

Bioaccumulation Factors for PCBs Revisited

KATRINE BORGÅ,^{*,†} AARON T. FISK,[‡] BARRY HARGRAVE,[§] PAUL F. HOEKSTRA,^{||} DEBORAH SWACKHAMER,[⊥] AND DEREK C. G. MUIR[#]

Norwegian Polar Institute, N-9296 Tromsø, Norway, Warnell School of Forest Resources, University of Georgia, Athens, Georgia, 30602-2152, DFO, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada, Syngenta Crop Protection Canada Inc., Guelph, Ontario, Canada, University of Minnesota, Minneapolis, Minnesota, and National Water Research Institute, Environment Canada, Burlington, Ontario, Canada

Bioaccumulation factors (BAFs) for individual polychlorinated biphenyl (PCB) congeners in Barents Sea and White Sea marine calanoid copepods were 1–3 orders of magnitude higher than BAFs in the same species in Canadian and Alaskan Arctic Ocean areas, and in freshwater plankton (Lake Ontario) reported from the mid- to early 1980s. The present study reviews variability in PCB BAFs from the North American Great Lakes and the Arctic Ocean, and discusses possible explanations for the large variation among different studies. BAFs are higher in recent Arctic marine and Great Lakes studies than previously reported, and they are at least 10 times higher than those predicted from the octanol–water partition coefficient (K_{OW}). If the recent high BAFs are realistic, it means that earlier reported BAFs are too low. This is likely due to earlier erroneously high quantification of water PCB concentrations, and it implies that bioaccumulation in zooplankton is more efficient than previously assumed. Evidence is presented supporting that also trophic transfer and biomagnification of PCBs in zooplankton leads to BAFs well above those predicted by simple equilibrium partitioning. Overall, miss-measurement of water PCB concentrations and biomagnification contribute significantly to variability in BAFs for PCBs within and among studies. This large variability of BAFs for PCBs in zooplankton illustrated in the present study is of importance for future assessments of potential new bioaccumulative chemicals that rely on measured BAFs, such as the European Union Registration, Evaluation and Authorization of Chemicals program (REACH).

Introduction

Bioaccumulation factors (BAF, $L \cdot kg^{-1}$ lipid, eq 1) have been used for the past 25 years to describe the net increase of organic contaminants such as persistent organic pollutants

(POPs) from water to biota due to uptake from all exposure routes (1, 2).

$$BAF = \frac{[POP \text{ in organism}]_{LIPID \text{ CORRECTED}}}{[POP \text{ in water}]_{DISSOLVED}} \quad (1)$$

The bioconcentration factor (BCF) is a special case of bioaccumulation seen in controlled laboratory experiments in which BCF is the equilibrium ratio between POPs in biota and the surrounding environment due to abiotic exposure; however, BAF and BCF are calculated the same way (eq 1). The Stockholm convention on POPs (3) and other chemical management programs categorize chemicals with BCF or BAF higher than 5000 (wet weight basis) as bioaccumulative. Although usually calculated on a wet weight basis (2, 4), BAFs and BCFs change depending on the lipid content of the organism. Therefore, lipid-normalized BAF and BCF values (eq 1) are more useful when comparing across animals, as the variation due to variable lipid content is eliminated.

BCF on a wet weight basis can be predicted from the chemical's octanol–water partition coefficient (K_{OW}), by models such as BCFWIN used by the U.S. Environmental Protection Agency (USEPA) (4). In absence of environmental measurements of a chemical in biota and water to calculate BAFs, BCFs predicted from K_{OW} s are useful tools for exposure and risk assessments of new chemicals; however, for animals with dietary exposure and uptake of POPs they may be underpredicted. Programs such as Registration, Evaluation and Authorization of Chemicals (REACH) in the European Union (5), the Canadian Environmental Protection Act (CEPA)'s Domestic Substances List (DSL) (6), and the USEPA high production chemicals assessments (7) are screening large numbers of chemicals for bioaccumulation potential using predicted BCFs. Future candidate POPs will likely be chemicals with BCF or BAFs greater than 5000, and field evidence for this will probably be essential to develop a strong case for inclusion on the POPs list. Thus, a detailed understanding of the uncertainties of BCF and BAF measurements is needed.

In the present study we have summarized data from arctic marine ecosystems and from the Great Lakes of North America to investigate uncertainties of field measurements of BAFs and factors affecting calculated values of BAFs. Factors influencing BAFs are those such as season or size-related characteristics of plankton (8–10), as well as differences in water sampling methods (such as filtration, sorbent material, on-board versus in situ sampling) that can affect estimates of dissolved POPs (11). Our focus is on water to zooplankton accumulation of polychlorinated biphenyls (PCB) for the following reasons. (i) PCBs are measured on a congener specific basis in many field programs, and their K_{OW} s are known (e.g., 12). (ii) Although feeding on phytoplankton and other zooplankton, by virtue of their size and physiology, zooplankton are believed to accumulate a significant fraction of POPs from the dissolved phase in water and to rapidly reach equilibrium with water (2, 8, 13), thus they are more straightforward to model than fish and other top predators. (iii) Because zooplankton are typically one trophic level lower than most invertebrate-feeding fish in marine and freshwater food webs, BAFs of POPs in zooplankton are generally the lowest field BAFs that can be routinely measured, and they are thus conservative values.

The main objective of the present study was to revisit the issue of whether “true” BAFs could be established for PCBs dissolved in water to zooplankton in lakes and marine

* Corresponding author tel: +47 777 505 00; fax: +47 777 505 01; e-mail: katrine.borga@npolar.no.

† Norwegian Polar Institute.

‡ University of Georgia.

§ Bedford Institute of Oceanography.

|| Syngenta Crop Protection Canada Inc.

⊥ University of Minnesota.

Environment Canada.

systems. In particular we wanted to understand whether BAFs for PCBs are in the range predicted by the congener's K_{OW} .

Materials and Methods

Comparison of BAFs. Unfortunately, relatively few field studies include PCB measurements in both zooplankton and the operational dissolved phase in water. We selected studies which report both water and zooplankton PCB concentrations (9, 14–22) as well as other work in which water and zooplankton were collected in the same region or lake but analyzed by separate groups (23–29) (Tables 1 and 2). All water samples were filtered to separate particle-bound and dissolved PCBs in all studies, except Oliver and Niimi (16) and Morrison et al. (17) which were centrifuged (removing particles larger than 0.2 μm) (30). BAFs (eq 1) for PCBs were calculated for zooplankton from the Arctic Ocean and from the Laurentian Great Lakes (Figure 1) and compared to the 1:1 relationship with K_{OW} (K_{OW} values from 12) (Figure 2).

There are few studies of PCBs in zooplankton and water that provide data from different seasons. BAF variability due to season was directly evaluated using data from one marine (9) and one freshwater study (21) (Figure 3). The influence of size was investigated within two marine studies, one comparing different calanoid copepod species (25) (Figure 2e), and one comparing zooplankton separated into different size fractions (9) (Figures 2a and 4a). A recurring problem in many studies is that water and biota were not sampled simultaneously or from the same locations. One example is an often-cited Lake Ontario study in which BAFs were calculated using water sampled in April 1984 and “net plankton” sampled by centrifugation in July 1982 (16).

