Effects of Organochlorine Contaminants on Thyroid Hormone Levels in Arctic Breeding Glaucous Gulls, *Larus hyperboreus*

Jonathan Verreault,[1,2] Janneche Utne Skaare,[3,4] Bjørn Munro Jenssen,[5] and Geir Wing Gabrielsen[1]

[1]Norwegian Polar Institute, Tromsø, Norway; [2]Department of Ecology and Zoology, University of Tromsø, Tromsø, Norway; [3]Norwegian School of Veterinary Science, Oslo, Norway; [4]National Veterinary Institute, Oslo, Norway; [5]Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

Studies on glaucous gulls (*Larus hyperboreus*) breeding in the Barents Sea have reported that high blood levels of halogenated organic contaminants in this species might cause reproductive, behavioral, and developmental stress. However, potential endocrine system modulation caused by contaminant exposure has yet not been reported in this Arctic apical predator. In this present study we aimed to investigate whether the current levels of a selection of organochlorines (OCs) were associated with altered circulating levels of thyroid hormones (THs) in free-ranging adult glaucous gulls breeding at Bear Island in the Barents Sea. Blood concentrations of 14 polychlorinated biphenyls, hexachlorobenzene (HCB), oxychlordane, and *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) were quantified, in addition to free and total thyroxine (T_4) and triiodothyronine (T_3), in plasma of 66 glaucous gulls in the spring of 2001. Negative correlations were found between plasma levels of T_4 and T_4:T_3 ratio, and blood levels of OCs in male glaucous gulls. Despite their relatively low contribution to the total OC fraction, HCB and oxychlordane were the most prominent compounds in terms of their negative effect on the variation of the T_4:T_3 ratio. Moreover, lower T_4 levels and T_4:T_3 ratios were measured in glaucous gulls breeding in a colony exposed to high levels of OCs, compared with a less exposed colony. Levels of T_3 were elevated in the high-OC–exposed colony. This may indicate that the glaucous gull is susceptible to changes to TH homeostasis mediated by exposure to halogenated organic contaminants. *Key words:* Arctic, contaminant, endocrine disruption, glaucous gull, organochlorine, T_3, T_4, thyroid hormone. *Environ Health Perspect* 112:532–537 (2004). doi:10.1289/ehp.6756 available via *http://dx.doi.org/* [Online 9 December 2003]

Endocrine-disrupting chemicals (EDCs) act by interfering with function(s) of the endocrine system (Colborn and Clement 1992). Sublethal levels of EDCs have been associated with developmental, behavioral, and reproductive abnormalities and alteration of endogenous hormone levels in laboratory and free-ranging avian species (Burger et al. 2002; Dawson 2000; NRC 1999; Tanabe 2002). Alteration of endocrine functions mediated by EDC exposure may act through interference with the synthesis, secretion, transport, binding, action, or elimination of endogenous hormones (Damstra et al. 2002). The major explanation evoked for the interaction between EDCs such as certain halogenated organic contaminants—for example, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), dichlorodiphenyldichloroethanes (DDTs), and hydroxylated metabolites of PCBs (OH-PCBs)—and the endocrine system is the structural similarity of EDCs with endogenous hormones.

In recent years, a wide body of evidence for interaction of halogenated organic contaminants with the thyroid hormone (TH) system has been reported. Abnormal TH concentrations and thyroid gland structure have been linked to exposure to halogenated organic contaminants in amphibians, reptiles, mammals, and birds (Dawson 2000; Leatherland 2000; Peakall 1992; Rolland 2000; Vos et al. 2000). Many studies have reported reduced levels of thyroxine (T_4) with increasing organochlorine (OC) levels, associated with either no or minimal effects on levels of triiodothyronine (T_3).

THs in birds regulate metabolic heat production (thermoregulation), growth, body weight, development of central nervous system, cell differentiation and maturation, hatching, molt, and reproduction (McNabb 2000; Merryman and Buckles 1998a, 1998b). Iodine, an essential element for TH synthesis, is stored in excess as iodide from dietary uptake (McNabb 1992). THs in birds are carried in the plasma bound to transport proteins, that is, albumin or transthyretin (TTR). Most of the T_4 in birds is associated with albumin, which has low-affinity binding sites with little specificity for T_4 or T_3 compared with TTR in mammals (Astier 1980; Davidson et al. 1978; Merryman and Buckles 1998a). Factors that influence thyroid functions include dietary iodine (I^-) availability, activity, ambient temperature, photoperiod, body condition, seasonality, and age (McNabb 2000).

