

Persistent Organochlorine Contaminants and Enantiomeric Signatures of Chiral Pollutants in Ringed Seals (*Phoca hispida*) Collected on the East and West Side of the Northwater Polynya, Canadian Arctic

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Abstract. To examine the influence of diet and age on organochlorine contaminant (OC) concentrations in two closely related ringed seal (*Phoca hispida*) populations enantiomeric fractions (EFs) of chiral contaminants and stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) were measured along with OCs in ringed seals collected from the east and west side of the Northwater Polynya. Seals from these two locations were feeding at the same trophic level based on $\delta^{15}\text{N}$ values in muscle but had slightly different sources of carbon based on $\delta^{13}\text{C}$ measurements in muscle. After removing the influence of age, sex, and blubber thickness, OC concentrations did not vary between ringed seals from the east and west side of the polynya. ΣPCB , ΣDDT , and $\Sigma\text{chlordanes}$ were found to increase with age for both male and female seals. The inclusion of older (>20 years) female seals, which may have a reduced reproductive effort, may influence the relationships in females. Stable isotopes failed to describe OC concentrations in ringed seals suggesting that diet was not a major factor in variation of OC concentrations within this ringed seal population. *Cis*- and *trans*-chlordanes, oxychlordanes, and heptachlor epoxide were all nonracemic in the ringed seal blubber but did not vary with age, sex, or collection site. $\alpha\text{-HCH}$ appeared racemic (enantiomeric fraction = 0.50 ± 0.01) in the seals, although this EF is different than those previously observed in their prey species, and was found to vary significantly with age. EF values in the ringed seals varied considerably from other Arctic marine mammals and seabirds, providing additional evidence that the type(s) and characteristic(s) of the enzymes involved in biotransformation of chiral OCs vary between these organisms.

The exposure of indigenous people to persistent organochlorine contaminants (OCs) through their traditional marine diet is of great concern in the Canadian, Alaskan, and Greenlandic Arctic. Although the use of OCs in the Arctic is extremely limited, these chemicals are transported to the Arctic by the atmosphere and, to a lesser extent, the ocean (Macdonald *et al.* 2000). The long food chains of the Arctic and the reliance on lipids by upper-trophic-level organisms result in significant concentrations of many OCs at upper trophic levels (Muir *et al.* 1988; Norstrom *et al.* 1998). High fat tissues, where OCs are mainly sequestered, are a staple in the diet of many Arctic Inuit. Therefore, Inuit experience greater exposure to OCs, and subsequently high tissue burdens (Sandau *et al.* 2000) through consumption of fatty tissue than people from more contaminated temperate regions (Gilman *et al.* 1997).

A number of techniques have been used recently to assess the dynamics of OCs within aquatic food webs. The ratio of the heavier to lighter stable isotopes of nitrogen ($^{15}\text{N}/^{14}\text{N}$), expressed as $\delta^{15}\text{N}$ values, generally increases with trophic position in aquatic food chains, providing a continuous variable with which to assess both trophic level (Hobson *et al.* 1995; Michener and Schell 1994) and food web transfer of OCs (Fisk *et al.* 2001a; Kidd *et al.* 1998). Enantiomeric ratios (ERs) or fractions (EFs) of chiral pollutants have also been used to assess bioaccumulation and biotransformation in marine food webs (Wiberg *et al.* 2000; Moisey *et al.* 2001). Chiral pollutants exist in two forms as optical isomers, called enantiomers, that have identical physical-chemical properties and abiotic degradation rates, but can have different rates of biotransformation (Buser and Muller 1993). Differences in biotransformation rates between species can often be detected by changes in EFs of chiral pollutants (Buser *et al.* 1992). The ability to biotransform OCs in aquatic food webs generally increases from invertebrates to fish to birds and mammals (Norstrom *et al.* 1978; Boon *et al.* 1989), and therefore EFs have the poten-

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tial to describe or quantify bioaccumulation and trophic transfer (Wiberg *et al.* 2000). The assessment of bioaccumulation using $\delta^{15}\text{N}$ values and EFs of chiral OCs in food web components has proven to be effective for the analysis of whole food webs, but assessment of their utility within a single species or between populations of the same species has been limited.

The ringed seal (*Phoca hispida*) was chosen as a sentinel species for monitoring of OCs in the Arctic because it is common and has a circumpolar distribution (King 1983), is a key link in many Arctic marine food chains (see Bradstreet 1982; Bradstreet and Cross 1982), and is an important component of the traditional diet of Arctic Inuit (McLaren 1958a; King 1983). Concentrations and patterns of OCs in ringed seals have recently been shown to vary across the Canadian, European, and Russian Arctic but with minimal variation in the Canadian high Arctic (Muir *et al.* 2000). Past studies have found that blubber thickness, sex and age were important variables in OC concentrations in ringed seals (Muir *et al.* 2000; Cameron *et al.* 1997). Despite a large dataset on OCs in ringed seals, there has been little effort to assess OCs and chiral OCs in populations of ringed seals that are close geographically but may be feeding differently. It has been suggested that feeding differences in ringed seals, such as diets consisting of fish versus zooplankton, could result in differences in PCB concentrations (Muir and Norstrom 2000). As well, there has been limited effort to analyze OCs in ringed seals that have a large range of ages (*i.e.*, more than 20 years) and knowledge of OC levels in very old seals is limited.