BAFs and Measured Zooplankton or Water PCB Concentrations. If the zooplankton PCB concentration is assumed to reflect the concentrations in its surrounding environment, the measured BAFs should be independent of exposure level. One difficulty in comparing BAFs from various studies and datasets, however, is the measurement of PCB in zooplankton and seawater samples. A correlation between calculated BAF and measured PCB in zooplankton and/or water might indicate a bias in the quantification of the respective PCB concentrations. Correlations between the calculated BAFs and measured ΣPCB (sum of all congeners quantified in both water and zooplankton within each study) and $\Sigma 10\text{PCB}$ (sum of congeners 28, 31, 52, 101, 105, 118, 149, 153, 138, and 180) concentrations was investigated separately for the freshwater and the marine studies (Spearman's rank order correlation). Although congeners included in ΣPCB and $\Sigma 10\text{PCB}$ differ among studies as the congeners included in each study varied, this does not influence the correlation, because within a study the same congeners were included in both zooplankton and water (Tables 1 and 2).

Statistical analyses (Pearson correlation and Spearman's rank order correlation) were performed in SAS V8 for Windows (31).

Results and Discussion

Variation in PCB BAFs. Both within and among studies, the marine and freshwater BAFs for individual PCB congeners ranged widely around the 1:1 relationship with K_{OW} (Figure 2). Calculated BAFs for Canadian and Alaskan Arctic marine studies were generally around or below the range predicted by K_{OW} , whereas Barents Sea and White Sea BAFs were approximately 1 to 3 orders of magnitude higher than those predicted by the K_{OW} (Figure 2a–e). Lakes Michigan, Ontario, and Erie BAFs are generally above the 1:1 line (Figure 2h–k), whereas the Experimental Lakes Area (L110 and L227) BAFs are below (Figure 2f–g), and Lake Superior BAFs are widely scattered around the line (Figure 2l). The difference between

maximum and minimum marine log BAFs ranged from 0.18 to 7.78 for the individual congeners, with largest difference for high K_{OW} congeners (Figure 2) ($n = 43$, Pearson $R = 0.370$, $p = 0.015$; Spearman's rank $R = 0.295$, $p = 0.054$). For the Great Lakes, differences between maximum and minimum BAF ranged from 0.002 to 4.01 for the individual congeners, and decreased slightly with increasing K_{OW} ($n = 86$, Pearson $R = -0.231$, $p = 0.032$; Spearman's rank $R = -0.155$, $p = 0.153$). Thus, the relationship between differences in observed freshwater log BAFs and log K_{OW} was opposite to that of the marine studies, but note that neither the marine nor the freshwater nonparametric correlations were significant.

When the relationship between calculated BAFs and the measured water and zooplankton PCB concentrations was investigated, marine BAFs were not correlated with marine zooplankton PCB concentrations (Spearman's rank: ΣPCB $r = 0.20$, $p = 0.704$, $\Sigma 10\text{PCB}$ $r = 0.25$, $p = 0.486$) (Figure 3). The lack of correlation between marine BAF and zooplankton PCB concentrations indicates that the PCBs in zooplankton were determined in a similar and comparable way among studies. Most of the marine zooplankton samples were comparable in that they were largely dominated by large calanoid copepods (14, 15, 19, 25). The quality of zooplankton PCB data is usually not considered problematic as lipids are relatively easy to separate and extract. Also, PCB levels in zooplankton are usually well above method detection limits, and interlaboratory comparisons for PCB quantification in marine biota suggest that between-laboratory accuracies of 15–20% can be achieved among experienced laboratories (32). Lake zooplankton PCB concentrations, however, were positively correlated with BAFs (Spearman's rank: ΣPCB $r = 0.93$, $p = 0.0002$; $\Sigma 10\text{PCB}$ $r = 0.96$, $p = 0.0005$). The positive correlation may be due to either compromised PCB measurements, which seems unlikely given the elevated PCB levels in biota and the high extraction efficiency of lipids and associated PCBs, or it may be due to inclusion of a wide variety of zooplankton size fractions and trophic guilds in the different studies.

BAFs decreased with increasing seawater and lake PCB concentrations (seawater: Spearman's rank ΣPCB $r = -0.72$, $p = 0.0427$; $\Sigma 10\text{PCB}$ $r = -0.81$, $p = 0.0041$; freshwater: ΣPCB $r = -0.75$, $p = 0.0199$; $\Sigma 10\text{PCB}$ $r = -0.79$, $p = 0.0362$) (Figure 3). The significant correlations between BAFs and water PCB concentrations suggest that either some PCB measurements were compromised or different sampling techniques (water collection, particle separation, volume extracted, extraction method) resulted in differences in fractionation of dissolved PCBs. Differences in water PCB measurements most likely explain the large variation in BAFs among the arctic marine studies, such as the Canadian Arctic and the Barents Sea, as PCB concentrations in similar zooplankton species did not differ between the studies (33), whereas the water concentrations differed widely (14, 23). When quantifying PCBs in water, water collection and contaminant extraction is the step associated with most uncertainties (34, 35). Passive respiratory uptake of contaminants from water is from the freely dissolved concentration, which should be used for BAF calculation to make them comparable among studies (2). Determination of the dissolved fraction of PCBs in water is challenging, and early work may not have thoroughly accounted for shipboard and laboratory contamination (36). Several methodological advances during the late 1990s, including use of clean rooms on ships, solid-phase extraction, and in situ samplers, have led to significantly lower dissolved PCBs reported both in the Arctic and in the Great Lakes. Overestimated dissolved water PCB concentrations were also noted by Harding (37) when reviewing BAFs from the early 1970s. In the early 1980s, when the Great Lakes' research was initiated, the general method used was to pump water onto the ship, centrifuge the water to separate out the

TABLE 1. Water Concentrations of Polychlorinated Biphenyls (PCBs) in the Reviewed Great Lakes and Arctic Marine Studies (Mean \pm SD $\mu\text{g/L}$)

	year	month	method ^b	filters	extraction	volume	n	clean room ^a		blank corr. ^c	ΣPCBs	ΣPCB_{10}	ref ^d	
								S	A					
Arctic Marine Studies														
White Sea	1999	August	Infiltrex	GF/A	XAD-2	< 100 L	4	-	+	+	119 \pm 61.2	40.1	20	
White Sea	2000	August	Infiltrex	GF/A	XAD-2	< 100 L	4	-	+	+	69.0 \pm 10.7	23.7	20	
Barents Sea	1999	May	submersible pump	GF/F	PUF	300–500 L	2	-	+	+		0.30	23	
Barents Sea	2001	July	submersible pump	GF/F	PUF	700–1100 L	2	+	+	+		0.95	24	
Northwater Polynya	1998	May	submersible pump	GF/F 145 μm	XAD-2	ca. 80L	6	-	+	+	290 \pm 158	121 \pm 68.9	14	
Canadian Ice Island	1986	May-June	Seastar/Infiltrex	GF/F 1 μm	XAD-2	180–400 L	6	-	-	-	7.0 \pm 5.0		54	
Resolute Bay	1993	April-June	Seastar/Infiltrex	GF/F 1 μm	XAD-2	200–400 L	4	-	-	-	15.1 \pm 6.5	10.3 \pm 4.6	9	
Barrow-Beaufort-Chukchi	1999/00	September	Infiltrex	GF/F 145 μm	XAD-2	ca. 100 L	5	-	-	+	87.1	20.6	15	
Holman	1999	June	Infiltrex	GF/F 145 μm	XAD-2	ca. 100 L	3	-	+	+	135 \pm 18.0		15	
Great Lakes Studies														
Lake Ontario	1984	April	March pump	centrifuge	solvent		7	-	-		1070	426	16	
Lake Ontario	2003	July	Infiltrex	GF/F 1 μm	XAD-2	100 L	2	+	+	+	59.6	18.6	22	
Lake Superior	1997	May	Infiltrex	GF/F 145 μm	XAD-2	200–400 L	17	+	+	+	117	67.4	27	
Lake Erie	1994	July-August	pump	centrifuge	solvent	50 L	3	-	-	-	220	89.0	17	
Lake Michigan	1994/95	whole year	submersible pump	GF/F 0.7 μm	XAD-2	300 L	68	-	-	-	150 \pm 95.0	42.2	18, 51	
Lake Michigan GTB	1997	April-June	submersible pump	GF/F 293 μm	XAD-2	200 L	3	-	-	-	107 \pm 12.6		21	
Lake Michigan GTB	1997	July-September	submersible pump	GF/F 293 μm	XAD-2	200 L	3	-	-	-	111 \pm 38.6		21	
Experimental Lake 110	1993	May and June	submersible pump	GF/F 0.7 μm	XAD-2	45–70 L	2	-	-	+	667	237 \pm 85.4	29	
			DOC corrected values using eq 3 (from 41)									327	96.5	
Experimental Lake 227	1993	May and June	submersible pump	GF/F 0.7 μm	XAD-2	45–70 L	2	-	-	+	518	157 \pm 4.4	29	
			DOC corrected values using eq 3 from 41									279	65	