A wide range and occasionally very high levels of halogenated organic contaminants have been reported in glaucous gulls (*Larus hyperboreus*; Bourne and Bogan 1972; Gabrielsen et al. 1995; Savinova et al. 1995), an apical predator breeding in the Barents Sea (Løvenskiold 1964). Studies on the glaucous gull have suggested that high blood levels of halogenated organic contaminants in this species might cause reproductive, behavioral, and developmental stress (Bustnes et al. 2001a, 2002, 2003b). In these studies, high levels of OCs have been associated with reduced parental attentiveness during incubation, higher rate of feather asymmetry, and decreased reproductive success. Contaminant-induced modulation of the bird's hormone and/or nervous system has been proposed as the underlying mechanism for the alteration of these biologic functions.

In this study we aimed to investigate whether the current blood levels of a selection of OCs were related to circulating plasma levels of T_4 and T_3 in free-ranging adult glaucous gulls breeding at Bear Island in the Barents Sea. Because halogenated organic contaminants have been linked to adverse biologic effects in free-ranging avian species (e.g., Barron et al. 1995), there might be a causative link between blood levels of these contaminants and the adverse reproductive, behavioral, and developmental effects reported in glaucous gulls (Bustnes et al. 2001a, 2002, 2003b). Levels of THs, which are involved directly or indirectly in the regulation/initiation of reproduction, behavior, and development, may be altered in glaucous gulls exposed to high concentrations of OCs through their diet. Assessment of circulating TH status has been suggested to be a useful biomarker of response in free-ranging animals exposed to contaminants (Fox 1993; Peakall 1992; Rolland 2000). According to Peakall (1992), the ratio between T_4 and T_3 (T_4:T_3) seems to be the most sensitive indicator revealing effects of contaminant exposure. A lower T_4:T_3 ratio, associated with increasing levels of halogenated organic contaminant in an organism, is likely to indicate alteration of TH homeostasis mediated by contaminant toxicity.

Address correspondence to J. Verreault, Norwegian Polar Institute, The Polar Environmental Centre, NO-9296 Tromsø, Norway. Telephone: 47-77-75-05-42. Fax: 47-77-75-05-01. E-mail: jonathan@npolar.no

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Materials and Methods

Study area. The field sampling was conducted at the south and southeast coast of Bear Island (74°21′ N, 19°05′ E) in the western Barents Sea during spring 2001. Bear Island has some of the largest seabird colonies in the Barents Sea, with several hundred thousand breeding pairs. The breeding population of glaucous gulls at Bear Island is estimated to be approximately 2,000 pairs (Mehlum and Bakken 2000). The breeding season at Bear Island is characterized by continuous daylight, an average temperature of 4–5°C, and periods of strong winds and even snowfall. Samples of glaucous gull were taken from two major breeding colonies: Evjebukta and Sørhamna. Blood levels of OCs in glaucous gulls from these two colonies are reported to be different, presumably due to different feeding ecology (Bustnes et al. 2000). Evjebukta is situated at the edge of the main seabird cliff, about 100–150 m above sea level, whereas Sørhamna is located farther away from the main seabird cliff and 20–30 m above sea level (Figure 1).

Field sampling. A total of 83 glaucous gulls were captured on their nests during the incubation period (Løvenskiold 1964), using a nest trap. The trap consisted of a snare placed on the edge of the nest bowl and attached to a mechanism triggered by a radio transmitter. Because nearly all nests in the breeding colonies were accessible, the nesting individuals were selected randomly for capture. The birds were given alpha-coded plastic leg bands and numbered steel rings. After capture, several biometric measurements were recorded: wing length (± 1 mm), bill, tarsus, and head length (± 0.1 mm), and body mass (± 10 g). Because all birds captured were incubating, they were assumed to be sexually mature, that is, at least 5 years of age (Gilchrist 2001). The age of the birds was assumed to be equally distributed between the two breeding colonies. The sex of the individuals was determined using the total head and bill length, as recommended by Coulson et al. (1983) for *Laridae* species. Adult male glaucous gulls from Bear Island have on average a bill longer than 61.5 mm and a total head and bill longer than 142 mm (Henriksen EO, unpublished data).