A large number of ringed seals ($n = 199$) from two populations utilizing the east and west side of the Northwater Polynya (NOW) were collected as part of a project on the ecology of ringed seals in the NOW. The NOW, in northern Baffin Bay, is the largest and most productive polynya in the Canadian Arctic, supporting large populations of marine mammals, including ringed seals and seabirds (Stirling 1980, 1997; Stirling *et al.* 1981). Polynyas are areas of open water, often surrounded by sea ice, which persist throughout the winter in polar seas and are one of the most important and least understood phenomena in polar ecology (Stirling 1980). The large sample of ringed seals allowed a subset of individual seals to be chosen for OC analysis that covered a large range of ages (0–42 years) for both sexes. This allowed an assessment of the influence of age and sex on OC concentrations and chiral OCs in two ringed seal populations that are close geographically. The feeding habits of these two populations (Holst *et al.* 2001) and the biological production of the polynya (Lewis *et al.* 1996) has been shown to vary between the east and west sides.

Materials and Methods

Field Collection and Sample Size

Tissue samples (muscle and blubber) from ringed seals were obtained from Inuit hunters from the west (Grise Fiord, Canada, $n = 27$) and the east side (Qânâq, Greenland, $n = 27$) (Figure 1), of the Northwater Polynya during the spring of 1998. All samples were placed in Whirl Pak bags, cryovials, or aluminum foil and frozen until analyzed for

stable isotopes (muscle) and OCs (blubber). Morphometric details were noted (sex, blubber thickness) and ages were determined using cementum growth layer groups in canine teeth from the lower jaw (Stewart *et al.* 1996).

Chemicals and Standards

All solvents (pesticide grade) and sodium sulfate (Na_2SO_4) were obtained from BDH Inc. (Toronto, ON). Pesticide grade Florisil, 60–100 mesh was obtained from the Floridin Corp. (Berkeley Spring, WV). Biobeads SX-3 used in the GPC column were purchased from Analytical Biochemistry Laboratories Ltd., (Columbia, MO). Nonracemic standards of α -HCH, oxychlorodane, heptachlor epoxide (HE), and *cis*- and *trans*-chlordane were obtained from EQ Laboratories (Atlanta, GA).

Extraction, Cleanup, and Analysis of Samples for OCs

Sample extraction and cleanup procedures have been published previously (Norstrom *et al.* 1988; Letcher *et al.* 1995). Briefly, a representative sample of tissue (approximately 2 g of fat, was ground with anhydrous sodium sulfate, spiked with a series of ^{13}C -labeled chlorobenzenes (tetra, penta, and hexachloro) and PCB congeners (PCBs 28, 52, 118, 153, 180, and 194). Samples were extracted with 100 ml (1:1) dichloromethane (DCM)/hexane and a fraction of the eluant was used to determine lipids gravimetrically. Lipids were subsequently removed from the samples by gel permeation chromatography (GPC). The lipid-free eluate, containing the OCs, was evaporated to 1 ml and applied to a Florisil column (8 g, 1.2% deactivated). All fractions were roto-evaporated, transferred to 2,2,4-trimethyl pentane, and evaporated to approximately 570 μl . ^{13}C -labeled PCB 138 was added as an instrument performance standard. Samples were analyzed on a Hewlett Packard 5890 gas chromatograph (GC) equipped with a 30 m \times 0.25 mm DB-5 MS column (J & W Scientific) and an HP 5970 MSD. A 3.0- μl sample volume was injected by splitless mode, and external standards were run after every six samples. The recovery of the ^{13}C -labeled internal standards was $83 \pm 1.6\%$ (mean \pm 1 SE) and concentrations were not corrected for these recoveries.

Chiral Analysis

A 30-m fused silica, 0.25 mm i.d. β -DEX 120 (20% nonbonded permethylated β -cyclodextrin) (Supelco Chromatography Products, ON) was used for chiral analysis. To reduce column bleed from entering the mass spectrometer a 1-m section of a DB5 MS column was joined to the MS end of the analytical column using a Supelco Glasseal capillary column connector.

Samples were analyzed on an HP 5890 Series II GC coupled with a 5972 mass selective detector (MSD). Splitless injection mode was used, with an injection volume of 3.0 μl . The temperature program used in the analysis was: injector temp 250°C, initial temperature and hold time 90°C for 1 min, first ramp at 15°C/min to 130°C, second ramp 2°C/min to 250°C and held for 7 min, total run time of 71 min. The MS was operated in the selected ion monitoring (SIM) mode, an interface temperature of 280°C, and a source temperature of 250°C. The compounds of interest with their respective retention times were determined from the fragmentation pattern of appropriate enriched and racemic standards. Peak height was used for quantification of all enantiomers because of the presence of a closely eluting compound with a similar fragmentation to α -HCH, but different ion ratio pattern (Moisey *et al.* 2001). In extreme instances, baseline resolution of the two peaks was not

