ER}}{1+\mathrm{ER}} = \frac{1}{1+\frac{1}{\mathrm{ER}}} \quad (1)$$

The enantiomeric ratios (ERs) were quantified as ER = (+)/(−) concentration ratios when enantiomer elution order was known on the chiral GC columns used (PCB-136, 149, 174, and 176) and as the areas of the first-eluting to the second-eluting enantiomers on Chirasil-Dex (for PCB-91, 95) and BGB-172 (for PCB-135, 183) (*13*, *22*, *23*).

Analytical Quality Assurance. Recoveries for the surrogate standards averaged (±1 SE) 89% ± 4.6 for PCB-30 and 82% ± 5.4 for PCB-204, and therefore no correction for recovery was made. The GC-ECD and GC-MSD detection limits were between 0.01 and 1 pg μL^{-1} for each PCB congener. Quality assurance protocol included the use of two standard reference materials from the National Institute of Standards and Technology (NIST, Gaithersburg, MD; SRM1588b Cod Liver Oil and SRM1945a Whale Blubber Homogenate). Standard reference materials and a racemic standard were analyzed concurrently with chiral PCB analysis to monitor enantiomer separation efficiency and elution order (*24*).

Data Analysis. The pattern of PCB congeners in bowhead whales was examined by calculating the accumulation relative to the recalcitrant PCB-153 and comparing bowhead blubber to *Calanus* samples

$$\text{relative ratios } (\mathrm{RR}_{153}) = ([\mathrm{PCB}_x]/[\mathrm{PCB}_{153}]_{\mathrm{blubber}})/([\mathrm{PCB}_x]/[\mathrm{PCB}_{153}]_{Calanus}) \quad (2)$$

where PCB_x and PCB_{153} represents the lipid-normalized concentrations of a specific PCB congener (*x*) and PCB-153 in bowhead blubber and zooplankton samples. Only congeners above the detection limit in >70% of all zooplankton and bowhead whale blubber samples were included in the calculations. Each PCB congener was assigned to one of five structural groups previously described (*25*, *26*): Group I, congeners without vicinal H atoms; II, congeners with vicinal H only in the *ortho* and *meta* positions and 2 *ortho* Cl atoms; III, same as II, but with 1 *ortho* Cl; IV, congeners with vicinal H in the *meta* and *para* positions with 2 *ortho* Cl, and V,

TABLE 1. Concentrations (Mean ± SE) of Chiral PCB Congeners in Zooplankton (*Calanus* spp.) and Bowhead Blubber and Liver Tissue (ng g^{-1} Lipid Adjusted)

PCB congener	zooplankton	bowhead liver	bowhead blubber
n[a]	13	20	40
% lipid	46.0 ± 7.51[b]	10.4 ± 0.17	70.4 ± 2.82
91	0.37 ± 0.18	1.61 ± 0.11	4.26 ± 0.44
95	1.47 ± 0.90	3.25 ± 0.23	9.63 ± 0.79
135	0.13 ± 0.05	1.42 ± 0.27	4.88 ± 0.83
136	0.39 ± 0.15	1.27 ± 0.10	8.76 ± 3.08
149	0.87 ± 0.26	4.42 ± 0.46	16.23 ± 2.26
174	0.16 ± 0.03	2.31 ± 0.29	3.64 ± 0.91
176	0.08 ± 0.03	0.59 ± 0.18	9.10 ± 1.46
183	0.20 ± 0.05	0.89 ± 0.09	5.71 ± 1.93
ΣPCB[c]	47.5 ± 14.7	159 ± 9.30	610 ± 54