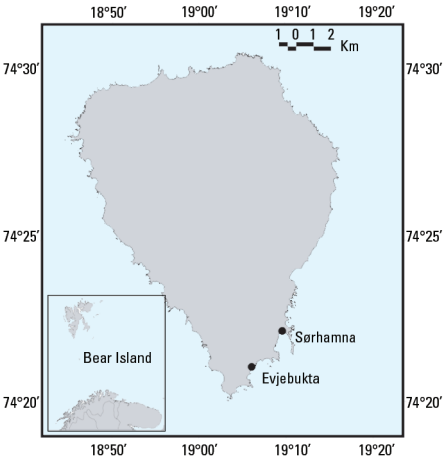

Figure 1. Map of the Barents Sea and Bear Island showing the two breeding colonies of glaucous gulls (Evjebukta and Sørhamna).

A blood sample (12 mL) was collected from the brachial vein with a heparinized 20-mL syringe and a 21-gauge hypodermic needle and was kept dark on ice during transport to the field laboratory. The whole-blood samples for OC analyses (6 mL) were transferred to 5-mL cryogenic vials and stored in a –20°C propane-driven freezer. The whole blood is documented to be a reliable matrix for quantification of OCs in incubating glaucous gulls (Bustnes et al. 2001b; Henriksen et al. 1998). The plasma for TH quantification was obtained by centrifugation of whole blood (6 mL; 5,000 rpm, 7 min), transferred to 1.2-mL cryogenic vials, and stored in liquid nitrogen. Both whole-blood and plasma samples were frozen within 6 hr of collection until thawed for analyses.

This project received approval from the Norwegian Animal Care Committee for research involving animals, and the permission to capture glaucous gulls at Bear Island was given by the Governor of Svalbard (Norway).

Contaminant analyses. The quantification of OCs in whole blood was performed at the Environmental Toxicology Laboratory at the Norwegian School of Veterinary Science, Oslo, Norway. This laboratory is accredited as a testing laboratory for OCs according to the requirements of NS-EN 45001 and ISO/IEC Guide 25.

The whole blood samples were weighed, and internal standards (PCB-29 and PCB-207) were added. The samples were extracted twice with cyclohexane and acetone, and the percentage of extractable plasma fat (± 0.01%) was determined gravimetrically. The extracted plasma fat in each sample was redissolved in cyclohexane and washed with ultrapure sulfuric acid, according to Brevik (1978). Aliquots of the fat-free extracts were injected automatically on a high-resolution gas chromatograph (HRGC; Agilent 6890 Series gas chromatography system; Agilent Technologies, Palo Alto, CA, USA), equipped with a split/splitless injector and two micro-electron capture detectors (^{63}N, 300°C). Two columns (SPB-5 and SPB-1701: 60 m, 0.25 mm inner diameter, and 0.25 µm film layer; Supelco Inc., Belleponte, PA, USA), of different polarity and selectivity, were used to obtain the desired chromatographic separation, both connected to a 1-m deactivated precolumn. Quantification was performed using PCB-29 and PCB-207 as internal standards in each sample. The OCs were identified on the basis of their retention time on the HRGC columns. Chromatographic data were interpreted using HP ChemStation Plus, Rev. A.07.01 (Hewlett-Packard Co., Palo Alto, CA, USA). Details on extraction, cleanup, chromatographic separation, and analytic quality are described by Bernhoft et al. (1997), with modifications by Andersen et al. (2001).

The following OCs were quantified: hexachlorobenzene (HCB), oxychlordane, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), and 14 PCB congeners with International Union for Pure and Applied Chemistry numbers (Ballschmiter and Zell 1980) 31, 52, 101, 99, 118, 114, 153, 105, 138, 156, 157, 180, 170, and 189, listed in order of their retention time. The 14 individual PCB congeners were significantly correlated with ΣPCB (Pearson *R*; $r^2 \geq 0.68$, $p < 0.000001$), although the correlation was weak for PCB-52 (Pearson *R*; $r^2 = 0.11$, $p = 0.006$). All OC concentrations were intercorrelated for both sexes.

