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SPATIAL TRENDS AND BIOACCUMULATION OF ORGANOCHLORINE POLLUTANTS IN MARINE ZOOPLANKTON FROM THE ALASKAN AND CANADIAN ARCTIC

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Abstract—Planktonic copepods (*Calanus glacialis* and *C. hyperboreus*; $n = 37$) and water ($n = 19$) were collected to examine the spatial distribution and bioaccumulation of organochlorine contaminants (OCs) in the Alaskan and Canadian Arctic. The rank order of total OC (Σ OC) group concentrations in *Calanus* samples was toxaphene ≥ Σ polychlorinated biphenyls (PCBs) > Σ hexachlorcyclohexane (HCH) > Σ DDT > Σ chlordane-related compounds (CHLOR) > Σ chlorobenzenes (ClBz). The dominant analyte was α-HCH in all water and zooplankton samples. The most abundant toxaphene congener in water and zooplankton samples was the hexachlorobornane B6-923. Organochlorine contaminant group concentrations in Alaskan zooplankton and water samples were lower than those in samples collected from sites in the eastern Canadian Arctic. Comparison of PCB and toxaphene congener profiles in zooplankton and water samples suggests that biotransformation by cytochrome P-4502B isozymes is low in *Calanus*, and limited phase I metabolism may occur. The log relationship of bioaccumulation factor (log BAF) versus octanol–water partition coefficient (log K_{ow}) relationship was near 1:1 for OCs within the log K_{ow} range of 3 to 6. A curvilinear model provided a better relationship between these two variables when OC compounds with log $K_{ow} > 6$ were included. These results suggest that hydrophobic OCs (log K_{ow} 3–6) in *Calanus* species are at equilibrium with the water concentrations and that physical partitioning, rather than biotransformation, is the major factor governing OC profiles in marine zooplankton.

Keywords—Biotransformation Invertebrate Partitioning Sea water Toxaphene

INTRODUCTION

Persistent organochlorine contaminants (OCs) are a structurally diverse group of agricultural and industrial compounds (or by-products) that are present in virtually every compartment in the Arctic biosphere and hydrosphere [1]. The transport of these compounds to cold, remote regions is due primarily to long-range atmospheric transport [2]. The persistence, toxicity, and bioaccumulation potential of OCs is particularly significant in the Arctic marine environment, where food chains are longer and many species have greater lipid content than their temperate counterparts [1]. As a result, OCs have reached high levels in top predatory marine species, such as polar bears (*Ursus maritimus*) [3]. Native peoples in the Canadian Arctic have greater OCs exposure than populations in southern Canada or the United States because of dietary exposure through consumption of lipid-rich traditional foods [4].

Calanoid copepods are dominant members of marine zooplankton communities, in terms of both number and biomass, in high-latitude regions. These organisms are important in polar food webs because of their high lipid reservoirs and biomass and provide higher trophic levels with a high-energy diet [5]. *Calanus hyperboreus* has an essentially epipelagic life history that includes high feeding rates in surface waters in the spring/summer months and a dormancy phase at greater depths in the winter, when breeding and egg release occur [6,7]. *Calanus glacialis* is also considered to be an omnivorous species, and its life cycle is suggested to span ≥ two years [8].

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Lipid dynamics in calanoid copepods likely influence the bioaccumulation of OCs. Zooplankton are constantly immersed in an aqueous environment, and partitioning plays an important role for the bioconcentration of OCs [9]. Recently, Fisk et al. [10] demonstrated that water solubility of the OCs is a key factor in explaining OC concentrations in Arctic marine zooplankton. These results require further field validation in other regions of the Arctic marine environment to better understand the dynamics and mechanisms of contaminant accumulation in lower trophic organisms.

While a small number of studies have investigated OCs in arctic zooplankton [10–14], limited data exist on OCs, in particular congener-specific toxaphene data, in the Alaskan marine environment [1]. This region is ecologically productive and is used extensively by native subsistence communities for traditional foods. This study addresses the spatial distribution of OCs in water and zooplankton samples from locations in the Alaskan Beaufort Sea coast and Canadian Arctic archipelago and investigates the bioaccumulation and potential biotransformation of OCs, with special emphasis on polychlorinated biphenyl (PCB) and toxaphene congeners.

METHODOLOGY

Field sampling

Calanus spp. samples were collected from five locations in northern Alaska and the Canadian Arctic (Fig. 1). Zooplankton samples were collected with an acetone-rinsed 100-μm plankton tow (1 m^2) in Holman, Northwest Territories, Canada (June 1999), and Barrow, Alaska, USA (September 2000). *Calanus hyperboreus* from the northern Baffin Bay