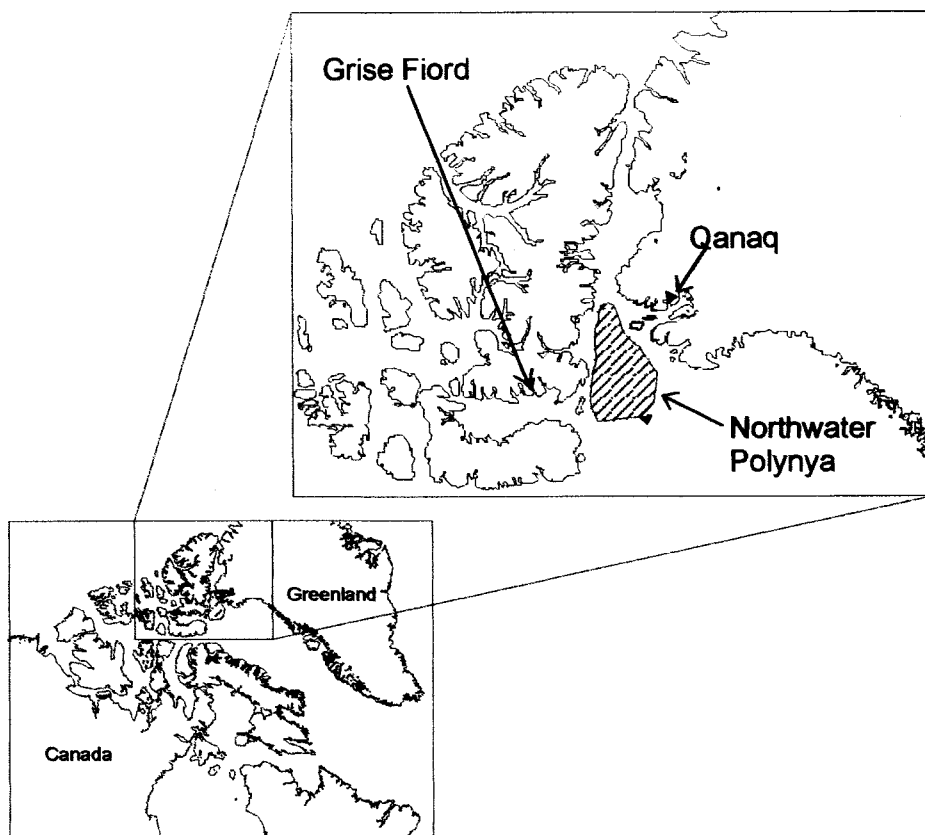


Fig. 1. Approximate location and size of the Northwater Polynya in May/June and the location of the communities of Grise Fiord, Canada and Qanaq, Greenland

possible. Since the target and the interfering compound were not baseline resolved, quantitation by height eliminated this partial peak overlap problem. Results of chiral analysis in samples that were not compromised by interfering compounds showed that using peak height produced the same results as peak area. Racemic or nonracemic standards were run after every seventh sample. The elution order of enantiomers was monitored with enantio-enriched standards.

Enantiomeric composition of chiral chemicals can be expressed as fractions (EFs) or ratios (ERs). EFs are more easily compared and used in mathematical equations (Harner *et al.* 2000). EF was used in this paper and was calculated using the following equation: $EF = (+) / [(+) + (-)]$, where (+) and (-) is the height of the corresponding enantiomer. For comparison with past work, where ERs are more commonly used, ERs were converted to EF with the equation: $EF = ER / (ER + 1)$.

Stable Isotope Analysis

Stable-carbon and 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 CO₂ and N₂ gases were then analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS) with every five unknowns separated by two laboratory standards. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000, \quad (1)$$

where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C/¹²C or ¹⁵N/¹⁴N. The R_{standard} values were based on the PeeDee Belemnite for ¹³C and atmospheric N₂ for ¹⁵N. Replicate measurements of internal laboratory standards (albumin) indicate measurement errors of $\pm 0.3\%$ for stable-nitrogen isotope measurements.

Statistical Analysis

ANCOVA was used to assess the influence of a number of variables on stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), ΣOC groups, and EFs of chiral pollutants in the seals using methods similar to those outlined in Muir *et al.* (2000) using SAS for Windows. Stable isotope values were examined to test the influence of the variables site, age, sex, blubber thickness (bt), the other stable isotope value (δOX) and all crosses. The ΣOC groups (ΣCIBz , ΣHCH , ΣCHLOR , ΣDDT , or ΣPCB) were examined for the effect of site, age, sex, blubber thickness (bt), $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and all crosses. EFs of chiral pollutants ($\alpha\text{-HCH}$, *cis*-chlordan, *trans*-chlordan, oxychlordan, or heptachlor epoxide) were examined to test the influence of the variables site, age, sex, and concentrations (lipid corrected) of ΣCIBz , ΣHCH , ΣCHLOR , ΣDDT , or ΣPCB but with no crosses. The significance of each variable was then assessed with type III sum of squares test and variables that were not significant at the 5% level were removed. The final model only included significant variables. Relationships between ΣOC and age and EFs of $\alpha\text{-HCH}$ and age were performed with simple linear regression using Systat for Windows (version 9.0, Evanston, IL).

Results

Age, Stable Isotopes, and OC Concentrations

Age, stable isotope values and OC concentrations for male and female ringed seals collected in Grise Fiord and Qânâq are summarized in Table 1.