^a S and A indicate whether clean room facilities were used during water extraction (S) and chemical analyses (A), respectively. ^b Infiltrex = AXYS Infiltrex II system in situ sampler; Seastar = Seastar in situ pump; AXYS Technologies, Sidney BC Canada; submersible pump = submersible pumping onto the ship (stainless steel system). ^c Blank corr indicates whether the results are blank-corrected. ^d Ref refers to reference list. See Table 1 in the supporting information for congeners included in the different studies.

TABLE 2. Zooplankton PCB Concentrations in the Reviewed Studies (Mean \pm SD ng/g Lipid Weight)

	year	month	method	S ^b	blank corr ^c	biota	size	n	lipid % ^d	Σ PCBs	Σ PCB ₁₀	ref ^e
Arctic Marine Studies												
White Sea	1999	August	VNH ^a (100 μ m)	–	+	Calanoid copepods		4	4.1 \pm 3.8	182 \pm 73.2	71.9 \pm 26.1	19
White Sea	2000	August	VNH (100 μ m)	–	+	Calanoid copepods		5	2.1 \pm 0.5	653 \pm 334	299 \pm 162	19
Barents Sea	1999	May	VNH (1000 μ m)	–	–	<i>Calanus glacialis</i>		3	2.2 \pm 1.0		53.8 \pm 19.5	25
Barents Sea	1999	May	VNH (1000 μ m)	–	–	<i>Calanus hyperboreus</i>		2	2.8 \pm 0.7		26.4 \pm 1.3	25
Northwater Polynya	1998	May	VNH (253 μ m)	–	+	<i>Calanus hyperboreus</i>		5	2.8 \pm 1.4	115 \pm 187	59.4 \pm 103	14
Canadian Ice Island	1986	May	VNH (20 and 253 μ m)	+	–	zooplankton	125–509 μ m	3	5.9 \pm 1.5	73.0 \pm 66.0		54
Canadian Ice Island	1986	May	VNH (20 and 253 μ m)	+	–	zooplankton	>509 μ m	9	3.7 \pm 1.4	46.0 \pm 27.0		54
Resolute Bay	1993	May-June	VNH (20 and 253 μ m)	+	–	zooplankton	125–509 μ m	3	3.3 \pm 1.1	28.9 \pm 6.0	18.9 \pm 5.4	9
Resolute Bay	1993	May-June	VNH (20 and 253 μ m)	+	–	zooplankton	>509 μ m	3	4.4 \pm 1.7	11.3 \pm 5.0	6.9 \pm 2.1	9
Barrow	2000	Sept	VNH (253 μ m)	–	+	<i>Calanus</i> spp.		3	27.3 \pm 0.9	37.5	6.9	15
Great Lakes Studies												
Lake Ontario	1982	July	centrifugation of 5000 L			mainly phytoplankton		3	0.5	46.4	15.6	16
Lake Ontario	2003	July	VNH (100 μ m)	–	+	zooplankton		3	1.1 \pm 0.9	349	173	22
Lake Superior	1998	May	VNH (100 μ m)	–	+	zooplankton		4	4.8 \pm 1.4	252	128	26
Lake Erie	1994	Julu-August	VNH (125 μ m)	–	–	zooplankton		5	1.2 \pm 0.2	2200	1120	17
Lake Michigan	1994/95	whole year	VNH (100 μ m)	+	–	zooplankton	100–300 μ m	70	20.0 \pm 10.0	832	282	18, 51
Lake Michigan GTB	1997	April-June	VNH (153 μ m)	–	–	zooplankton		3	0.6 \pm 0.2	4310 \pm 756		21
Lake Michigan GTB	1997	July-Sept	VNH (153 μ m)	–	–	zooplankton		3	2.1 \pm 0.4	473 \pm 46.2		21
Experimental Lake 110	1992/93	May/July	VNH (100 μ m)	–	–	zooplankton		2	23.6 \pm 0.6	194	71.2 \pm 8.7	28
Experimental Lake 227	1992/93	May/July	VNH (100 μ m)	–	–	zooplankton		2	28.0 \pm 1.8	276	73.7 \pm 9.9	28

^a VNH = vertical net hauls (mesh size). ^b S = field equipment was solvent-rinsed between samplings. ^c Blank corr = whether measured values were blank-corrected. ^d Lipid content is % of wet weight (except for Barrow, Lake Michigan and experimental lakes (ELA110 and ELA227), where they are % of dry weight, thus italicized). ^e Ref refers to reference list. See Table 2 in the supporting information for congeners included in the different studies.

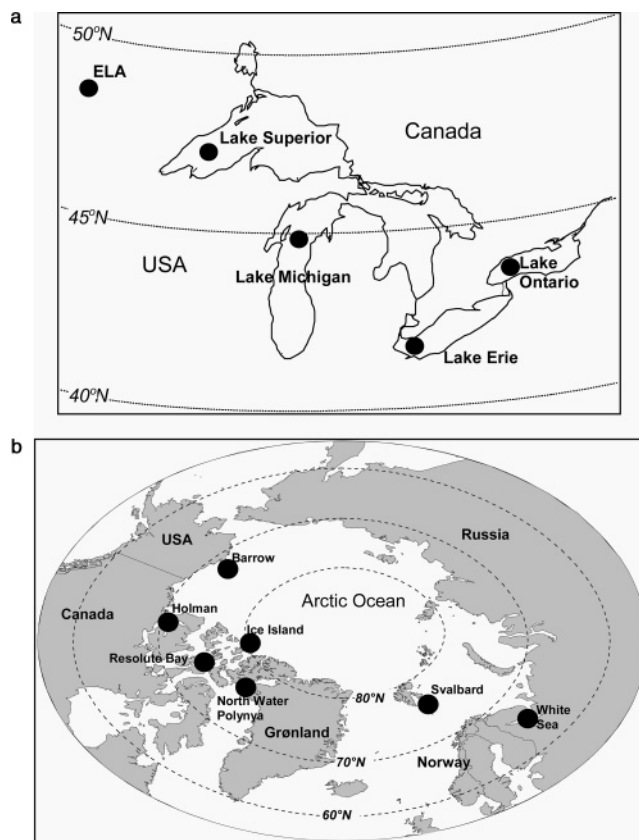


FIGURE 1. Sites of the different reviewed (a) freshwater and (b) marine studies. Black dots indicate approximate sampling areas within each lake and in the Arctic Ocean. ELA = Experimental Lakes Area.

particles, and use dichloromethane extraction to recover the dissolved PCBs (e.g., 16). This method however, can also accumulate PCBs shipboard and cause laboratory contamination, and was largely abandoned in favor of XAD-2 resin extraction (29, 38) or polyurethane foam (PUF), although in some laboratories with low PCB backgrounds dichloromethane extraction yielded results similar to those from XAD-2 resins (39).