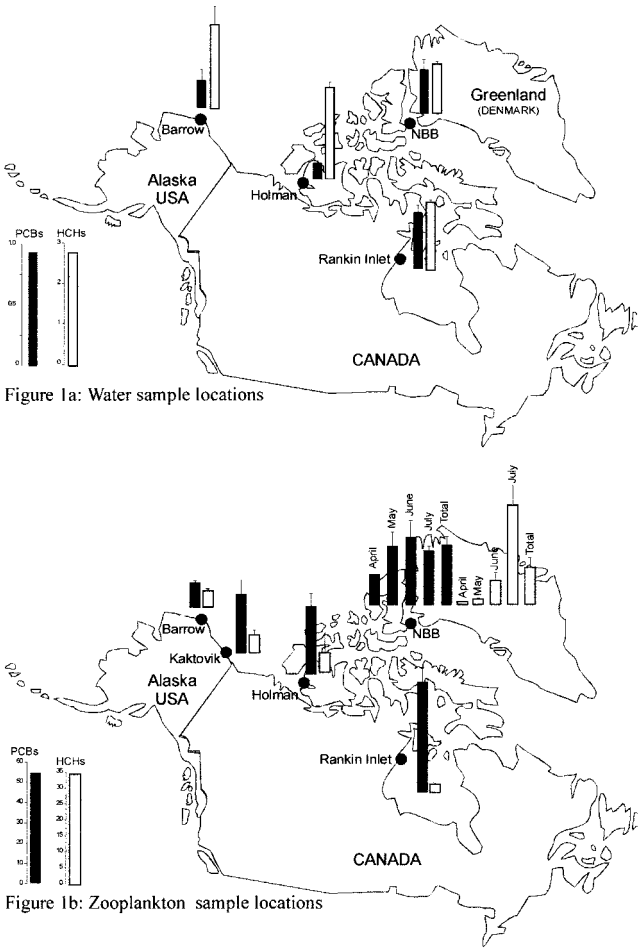

Fig. 1. Water (**a**) and zooplankton (**b**) sample locations in Barrow and Kaktovik, Alaska; Holman, Northwest Territories; Rankin Inlet, Nunavut; and north Baffin Bay (NBB). Mean Σ polychlorinated biphenyls (PCB) and Σ hexachlorcyclohexane (HCH) (±1 standard error) in water (ng/L) and zooplankton (ng/g dry wt) are included. Data from Rankin Inlet are from Muir and Lockhart [15]; data for NBB are from Fisk et al. [10].

(NBB) were collected in July 1998 using previously described methods [10]. Additional copepod samples were collected over three weeks from the eastern Beaufort Sea (near Kaktovik, AK, USA) in September 1998 and generously donated by LGL Limited (Sidney, BC, Canada). Zooplankton in the samples from Barrow and Holman were sorted by taxonomic classification using voucher specimens. All invertebrate samples were dominated by *C. glacialis* and *C. hyperboreus*. Zooplankton samples were collected at Rankin Inlet, Nunavut (NU), Canada, but not taxonomically identified [15].

We expected that the concentrations of OCs in the zooplankton would be low. This presumption led us to pool *Calanus* samples from the same geographical location and time of sampling. Samples were stored at −20°C during transport to the analytical laboratories at the National Water Research Institute (Environment Canada, Burlington, ON, Canada).

Water samples from Holman and Barrow were collected in the same location as *Calanus* spp. samples using solid-phase extraction with an automated in situ sampler (Infiltrex II system, Axys Environmental, Sidney, BC, Canada). Water samples from NBB were collected in May 1998 using similar techniques [10]. As well, water samples were collected directly onto a XAD-2 (Sigma-Aldrich, Oakville, ON, Canada) column using an Axys SeaStar at Rankin Inlet, NU (same parameters as Infiltrex II system). Automated water samplers were deployed at a depth of 15 m, and approximately 100 L of water (flow rate: 100 ml/min) were sampled. The suspended particulate matter in the water column was removed using glass-microfiber filters (145 μm) prefired at 450°C to remove potential OC contamination. Dissolved OC analytes were extracted by a XAD-2 resin column and stored at 2°C until time of analysis.

Chemicals and standards

All solvents (pesticide grade) were obtained from Caledon Laboratories (Georgetown, ON, Canada). American Chemical Society–grade granular sodium sulfate (Na_2SO_4) was obtained from EM Science (Gibbstown, NJ, USA). Pesticide-grade dry silica (60–200 mesh) was obtained from ACP (Montréal, PQ, Canada). The SX-3 Biobeads (200–400 mesh) used in gel permeation chromatography columns were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

Extraction and cleanup of biotic samples

Zooplankton samples (~10–20 g wet wt) from NBB and Rankin Inlet were extracted using previously described techniques [10,15]. Samples from Alaska and Holman were homogenized with sodium sulfate and spiked with two polychlorinated biphenyl (PCB) internal standards (CB-30 and CB-204) to monitor the efficiency of the extraction protocol. Samples were extracted with dichloromethane (DCM) using Soxhlet (Millville, NJ, USA) extraction for 16 h and subsequently passed through an Allihn funnel containing sodium sulfate and concentrated. Lipids and other bio-organic materials in each sample were removed using gel permeation chromatography. The analyte sample was concentrated and separated on 8 g of 100%-activated silica gel into two fractions: 65 ml of 100% hexane (F1) and 90 ml of 50% hexane:50% dichloromethane (F2). Endrin ketone and 1,3-dibromobenzene were added as laboratory spiking surrogates to determine fractionation performance. Samples were roto-evaporated, transferred to 2,2′,4-trimethylpentane (isooctane), and concentrated to approximately 100 μl. The CB-166 was added to volume correct sample analysis.

Extraction of water samples

Water samples were extracted using the same protocol under clean-room laboratory conditions (carbon and HEPA™ filters, Anaheim, CA, USA, positive pressure) at the National Water Research Institute (Environment Canada). Field and laboratory blanks for each sampling location were extracted and analyzed concurrently. The XAD-2 resin columns were transferred and eluted with methanol (200 ml) and then DCM (250 ml). The combined eluant was shaken with 3% NaCl solution, and the organic (DCM) lower layer was separated. The DCM extracts were concentrated, transferred with isooctane, and reduced to 1 ml under a gentle stream of high-purity nitrogen. Samples were subsequently separated on silica gel into two fractions (see the previous discussion). Each fraction was transferred to isooctane and concentrated to 100-μl for gas chromatography–electron capture detector (GC–ECD) analysis.