[a] Number of samples analyzed. [b] Dry weight basis. [c] ΣPCB = sum of 4/10, 7/9, 6, 8/5, 19, 12/13, 18, 15/17, 24/27, 16, 32, 54/29, 26, 25, 50, 31/28, 33/21/53, 51, 22, 45, 46, 52/49, 43, 47/48, 44, 59, 42, 64, 41/71, 40, 100, 63, 74, 76/98, 70, 95/66, 91, 55, 56/60, 92/84, 101, 99, 119, 83, 97, 87, 81, 85, 136, 110, 82, 151, 135, 144, 107/147, 149, 133, 118, 114, 143, 141, 145, 153, 132, 105, 141/179, 137, 176, 130, 163, 138, 158, 129/178, 175, 187, 182, 183, 128, 167, 185, 174, 177, 171, 156, 202/173, 172, 197, 180/193, 191, 199, 170/190, 198, 201, 176/203, 189, 206, 195, 207, 194, 205, 208, and 209 (IUPAC designation).

same as IV, but with 3 *ortho* Cl atoms. A Model-I ANOVA ($\alpha = 0.05$) was used to examine differences in the relative ratios (RR_{153}) among structural groups. Tukey's multiple comparisons test was used to examine significant differences in congener distributions (overall $\alpha = 0.05$).

The influence of body length, gender, and all first-order interaction effects on EF values for chiral PCBs in bowhead blubber and liver were examined by the Analysis of Covariance (ANCOVA) model

$$EF = \mu + \text{length} + \text{gender} + (\text{length} \times \text{gender}) + \epsilon \quad (3)$$

where ϵ is the error vector and μ is a constant value. This model was reduced for factors not significant according to Type III Sums of Squares test ($\alpha = 0.05$). Potential interactions between enantiomer distribution and body length, a surrogate for age, were evaluated using first-order linear regression analysis. The EF values for the zooplankton and bowhead whale liver and blubber were compared using a Model-I ANOVA ($\alpha = 0.05$). All statistical analysis was performed using Systat for Windows, Version 8.0 (SPSS, Chicago, IL).

Results and Discussion

Total PCB Concentrations. The sum PCB concentrations (ΣPCB) in bowhead whale blubber (610 ± 54 ng g^{-1} lipid adjusted) were approximately four times higher than ΣPCB concentrations in liver (Table 1). These results are consistent with other published lipid-adjusted concentrations for ΣPCBs (average ± 1 SE: 485 ± 46 ng g^{-1}) in bowhead blubber (*16*−*18*). However, ΣPCB in this study is based on 102 congeners compared to 10−20 in previous work.

Previous investigations have revealed that concentrations of ΣPCBs and other OCs in bowhead blubber are gender and age-dependent, possibly due to the transfer of contaminants from reproductively active females to the fetus and neonate (*18*, *21*). Hoekstra et al. (*21*) found that ΣPCB levels in male and female whales <13 m in length was not statistically different. However, ΣPCB burden in blubber samples from adult female whales (>13 m in length) were significantly lower than males from the same body length cohort, suggesting transfer of PCB burden during periods of reproduction (*21*). In other cetaceans, it has been estimated that 60−90% of the OC burden is transferred from the female to the nursing calf either through trans-placental transfer and/or lactation (*27*, *28*).

The bowhead is a baleen whale that primarily feeds on low trophic level prey items such as zooplankton (*15*). As a result, ΣPCB in the bowhead whale are relatively low compared to levels found in other cetaceans. The ΣPCBs were significantly greater in the Northeast Atlantic and North Pacific minke whale (*Balaeoptera acutorostrata*) populations (mean: 3750 ng g^{-1} lipid and 2300 ng g^{-1} lipid; respectively) (*29*, *30*). Organochlorine concentrations, including ΣPCB, in the Northwest Atlantic right whale (*Eubalaena glacialis*) (*31*) were approximately nine times greater (mean ΣPCB: 5700 ng g^{-1} lipid) than in bowhead whale blubber. Differences between these species of mysticetes and the bowhead whale are likely due to variations in proximity of habitat to sources of contamination and prey item selection.