Petrick et al. (34) estimated that 500–1000 L of water should be extracted to obtain reliable dissolved PCB concentrations without interference of analytical problems. In the reviewed studies, lower seawater dissolved PCB concentrations ($\text{fg}\cdot\text{L}^{-1}$ range) were reported when 200–400 L was extracted using XAD-2 resins (9), and when more than 400 L was extracted using PUFs (23, 24) (Table 1). Higher seawater dissolved PCB concentrations ($\text{pg}\cdot\text{L}^{-1}$ range) were reported when less than, or close to, 100 L was extracted using XAD-2 resins (14, 15). Thus, it appears that extraction by XAD-2 and PUFs yields different measurements of dissolved PCBs, and/or that extraction of low water volume may result in greater interference from background PCB contamination. Breakthrough of the cartridge in use by large volume sampling is considered negligible as extracting different high volumes of water resulted in comparable PCB concentrations (40).

Although all the studies considered in this analysis filtered or centrifuged water to remove particulate organic carbon (POC) and POC-associated PCBs, not all of the chemicals were freely dissolved as filtering and centrifugation are only operational definitions (30, 37). PCBs associated with dissolved organic carbon (DOC) will pass through the filter, and are not freely dissolved and bioavailable for passive uptake to lower trophic level biota. By adjusting for DOC, the freely dissolved concentration can be obtained, which is lower than the operationally dissolved, resulting in higher BAFs.

Burkhard (41) suggest calculating the freely dissolved fraction by eq 2:

$$f_d = 1 / (1 + \text{POC} \times K_{OW} + \text{DOC} \times 0.08K_{OW}) \quad (2)$$

where f_d is the freely dissolved fraction, DOC is the average dissolved organic carbon concentration in the water column (kg of organic carbon $\cdot\text{L}^{-1}$ of water), POC is the average particulate organic carbon concentration in the water column (kg of organic carbon $\cdot\text{L}^{-1}$ of water), K_{OW} estimates the partition between POC and freely dissolved chemical, and $0.08K_{OW}$ estimates the partition between DOC and freely dissolved chemical. Assuming POC is negligible for filtered or centrifuged water, the fraction dissolved is

$$f_d = 1 / (1 + \text{DOC} \times 0.08K_{OW}) \quad (3)$$

As more hydrophobic PCBs, usually with $\log K_{OW}$ higher than 6, have a larger proportion bound to or associated with dissolved organic carbon (2), the difference between BAFs based on operational and freely dissolved water PCB concentration is higher for these congeners. Likewise, the variability in measured BAFs would likely be higher with increasing K_{OW} , as different collection techniques (in which some are more likely to have captured the freely dissolved concentrations) would be more influential on the more hydrophobic congeners. Some extraction devices, such as XAD-2 resins, do not capture DOC and associated contaminants unless the DOC concentrations are high ($> 10 \text{ mg}\cdot\text{L}^{-1}$) (41), and DOC correction may therefore not be required. DOC values in Great Lakes waters ranged from 1 to 2 $\text{mg}\cdot\text{L}^{-1}$ in Lake Superior, Michigan, Huron, and Ontario in water collected for PCB analysis in 1993 (42) and similar DOC concentrations were found in mid-lake sites in 1997 (27). Thus, f_d for a PCB congener with $\log K_{OW}$ of 7 is 99% after