PCB/OC pesticide analysis

Polychlorinated biphenyl and OC pesticide analysis on both fractions from zooplankton samples was performed using a Hewlett-Packard (Wilmington, DE, USA) 5890 gas chromatograph with a ^{63}Ni-electron capture detector. Splitless injections

of 1-μl volumes were performed by a Hewlett-Packard 7673 autosampler with a splitless time of 2.0 min (injector temperature set at 220°C). Compound separation was completed using a 60-m × 0.25-mm (i.d.) DB-5 column (internal film thickness 0.25 μm; J&W Scientific, Folsom, CA, USA) with H_2 carrier gas (at a constant flow rate of 0.91 ml/min). Nitrogen was used as the makeup gas for the ECD (detector temperature: 325°C). The oven temperature program was initiated at 80°C (held 2.0 min, ramped to 150°C at 10°C/min, then ramped at 2°C/min to 280°C, 10-min hold time) and maintained until the completion of the 90-min run. Water samples were analyzed with a dual column (DB-1 and DB-5), single injection GC–ECD with conditions similar to those noted previously. Sample quantification was performed using multiple external standards obtained from the National Laboratory for Environmental Testing (Environment Canada).

Toxaphene analysis

Fraction 2 was analyzed for toxaphene by gas chromatography–mass spectroscopy (GC–MS) in electron capture negative ion mode. Those analyses were performed using a Hewlett-Packard 6890 GC with a 30-m × 0.25-mm (i.d.) HP5-MS column (internal film thickness 0.25 μm) with a Hewlett-Packard 5973 MS detector. The quantification methods and GC–MS operating parameters used have been previously described [16]. Quantification of total toxaphene and its homologue groups was accomplished using a technical toxaphene standard. Individual congeners (Parlar 11/12, Parlar 15, Parlar-25, Parlar-31, B6-923, B7-515, B7-1001, B8-531, B8-789, B8-806/809, B8-1413, B8-1414/1945, B8-2229, B9-715, B9-1025, B9-1046, B9-1049, B9-1679, and B10-1110) were quantified using a series of authentic external standards of each compound [16]. Polychlorinated bornanes were assigned nomenclature as described by Andrews and Vetter [17]. In brief, the letter B refers to chlorobornane, and the number indicates Cl substitution. The four-digit numeric code describes the arrangement of Cl substitution on the bornane carbon skeleton. The Parlar numeric system was used to classify congeners with undetermined structure.

Analytical quality assurance

Recoveries for the surrogate standards in zooplankton samples were averaged and considered acceptable if greater than 70% (average ± 1 standard error: PCB-30, 89% ± 4.6; PCB-204, 82% ± 5.4). The XAD-2 resin columns were spiked with 1,3,5-tribromobenzene, CB-30, and octachloronaphthalene prior to deployment to monitor sample extraction. All results were volume corrected using CB-166 and then blank corrected. Detection limits were approximately 0.01 ng/g for zooplankton and 0.1 pg/L for water for all the OC compounds. Calibration standards were analyzed after every 10 samples. Quality assurance procedures for organochlorine analysis included the use of standard reference materials (SRM1588 Cod Liver Oil) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and participation in an international interlaboratory comparison program on toxaphene and PCB analysis (QUASIMEME, Aberdeen, UK).

Statistical analysis

Variation in contaminant class concentrations was examined by summing (Σ) the main OC groups of chlorobenzenes (Σ ClBz), hexachlorocyclohexanes (Σ HCH), chlordane-related compounds (Σ CHLOR), DDT and DDT metabolites (Σ DDTs), and polychlorinated biphenyls (Σ PCBs) congeners. Table 1 provides a more detailed account of the composition of each sum OC group. Data on OC water concentrations were compared by location using a model I analysis of variance (ANOVA) with Tukey's test for post hoc comparisons ($\alpha = 0.05$). Water and zooplankton data were log transformed to reduce the skewness and kurtosis of the data and yield data that meet the assumptions for ANOVA.

The influence of sample location, lipid content, and water content in zooplankton samples and all first-order interaction effects on Σ OC concentrations were examined using a general linear model.

$$\text{Log}_e \sum \text{OC} = \mu + \text{location} + \text{water} + \text{lipid} + (\text{location} \times \text{water}) + (\text{location} \times \text{lipid}) + \epsilon$$

Interaction terms were not significant and therefore ignored. The reduced general linear model used was

$$\text{Log}_e \sum \text{OC} = \mu + \text{location} + \text{water} + \text{lipid} + \epsilon$$

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 ($\alpha = 0.05$). Differences in Σ OC concentrations in filtered water samples were investigated using a model I ANOVA ($\alpha = 0.05$) and Tukey's post hoc comparison tests.

The potential biotransformation of PCB congeners and other OC analytes was examined by calculating the accumulation of PCBs and OCs in zooplankton samples to water relative to the recalcitrant CB-153 (RR_{153}):

$$RR_{153} = ([\text{Compound}_X]/[CB_{153}]_{\text{Calanus}}) \div ([\text{Compound}_X]/[CB_{153}]_{\text{water}})$$

where Compound_x and CB_{153} represent the concentrations of a specific OC and CB-153 in zooplankton and water samples. Each PCB congener was assigned to one of five structural groups previously described [18]: group I, congeners without vicinal H atoms; group II, congeners with vicinal H only in the *ortho* and *meta* positions and two *ortho* Cl atoms; group III, same as group II, but with one *ortho* Cl; group IV, congeners with vicinal H in the *meta* and *para* positions with two (or less) *ortho* Cl; and group V, same as group IV, but with three *ortho* Cl atoms. A model I ANOVA ($\alpha = 0.05$) was used to examine differences in the relative ratios (RR_{153}) between structural groups. Tukey's multiple comparison test was used to examine significant differences in congener proportions.