Standard procedures were used to ensure quality assurance and control, and the results were within the laboratory's accredited requirements for precision, linearity, and sensitivity. Detection limits for individual compounds were determined as three times the noise level and were between 0.003 and 0.013 ng/g wet weight (ww). All calculations were done within the linear range of the detector's five-level calibration curve. The reproducibility was tested continuously by analyzing PCB levels in the laboratory's own reference sample (seal blubber). The results were within the mean coefficient of variance for the year 2000 (8.7%). The repeatability of the HRGC performance was tested by repeated injection of standard compounds at regular time intervals. Percent recoveries and coefficients of variance of OCs in spiked sheep blood varied from 83 to 119% and from 0.62 to 10.35%, respectively. Blank samples were included in each series to test for interference.

TH analyses. The TH quantification was performed at the Department of Biology at the University of Science and Technology, Trondheim, Norway.

Radioimmunoassays were used to determine the plasma concentration of total (T) and free (F) T_4 and T_3 with commercially available kits (Coat-A-Count; Diagnostic Products Corporation Inc., Los Angeles, CA, USA). The detection limits of the TT_3, TT_4, FT_3, and FT_4 kits were 0.11 nmol/L, 3.22 nmol/L, 0.31 pmol/L, and 0.13 pmol/L, respectively. Analytic results under the minimum detectable concentration were set to half the respective detection limit. The repeatability of the assays was tested by running samples in duplicates, and readings with a coefficient of variation > 15% were excluded from the final data set. The range limit of 15% was set according to the laboratory's quality assurance and control routine. Thus, from a total of 83 captured birds, 10 birds from Sørhamna and 7 birds

from Evjebukta were removed from further analyses, for a remaining total of 66 birds.

In each kit, two human serum controls (Immunoassay Plus Control level 2, Biorad Laboratories, Liquichek and Lyphochek, Bio-Rad Laboratories, Hercules, CA, USA) were also assayed to test for repeatability. Results were in the acceptance range of the kits and met the laboratory's established requirements for precision.

Data analyses. Statistical analyses were carried out using the statistical packages Statistica, version 6 (StatSoft 2002), and SAS, release 6.09 (SAS Institute, Cary, NC, USA). Statistical significance was set at $p \leq 0.05$. Data were analyzed for normality by Shapiro-Wilk's *W*-test and the Kolmogorov-Smirnov and Liliefors test (Zar 1999). Variables that did not approximate the normal distribution were $\log_{10}$ transformed.

Because body condition in avian species may influence OC levels (e.g., Henriksen et al. 1998), TH levels (e.g., McNabb 2000), and other biologic parameters such as reproductive success (e.g., Saether 1997), adjusting for body condition was necessary. A single measure of body size (body size index) was obtained using principal component analysis (Hair et al. 1998). The body size index of glaucous gulls, which was expressed by the scores on the first principal component (PC1), was extracted from two morphologic measurements: wing length and total head and bill length (Henriksen et al. 2000). The body condition of an individual was defined as the residuals obtained when body mass was regressed against the body size index, that is, the difference between the observed body mass and the mass predicted from the body size index (Green 2001). The body size index and body condition were calculated separately for each sex because the glaucous gull is sexually dimorphic (Gilchrist 2001; Løvenskiold 1964).

General linear models (GLMs) were used to analyze the effect of any combinations of categorical and continuous variables (StatSoft 2002). Following Rothman (1990), *p*-values were not adjusted despite the use of multiple comparisons. The Levene's test (StatSoft 2002) was computed to test for homogeneity of variance. Backward elimination (Zar 1999) was used to select the variables in the model that contributed significantly to the variation of OC and TH levels and TH ratios in the whole model, which included the following variables: sex, breeding colony, extractable plasma fat percentage, day of capture, and body condition. Day of capture refers to the day in the incubation period (1–30 days) when the birds were captured. Because timing in the reproductive cycle plays a major role in the natural oscillations of circulating hormones, correlations (coefficients) between TH and OC levels were adjusted for day of capture in the incubation period. Likewise, OC levels (ww basis) were corrected for plasma fat content because of their lipophilic properties, using extractable plasma fat (percent of whole blood; Hebert and Keenleyside 1995). Correlations are expressed using the Pearson correlation coefficient (*r*).