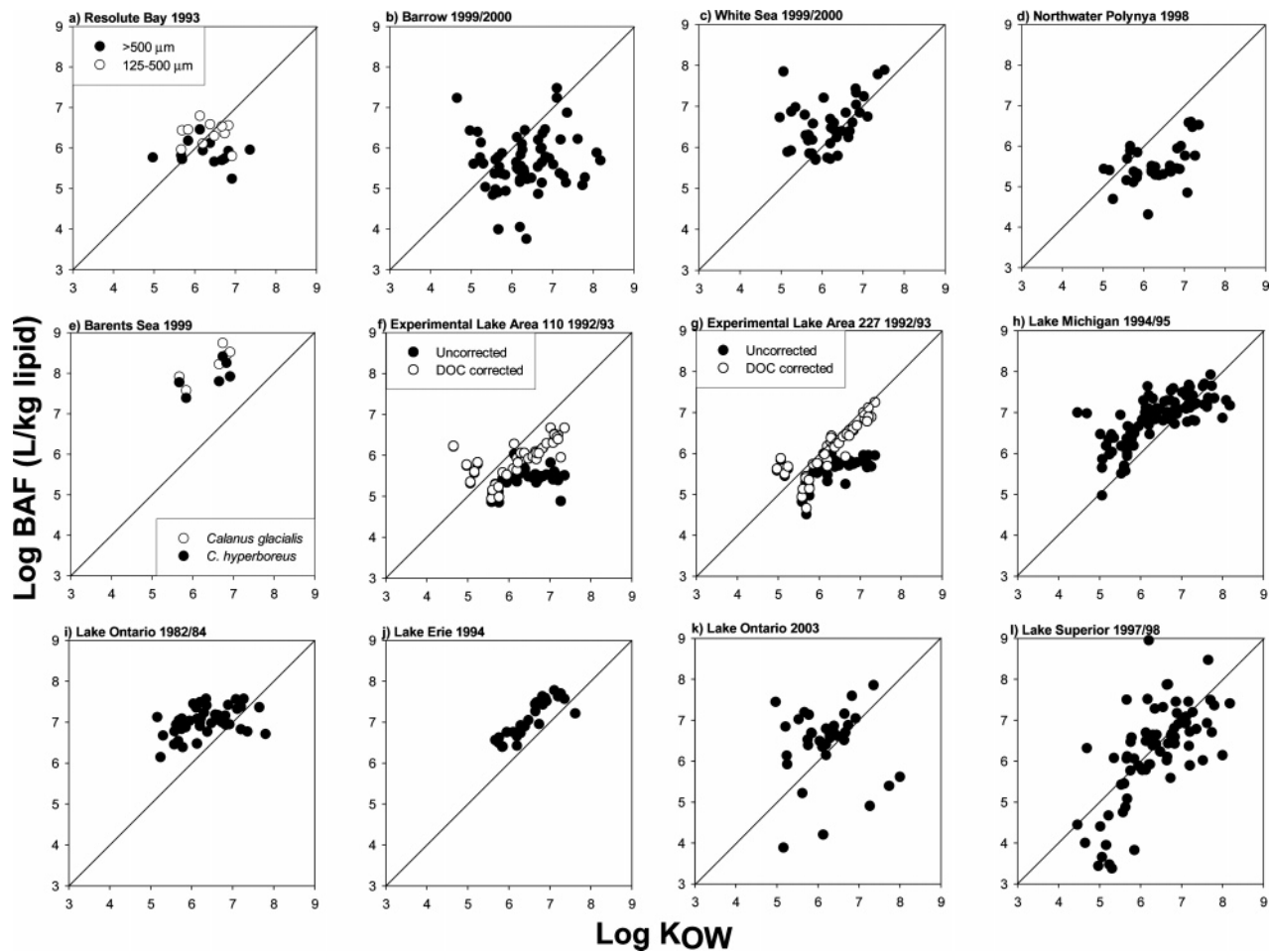


FIGURE 2. Bioaccumulation factors (BAFs) for individual PCB congeners related to the octanol–water partitioning coefficient (K_{OW} , from 12) in marine (a–e) and freshwater systems (f–l). Diagonal line indicates 1:1 relationship between log BAF and log K_{OW} as predicted by equilibrium partitioning. References for the respective studies are listed in Table 1 for water data and Table 2 for zooplankton data.

filtration, and no correction of BAFs for DOC sorption is necessary for oligotrophic freshwater systems, or for arctic marine waters where DOC concentrations are also low ($0.5\text{--}1.5\text{ mg}\cdot\text{L}^{-1}$, 43). An exception would be small inland lakes where DOC is higher. Jeremason et al. (29) compared the PCB cycling in two ELA lakes, one (L227) that was eutrophic as a result of continuous nitrogen and phosphorus additions, and the other (L110) that was oligotrophic. Because of elevated DOC ($7\text{--}13\text{ mg}\cdot\text{L}^{-1}$), Jeremason et al. (29) corrected dissolved PCB concentrations for DOC sorption leading to a reduction in ΣPCB concentrations by 40% in both lakes. This would raise BAFs for ΣPCBs in zooplankton in those lakes by about a factor of 2. When the individual PCB congeners in L110 and L227 were DOC-corrected in the same way, the DOC-corrected BAFs were up to 20 times higher than the noncorrected ones, with largest difference for high K_{OW} congeners (Figure 2f, g).

The present comparison of BAFs for PCBs is based on studies representing different areas, seasons, years, and zooplankton species, which may all contribute to variation in BAFs, in addition to variation caused by different water sampling and extraction methods. Both marine and freshwater BAFs vary seasonally due to variations in zooplankton's lipid content as well as the suspended particulate matter concentrations which influence the chemical's bioavailability (8, 9, 18, 21), but the seasonal variation in log BAFs for ΣPCBs was less than 1 order of magnitude (Figure 4). Less than 1 order of magnitude in log BAFs due to seasonal variation was also seen for particulate organic matter in the Baltic Sea (10). Log BAFs for ΣPCBs varied with size fraction of marine

zooplankton, but by less than 0.5 orders of magnitude (Figure 4a). Also, Barents Sea calanoid copepod species (*C. glacialis* and *C. hyperboreus*) with different body size showed different BAFs, however, usually less than 0.5 orders of magnitude (Figure 2). Thus, seasonal, zooplankton, or size specific BAF variation (8, 9, 21) (Figure 4) was much lower than the BAF difference of 2–3 orders of magnitude observed between studies carried out at the same time of year including the same zooplankton species (*C. hyperboreus*) (14, 25) (Figure 2d, e).

Equilibrium Partitioning, Trophic Interactions, and BAFs. Several field studies with plankton have demonstrated a curvilinear relationship between log BAF and log K_{OW} (e.g., 14, 15, 44). Higher BAFs than predicted by K_{OW} , and a curvilinear relationship between log BAF and log K_{OW} , suggest that PCB concentrations in zooplankton are not in equilibrium with water (2, 14, 25, 37). In some of the reviewed studies BAF for PCBs is close to K_{OW} (e.g., 14, 15), however, as discussed above there is reason to believe that these BAFs are underestimated due to overestimated water PCB concentrations, as also discussed by Harding (37). The most challenging assumptions of a 1:1 linear relationship between BAF and K_{OW} (= equilibrium partitioning) are that PCBs partition mainly into the neutral lipid pool of the organism, and that dietary PCB uptake leading to biomagnification is negligible so that the animal's PCB level is in equilibrium with that in water (2, 17).

In organisms, hydrophobic contaminants may partition into other organic phases in addition to lipid (44–46). PCB partitioning into other organic phases could lead to linearity

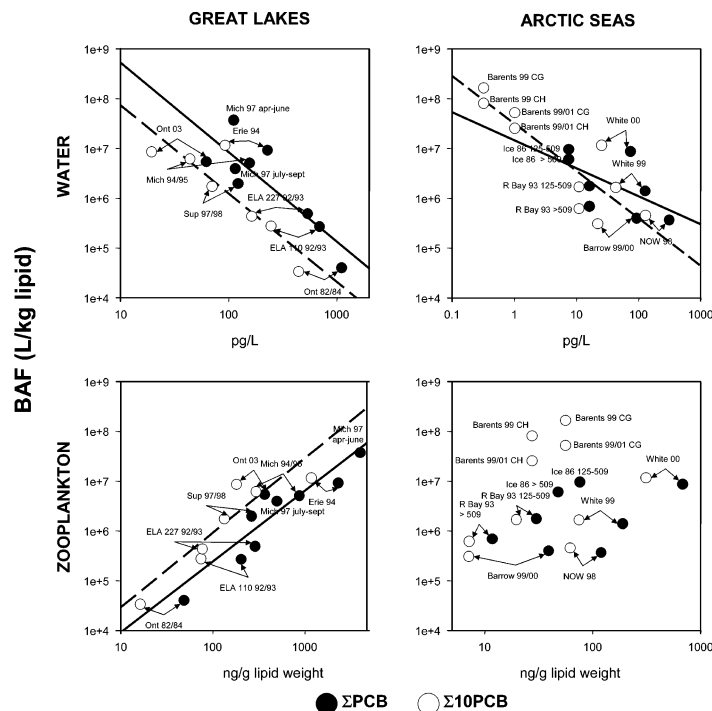


FIGURE 3. Relationship between BAFs and measured zooplankton and water concentrations in marine and freshwater studies: Ont = Ontario, Mich = Michigan, ELA = Experimental Lake Area, Sup = Superior, Barents = Barents Sea, White = White Sea, Ice = Ice Island, R Bay = Resolute Bay, NOW = Northwater Polynya, CG = *Calanus glacialis*, CH = *Calanus hyperboreus*, numbers represent sampling year. References for the respective studies are listed in Table 1 for water data and Table 2 for zooplankton data.

between log BAF and log K_{OW} , but deviation from a 1:1 relationship, if the other organic phase is well described by K_{OW} . Several laboratory and field studies have reported BAFs (organic carbon normalized) higher than K_{OW} (45, 47, 48), indicating greater partitioning of PCBs into organic carbon than octanol.