Results of the ANCOVA model showed that $\delta^{15}\text{N}$ values only varied with $\delta^{13}\text{C}$ values ($p < 0.01$) and were not dependent on site, age, sex, or blubber thickness (all p values > 0.05). Values of $\delta^{15}\text{N}$ were found to increase with $\delta^{13}\text{C}$ values. Values of $\delta^{13}\text{C}$ were found to depend on site, age, and $\delta^{15}\text{N}$ values (ANCOVA, all p values < 0.05) but not sex or blubber thickness (p values > 0.05) resulting in a model of:

$$\delta^{13}\text{C} = \mu + \text{site} + \text{age} + \delta^{15}\text{N} + (\text{site} * \text{sex}) \\ + (\text{site} * \delta^{15}\text{N}) + \epsilon \quad (2)$$

Values of $\delta^{13}\text{C}$ increased with age and $\delta^{15}\text{N}$ values and were greater in the Qânâq ringed seals. Female seals had lower $\delta^{13}\text{C}$ values than males in Qânâq, and the influence of $\delta^{15}\text{N}$ values on $\delta^{13}\text{C}$ values was greater in Grise Fiord seals.

Despite lower concentrations in female ringed seals of Qânâq, concentrations of ΣCIBz were not found to differ with any of the variables examined. Concentrations of ΣHCH were found to depend on the age * site interaction, with concentrations increasing significantly with age in the Grise Fiord population but not in the Qânâq population. ΣCHLOR , ΣDDT , and ΣPCB concentrations depended on age and the interaction of age and sex but did not vary with site. Concentrations of these OCs increased with age, but older aged female seals had lower concentrations than males.

Mean EFs of the chiral OCs are given in Table 2. EFs of α -HCH were found to significantly decline with age in the ringed seal population (Figure 2) but were not influenced by any other variables (all p values > 0.05). EFs of *cis*-chlordane were positively related to $\delta^{13}\text{C}$ values and concentrations of ΣDDT but negatively related to ΣCHLOR concentrations (p values < 0.05). EFs of *trans*-chlordane significantly declined with $\delta^{15}\text{N}$ values ($p < 0.05$), but no other variables were significant. EFs of oxychlordane and HE did not vary with any of the parameters (site, age, sex, or the concentrations [lipid corrected] of ΣCIBz , ΣHCH , ΣCHLOR , ΣDDT , or ΣPCB) (all p values > 0.05).

Discussion

Feeding Ecology

Based on the samples chosen for the OC work the feeding ecology of ringed seals varied slightly between the Qânâq and Grise Fiord populations and between sexes. The trophic level at which the seals fed did not vary between populations or with sex based on $\delta^{15}\text{N}$ values, but the sources of carbon did significantly vary between sites and with age based on $\delta^{13}\text{C}$ values. Stable isotope signatures for food webs at Qânâq and Grise Fiord were similar (Hobson *et al.* 2001), so direct trophic comparisons of seals between these locations can be made.

$\delta^{15}\text{N}$ is correlated to trophic level in aquatic food webs (Hobson and Welch 1992; Hobson *et al.* 1994, 1997), including the Northwater Polynya (Hobson *et al.* 2001), and $\delta^{13}\text{C}$ can be used as a tracer of carbon source (McConnaughey and McRoy 1979; Rau *et al.* 1983; Dunton *et al.* 1989; Hobson 1993). Pelagic food items, based on more depleted $\delta^{13}\text{C}$ values, made up a greater percentage of the diet of seals from Qânâq and increased with age of the seals. In a more comprehensive evaluation of the feeding ecology of these ringed seal populations based on stomach content analysis, Holst *et al.* (2001) determined that the diets of ringed seals from Grise Fiord and Qânâq differed in the types and proportion of prey consumed. Nonetheless, both diets consisted mainly of arctic (*Boreogadus saida*) and polar cod (*Arctogadus glacialis*), although the seals from Grise Fiord had proportionally more polar cod in their diet. Seals from Grise Fiord had higher $\delta^{13}\text{C}$ than those from Qânâq, suggesting that they were taking more benthic prey (Holst *et al.* 2001). Holst *et al.* (2001) proposed that polar cod might have contributed to higher $\delta^{13}\text{C}$ values.

OC Levels

The concentrations of OCs found in the ringed seals of this study are in the range reported previously for Canadian Arctic ringed seals (Muir *et al.* 1999, 2000). Concentrations of ΣDDT and ΣPCB in seals from this study were lower than those reported for this species in the European Arctic, such as Svalbard (Wolkers *et al.* 1998) and Finland and White Sea Russia (Kostamo *et al.* 2000). HCH concentrations were higher in the NOW ringed seals than those reported for ringed seals of the European Arctic. This pattern of higher concentrations of DDTs and PCBs and lower concentrations of HCH in the European Arctic is consistent with past compilations of circumpolar data in ringed seals (Muir *et al.* 2000) and polar bears (Norstrom *et al.* 1998).

NOW East versus West Populations

After removing the influence of age, sex, and blubber thickness, the concentrations of OCs did not vary between the ringed seals of Qânâq and Grise Fiord. Muir *et al.* (2000) found that OC concentrations varied in ringed seals over large distances in the Arctic, such as between east Greenland and the Canadian Arctic, but not between geographically close populations, such as within the Canadian eastern Arctic. Although slight differences in $\delta^{13}\text{C}$ between these populations were found, in general the two populations were feeding at the same trophic level, based on $\delta^{15}\text{N}$, and essentially on the same food items, especially fish (polar and arctic cod) (Holst *et al.* 2001). Ringed seals generally remain in a given area for significant lengths of time but are known to move significant distances (Heide-Jørgensen *et al.* 1992; Teilmann *et al.* 1999) and it is likely that some seals moved between the two sampling locations and thus potentially obscured any difference in regional OC levels if they exist. Preliminary analyses indicate that the two ringed seal populations appear genetically similar (Davis *et al.* 1999).