PCB Congener Pattern. The bioaccumulation of PCB congeners in marine mammals is influenced by numerous factors, such as hydrophobicity, degree of chlorination, and substitution pattern on the biphenyl moiety (*32*), and factors associated with diet and physiology of the target species (*33*). PCB congeners (normalized to PCB-153) from zooplankton to bowhead blubber were grouped according to Cl-substitution in order to investigate potential biotransformation in the bowhead whale (Figure 2). This structure−activity relationship approach to quantify PCB metabolism has been applied to other marine species (*34*), including cetaceans (*35*) to describe potential metabolism via cytochrome P-450 (CYP) related isozymes.

Significant differences in the RR_{153} among structural groups were observed ($p < 0.001$) for the bowhead whale. Congeners belonging to structural Group III, and to a lesser extent Group IV, were less recalcitrant ($RR_{153} < 1$) than Groups I, II, and V ($RR_{153} > 1$; Figure 2). The relative accumulation of Group III congeners was significantly different from Group I, II, IV, and V ($p < 0.001$ for all comparisons). Group IV congeners were significantly different from Groups I and III only ($p = 0.02$ and $p < 0.001$, respectively). While the RR_{153} values for PCBs were weakly correlated with log K_{ow} ($r^2 = 0.10$, $p < 0.05$; data not shown), the low coefficient of variation for this relationship suggests that factors other than physical-chemical partitioning influence the overall PCB profile within the bowhead whale.

The PCB congeners included in structural Groups III and IV are considered the least persistent in biota due to the presence of vicinal H atoms in *ortho-meta* and *meta*−*para* positions and limited degree of *ortho* Cl substitution. Congeners represented by these groups are typically biotransformed via CYP-related pathways, possibly by CYP1A-related enzymatic degradation (*32*, *36*). Activity of CYP1A and 2B isozymes has been observed in other cetaceans (*37*, *38*) or suggested based on contaminant profiles (*31*, *39*). The PCB congener profile in bowhead whale blubber suggests that this species has the ability to metabolize Group III and IV congeners via CYP1A-related pathways.

Group I congeners are considered persistent PCBs, while Group II congeners may be metabolized by the CYP2B subfamily (*25*). The PCB congeners that lack *ortho/meta* or *meta/para* vicinal H atoms are typically not easily metabolized (*32*, *36*). The $RR_{153} > 1$ for Group I, II, and V congeners implies little or no CYP2B-mediated biotransformation by the bowhead whale. The relative accumulation of these congeners in the blubber of the bowhead whale is consistent with other studies on PCB congener patterns in cetaceans (*40*) and is reduced relative to terrestrial mammals and polar bears (*35*).

Enantiomeric Fractions (EFs) of Chiral PCBs. The EF values for PCB atropisomers in zooplankton and bowhead liver were not significantly correlated with ΣPCB or individual PCB atropisomer concentrations ($p > 0.15$; data not shown). All congeners detected in zooplankton were racemic or near