Biomagnification may occur between the lowest trophic levels of the food web (48–51), leading to higher BAFs than predicted from K_{OW} (e.g., 17). One reason why this has often been overlooked may be erroneously high quantification of PCBs in water, as indicated by the negative relationship between BAFs and measured water PCB concentrations. To understand PCB dynamics in aquatic ecosystems it is important to understand energy and contaminant flux also in the lower part of the food web, among organisms such as bacteria, heterotrophic algae, protozoans, and others (48). For example, although mostly considered strictly herbivorous, various copepods selectively feed on protozoa rather than phytoplankton (52). High feeding rates in zooplankton may significantly and rapidly elevate PCB concentrations above those predicted by equilibrium partitioning and K_{OW} (37). In benthic invertebrates, dietary uptake of contaminants resulted in increased bioaccumulation compared to that predicted by K_{OW} (17, 53). One way to evaluate dietary PCB enrichment in an organism is to calculate biomagnification factors (BMF, eq 4) (2).

$$BMF = \frac{[PCB \text{ in predator}]_{LIPID \text{ ADJUSTED}}}{[PCB \text{ in prey}]_{LIPID \text{ ADJUSTED}}} \quad (4)$$

BMF equals 1 when the PCB concentrations in predator ([PCB in predator]) and its prey ([PCB in prey]) are equal, whereas BMF higher or lower than 1 indicates biomagnification or elimination, respectively. Zooplankton BMFs from studies carried out in the early 1970s were higher than or equal to 1, indicating biomagnification also at lower trophic levels (37). Unfortunately, it was not possible to calculate

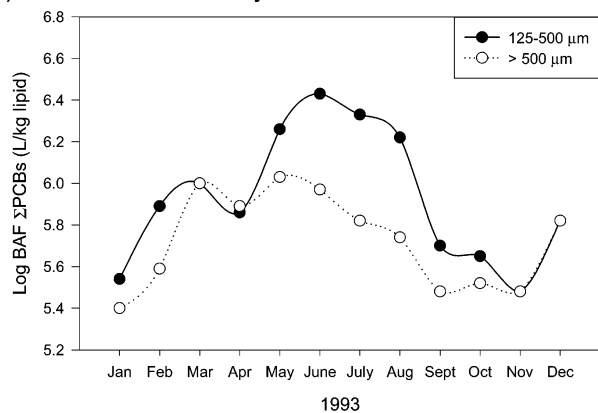
BMFs in the present study because prey items of the zooplankton were not analyzed, and because different size fractions of zooplankton consist of various trophic guilds (e.g., 9, 54) between which the trophic links are not well established. In addition, zooplankton size influences the bioaccumulation rates (55, 53) independently of trophic position, and therefore confounds the interpretation of trophic transfer. When comparing predominantly herbivorous and carnivorous zooplankton the latter had higher concentrations of more hydrophobic POPs, which was attributed to diet differences rather than size or age (56). This is in agreement with earlier studies illustrating the importance of dietary uptake for hydrophobic POPs (53). Similarly, a feeding zooplankton community showed rapid PCB uptake and increased bioaccumulation compared to nonfeeding zooplankton community (48), probably related to over-saturation of PCBs since the elimination rate is slow and equilibrium between water and biota is therefore not reached (14). In the feeding zooplankton community, most BAFs (organic carbon adjusted) were 1–2 orders of magnitude higher than K_{OW} , whereas they were in the range of K_{OW} s in nonfeeding situations.

Several bioaccumulation models have a nonequilibrium solution, taking into account not only partitioning uptake (k_1) and elimination (k_2) of POPs from the surrounding environment, but also uptake from diet (k_D) and elimination by fecal egestion (k_E), metabolism (k_M), and growth dilution (k_G) (e.g., 17). k is the rate constant describing the respective uptake or elimination process. At steady state the animal's POP concentration is:

$$[POP \text{ in organism}] = (k_1 \times [POP \text{ in water}]_{DISSOLVED} + k_D \times [POP \text{ in diet}]) / (k_2 + k_E + k_M + k_G) \quad (5)$$

Of the elimination pathways, metabolism is often considered negligible in invertebrates (15, 17), whereas growth rates vary considerably (e.g., 57) and may greatly influence the bioaccumulation in the animal (e.g., 53). Elimination

a) Marine: Resolute Bay



b) Lake Michigan: Grand Traverse Bay

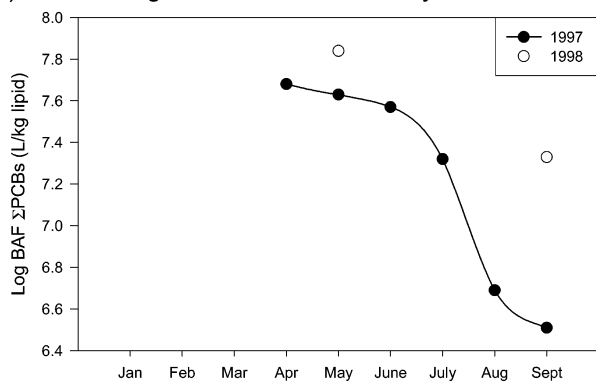


FIGURE 4. Seasonal variation in (a) marine (9) and (b) freshwater (27) zooplankton BAF. Missing data for Lake Michigan zooplankton in May 1997 and water in July 1997 were replaced by the average of the preceding and following months.

through growth dilution is, however, often not considered when modeling contaminant bioaccumulation (e.g., 17). When a bioaccumulation model including growth dilution was parametrized for PCB bioaccumulation in the Barents Sea calanoid copepods, growth rate was indeed one of the most sensitive parameters (25). However, even by varying the growth rates in different bioaccumulation scenarios, resulting BAFs were not similar to those predicted by K_{OW} (25).

The present study has demonstrated that BAFs for PCBs are greater in recent arctic marine and Great Lakes studies than previously reported in the same regions, and that they are at least 10 times higher than predicted from K_{OW} . It seems difficult to establish exact BAFs for PCBs in marine and freshwater zooplankton, as the variability of the system and the use of different PCB sampling and quantification methods results in BAFs that vary more than 1 order of magnitude. The BAF variation among studies is greater than can be accounted for by seasonal or size related differences. The negative dependence of BAFs to PCB exposure from water, in combination with the wide variety of methods used in water PCB measurements, suggest that earlier BAFs were too low due to overestimated water measurements. In addition, partitioning into other organic phases and dietary uptake of contaminants may lead to BAFs for PCBs above the ones predicted by K_{OW} . Whereas issues of water PCB measurements and partitioning medium do not require a process in addition to equilibrium partitioning to explain bioaccumulation in zooplankton, trophic transfer and biomagnification of contaminants suggests that bioaccumulation in zooplankton exceeds what is predicted by equilibrium partitioning between water and lipids. All three contribute

significantly to variability in PCBs BAFs both within and between studies, and may confound conclusions about zooplankton bioaccumulation and contaminant flux if assumed negligible.

The large variability of BAFs for PCBs in zooplankton illustrated in the present study needs to be considered in future assessments of potential new bioaccumulative chemicals that rely on laboratory or field measured BAFs, such as the European Union REACH program, the nomination of chemicals as future POPs under the Stockholm Convention, and other assessment programs. BAFs predicted from chemical structures and/or K_{OW} may not give a sufficient estimate of bioaccumulation at the level of invertebrate communities, and overestimation of water concentrations due to contamination by candidate chemicals will need to be considered.

Acknowledgments

Colleagues attending the SETAC World Conference in Portland Oregon are thanked for valuable discussion and feedback. During the present study K.B. was supported by Norwegian Research Council's project 159417/S30.

Supporting Information Available

Two tables showing congeners included in water and zooplankton data (pdf). This material is available free of charge via the Internet at <http://pubs.acs.org>.

