EXAMINATION OF THE BIOACCUMULATION OF HALOGENATED DIMETHYL BIPYRROLES IN AN ARCTIC MARINE FOOD CHAIN USING NITROGEN STABLE ISOTOPE ANALYSIS

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Introduction

Halogenated dimethyl bipyrroles (HDBPs) form a family of novel, mixed halogenated, heterocyclic compounds. Their source is currently unknown, but HDBPs are thought to be of biogenic origin[1]. Few data presently exist on environmental concentrations of HDBPs. However, the most abundant HDBP congener, HDBP-Br_4Cl_2, has been found at low ppm concentrations in higher trophic level organisms such as bald eagles[1]. These occurrences of high concentrations show that HDBPs are bioaccumulative, that is they may be accumulated by an organism via uptake from food and water.

Physical property data for HDBPs, specifically high octanol/water partition coefficients (K_{ow}s), suggest that HDBPs may be transferred through aquatic food chains. Food chain transfer is a major exposure pathway of aquatic organisms to hydrophobic organohalogens[2]. In fact, the high K_{ow}s of these compounds suggest that they have the potential to biomagnify, or increase in concentration from one trophic level to the next. Biomagnification in invertebrates and fish has been shown to be related to K_{ow}[3-5].

Stable isotope analysis provides a continuous variable with which to assess both trophic level[6,7] and food chain transfer of persistent organic pollutants[3]. In the case of nitrogen stable isotope analysis, the ratio of the heavier to lighter stable isotopes of nitrogen ($^{15}N/^{14}N$) generally increases with trophic position in aquatic food chains. Biomagnification factors (BMFs) can then be estimated from slopes of logarithmic concentration of contaminants vs. trophic level[4].

In this study, two HDBP congeners – 1,1'-dimethyl-3,3',4,4'-tetrabromo-5,5'-dichloro-2,2'-bipyrrole (HDBP-Br_4Cl_2) and 1,1'-dimethyl-3,3',4,4',5,5'-hexabromo-2,2'-bipyrrole (HDBP-Br_6), and two congeners of unknown structure, HDBP-Br_3Cl_3 and HDBP-Br_5Cl, were analyzed in an aquatic food chain from the Northwater (NOW) Polynya. The trophic levels of the zooplankton – fish – seabird – marine mammal food chain were characterized using nitrogen stable isotope analysis.

Methods and Materials

Sample Collection. Zooplankton samples (*Calanus hyperboreus, Mysis occulata,* and *Sagitta sp.*) were collected between May and July of 1998 using large zooplankton nets (1 m^2). Arctic cod (*Boreeogadus saida*) were opportunistically collected with hand-held nets. Seabirds [dovekie

(*Alle alle*), black guillemot *(Cepphus grylle),* black-legged kittiwake *(Rissa tridactyla),* and glaucous gull (*Larus hyperboreus*)] were collected from the Northwater Polynya in May and June of 1998 by shotgun. Samples of ringed seal (*Phoca hispida*) blubber were obtained from local hunters from Qaanaaq, Greenland during the same period.

Stable Isotope Analysis. Prior to stable isotope analyses, all tissue samples were washed in distilled water and then freeze dried, powdered and treated to remove lipids. Whole body homogenates were the tissue substrates used for zooplankton and fish, whereas muscle was analyzed for birds and seals respectively. Zooplankton samples were soaked in 0.1N HCl to remove carbonates and allowed to dry without rinsing.

Stable nitrogen isotope assays were performed on 1 mg subsamples of homogenized materials by loading into tin cups and combusting at 1,800°C in a Robo-Prep elemental analyzer. Resultant N_2 gas was then analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer with every 5 unknowns separated by 2 laboratory standards (albumen). Atmospheric N_2 was used as the ^{15}N standard.

Organohalogen Analysis. All samples were worked up according to a similar method described in Tittlemier *et. al.*[1] The following tissues were analyzed for organohalogens: zooplankton (whole body), fish (whole body), seabirds (liver), and seal (blubber). PCB and OC analysis was performed by GC-EI-SIM-MS. HDBPs were analyzed using GC-ECNI-SIM-MS.

Results and Discussion

Stable Isotope Analysis. Results of the ^{15}N stable isotope analysis are listed in Table 1. Values are expressed as $\delta^{15}N$, defined in Hobson *et al.*[7] Since captive-rearing studies suggest that nitrogen turnover rates differ between birds and other organisms[8], $\delta^{15}N$ values were transformed to trophic level values, TL[7].

HDBP Concentrations. Concentrations of organohalogens were recovery corrected since recoveries of the internal standards ranged from 60-110%. The recovery corrected and lipid normalized concentrations of the HDBP congeners are given in Table 1. HDBP-Br_4Cl_2 was detected in all of the samples studied from the NOW polynya. In general, levels of HDBP-Br_4Cl_2 were similar to those recorded in seabird samples from Atlantic Canada[1]. For this comparison, the HDBP-Br_4Cl_2 egg wet weight concentrations were transformed to lipid normalized liver concentrations using an egg-to-liver organochlorine ratio calculated from Braune and Norstrom[9]. Such a comparison cannot be made for the other congeners since the present study contains the first reported concentration data for the congeners.