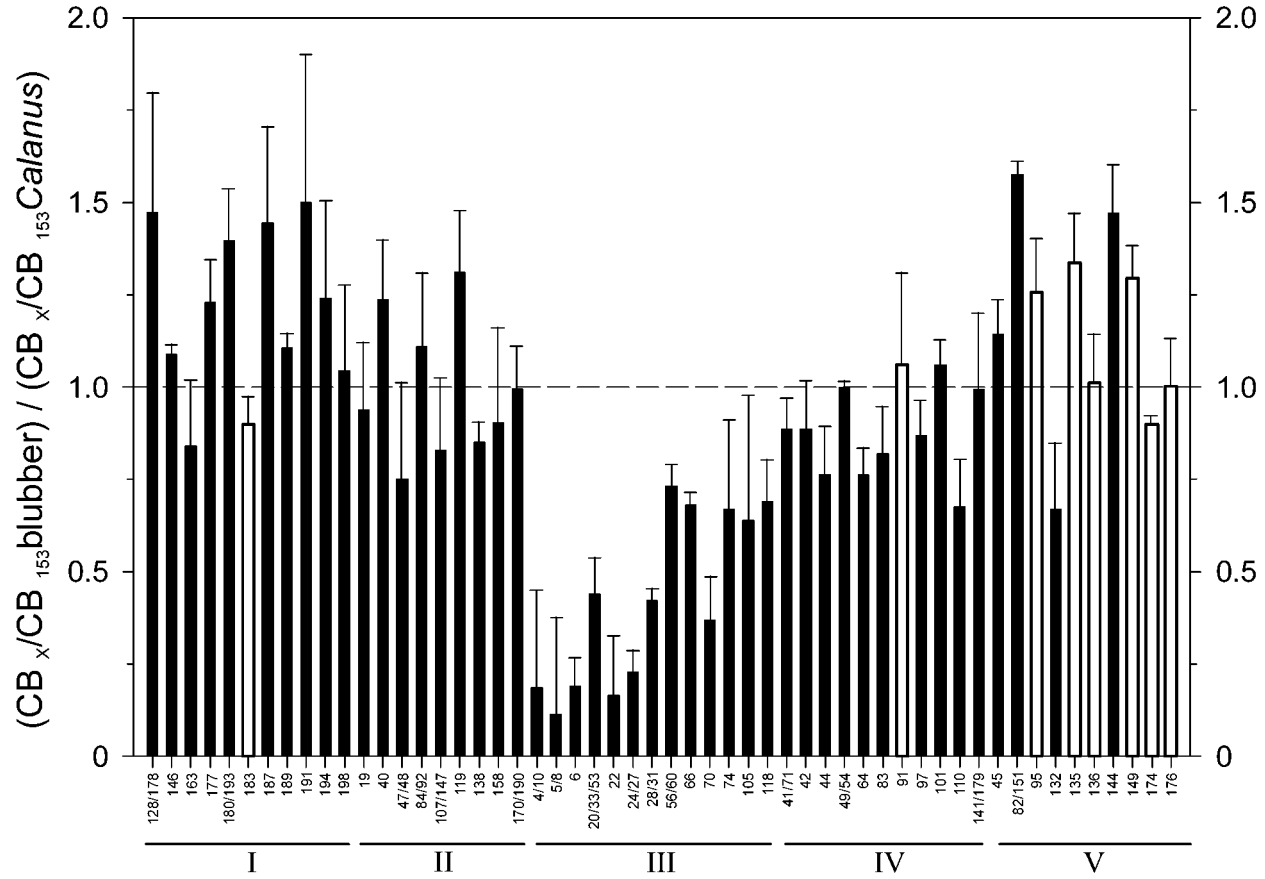

FIGURE 2. Mean relative ratios of PCB congeners relative to PCB-153 [bowhead blubber ($n = 40$)/*Calanus* spp. ($n = 13$)] arranged by structural classification: Group I, congeners without vicinal H atoms; II, congeners with vicinal H only in the *ortho* and *meta* positions and 2 *ortho* Cl atoms; III, same as II, but with 1 *ortho* Cl; IV, congeners with vicinal H in the *meta* and *para* positions with 2 (or less) *ortho* Cl, and V, same as IV, but with 3 *ortho* Cl atoms. Dashed line represents unity (PCB-153 = PCB$_x$ = 1). The open bars represent RR$_{153}$ values for chiral PCBs examined in this study.