Table 1. Age, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in muscle, lipid content and concentrations (ng/g, wet weight, not corrected for any variables) of persistent organochlorine contaminants in blubber (mean \pm 1 SE) of ringed seals collected on the east and west side of the Northwater Polynya in 1998 (note that only the 10 PCB congeners with the highest concentrations are reported and are ranked by concentration)

	Grise Fiord		Qânâq	
	Female	Male	Female	Male
n	15	12	14	13
age	19.0 \pm 3.9	14.8 \pm 3.0	15.3 \pm 3.6	14.8 \pm 3.3
$\delta^{15}\text{N}$	17.4 \pm 0.1	17.3 \pm 0.3	17.2 \pm 0.2	17.4 \pm 0.1
$\delta^{13}\text{C}$	-18.3 \pm 0.1	-18.2 \pm 0.1	-19.1 \pm 0.2	-19.4 \pm 0.3
lipid %	90.4 \pm 1.8	91.2 \pm 1.4	85.6 \pm 3.6	87.8 \pm 4.7
ΣCIBz^a	42 \pm 4.5	47 \pm 10	25 \pm 3.0	38 \pm 6.0
1,2,3,4-tetraCBz	4.2 \pm 1.0	3.3 \pm 1.0	4.3 \pm 2.1	4.3 \pm 0.7
1,2,4,5-tetraCBz	8.1 \pm 1.1	14 \pm 4.8	4.6 \pm 0.6	13 \pm 2.3
pentaCBz	8.4 \pm 1.1	7.3 \pm 1.9	5.0 \pm 0.5	7.0 \pm 1.5
hexaCBz	22 \pm 2.6	22 \pm 3.9	11 \pm 1.0	14 \pm 2.6
ΣHCH^b	148 \pm 12	179 \pm 38	68 \pm 5.9	136 \pm 31
α -HCH	106 \pm 9.0	107 \pm 22	49 \pm 3.9	88 \pm 20
β -HCH	35 \pm 3.0	64 \pm 16	17 \pm 1.9	41 \pm 9.5
γ -HCH	7.6 \pm 2.5	8.8 \pm 2.1	2.8 \pm 0.5	6.8 \pm 1.3
ΣCHLOR^c	338 \pm 38	854 \pm 168	187 \pm 27	546 \pm 156
<i>trans</i> -chlordane	1.7 \pm 0.2	4.5 \pm 0.9	1.7 \pm 0.4	2.8 \pm 0.6
<i>cis</i> -chlordane	13 \pm 2.0	30 \pm 8.7	6.5 \pm 1.2	11 \pm 2.0
<i>cis</i> -nonachlor	17 \pm 2.7	37 \pm 10	8.9 \pm 2.0	17 \pm 4.1
<i>trans</i> -nonachlor	120 \pm 16	291 \pm 72	63 \pm 15	163 \pm 60
MC-5	13 \pm 1.4	25 \pm 4.8	8.2 \pm 0.8	24 \pm 8.0
MC-7	6.0 \pm 0.9	18 \pm 4.5	4.3 \pm 0.4	12 \pm 2.7
Oxychlordane	131 \pm 20	363 \pm 79	75 \pm 7.8	261 \pm 78
Heptachlor epoxide	36 \pm 3.9	85 \pm 16	19 \pm 1.8	55 \pm 14
ΣDDT^d	394 \pm 89	963 \pm 271	390 \pm 63	944 \pm 229
<i>p,p'</i> -DDT	87 \pm 16	179 \pm 47	69 \pm 12	190 \pm 64
<i>p,p'</i> -DDD	11 \pm 1.4	24 \pm 4.3	11 \pm 1.8	20 \pm 4.0
<i>p,p'</i> -DDE	296 \pm 73	760 \pm 230	310 \pm 51	733 \pm 171
ΣPCB^e	483 \pm 86	963 \pm 206	476 \pm 78	1,478 \pm 348
CB 153	100 \pm 23	237 \pm 64	93 \pm 18	346 \pm 89
CB 138	65 \pm 15	152 \pm 33	59 \pm 10	243 \pm 64
CB 99	35 \pm 6.2	86 \pm 19	33 \pm 5.1	142 \pm 40
CB 101/90	30 \pm 4.1	63 \pm 12	27 \pm 4.0	79 \pm 17
CB 118	27 \pm 4.2	53 \pm 11	30 \pm 5.0	56 \pm 7.8
CB 180	22 \pm 5.2	40 \pm 11	25 \pm 5.9	80 \pm 22
CB 52	18 \pm 1.8	33 \pm 5.9	12 \pm 1.3	39 \pm 9.3
CB 146	14 \pm 3.4	34 \pm 9.1	15 \pm 2.9	53 \pm 13
CB 187	13 \pm 2.4	22 \pm 4.6	14 \pm 2.9	39 \pm 11
CB 74	11 \pm 1.7	22 \pm 3.8	11 \pm 1.1	30 \pm 6.2
Other OCs				
Dieldrin	55 \pm 4.2	105 \pm 19	33 \pm 3.0	74 \pm 17
Mirex	6.8 \pm 1.4	7.9 \pm 2.5	5.2 \pm 1.1	10 \pm 3.0
Photomirex	6.0 \pm 1.3	10 \pm 2.6	6.4 \pm 1.1	13 \pm 3.6
OCS	1.2 \pm 0.2	2.3 \pm 0.6	1.7 \pm 0.6	1.6 \pm 0.3

^a ΣCIBz (chlorobenzene) = sum of 1,2,4,5-tetraCIBz, 1,2,3,4-tetraCIBz, pentaCIBz, and hexaCIBz.