The RR_{153} versus log K_{ow} relationships were investigated by linear (first-order) regression. The relationships between bioaccumulation factors (BAFs) of selected OCs and octanol–water coefficients (K_{ow}) were examined in the same manner. Log K_{ow} values used for this purpose were taken from previous reports [19,20]. The BAFs were determined for *Calanus* spp. and water data from the same location. The BAF calculations were performed for selected OCs compounds in all samples using the equation

$$\text{BAF (for OC}_x\text{)} = [\text{OC}_x \text{ in } \textit{Calanus} \text{ spp.}]/[\text{OC}_x \text{ in water}]$$

where [*Calanus* spp.] are the mean lipid-adjusted OC concentrations in zooplankton samples (ng/g lipid) and [water] is the mean OC concentration in water samples (ng/ml). All statistical analysis was performed using Systat® for Windows, Ver 8.0 (SPSS, Chicago, IL, USA).

Table 1. Concentrations (mean ± 1 standard error) of major Σ organochlorine contaminants (OCs) in *Calanus* spp. (ng/g dry wt) and water samples (ng/L)

Location	*n*	Lipid %[a]	Water %	Σ ClBz[b]	Σ HCH[c]	Σ CHLOR[d]	Σ DDT[e]	TOX[f]	Σ PCB[g]
Calanus spp.									
Barrow, Alaska	3	27.3 ± 0.9	90.6 ± 0.3	2.89 ± 0.35	5.08 ± 0.64	0.79 ± 0.45	1.18 ± 0.95	26.0 ± 7.50	12.6 ± 0.74
Kaktovik, Alaska	5	43.3 ± 4.2	85.8 ± 0.4	2.16 ± 0.28	5.61 ± 1.66	1.13 ± 0.19	3.22 ± 0.62	32.4 ± 9.26	29.2 ± 7.26
Holman, Northwest Territories	5	48.9 ± 6.6	73.4 ± 1.0	3.83 ± 0.58	6.21 ± 1.08	3.32 ± 0.85	5.33 ± 0.94	38.4 ± 3.30	33.8 ± 6.09
Northern Baffin Bay	20	39.3 ± 2.1	83.0 ± 1.2	2.42 ± 0.18	11.8 ± 3.23	4.44 ± 1.00	4.74 ± 0.74	56.0 ± 16.3	30.2 ± 4.03
Rankin Inlet, Nunavut	4	26.3 ± 7.2	NA[h]	1.88 ± 0.33	2.54 ± 0.44	4.61 ± 0.49	5.57 ± 0.58	20.3 ± 3.29	54.5 ± 7.49
Water									
Barrow, Alaska	7	—	—	0.120 ± 0.006	2.106 ± 0.460	0.010 ± 0.004	0.019 ± 0.006	0.188 ± 0.046	0.231 ± 0.090
Holman, Northwest Territories	3	—	—	0.043 ± 0.004	2.277 ± 0.146	0.008 ± 0.002	0.005 ± 0.001	0.150 ± 0.040	0.135 ± 0.018
Northern Baffin Bay	6	—	—	0.025 ± 0.002	1.233 ± 0.078	0.015 ± 0.002	0.008 ± 0.004	0.253 ± 0.013	0.368 ± 0.080
Rankin Inlet, Nunavut	3	—	—	0.014 ± 0.002	1.687 ± 0.062	0.011 ± 0.003	0.003 ± 0.001	NA	0.469 ± 0.058

[a] Lipid % is dry weight.
[b] Σ ClBz (chlorobenzenes) = sum of 1,2-diClBz, 1,4-diClBz, 1,2,3-triClBz, 1,2,4-triClBz, 1,3,5-triClBz, 1,2,3,4-tetraClBz, 1,2,3,5-tetraClBz, pentaClBz, and hexaClBz (HCB).
[c] Σ HCH (hexachlorocyclohexanes) = sum of α-HCH, β-HCH, γ-HCH, and δ-HCH.
[d] Σ CHLOR (chlordanes) = sum of *cis*-chlordane, *trans*-chlordane, oxychlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor, and *cis*-heptachlor epoxide.
[e] Σ DDT (dichlorodiphenyltrichloroethane isomers) = sum of *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT, and *p,p′*-DDT.
[f] TOX (toxaphene) = total toxaphene.
[g] Σ PCB (polychlorinated biphenyls) = sum of congeners 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.
[h] NA = not available.

RESULTS AND DISCUSSION

Lipid, water content, and sample location

Copepod samples with highest lipid content (Kaktovik, Holman, NBB) were collected in summer to early fall. Fisk et al. [10] found that lipid levels increased from spring through the summer months. These observations are consistent with previous studies that have monitored lipids in *Calanus* species during the summer months in the Arctic [21]. Typically, copepods aggregate near the top of the water column during spring and summer to feed, increase lipid quantity, and prepare for reproduction and return to deeper levels in winter to lay eggs [6,7]. Lipid composition in zooplankton samples from Barrow and Rankin Inlet collected in September did not match the seasonal enrichment profile described previously. These fluctuations may also be associated with age composition and feeding behavior of zooplankton [7]. Further research is required to investigate potential spatial differences of feeding ecology and productivity of zooplankton species in the Arctic.