TABLE 2. Enantiomeric Fractions (EF, Mean ± 1 SE) of Chiral PCBs in the Racemic Standard, Zooplankton (*Calanus* Species; $n = 10$) and Bowhead Whale Blubber ($n = 40$) and Liver Samples ($n = 20$)[a]

					bowhead whale	
PCB	Cl substitution	vicinal H-atoms	racemic standard EF$_{\pm std}$	*Calanus* spp. EF$_{zoo}$	EF$_{liver}$	EF$_{blubber}$
91	236−24	1 *ortho-meta*, 1 *meta−para*	*0.500 ± 0.002*	*0.500 ± 0.001*	*0.495 ± 0.003*	0.456 ± 0.021
95	236−25	2 *meta−para*	*0.493 ± 0.001*	*0.492 ± 0.008*	*0.490 ± 0.002*	*0.489 ± 0.010*
135	235−236	1 *meta−para*	0.502 ± 0.002	NQ	0.533 ± 0.002	0.571 ± 0.002
136	236−236	2 *meta−para*	*0.498 ± 0.003*	*0.500 ± 0.002*	*0.495 ± 0.002*	*0.498 ± 0.002*
149	236−245	1 *meta−para*	*0.499 ± 0.004*	*0.502 ± 0.003*	*0.501 ± 0.003*	0.511 ± 0.014
174	2345−236	1 *meta−para*	*0.498 ± 0.002*	0.501 ± 0.006	ND	0.511 ± 0.002
176	2346−236	1 *meta−para*	0.500 ± 0.005	ND	ND	0.551 ± 0.007
183	2346−245	none	*0.499 ± 0.001*	0.498 ± 0.004	ND	0.475 ± 0.003

[a] ND, nondetected (signal/noise < 3); NQ, nonquantified (signal/noise <5). Italicized EFs are not significantly different ($\alpha = 0.05$). PCB chlorine substitution pattern (ring 1 − ring 2) and the vicinal H-atoms present in each congener are provided.

racemic for all samples analyzed, suggesting that *Calanus* do not accumulate or biotransform PCBs enantioselectively (Table 2).

The EF values in liver closely approximated the values in zooplankton, suggesting that the uptake of PCB atropisomers from low trophic level prey to the bowhead whale is not enantioselective. The adsorption and elimination of OCs via gastrointestinal and fecal transport is generally regarded as being controlled by partition-based processes and is unlikely to be stereospecific (*41, 42*). However, the modification of EFs from liver to blubber (PCB-91, 135, and 149) and zooplankton to blubber (PCB-174 and 183) were significant ($p < 0.022$) and suggests that bowhead whales have the ability to enantioselectively biotransform and accumulate several PCB atropisomers.

The chiral signatures of PCB atropisomers in bowhead blubber appear to be structure-dependent based on EF values different from 0.5 (racemic) and suggest that CYP-mediated metabolism as well as other processes that influence bioaccumulation may be enantioselective. The stereochemical selectively of drug metabolizing enzymes is well documented (*43*), including CYP-dependent monooxygenase activity (*44*), CYP1A, and epoxide hydrolase isozymes (*44, 45*). To our knowledge, the stereoselectivity of PCB metabolism has not been addressed. However, highly selective, non-CYP mediated processes also influence the stereochemically specific bioactivity of chiral molecules (*4, 47, 48*). As a result, the term "biotransformation" in this manuscript will refer to all possible processes related to CYP-mediated metabolism, receptor-mediated disposition, and other unidentified mech-