^b ΣHCH (hexachlorocyclohexane) = sum of α -HCH, β -HCH, and γ -HCH.

^c ΣCHLOR (chlordanes) = sum of heptachlor, heptachlor epoxide, *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, MC-5, MC-7 and oxychlordane.

^d ΣDDT = sum of *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT.

^e ΣPCB = sum of congeners 1, 3, 4/10, 7, 6, 8/5, 19, 18, 17, 24/27, 16/32, 26, 25, 31, 28, 33, 22, 45, 46, 52, 49, 47, 48, 44, 42, 41/71, 64, 40, 74, 70/76, 95/66, 56/60, 91, 84/89, 101, 99, 83, 97, 87, 85, 136, 110, 82, 151, 144/135, 149, 118, 134, 114, 131, 146, 153, 132, 105, 141, 130/176, 179, 137, 138, 158, 178/129, 175, 187, 183, 128, 185, 194, 196/203, 189, 208, 195, 207, 194, 205, 206, and 209.

Age, Sex, and Blubber Thickness

Concentrations of ΣPCBs , ΣDDTs , and ΣCHLOR compounds were found to increase with age, for both male and female ringed seals (Figure 3). Sex was an important variable within

the context of seal age; with age the concentrations of OCs became greater in males than in females. These relationships were not observed for ΣCBz and ΣHCHs , although there was an age-within-site significance for ΣHCHs . Positive relationships between OC concentration and age has been observed

Table 2. Enantiomeric fractions (mean ± 1 SE) of chiral OCs in ringed seals collected on the east (Grise Fiord) and west (Qânâq) side of the Northwater Polynya in 1998

	n ^a	α-HCH	cis-Chlordane	trans-Chlordane	Oxychlordane	Heptachlor Epoxide
Racemic standard	3, 3	0.50 ± 0.01	0.49 ± 0.003	0.50 ± 0.003	0.52 ± 0.01	0.48 ± 0.003
All ringed seals	50, 47	0.50 ± 0.01	0.72 ± 0.03	0.43 ± 0.03	0.62 ± 0.02	0.45 ± 0.01
Grise Fiord	25, 24	0.51 ± 0.01	0.72 ± 0.04	0.38 ± 0.03	0.63 ± 0.03	0.47 ± 0.01
Qânâq	25, 23	0.49 ± 0.02	0.73 ± 0.03	0.49 ± 0.05	0.62 ± 0.02	0.43 ± 0.01

^a The first number represent the sample size for analysis of α-HCH, oxychlordane, and HE; the second the sample size for cis- and trans-chlordane.

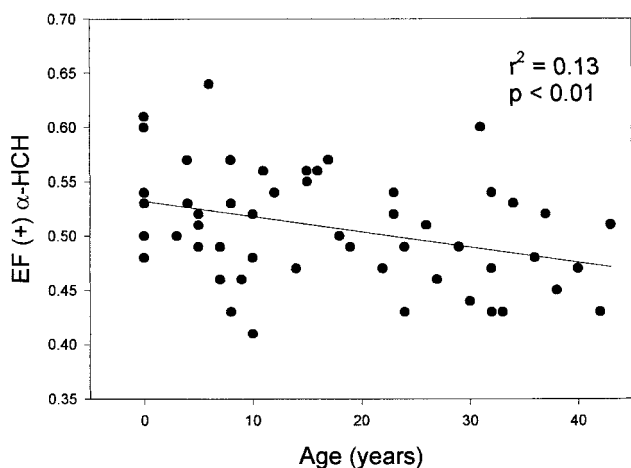


Fig. 2. Relationship between EF of α-HCH and age in ringed seals collected in the Northwater Polynya. Line represents the linear regression for all data

previously for male ringed seals (Muir *et al.* 2000; Wolkers *et al.* 1998) but is less commonly observed in female seals (Kostamo *et al.* 2000). Reproduction, including birth and lactation, provides a means for female seals to reduce body burdens of OCs, and counters the effect of accumulation of OCs with age. Wolkers *et al.* (1998) found PCBs increased with age in Svalbard ringed seals and that sex was not an important variable. They suggested that the continued feeding of ringed seals during lactation compensates for loss of OCs due to lactation. The difference in the slope of age-OC concentration relationships between our male and female ringed seals suggests that birth and lactation are important variables in female ringed seal OC concentrations.