Spatial distribution of OCs

In zooplankton samples from Holman, Barrow, and Kaktovik, the ranking of OC group concentrations (ng/g, dry-wt basis) was toxaphene (TOX) $\geq$ Σ PCB $>$ Σ HCH $>$ Σ DDT $>$ Σ CHLOR $>$ Σ ClBz (Table 1). This rank order of OC concentrations in zooplankton samples was generally consistent for all sites sampled in this study. No significant differences were found for any Σ OC groups among the western Arctic sites (Holman, Kaktovik, and Barrow) when the effect of lipid and water content covariates was removed ($p > 0.05$ for all comparisons). However, significant differences were observed between the Alaskan/Holman sample sites and NBB/Rankin Inlet for TOX, Σ PCBs, Σ DDT, Σ HCH, and Σ CHLOR ($p < 0.05$).

In general, the most common OCs in both water and zooplankton samples were the more water soluble, less hydrophobic compounds. The most predominant OC analyte found in *Calanus* and water samples was α-HCH. More highly chlorinated OC compounds, such as octa- and nonachlorobiphenyls, are less frequently observed in water and zooplankton in the Arctic [10] or even in temperate marine environments [22]. In *Calanus* from Alaska and Holman, the most abundant chemicals ranked in order were α-HCH $>$ hexachlorobenzene (HCB) $>$ CB-118 $>$ CB-138 $>$ CB-95/66 $>$ γ-HCH $>$ B6-923 $>$ B7-1001 $>$ CB-28/31 $>$ B9-1679 $>$ *cis*-chlordane $>$ *p,p′*-DDE. The greater abundance of toxaphene congeners relative to other OCs in zooplankton is consistent with OC concentrations in zooplankton collected in the Arctic Ocean offshore from Axel Heiberg Island in the Canadian Arctic [11].

The specific ranking order differed in the water samples where Σ HCH was the dominant analyte group, followed by Σ PCB $>$ TOX $>$ Σ ClBz $>$ Σ CHLOR $>$ Σ DDT. α-HCH was the predominant OC compound, followed by γ-HCH, CB-95/66, B6-923, B7-1001, CB-43/49, B8-789, HCB, CB-101, and CB-44, respectively. The relative abundance of α- and γ-HCH isomers observed in this study is similar to other investigations that have demonstrated that HCH is generally the most common of the OCs in Arctic seawater [13,23]. The concentrations of OC compounds in water and zooplankton samples, including *C. hyperboreus* collected in the late 1980s, were in good general agreement with this study [11]. Recent investigations in the high Canadian Arctic report similar OC levels in water and zooplankton samples as in this study [10–13,23]. This suggests that OC concentrations in the western Arctic water and zooplankton have remained constant over the decade.

Spatial trends of OCs in zooplankton and water reported here are similar to trends observed previously in marine mammals [1]. The Σ PCB, Σ DDT, Σ CHLOR, and total toxaphene concentrations from Alaska and western Canada are lower than those in samples from NBB and Rankin Inlet, NU (Fig. 1a and b and Table 1). Previous studies have revealed a gradient of Σ PCBs and Σ DDT from west to east in abiotic compartments in the Arctic marine environment of North America [1] and circumpolar observations in higher-trophic-level organisms, such as ringed seals [24].

The β-, γ-, and δ-isomers of HCH and HCB in zooplankton and water samples from the Alaskan and western Canadian Arctic were relatively higher than those in corresponding samples from Rankin Inlet and the NBB. The abundance of HCB and HCH in Alaskan and western Canadian water and zooplankton relative to the eastern Canadian Arctic reflects the long-range atmospheric transport of these chemicals and geographic proximity to areas of recent application in Asia [25,26]. Concentrations of α-HCH in seawater from the Canadian Basin were three to four times higher than those in seawater from the Beaufort Sea. These high concentrations in the Canadian Archipelago, relative to the Beaufort Sea, may be due to relatively long residence time and extended periods of ice cover, thereby restricting the potential removal of α-HCH via atmospheric and/or marine transport [27]. Temporal variation in Σ HCH concentrations within zooplankton was substantial at NBB (Fig. 1), suggesting the possibility for strong seasonal changes in OC concentrations by one or more factors related to zooplankton lipid content, life stage, and physiological status that have not been accounted for in this study [10].

Total toxaphene levels in zooplankton and water were lower at Alaskan sites compared to the eastern Canadian Arctic. Levels are generally consistent with previously reported values in the high Canadian Arctic [11–13,23,28]. Congener profiles in water and zooplankton samples from Barrow and Holman were similar. The most abundant toxaphene congeners in both water and zooplankton samples was B6-923 (Fig. 2). However, the relative abundance of several hepta-, octa-, and nonachlorobornanes (B7-1001, B8-789, B8-2229, and B9-1679) increased from water to zooplankton.

Although congener-specific toxaphene data in marine biota have been previously described [29], quantitative analysis has been restricted to higher-trophic-level organisms, such as ringed seals (*Phoca hispida*), beluga whales (*Delphinapterus leucas*), and polar bears [30–32]. The major toxaphene congeners in several marine mammalian species are B8-1413 and B9-1679, which represent as much as 80% of total toxaphene levels [29,33,34]. The stability of these two congeners in mammalian systems and subsequent bioaccumulation has been attributed to an alternating *endo-exo-endo-exo* Cl conformation on the six-member bornane ring and lower polarity relative to other toxaphene congeners [35,36].