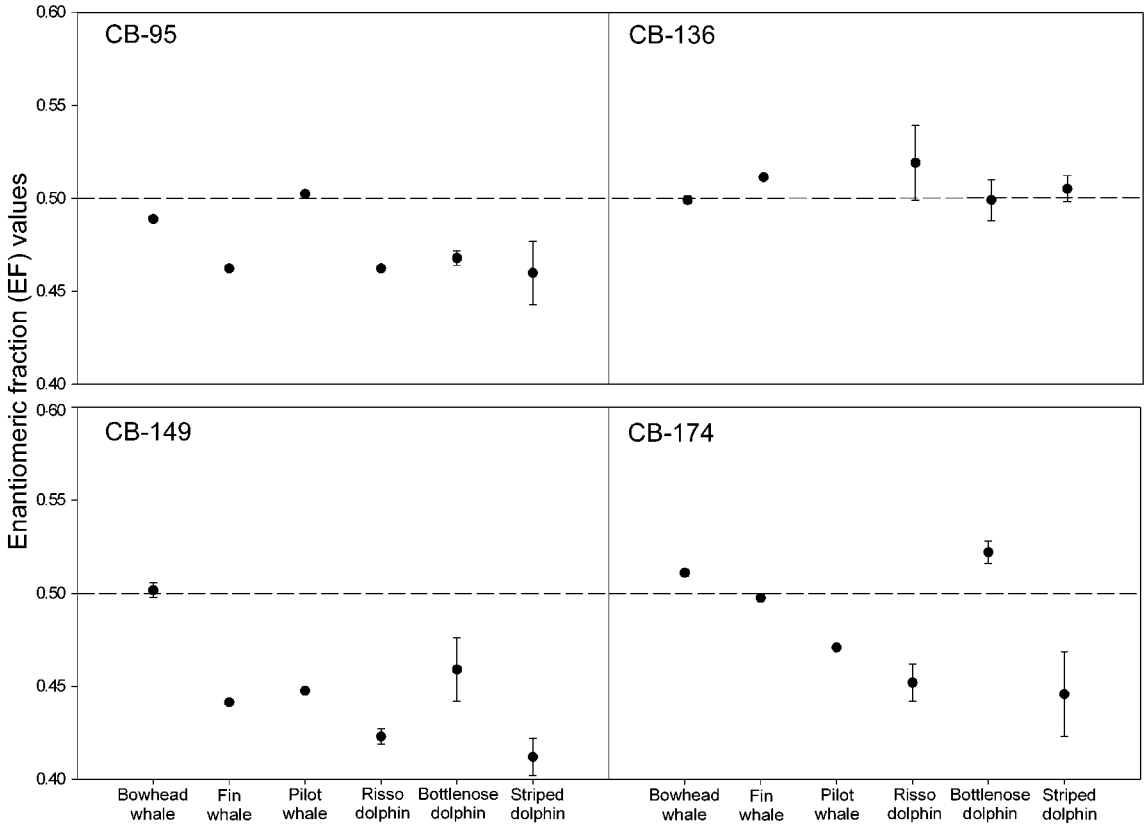

FIGURE 3. Mean (± 1 SE) enantiomeric fractions (EFs) of four PCB atropisomers in bowhead liver (PCB-95, 136, 149) and blubber (PCB-174) compared to EF liver values in cetaceans from the Mediterranean Sea (*11, 12*). Dashed lines represent a racemic signature (EF = 0.50). The enantiomer ratio values for PCB-136 and 149 from refs *11* and *12* were inverted to correspond with the elution order of the (+) and (−) enantiomers quantified in this study.

anisms responsible for enantiomer-specific accumulation of chiral PCBs in the bowhead whale.

The change in the EF values of chiral PCBs from *Calanus* to the bowhead was less than the change in EF values of chiral OCs observed between prey and predator in other studies (*7*, *49*, *50*). The apparent biotransformation of chiral PCBs as interpreted from enantiomer-specific accumulation is not consistent with the relative accumulation profile of PCB congeners (Figure 2). While the EF values for PCB-91, 135 and 149 were nonracemic in bowhead blubber, these congeners had RR_{153} values > 1, indicating enantiomer-specific recalcitrance to biotransformation relative to PCB-153. Combined with the racemic EFs in prey and nonenantioselective uptake inferred from EFs in bowhead liver, this indicates that biotransformation of these congeners by the bowhead whale is enantioselective.

The accumulation of PCB atropisomers with 1 (or 0) vicinal pair of H-atoms in the *meta/para* position in bowhead blubber was enantiomer-specific. Congener PCB-183 lacks vicinal hydrogen atoms in both *meta/para* and *ortho/meta* positions and is generally regarded as difficult for biota to metabolize. Nonetheless, EF values for PCB-183 in bowhead blubber were nonracemic and significantly different than values recorded in zooplankton.