The age range of the seals (and the oldest age collected) in this study was much larger than those collected previously for contaminant research and may have accounted for the relationships observed in female seals. This study included 12 female seals older than 20 years, 8 older than 30 years, and 2 older than 40 years, whereas past studies have rarely used individuals over 20 years of age. The age at which reproductive effort in female ringed seals begins to decline is not clearly understood. Lydersen and Gertz (1987) found that two female ringed seals of age 40 and 45 years were no longer fertile, but another pair, 39 and 40 years old, appeared to have normal reproductive function. Smith (1987) observed no change in fecundity with age in ringed seals examined from the western Canadian Arctic. McLaren (1958b) concluded that a single female ringed seal of age 30 was no longer reproductively active. Although

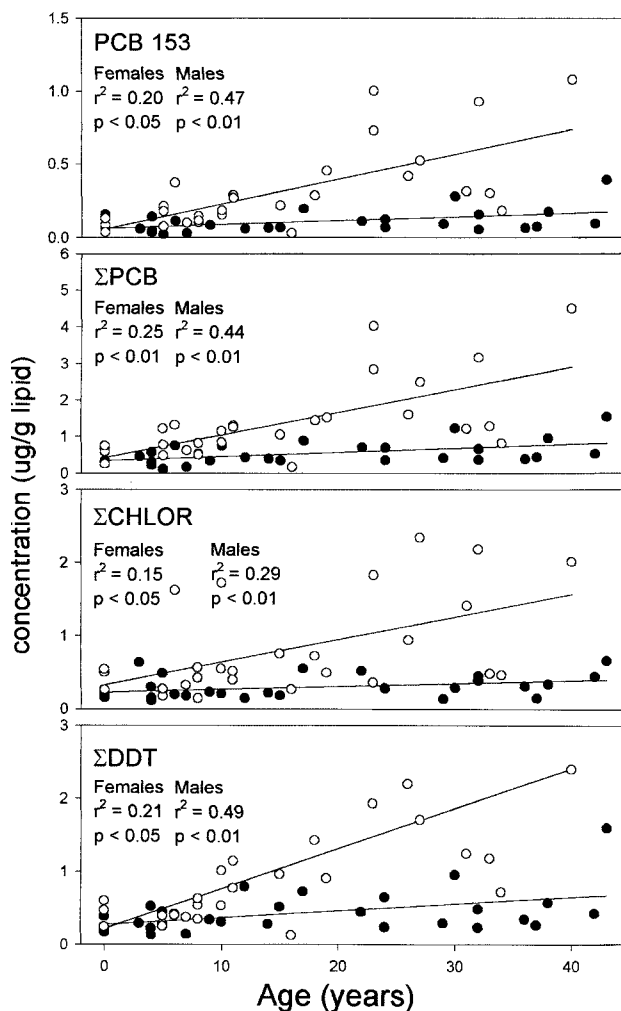


Fig. 3. Relationship between OC concentrations (µg/g, lipid corrected) and age in male (○) and female (●) ringed seals utilizing the the Northwater polynya

the age-OC concentration relationships observed in the NOW seal cannot provide a definitive answer, it suggests that reproduction declines at older ages in female ringed seals.

Blubber thickness was not a significant variable in OC concentrations in the NOW ringed seals once the influence of age and sex were removed from the dataset. Blubber thickness has been found to influence OC concentrations in ringed seal populations (Muir *et al.* 2000). The lack of a significant relationship between OC and blubber thickness in the NOW ringed

seals may be due to low variation in blubber thickness among individuals as they were collected in the same season and region.

Stable Isotopes

Stable isotopes appear to have limited ability to describe OC concentrations within a single population or two closely associated populations of ringed seals. $\delta^{15}\text{N}$ values have been shown to be positively correlated with OC concentrations in aquatic food webs (Jarman *et al.* 1996; Kidd *et al.* 2001), including the NOW food web (Fisk *et al.* 2001a). Although OC concentrations varied considerably with age and sex in our seal populations, the processes that result in these differences did not influence stable isotope values. ΣPCBs , ΣDDTs , and ΣCHLOR were found to vary with age and sex, which was not due to different exposure but is related to contaminant elimination in the seals. The increase in OC concentrations with age that was observed in ringed seals of this study suggests that the elimination of OCs is slower than uptake and that concentration of OCs in ringed seal blubber is not in equilibrium with diet items. Female seals can eliminate OCs through the production of offspring and subsequent lactation as discussed above. Neither age nor likely reproductive effort varied with $\delta^{15}\text{N}$ values in the NOW ringed seals, and therefore $\delta^{15}\text{N}$ values did not describe OC concentrations within these ringed seal populations. Although variation in $\delta^{13}\text{C}$ values varied with age and site in ringed seals, it did not provide any insight into differences in OC concentrations among seals.

Chiral Contaminants

Four of the five chiral contaminants measured in the ringed seals were nonracemic. α -HCH did not appear to be enantioselectively biotransformed by the ringed seals as the EFs were 0.50 ± 0.01 . EFs of α -HCH in water, zooplankton and Arctic cod from the NOW were slightly depleted in (+) α -HCH (EFs ≈ 0.45) (Moisey *et al.* 2001), which suggests that there may be some selective metabolism of the (-) α -HCH enantiomer in ringed seals. An overall food web assessment of α -HCH in the NOW, which included the ringed seal data of this study, concluded that ringed seals do not metabolize α -HCH efficiently (Moisey *et al.* 2001). An excess of (+) α -HCH (EFs = 0.58) were found in the blubber of harbor (*P. vitulina*) and grey (*P. grypus*) seals, although no data was provided for their food (Klobes *et al.* 1998). In 10 species of small cetaceans there was an excess of the (+) α -HCH in blubber but the relative value of EFs varied between species (Tanabe *et al.* 1996). From these results it appears that the enantio-selective biotransformation of α -HCH varies between marine mammal species.