Results

Organochlorines. The ΣPCB concentration in this study ranged from 31.9 to 1,927 ng/g ww (Table 1) and accounted for 76% of the total OC fraction for both sexes. The most abundant PCBs were PCB-153, PCB-138, and PCB-180, making up 57% of the PCB fraction. Levels of OCs (all compounds) were on average 42% (range, 17–77%) higher in males than in females, but the difference was nonsignificant for HCB and oxychlordane (Table 2). Levels of HCB, *p*,*p*′-DDE, and ΣPCB were higher in birds from the breeding colony Evjebukta than in birds from Sørhamna (Table 2). The effect of breeding colony on the variation of these OCs was stronger than the effect of sex in the whole model including day of capture and extractable plasma fat percentage. Body condition did not influence the variation of OC levels.

Thyroid hormones. Levels of circulating FT_3 and TT_3 were 28% higher in males than in females. Levels of T_4 were lower in males than in females, 26% for FT_4 and 16% for TT_4, although not significant for TT_4 (Tables 2 and 3). Concurrently, males had 37 and 44% lower FT_4:FT_3 and TT_4:TT_3 ratios, respectively, than did females. The total to free T_4 and T_3 ratios did not differ significantly between sexes (Table 2).

From a population-wide perspective, the levels of FT_3 and TT_3 were higher in birds from the breeding colony at Evjebukta compared with birds from Sørhamna, whereas levels of FT_4 and TT_4 were lower in birds from Evjebukta, although not significant for FT_4 (Table 2). The FT_4:FT_3 and TT_4:TT_3 ratios were lower in birds from Evjebukta compared with birds from Sørhamna. The ratios of total to free T_4 and T_3 did not differ between the breeding colonies (Table 2). The effect of breeding colony on the variation of FT_3, TT_4, and TT_4:TT_3 was stronger than the effect of sex in the whole model including day of capture. Body condition and extractable plasma fat percentage did not influence the variation of TH levels and TH ratios.

Relationships. In females, no correlation was found between OC and TH concentrations and thyroid ratios. However, in males, negative correlations were found between the FT_4:FT_3 and TT_4:TT_3 ratios and most OCs quantified (Table 4, Figure 2). Negative relationships were also found between FT_4 and TT_4 and OCs, although these were statistically significant for HCB and oxychlordane only (Pearson *R*; $r \geq -0.37$, $p \leq 0.043$; Figure 3). Additionally, there was a trend for increasing FT_3 and TT_3 levels with increasing OC levels, although not significant. The most prominent negative correlations were found between the TT_4:TT_3 ratio and HCB, oxychlordane, and the three *mono-ortho* PCBs (congeners 118, 114, and 105; Pearson *R*; $r \geq -0.43$, $p \leq 0.017$; Table 4). Levels of HCB, which made up only 3.4% of the total OC fraction in males, accounted for 20.6% of the variation of TT_4:TT_3 ratio in the whole model ($p = 0.012$). The effect of breeding colony on the variation of TT_4:TT_3 in males was inhibited by the effect of HCB in the same model (GLM; $F_{1,22} = 0.00$, $p = 0.99$). Furthermore, negative relationships were found in males between TT_4:FT_4 and OCs, although this was statistically significant for oxychlordane only ($r = -0.40$, $p = 0.026$). No association was

Table 1. Concentrations of organochlorines (ng/g ww) and extractable plasma fat percentage for male and female glaucous gulls (*Larus hyperboreus*) breeding at Bear Island.