In contrast, the accumulation of PCB-95 and 136, both with two pairs of vicinal H-atoms in the *meta/para* position in bowhead whale blubber, was not enantiomer-specific relative to both liver and *Calanus* species. However, these congeners are among the more readily metabolizable PCBs by CYP2B isozymes (*32*, *36*). These observations cannot be explained by chemical structure and suggests that the metabolic capacity and/or other non-CYP mediated pathways that influence the accumulation of chiral OCs in the bowhead may be more complex than previously expected from reported achiral PCB profiles in other cetaceans (*35*, *40*, *51*).

Figure 3 presents the results for EFs from other studies on cetaceans demonstrating that interspecies differences exist in accumulation of chiral PCBs. The EF values for PCB-136 derived from bowhead liver and PCB-174 in blubber were similar to values determined in liver samples from the fin whale (*Balaenoptera physalus*) which suggests similar enantioselective biotransformation between these two mysticete species. However, bowhead whale EF values were significantly different from those reported for odontocetes, such as the long-finned pilot whale (*Globicephala melanena*), Risso's dolphin (*Grampus griseus*), bottlenose dolphin (*Tursiops truncates*), and striped dolphin (*Stenella coeruleoalba*) (*11*, *12*).

The differences between baleen and small toothed whales may be attributed to species-specific stereochemically sensitive processes, the degree of contamination, and the different food chains occupied by these piscivorous cetaceans (odontocetes and fin whale) as the bowhead whale feeds almost exclusively on invertebrates (*15*). Nonracemic proportions of chiral PCBs have been documented in some fish species (*14*). Therefore the accumulation of nonracemic congeners from prey items has to be taken into account with any interpretation of chiral biotransformation process (or processes) in higher trophic organisms.

Gender, Body Length, and EF Values. Bowhead whale gender and body length were not significantly correlated with the EFs for PCB-135, 136, 174, 176, and 183 ($p > 0.05$ for both main effects; all comparisons). The EFs for PCB-91, 95, and 149 were significantly correlated with increasing body length ($p < 0.05$; Figure 4). However, gender did not influence the EF values for both PCB-95 and PCB-149. The results