EFs of α -HCH and other chiral contaminants in seal blubber may not reflect the metabolic capability of seals. Wiberg *et al.* (1998, 2000) noted near racemic α -HCH (EFs = ~ 0.52) in blubber of ringed seals but nonracemic values in liver (EFs = ~ 0.6). This phenomenon was observed with other chiral pollutants, such as *trans*-chlordane, but in some cases the EF was greater in blubber (Wiberg *et al.* 2000). Wiberg *et al.* (1998) attributed this difference to greater metabolic activity in the

liver as compared to the blubber. This would imply that the proportion of the α -HCH body burden that is transformed is small and consequently the EF in ringed seal blubber is closer to that in the diet than in liver. This is not always the case, since EFs of many chiral OCs in ringed seal blubber have been found that do not match their main prey item, Arctic cod (Wiberg *et al.* 2000; Moisey *et al.* 2001). In seven species of seabirds, animals that do not retain such a large reserve of fat as found in ringed seals, there were no differences in EFs of chiral chlordanes between liver and fat (Fisk *et al.* 2001b). Differences in EFs of chiral pollutants between tissues of seals, and potentially other marine mammals, require further study.

A significant negative trend in EFs of α -HCH was observed with age in this study (Figure 3), although the amount of variability explained was low ($r^2 = 0.13$). The change in EFs of α -HCH in ringed seals occurred despite strong evidence that these mammals have limited capacity to metabolize α -HCH (see above). Wiberg *et al.* (2000) noted no differences in EFs of α -HCH with sex or age in ringed seals, although the number of samples analyzed and the range of ages were more limited than the present study.

All chlordanes components were found to be nonracemic in ringed seals. There was enrichment and depletion of the (+) enantiomer of *cis*- and *trans*-chlordane, respectively, consistent with past results reported in ringed seal blubber (Wiberg *et al.* 2000). A depletion of (+) enantiomer of both *cis*- and *trans*-chlordane was reported in six of seven seabird species (Fisk *et al.* 2001b), suggesting variation in the type or characteristics of enzyme(s) responsible for the chiral signatures of the chlordanes components observed in ringed seals and seabirds. The EFs of *trans*-chlordane varied with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively, suggesting that diet and exposure may play a role in EFs observed in ringed seals. However, *cis*- and *trans*-chlordane were racemic in the main prey items of the ringed seal (Arctic cod and *Themisto libellula*) (Fisk, unpublished data) suggesting that nonracemic values in the seals are due to their metabolic activity. Relationships between *cis*-chlordane EFs and concentrations of ΣDDT and ΣCHLOR suggest that induction of metabolizing enzyme systems by these chemicals may be an important variable in EFs. However, there was a negative relationship with ΣCHLOR and positive with ΣDDT despite similar relationships with age and sex for these chemicals. It may be that the range of concentrations observed in the ringed seals was not sufficient to cause significant differences in enzyme induction or activities.

There was an enrichment of the (+) enantiomer of oxychlordane, consistent with past results in ringed seal blubber (Wiberg *et al.* 2000). Enrichment of the (+) enantiomer has also been observed in polar bear (Wiberg *et al.* 2000) and seabirds (Fisk *et al.* 2001b). Oxychlordane is a metabolite of *cis*- and *trans*-chlordane, produced in mammals and birds. The relationship between the enantiomer of *cis*- and *trans*-chlordane that is metabolized and the corresponding enantiomer of oxychlordane that is formed is unknown. Fisk *et al.* (2001a) suggested that depletion of the (+) enantiomer of both *cis*- and *trans*-chlordane and the enrichment of the (+) oxychlordane in seabirds suggested a conversion of (+) *cis*- and/or *trans*-chlordane into (+) oxychlordane. Such a relationship is not obvious in the ringed seals because of the differences in depletion of the *cis*- and *trans*-chlordane enantiomers. However, it may be that not all of the *cis*- and *trans*-chlordane that is

metabolized is converted to oxychlordanes, and a preference for the conversion of the (+) *trans*-chlordanes enantiomer to (+) oxychlordanes may still occur. Oxychlordanes are also accumulated from diet and the chiral signature in food needs to be considered. However, Wiberg *et al.* (2000) found that the biomagnification factor of both (+) and (–) oxychlordanes was two to three times that of PCB 153, indicating that most of the compound was formed by metabolism rather than accumulated from diet. Ringed seals appear to metabolize the *cis*-chlordanes more efficiently than *trans*-chlordanes based on BMFs normalized to PCB 153 (Wiberg *et al.* 2000).

There was a slight depletion of the (+) enantiomer of HE in the ringed seals, consistent with past EFs reported for ringed seal blubber (Wiberg *et al.* 2000). This is opposite of what has been observed in Arctic seabirds (Fisk *et al.* 2001b) and polar bears (Wiberg *et al.* 2000), where an enrichment of the (+) enantiomer was found. HE is a metabolite of heptachlor, and recent work has suggested that levels in marine mammals and seabirds are influenced by formation within the organism (Fisk *et al.* 2001a). Heptachlor epoxide EFs were 0.61 ± 0.003 in Arctic cod from this region (Fisk, unpublished data), suggesting either significant biotransformation of the (+) enantiomer acquired from the consumption of the cod or significant formation of the (–) enantiomer from heptachlor by the seals. Regardless, the opposite chiral signature suggests that the either the biotransformation or formation of HE in ringed seals appears to be different than in other Arctic organisms.

The EFs of oxychlordanes and HE did not vary with sex, age, site, or concentrations of the major OC groups. Klobes *et al.* (1998) noted no difference in EFs of oxychlordanes with sex or age in harbor or gray seals.

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