Literature Cited

- (1) Mackay, D. Correlation of bioconcentration factors. *Environ. Sci. Technol.* **1982**, *16*, 274–278.
- (2) Gobas, F. A. P. C.; Morrison, H. A. Bioconcentration and biomagnification in the aquatic environment. In *Handbook of Property Estimation Methods for Chemicals: Environmental and Health Sciences*; Boethling, R. S., Mackay, D., Eds.; Lewis Publishers: Boca Raton, FL, 2000; pp 189–231.
- (3) UNEP (United Nations Environment Program). *Final Act of the Conference of Plenipotentiaries on The Stockholm Convention On Persistent Organic Pollutants*; UNEP: Geneva, Switzerland, 2001, 44 pp.
- (4) Meylan, W. M.; Howard, P. H.; Boethling, R. S.; Aronson, D.; Printup, H.; Gouchie, S. Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. *Environ. Toxicol. Chem.* **1999**, *18*, 664–672.
- (5) European Commission. *Why do we need REACH? REACH in brief*; European Commission, Environment Directorate General: Brussels, 2004; 18 pp.
- (6) Environment Canada. *Existing Substances Evaluation Bulletin*; Ottawa ON, 2003; 9 pp. http://www.ec.gc.ca/Substances/ese/eng/what_new.cfm.
- (7) Walker, J. D.; Knaebel, D.; Mayo, K.; Tunkel, J.; Gray, D. A. Use of QSARs to promote more cost-effective use of chemical monitoring resources. 1. Screening industrial chemicals and pesticides, direct food additives, indirect food additives and pharmaceuticals for biodegradation, bioconcentration and aquatic toxicity potential. *Water Qual. Res. J. Can.* **2004**, *39*, 35–39.
- (8) Harding, G. C.; LeBlanc, R. J.; Vass, W. P.; Addison, R. F.; Hargrave, B. T.; Pearre, S.; Dupuis, A., Jr.; Brodie, P. F. Bioaccumulation of polychlorinated biphenyls (PCBs) in the marine pelagic food web, based on a seasonal study in the southern Gulf of St. Lawrence, 1976–1977. *Mar. Chem.* **1997**, *56*, 145–179.
- (9) Hargrave, B. T.; Phillips, G. A.; Vass, W. P.; Bruecker, P.; Welch, H. E.; Siferd, T. D. Seasonality in bioaccumulation of organochlorines in lower trophic level arctic marine biota. *Environ. Sci. Technol.* **2000**, *34*, 980–987.
- (10) Bruhn, R.; McLachlan, M. S. Seasonal variation of polychlorinated biphenyl concentrations in the southern part of the Baltic Sea. *Mar. Poll. Bull.* **2002**, *44*, 156–163.
- (11) Muir, D.; Strachan, W. Persistent organic pollutants in seawater. In *Sources, Occurrence, Trends and Pathways in the Physical Environment*; Bidleman, T., Macdonald, R., Stow, J., Eds.; The Canadian Arctic Contaminants Assessment Report II. Indian and Northern Affairs Canada: Ottawa, Canada, 2003; pp 92–99.