In water, and presumably in organisms within lower trophic levels, the degradation of toxaphene is limited. The most dominant congener in both water and zooplankton was B6-923, followed by B7-1001. In water, these two congeners comprised 57 and 10% of the total congener-specific concentrations. However, the relative abundance of B6-923 decreased in zooplankton to only 21% of congener-specific concentrations be-

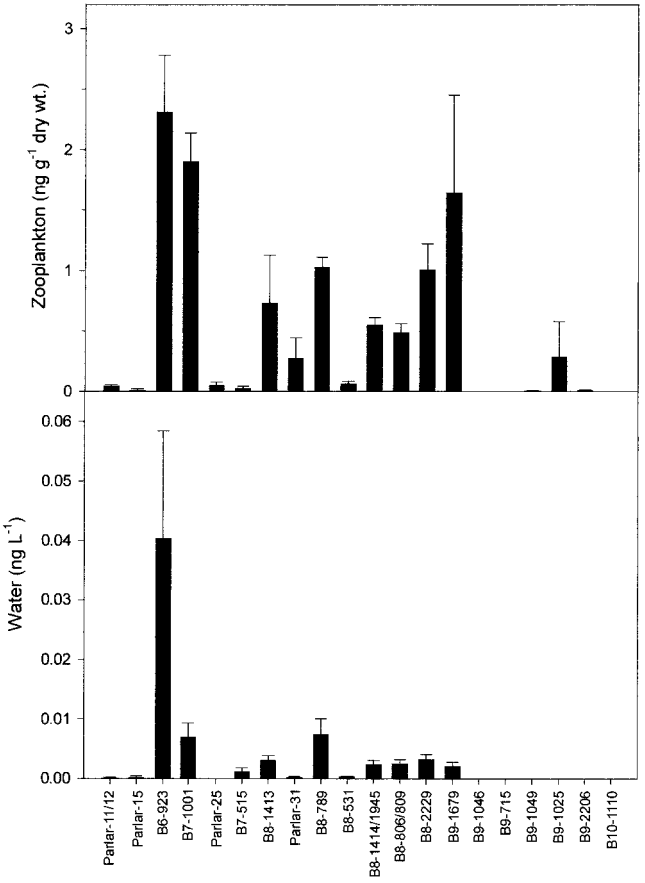

Fig. 2. Mean (±1 standard error) concentrations of individual toxaphene congeners in water (ng/L) and zooplankton samples (ng/g dry wt) from Holman, Northwest Territories, and Barrow, Alaska (combined).

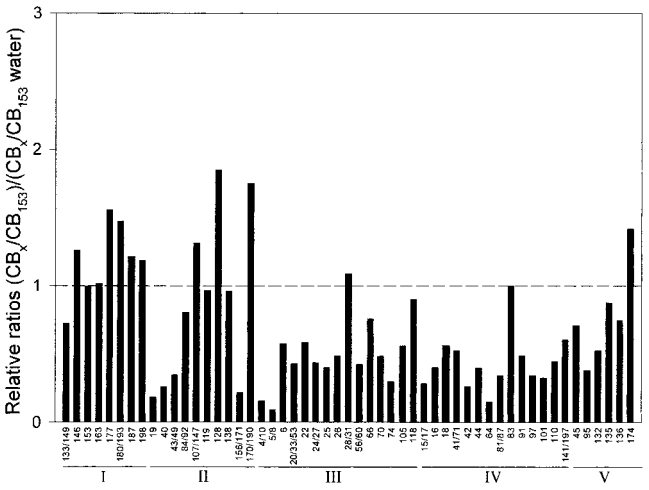

Fig. 3. Relative ratios of polychlorinated biphenyl (PCB) congeners (zooplankton/water) arranged by structural classification: group I, congeners without vicinal H atoms; group II, congeners with vicinal H only in the *ortho* and *meta* positions and two *ortho* Cl atoms; group III, same as group II, but with one *ortho* Cl; group IV, congeners with vicinal H in the *meta* and *para* positions with two (or fewer) *ortho* Cl; and group V, same as group IV but with three *ortho* Cl atoms. Dashed line represents unity (PCB-153 = PCB_x = 1).

cause of increasing abundance of B7-1001 (17%), B9-1679 (15%), B8-1413 (9%), and B8-2229 (9%). These results are consistent with the changing toxaphene profiles within the Arctic marine water–zooplankton–arctic cod (*Boreogadus saida*) food chain [37]. While the decrease of B6-923 in zooplankton relative to other toxaphene congeners might be attributed to biotransformation, it is more likely due to increased bioaccumulation of the more hydrophobic toxaphene congeners.

OC profiles and K_{ow} *relationships*

The accumulation of PCBs in zooplankton from water (relative to CB-153) is shown in Figure 3. Our results suggest that *Calanus* species may not detectably metabolize most OCs, perhaps because of a lack of appropriate enzymes or because OC exposure is insufficient to induce the appropriate metabolic degradation pathways. In biological systems, OC metabolism is mediated by induction of cytochrome P450 isozymes. Much of the research on P450 characterization and expression due to contaminant exposure in aquatic organisms has been limited to fish, birds, and marine mammals [38]. While recent investigations have identified CYP450-related gene families in several invertebrates [39], the potential metabolism of PCB congeners by marine invertebrates still remains largely unknown. Congeners were grouped according to Cl-substitution patterns to infer potential biotransformation by different cytochrome P450 isozymes from chemical analysis (Fig. 3). This approach for investigating structure–activity relationships of PCB metabolism in marine biota has been applied to species from various trophic positions [40].