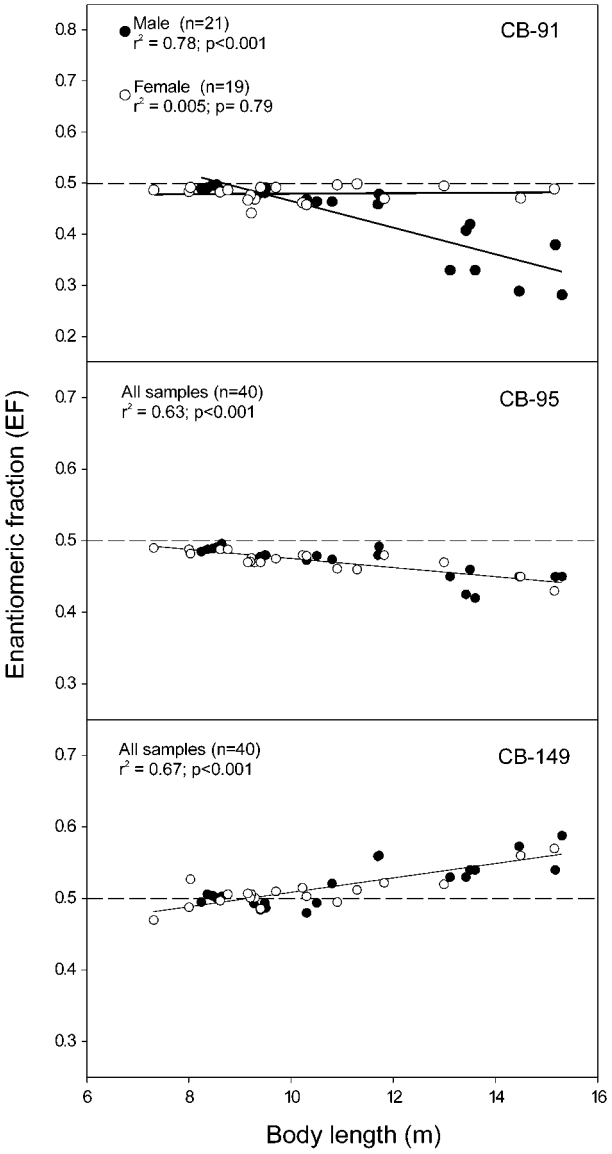

FIGURE 4. Relationship between enantiomeric fractions of PCB-91, 95, and 149 in blubber from male (●) and female (○) bowhead whales with body length. Dashed line represents racemic signature (EF = 0.50). The solid lines provide the first-order linear regression of EF values and body length for each gender. The EF values for PCB-95 (EF_{CB-95} = −0.0064(length) + 0.539) and PCB-149 (EF_{CB-149} = 0.01(length) + 0.408) in males and females were pooled since the correlation with gender was not significant.

suggest that enantiomer-specific accumulation of these congeners by the bowhead whale occurs gradually over time. The changes in EF values with age likely reflect the limited capacity of the bowhead to preferentially accumulate or degrade a specific enantiomer of PCB-95 and 149.

Gender and the gender-length interaction term for PCB-91 were significant under the ANCOVA model (eq 3; $p < 0.05$). The EF values for PCB-91 were not significantly correlated with body length in females (EF_{CB-91} = 0.001-(length) + 0.47; $p = 0.78$; first-order linear regression) but were significantly correlated in males (EF_{CB-91} = −0.03(length) + 0.73; $p < 0.001$) (Figure 4). Additionally, males and females that were <13 m in length, and considered sexually immature (*20*), did not have significantly different EF values for PCB-91 ($p = 0.63$). The EF values for PCB-91 were inversely correlated with PCB-91 concentrations in blubber samples from male whales (EF_{CB-91} = −0.02[PCB-91] + 0.53; $F_{1,19}$ = 38.0, $p < 0.001$; $r^2 = 0.67$). However, this relationship was not evident in the female whales (EF_{CB-91} = -4.3×10^{-6}[PCB-91] + 0.48; $F_{1,17} = 0.02$, $p = 0.88$; $r^2 = 0.001$). The enantiomer-specific accumulation of the first eluting enantiomer of PCB-91 was also significantly correlated with the total PCB burden in blubber ($p < 0.001$).

The relationship of EF values for PCB-91 with length, gender, and concentration is unique to our knowledge as previous investigations on enantiomer-specific accumulation of chiral PCBs in a limited number of cetaceans did not observe any correlation with PCB concentrations in liver tissue and biological characteristics (gender, age, etc.) (*11, 12*). The relationship between EF for PCB-91 and body length in mature male bowhead whales (>13 m in length) is either influenced by increasing PCB concentrations with time and/or the modification of an uncharacterized enantioselective process (or processes) during the sexual maturity of male bowhead whales.

Chiral analysis has been used in this study to provide detailed information on the biotransformation of PCBs in cetaceans that would have been overlooked by conventional interpretation of relative achiral PCB profiles. The accumulation of several chiral PCBs in the bowhead whale was enantioselective. Body length (i.e. age) and gender are important factors that appear to affect enantioselective accumulation of PCB atropisomers in the bowhead whale. This investigation provides evidence that the processes governing PCB biotransformation, and ultimately, bioaccumulation in cetaceans is more complex than previously believed. Our results imply that stereochemical processes other than CYP metabolism affect PCB bioaccumulation and disposition in marine mammals.

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