	Females (*n* = 34)			Males (*n* = 32)		
	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Extractable plasma fat (%)	0.48 ± 0.20	0.13–0.96	0.47	0.54 ± 0.18	0.22–0.89	0.54
HCB	18.0 ± 11.2	3.84–45.7	13.3	22.3 ± 12.0	6.31–52.8	22.9
Oxychlordane	12.1 ± 9.58	2.66–50.1	9.55	14.2 ± 7.51	4.66–36.0	12.3
p,p´-DDE	69.1 ± 47.4	10.0–221	63.4	122 ± 92.6	29.2–505	103
PCB-31	0.34 ± 0.22	0.10–1.13	0.27	0.36 ± 0.17	0.10–0.75	0.41
PCB-52	0.37 ± 0.25	0.06–0.97	0.32	0.36 ± 0.23	0.06–1.04	0.30
PCB-101	1.17 ± 1.39	0.13–8.20	0.77	1.69 ± 1.46	0.44–6.89	1.31
PCB-99	16.4 ± 14.1	2.30–63.3	11.9	22.6 ± 16.7	4.44–70.4	17.1
PCB-118	27.3 ± 24.4	3.74–121	19.1	41.5 ± 29.4	6.71–139	31.4
PCB-114	0.90 ± 0.91	0.13–4.91	0.60	1.29 ± 1.06	0.24–5.35	1.07
PCB-153	118 ± 112	9.87–499	73.3	179 ± 166	25.6–780	124
PCB-105	7.73 ± 6.22	1.24–29.5	5.79	11.1 ± 7.80	2.19–40.7	9.55
PCB-138	67.4 ± 64.8	7.19–276	43.6	101 ± 84.0	16.9–379	68.6
PCB-156	9.34 ± 9.49	0.94–47.2	5.92	13.7 ± 10.4	2.03–42.1	10.3
PCB-157	2.69 ± 2.53	0.42–11.9	1.73	3.45 ± 2.49	0.57–11.4	2.68
PCB-180	58.8 ± 61.6	4.46–291	31.5	89.0 ± 82.4	12.2–357	57.5
PCB-170	17.6 ± 18.5	1.31–84.9	9.73	25.4 ± 22.6	3.51–90.8	17.4
PCB-189	0.88 ± 0.91	0.07–4.44	0.51	1.38 ± 1.20	0.19–5.56	1.06
ΣPCB (14)	329 ± 314	31.9–1,443	208	492 ± 421	75.3–1,927	351

found between the TT_3:FT_3 ratio and any of the OCs quantified.

Discussion

In the present study, significant negative associations were found between blood levels of a selection of OCs and circulating THs and TH ratios in plasma of male glaucous gulls breeding at Bear Island in the Barents Sea. In this study we also documented that glaucous gulls breeding in a high-OC–exposed colony had lower plasma levels of T_4 and T_4:T_3 ratios compared with a less-exposed colony. Concurrently, levels of plasma T_3 were elevated in birds breeding in the high-OC–exposed colony. This indicates that the glaucous gull is susceptible to changes to TH homeostasis mediated by exposure to halogenated organic contaminants in the Barents Sea food chain.

The levels of circulating T_4 reported in this study on glaucous gulls were in the lower end of the corresponding levels reported for other adult precocial species, whereas the levels of T_3 were within the range previously reported for these species (Astier 1980; McNabb 2000). Furthermore, blood levels of the OCs quantified in this study were comparable with previously reported blood levels in glaucous gulls breeding at Bear Island, and the levels were higher in males compared with females, which also is in accordance with the same studies (Bustnes et al. 2000, 2001a, 2001b, 2002, 2003a). The lower OC levels measured in incubating females may be explained partly by excretion of OCs deposited in the egg yolk during egg formation (Bargar et al. 2001; Ingebrigtsen et al. 1984; Norstrom et al. 1986).

Interbreeding colony comparison. In line with findings by Bustnes et al. (2000), glaucous gulls breeding in the colony Evjebukta had higher blood levels of OCs than those from Sørhamna for both males and females. In an attempt to compare TH levels between individuals with various degrees of contamination, it was necessary to perform an interbreeding colony comparison to control for potential confounding factors that may influence TH levels, that is, sex, age, activity, body condition, and diet (I^- availability; e.g., McNabb 1992, 2000). Because sex, day of capture in the incubation, and body condition were controlled for in the statistical models, the age of the birds was assumed to be similarly distributed between the two colonies. However, activity and diet, associated with the glaucous gull's specialization in terms of feeding strategy, is known to differ between these two breeding colonies (Bustnes et al. 2000). Food items included in the diet of glaucous gulls, which are selected at different trophic levels in the food chain, differ in their contaminant burden (Borgå et al. 2001; Sagerup et al. 2002) and a complex array of constituents such as iodine, an essential component of THs (e.g., McNabb 1992). The T_4:T_3 ratio is known to be altered by changing iodine availability where high iodine favors T_4 formation (increasing T_4:T_3 ratio), and low iodine favors T_3 formation (decreasing T_4:T_3 ratio; McNabb 1992). However, studies on fish-eating birds such as the herring gull (*Larus argentatus*) from the Great Lakes in North America have provided strong evidence that hypothyroidism in this species was due not to iodine deficiency but to exposure to halogenated organic contaminants, although the disruptive mechanisms suggested were speculative (Fox 1993; McNabb and Fox 2003; Moccia et al. 1986). Based on these results, and because marine environments (e.g., the Barents Sea) are documented to be rich in iodine compared with freshwater environments (e.g., the Great Lakes; Fox 1993; Peakall 1992), iodine deficiency is not likely to occur in glaucous gulls breeding at Bear Island. Therefore, even though it cannot be completely disregarded, the difference in diet between individuals in the two breeding colonies is not likely to affect levels of circulating or stored THs.