The ANOVA revealed significant differences in RR_{153} between structural groups of PCB congeners in the *Calanus* species ($F_{4,48} = 7.09$, $p < 0.001$). The RR_{153} values for group IV PCB congeners were significantly different from group I ($p = 0.001$) and group II ($p < 0.001$) congeners. As well, group I congeners were significantly different from group III congeners ($p = 0.001$). None of the other combinations differed significantly ($p > 0.15$). Group I and II congeners generally had $RR_{153} > 1$, suggesting that these congeners are not readily biotransformed by *Calanus* species. Group I congeners are considered persistent PCBs, while group II congeners may be metabolized by the CYP2B subfamily. The degree of CYP2B gene expression in fish and invertebrates may be limited [41]. Previous investigations found that non- and mono-*ortho* (coplanar) Cl-substituted PCB congeners readily accumulated in phytoplankton and zooplankton species to a greater degree than other congeners [42]. This observation implies that CYP450 gene activity is low, or perhaps that CYP450 genes are not present, in planktonic marine organisms. The $RR_{153} > 1$ for each congener suggests little or no CYP2B-mediated biotransformation for groups I and II PCB congeners by *Calanus* species.

Congeners from groups III, IV, and V had $RR_{153} < 1$, indicating potential biotransformation by zooplankton. Congeners in these three groups are considered the least recalcitrant because of the presence of vicinal H atoms in adjacent *ortho-meta* and *meta-para* positions. However, the relative accumulation of PCB congeners and the high variability associated with RR_{153} values in structurally grouped PCBs implies that PCB congener distribution may be dependent on partition-based elimination rather than biotransformation.

The RR_{153} values for PCB congeners in groups I, II, III, and V were positively correlated with log K_{ow} (Fig. 4), further supporting the idea that physical properties are the main factor governing patterns of PCB congeners in zooplankton. The linear relationship between RR_{153} − log K_{ow} was significant for

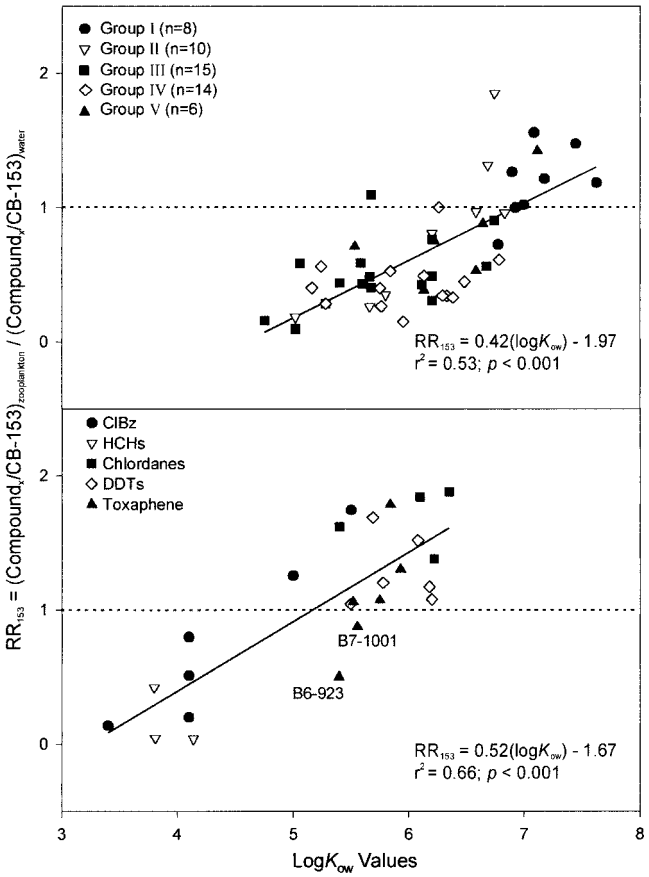

Fig. 4. RR_{153} − log K_{ow} relationship for *Calanus* samples for polychlorinated biphenyl (PCB) and other organochlorine contaminant (OC) compounds. Linear regression for PCB congeners by structural classification and OC groups is illustrated.

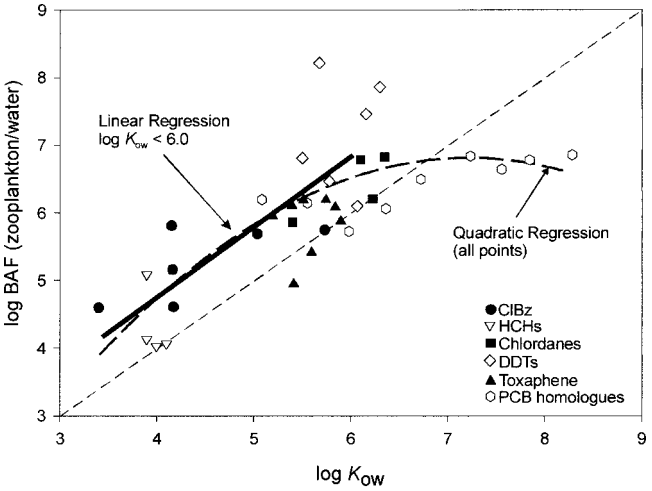

Fig. 5. Log BAF − log K_{ow} relationships for *Calanus* samples (n = 8) on lipid basis and water samples (n = 10). The straight dashed line represents unity for log bioaccumulation factor (BAF) − log K_{ow}. Quadratic regression of all points is represented by the curved dashed line (r^2 = 0.67). Linear regression for organochlorine contaminant (OC) compounds with a log K_{ow} < 6 are identified by the solid line (r^2 = 0.63).

PCB congeners (RR_{153} = 0.42log K_{ow} − 1.97, r^2 = 0.53, p < 0.001). The RR_{153} for group II congeners (open *ortho-meta* positions) also correlated with log K_{ow}, providing additional evidence that CYP2B activity is low or nonexistent in *Calanus* species. However, this relationship was not observed for congeners in group IV. These congeners are characterized by open *meta-para* positions and suggest that limited phase I metabolism may occur within zooplankton.