- (12) Hawker, D. W.; Connell, D. W. Octanol–water partition coefficients of polychlorinated biphenyl congeners. *Environ. Sci. Technol.* **1988**, *22*, 382–387.
- (13) Hamelink, J. L.; Waybrandt, R. C.; Ball, R. C. Proposal: Exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. *Trans. Am. Fish. Soc.* **1971**, *100*, 207–214.
- (14) Fisk, A. T.; Stern, G. A.; Hobson, K. A.; Strachan, W. J.; Loewen, M. D.; Norstrom, R. J. Persistent organic pollutants (POPs) in a small, herbivorous, arctic marine zooplankton (*Calanus hyperboreus*): trends from April to July and the influence of lipids and trophic transfer. *Mar. Pollut. Bull.* **2001**, *43*, 93–101.
- (15) Hoekstra, P. F.; O'Hara, T. M. O.; Teixeira, C.; Backus, S.; Fisk, A. T.; Muir, D. C. G. Spatial trends and bioaccumulation of organochlorine pollutants in marine zooplankton from the Alaskan and western Canadian Arctic. *Environ. Toxicol. Chem.* **2002**, *21*, 575–583.
- (16) Oliver, B. G.; Niimi, A. J. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environ. Sci. Technol.* **1988**, *22*, 388–397.
- (17) Morrison, H. A.; Gobas, F. A. P. C.; Lazar, R.; Haffner, G. D. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. *Environ. Sci. Technol.* **1996**, *30*, 3377–3384.
- (18) Trowbridge, A. G. Polychlorinated biphenyls in the lower trophic levels of the Lake Michigan foodweb. Ph.D. Thesis, University of Minnesota, 2001; 214 pp.
- (19) Muir, D. C. G.; Savinova, T.; Savinov, V.; Alexeeva, L.; Potelov, V.; Svetoch, V. Bioaccumulation of PCBs and chlorinated pesticides in seals, fishes and invertebrates from the White Sea, Russia. *Sci. Total Environ.* **2003**, *306*, 111–131.
- (20) Savinov, V.; Muir, D.; Savinova, T.; Alexeeva, L. PCBs and chlorinated pesticides in sediments, seawater and plankton from the White Sea, Russia. Manuscript in prep.
- (21) Stapleton, H. M.; Skubinna, J.; Baker, J. E. Seasonal dynamics of PCB and toxaphene bioaccumulation within a Lake Michigan food web. *J. Great Lakes Res.* **2002**, *28*, 52–64.
- (22) Muir, D.; Teixeira, C. *Results of legacy organochlorine measurements in Lake Ontario using in situ and clean sampling techniques, 2003–2004*. Internal report, National Water Research Institute: Burlington, ON, 2004.
- (23) Olsson, K.; Kallenborn, R.; Borgå, K.; Thorgren, J. O. *Organochlorines in the Eurasian Arctic: transport and uptake into ice-associated ecosystems, Rovaneimi, Finland, October 2002*; Arctic Monitoring and Assessment Programme (AMAP) Report, 2: Oslo, Norway, P-P12 (ISBN 82-7971-021-3), 2002. Available from: www.amap.no.
- (24) Gustafsson, O.; Andersson, P.; Axelman, J.; Bucheli, T. D.; Komp, P.; McLachlan, M.; Sobek, A.; Thorgren, J. O. Observations of the PCB distribution within and between ice, snow, and meltwater in the Barents Sea marginal ice zone and the North Pole area during the ODEN 2001 expedition. *Sci. Total Environ.* **2005**, in press.
- (25) Borgå, K.; Di Guardo, A. Using zooplankton PCB concentrations and theoretical models to estimate Barents Seawater PCB concentrations. *Sci. Total Environ.* **2005**, in press.
- (26) Muir, D. C. G.; Whittle, D. M.; De Vault, D. S.; Bronte C. R.; Karlsson, H.; Backus, S.; Teixeira, C. Bioaccumulation of toxaphene congeners in the Lake Superior food web. *J. Great Lakes Res.* **2004**, *30*, 316–340.
- (27) USEPA. Unpublished PCB data in water, 2001. Provided by M. Hulting; Great Lakes National Program Office (GLNPO): Chicago, IL, 2004.
- (28) Muir, D.; Grift, N. PCBs and OC pesticides in zooplankton from ELA lakes 110 and 227, Internal report; Department of Fisheries and Oceans: Winnipeg, MB, 1995; 5 pp.
- (29) Jeremiason, J. D.; Eisenreich, S. J.; Paterson, M. J.; Beatty, K. G.; Hecky, R.; Elser, J. J. Biogeochemical cycling of PCBs in lakes of variable trophic status: A paired lake experiment. *Limnol. Oceanogr.* **1999**, *44*, 889–902.
- (30) L'Italien, S.; Fay, L. A. *Great Lakes Surveillance Program Field Methods Manual*; WQB/IWD-OR-93-01/1; Water Quality Branch Inland Waters Directorate, Environment Canada: Burlington, ON, 1993.
- (31) SAS Institute Inc. *SAS/STAT User's Guide*, Version 6, 4th ed.; Cary, NC, 1989; Vols 1 and 2.
- (32) de Boer, J.; Law, R. J. Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic hydrocarbons (PAHs). *J. Chromatogr. A* **2003**, *1000*, 223–251.
- (33) Borgå, K.; Gabrielsen, G. W.; Kleivane, L.; Skaare, J. U.; Norstrom, R.; Fisk, A. Why do semi-circumpolar trends of OCs differ among Arctic trophic levels? *Environ. Sci. Technol.* **2005**, *10*, 3464–3473.
- (34) Petrick, G.; Schulz-Bull, D. E.; Martens, V.; Scholz, K.; Duinker, J. C. An in-situ filtration/extraction system for the recovery of trace organics in solution and on particles tested in deep ocean water. *Mar. Chem.* **1996**, *54*, 97–105.
- (35) IOC. *Chlorinated biphenyls in open waters: sampling, extraction, cleanup and instrumental determination*; Intergovernmental Oceanographic Commission, Man Guides 27, UNESCO Press: Paris, France, 1993.
- (36) Lohmann, R.; Jaward, F. M.; Durham, L.; Barber, J. L.; Ockenden, W.; Jones, K. C.; Bruhn, T.; Lakaschus, S.; Dachs, J.; Booij, K. Potential contamination of shipboard air samples by diffusive emissions of PCBs and other organic pollutants: implications and solutions. *Environ. Sci. Technol.* **2004**, *38*, 3965–3970.
- (37) Swackhamer, D. L.; Eisenreich, S. J. Intercomparison of methodologies for measuring PCBs in particulate and dissolved phases in Green Bay Water; USEPA, Great Lakes National Program Office: Chicago, IL, 1989.
- (38) Harding, G. C. Organochlorine dynamics between zooplankton and their environment, a reassessment. *Mar. Ecol. Prog. Ser.* **1986**, *33*, 167–191.
- (39) Pearson, R. F.; Hornbuckle, K. C.; Eisenreich, S. J.; Swackhamer, D. L. PCBs in Lake Michigan water revisited. *Environ. Sci. Technol.* **1996**, *30*, 1429–1436.
- (40) Sobek, A.; Gustafsson, O.; Hajdu, S.; Larsson, U. Particle–water partitioning of PCBs in the photic zone: a 25-month study in the open Baltic Sea. *Environ. Sci. Technol.* **2004**, *38*, 1375–1382.
- (41) Burkhard, L. P. Estimating dissolved organic carbon partition coefficients for nonionic organic chemicals. *Environ. Sci. Technol.* **2000**, *34*, 4663–4668.
- (42) Anderson, D. J.; Bloem, T. B.; Blankenbaker, R. K.; Stanko, T. A. Concentrations of polychlorinated biphenyls in the water column of the Laurentian Great Lakes: spring 1993. *J. Great Lakes Res.* **1999**, *25*, 160–170.
- (43) Dafner, E. V.; Wangersky, P. J. A brief overview of modern directions in marine DOC studies. Part II—Recent progress in marine DOC studies. *J. Environ. Monit.* **2002**, *4*, 55–69.
- (44) Axelman, J.; Broman, D.; Naf, C. Field measurements of PCB partitioning between water and planktonic organisms: Influence of growth, particle size, and solute–solvent interactions. *Environ. Sci. Technol.* **1997**, *31*, 665–669.
- (45) Swackhamer, D. L.; Skoglund, R. S. Bioaccumulation of PCBs by algae: kinetics versus equilibrium. *Environ. Toxicol. Chem.* **1993**, *12*, 831–838.
- (46) Skoglund, R. S.; Swackhamer, D. L. Evidence for the use of organic carbon as the sorbing matrix in the modeling of PCB accumulation in phytoplankton. *Environ. Sci. Technol.* **1999**, *33*, 1516–1519.
- (47) Broman, D.; Naf, C.; Axelman, J.; Bandh, C.; Pettersen, H.; Johnstone, R.; Wallberg, P. Significance of bacteria in marine waters for the distribution of hydrophobic organic contaminants. *Environ. Sci. Technol.* **1996**, *30*, 1238–1241.
- (48) Wallberg, P.; Jonsson, P. R.; Andersson, A. Trophic transfer and passive uptake of a polychlorinated biphenyl in experimental marine microbial communities. *Environ. Toxicol. Chem.* **2001**, *20*, 2158–2164.
- (49) Wallberg, P.; Bergqvist, P. A.; Andersson, A. Potential importance of protozoan grazing on the accumulation of polychlorinated biphenyls (PCBs) in the pelagic food web. *Hydrobiologia* **1997**, *357*, 53–62.
- (50) Borgå, K.; Gabrielsen, G. W.; Skaare, J. U. Differences in contamination load between pelagic and sympagic invertebrates in the Arctic marginal ice zone: influence of habitat, diet and geography. *Mar. Ecol. Prog. Ser.* **2002**, *325*, 157–169.
- (51) Trowbridge, A. G.; Swackhamer, D. L. Preferential biomagnification of aryl hydrocarbon hydroxylase-inducing polychlorinated biphenyl congeners in the Lake Michigan, USA, lower food web. *Environ. Toxicol. Chem.* **2002**, *21*, 334–341.
- (52) Verity, P. G.; Paffenhöfer, G. A. On assessment of prey ingestion by copepods. *J. Plank. Res.* **1996**, *18*, 1767–1779.
- (53) Thomann, R. V. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ. Sci. Technol.* **1989**, *23*, 699–707.
- (54) Hargrave, B. T.; Harding, G. C.; Vass, W. P.; Erickson, P. E.; Fowler, B. R.; Scott, V. Organochlorine pesticides and poly-

- chlorinated-biphenyls in the Arctic Ocean food web. *Arch. Environ. Contamin. Toxicol.* **1992**, *22*, 41–54.
- (55) Landrum, P. F. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*—role of physiological and environmental variables. *Aquatic Toxicol.* **1988**, *12*, 245–271.
- (56) Borgå, K.; Gabrielsen, G. W.; Skaare, J. U. Differences in contamination load between pelagic and sympagic invertebrates in the Arctic Marginal Ice Zone: influence of habitat, diet and geography. *Mar. Ecol. Prog. Ser.* **2002**, *235*, 157–169.
- (57) Hopcroft, R. R.; Clarke, C.; Nelson, R. J.; Raskoff, K. A. Zooplankton communities of the Arctic's Canada Basin: the contribution by smaller taxa. *Polar Biol.* **2005**, *28*, 198–206.

Received for review February 23, 2005. Revised manuscript received March 29, 2005. Accepted March 30, 2005.

ES050376I