Relationships between OCs and THs. In females, no correlation was found between OC and TH concentrations and thyroid ratios. In males, however, several negative correlations were found showing decreasing plasma T_4 levels and T_4:T_3 ratios with increasing blood levels of most OCs quantified. In males, HCB and oxychlordane seemed to have a particularly negative effect on the T_4:T_3 ratio and on the circulating levels of T_4. HCB and oxychlordane accounted for a minimal proportion (< 3.4%) of the total OC fraction. In an experimental study, Pisarev et al. (1990)

Table 2. Results from analyses of covariance (GLM, type III) testing the difference in blood levels of organochlorines (log_{10} ng/g ww), plasma levels of thyroid hormones (mol/L) and thyroid ratios (log_{10} mole ratio) between two breeding colonies of glaucous gulls (*Larus hyperboreus*) at Bear Island.

	Breeding colony			Sex			Whole model	
	df	*F*-Value	*p*-Value	df	*F*-Value	*p*-Value	r^2	*p*-Value
HCB	1, 61	23.66	< 0.00001	1, 61	1.75	0.190	0.43	< 0.000001[a]
p,p´-DDE	1, 61	11.37	0.001	1, 61	10.54	0.002	0.37	< 0.00001[a]
Oxychlordane	1, 61	2.04	0.158	1, 61	2.01	0.161	0.13	0.068[a]
ΣPCB (14)	1, 61	6.00	0.017	1, 61	5.24	0.026	0.20	0.009[a]
Free T_3	1, 62	8.47	0.005	1, 62	6.41	0.014	0.21	0.002[b]
Total T_3	1, 62	7.65	0.007	1, 62	9.57	0.003	0.25	< 0.001[b]
Free T_4	1, 62	1.15	0.287	1, 62	11.26	0.001	0.18	0.006[b]
Total T_4	1, 62	4.52	0.037	1, 62	2.81	0.098	0.19	0.004[b]
Total T_4:free T_4	1, 62	0.45	0.502	1, 62	2.71	0.105	0.11	0.071[b]
Total T_3:free T_3	1, 62	2.76	0.101	1, 62	0.01	0.903	0.05	0.333[b]
Total T_4:T_3	1, 62	3.98	0.050	1, 62	3.18	0.079	0.19	0.004[b]
Free T_4:T_3	1, 62	8.69	0.005	1, 62	18.35	< 0.001	0.33	< 0.001[b]

Abbreviations: df; degree of freedom; r^2; fit of the model.
[a]The whole model included breeding colony, sex, day of capture, and extractable plasma fat (%). [b]The whole model included breeding colony, sex, and day of capture.

Table 3. Concentrations of free (pmol/L) and total (nmol/L) T_3 and T_4 and TT_4:FT_4, TT_3:FT_3, TT_4:TT_3, and FT_4:FT_3 ratios (mole ratio) for male and female glaucous gulls (*Larus hyperboreus*) breeding at Bear Island.

	Females (*n* = 34)			Males (*n* = 32)		
	Mean ± SD	Range	Median	Mean ± SD	Range	Median
Free T_3	3.38 ± 1.32	1.25–5.9	3.15	4.32 ± 1.75	1.50– 9.3	4.00
Total T_3	3.02 ± 1.02	1.38–5.25	2.69	3.86 ± 1.20	1.80–7.63	3.92
Free T_4	36.7 ± 12.7	11.4–65.1	36.7	27.1 ± 9.63	14.4–51.4	26.7
Total T_4	34.3 ± 13.4	1.61–56.5	37.9	28.9 ± 11.6	1.61–62.6	28.1
Total T_4:free T_4	0.92 ± 0.27	0.11–1.32	0.97	1.07 ± 0.29	0.11–1.55	1.02
Total T_3:free T_3	0.93 ± 0.17	0.68–1.43	0.89	0.94 ± 0.16	0.67–1.34	0.93
Total T_4:T_3	13.1 ± 7.85	0.54–41.0	13.6	8.25 ± 4.70	0.54–26.7	8.04
Free T_4:T_3	12.9 ± 7.68	2.79–40.7	11.8	7.18 ± 3.96	1.94–21.4	5.72

Table 4. Pearson correlation coefficients, adjusted for extractable plasma fat (%) and day of capture, and significance levels for relationships between organochlorine levels (log_{10} ng/g ww) and T_4:T_3 ratio (log_{10} mole ratio) measured in male glaucous gulls (*n* = 32) breeding at Bear Island (GLM, type III).