The RR_{153} − log K_{ow} relationship for other OC compounds was similar to that observed for PCBs (RR_{153} = 0.52 log K_{ow} − 1.67, r^2 = 0.66, p < 0.001; Fig. 4). The relative accumulation of congeners B8-1413, B8-2229, and B9-1679 (RR_{153} > 1) in zooplankton is consistent with current knowledge of toxaphene biotransformation and physical partitioning properties [43]. These three congeners have 2-*exo*,3-*endo*,5-*exo*,6-*endo*,8,8,10,10 Cl substitutions [35]. It has been hypothesized that alternating *exo-endo* Cl substitution at positions C2, C3, C5, and C6 increases the stability of these congeners [34].

The relative accumulation of B7-1001 was less than unity (RR_{153} = 0.94), indicating that this congener does not readily bioaccumulate relative to other recalcitrant compounds (CB-153). The structure of B7-1001, which is similar to the staggered 2-*exo*,3-*endo*,5-*exo* ring configuration of B8-1413, is hypothesized to make this congener less vulnerable to the oxidative process and/or anaerobic reductive dechlorination than B6-923 [44].

The RR_{153} − log K_{ow} < 1 for the 2-*exo*,3-*endo*,6-*exo*,8,9,10-hexachlorobornane B6-923 [44] suggests that this toxaphene congener may be degraded by zooplankton. This congener is believed to be more vulnerable than B7-1001 to oxidative metabolism because of the presence of two vicinal *exo*-chlorine atoms at C1 and unsubstituted C5 position [45]. However, the relatively high aqueous solubility of B6-923 and B7-1001 also may reduce the bioaccumulation of these congeners relative to more lipophilic, highly chlorinated bornanes [46]. The large proportion of B6-923 in seawater was surprising given that this congener is associated mainly with anaerobic dechlorination of geminal-substituted chlorobornane congeners in sediments [47]. Although B6-923 has not been previously reported in seawater, it is worth noting that hexachlorinated homologues of toxaphene are known to be prevalent in Great Lakes waters [48].

BAF − K_{ow} relationships

Bioaccumulation factors were plotted against log K_{ow} values to determine if OC compounds in zooplankton were at equilibrium with the marine environment (Fig. 5). The BAF − K_{ow} relationship observed for *Calanus* samples in this study from Barrow and Holman was approximately ≥1 for all OCs. The relationship was significant, but a linear model (log BAF = 0.52 log K_{ow} + 3.10, p = 0.01) provided a poor correlation between the two variables (r^2 = 0.29). Quadratic (second-order) regression revealed a stronger correlation between log BAF − log K_{ow} for the OCs detected (log BAF = −3.61 + [2.89 log K_{ow}] − [0.20 log K_{ow}]^2, r^2 = 0.63, p < 0.001), when more hydrophobic OCs (log K_{ow} > 6.0) were included.

The OC concentrations in equilibrium between lipids of an aquatic organism and water should result in a one-to-one log-linear relationship between lipid-adjusted BAF data from the organism and K_{ow} of the compound [49]. Laboratory experiments and field observations have shown that the BAF for very hydrophobic OCs (log K_{ow} > 6.0) deviates below this linear relationship and that a curvilinear model better describes the association [50]. The log-linear relationship between BAF − K_{ow} for OC compounds with log K_{ow} > 6.0 was less than 1:1, which be attributed to factors such as overestimation of

bioavailable OC concentrations, inaccurate octanol–water coefficients, and insufficient time to achieve equilibrium [51].

For OC compounds with a log K_{ow} 3 to 6, the approximate 1:1 relationship suggests that partition-based uptake and elimination of these OCs from *Calanus* samples is the most important factor governing OC accumulation in zooplankton. Linear regression for OC compounds with a log $K_{ow} < 6$ also revealed a significant correlation between the two variables (log BAF = 1.04 log K_{ow} + 0.57, $r^2 = 0.57$, $p < 0.001$). Similar relationships were found for Arctic and North Pacific marine zooplankton, suggesting that OCs in *Calanus* species are at equilibrium with OC concentrations in seawater [10,12,52]. The BAF − K_{ow} relationship further supports our findings that OC biotransformation by zooplankton is very limited. Moisey et al. [14] found that HCH-isomer patterns in pelagic arctic marine zooplankton were similar to those in seawater. In this study, all HCH isomers analyzed had a log BAF − log $K_{ow} \geq 1$, suggesting limited biotransformation of these compounds by *Calanus* spp. All toxaphene congeners (except B6-923 and B7-001) assessed in this study expressed the similar log BAF − log $K_{ow} \geq 1$, suggesting that contaminant partitioning processes, as interpreted from log K_{ow}, are the overriding factors governing OC accumulation between zooplankton and the marine environment.

The log BAF − log $K_{ow} < 1$ for B6-923 and B7-1001 indicates that these congeners may be metabolized by *Calanus* or that the relative rate of accumulation may be driven by factors other than physical partitioning and/or elimination factors. Fisk et al. [46] found a bioconcentration factor <1 for heptachlorobornanes, confirming previous observations of negligible bioaccumulation of B6-923 and B7-1001 by fish in toxaphene-treated lakes [53]. This evidence, in combination with a log K_{ow} relationship and relative accumulation of recalcitrant OCs, suggests that bioaccumulation of B6-923 and B7-1001 may be influenced by both partition-based elimination and biotransformation processes in *Calanus* species.

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