The one obvious exception to the similarities between the NOW and Atlantic seabird data occurs with the dovekies. The dovekie HDBP-Br_4Cl_2 concentrations (1.76 ppb) are approximately ten times lower than the Atlantic puffin (*Fratercula artica*, 20 ppb), a species which has a similar winter habitat and diet. This difference may be explained by the change in dovekie diet just prior to their collection in early summer[10]. During late spring, as the dovekies return to the NOW Polynya from the St. Lawrence Estuary and Atlantic Canada, they feed exclusively on lipid-rich relatively clean zooplankton, rather than fish (N. Karnovsky, unpublished data). Organohalogen levels in their livers are then diluted by the rapid influx of clean lipids at this time, and may not reflect whole body concentrations.

Biomagnification of HDBPs. In general, concentrations of HDBPs were found to increase with a corresponding increase in trophic level. However, ringed seals did not follow this trend. This deviation was also found with OCs and PCBs quantitated in the same samples, which was due to the concentration differences between male and female samples. Females generally

have lower concentrations of OCs and PCBs because they have increased elimination capacities through maternal transfer and lactation[11]. However, this does not seem to be the case for HDBPs since there was no significant difference between HDBP concentrations in the male and female seals. A more plausible explanation is that seals are more proficient than birds at metabolizing HDBPs. In general, seals have a higher capability than zooplankton or fish, but lower than birds, for metabolism of organohalogen compounds like PCBs[12].

Integrated biomagnification factors (BMF_{TL}s) were calculated over all trophic levels of the food chain for HDBPs and selected recalcitrant OCs and PCBs from the slopes of the linear regressions of ln(concentration) versus trophic level (excluding seal data). The calculated BMF_{TL} values were similar to those for recalcitrant compounds such as CB- 153 and *p,p'*-DDE[13], and indicate that all four HDBP congeners biomagnify. The slopes for the HDBPs were not significantly different, but it does appear that HDBP-Br_4Cl_2 has a higher BMF (14.1) than HDBP-Br_3Cl_3 (5.1), HDBP-Br_5Cl (6.0), and HDBP-Br_6 (6.9). HDBP-Br_4Cl_2 would thus be expected to biomagnify through the NOW food chain to a greater extent than the other congeners.

This is a somewhat unexpected result since it has been demonstrated that BMF_{TL}s tend to increase with an increase in recalcitrance and K_{ow}[3,4]. Feeding studies with rainbow trout have also shown such a relationship between BMF and K_{ow}.[5] The log K_{ow} of HDBP-Br_4Cl_2, estimated at 7.0, is lower than the log K_{ow}s of HDBP-Br_5Cl, estimated at 7.2, and HDBP-Br_6, experimentally determined to be 8.9±0.3[14] and estimated at 7.3 using the fragment constant method[15].

The change in the BMF_{TL} behaviour for the HDBPs may be attributed to differences in metabolism and possibly differences in assimilation efficiencies. Since BMF_{TL} can be considered a ratio of assimilation to elimination rates at steady state, a decrease in assimilation efficiency or an increase in elimination will cause a corresponding decrease in the BMF_{TL}. Fisk *et. al.*[5] demonstrated that assimilation efficiencies of OCs for rainbow trout decreased at high K_{ow} (log $K_{ow} > 7.0$).

Conclusions. This study provides the first evidence that four, possibly naturally-produced, organohalogens biomagnify in aquatic food chains. The HDBP congeners were found to increase with trophic level, except in ringed seals, which appear more capable than birds in metabolizing HDBPs. The HDBP BMF_{TL}s did not follow a relationship between BMF and K_{ow}, likely due to differences in metabolism or assimilation efficiencies.

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Table 1. HDBP congener and stable isotope analysis results, mean ± standard deviation. Concentrations are lipid corrected, ng/g.

org.[a]	n	$\delta^{15}N$	TL	Br_3Cl_3	Br_4Cl_2	Br_5Cl	Br_6
HB	3	9.0±0.2	1.96±0.05	nd[b]	0.021±0.02	nd	nd
BA	3	10.8±0.4	2.41±0.09	0.11±0.10	0.22±0.03	0.033±0.005	0.58±0.23
DOVE	3	11.9±0.9	3.1±0.2	0.55±0.15	1.8±0.3	0.31±0.07	0.69±0.33
CH	3	10.9±0.2	2.46±0.04	nd	0.057±0.027	nd	nd
BLKI	5	13.2±0.4	3.5±0.1	3.3±1.5	15±6	2.1±0.9	13±23
COD	5	14.1±0.5	3.3±0.1	0.17±0.28	0.38±0.39	0.096±0.099	0.53±0.60
BLGU	6	14.9±0.7	3.9±0.2	0.62±0.44	1.9±1.5	0.34±0.14	6.9±4.7
GLGU	4	16.0±0.8	4.2±0.2	11±7	42±28	3.5±1.7	13±4
RS	10	17.4±0.5	4.2±0.2	0.027±0.022	0.017±0.008	nd	0.046±0.044

[a]HB=*Calanus hyperboreus*, BA=*Mysis occulata*, DOVE=dovekie, CH=*Sagitta sp.*, BLKI=black-legged kittiwake, COD=Arctic cod, BLGU=black guillemot, GLGU=glaucous gull, RS=ringed seal

[b]not detected