	Total T_4:T_3		Free T_4:T_3	
	R	*p*-Value	*R*	*p*-Value
HCB	–0.45	0.012	–0.39	0.035
Oxychlordane	–0.44	0.014	–0.26	0.168
p,p´-DDE	–0.38	0.041	–0.35	0.056
PCB-31	–0.37	0.042	–0.32	0.085
PCB-52	–0.16	0.406	–0.35	0.059
PCB-101	–0.26	0.159	–0.30	0.108
PCB-99	–0.41	0.026	–0.27	0.141
PCB-118	–0.43	0.017	–0.34	0.069
PCB-114	–0.44	0.016	–0.36	0.053
PCB-153	–0.40	0.030	–0.26	0.166
PCB-105	–0.45	0.013	–0.38	0.039
PCB-138	–0.39	0.032	–0.26	0.158
PCB-156	–0.41	0.025	–0.31	0.089
PCB-157	–0.42	0.022	–0.33	0.077
PCB-180	–0.35	0.055	–0.26	0.172
PCB-170	–0.38	0.038	–0.27	0.154
PCB-189	–0.38	0.039	–0.30	0.112
ΣPCB (14)	–0.40	0.031	–0.28	0.141

R; slope.

observed a 25% reduction of serum levels of T_4 in rats exposed to a relatively high dose of HCB, whereas the levels of T_3 were not significantly affected. Moreover, in glaucous gulls breeding at Bear Island, it has been documented that HCB had the strongest correlative effect on feather asymmetry and reproductive parameters, whereas oxychlordane was negatively correlated with survival probability (Bustnes et al. 2002, 2003b); these studies have argued that HCB and oxychlordane were the OCs that possibly produced most stress in glaucous gulls. However, because the blood levels of many lipophilic halogenated organic contaminants are intercorrelated, the contributing or causative agent(s) to TH depletion in this species might not necessarily have been detected here.

Because dose–response toxicologic experiments were not performed between male and female glaucous gulls, the associations observed here between THs and OCs in males only cannot be explained from a sex-specific perspective, although they may lead to speculation toward that eventuality.

Potential mechanisms of disruption. Because the negative associations reported in this study do not allow the demonstration of causality of TH depletion in glaucous gulls, extrapolations of laboratory results from other vertebrate species are necessary to suggest potential mechanisms of disruption, although these mechanisms remain speculative.

Based on experimental studies, our findings could support the mechanism involving the interference of contaminants with TH plasma carrier proteins (e.g., Brouwer et al. 1986, 1998). Because of structural resemblance to THs, certain halogenated organic contaminants, and especially some OH-PCBs, may disturb circulating TH levels by competing for binding sites on their transport proteins. Instances of binding affinity of PCBs, OH-PCBs, PCDDs, and polychlorinated dibenzofurans to the T_4 transport protein TTR have been reported in a number of studies (Brouwer 1989; Brouwer and van den Berg 1986; Brouwer et al. 1986; Lans et al. 1994; Letcher et al. 2000; van den Berg et al. 1991). The displacement of T_4 from TTR is presumed to facilitate the excretion of the free T_4 fraction in urine or bile, thereby decreasing circulating total T_4 levels (Brouwer et al. 1986; Lans et al. 1994). Recent analyses have revealed the presence of several major OH-PCBs and methyl sulfone metabolites of PCBs in plasma of glaucous gulls breeding at Bear Island (Verreault J, unpublished data).

The laboratory studies cited here have been generally performed in rodents, in which TTR is the principal T_4 carrier protein in the plasma (McNabb 1992; Refetoff et al. 1970). The situation in birds may be different. Most of the T_4 in birds is associated with the plasma-carrier protein albumin, which has low-affinity binding sites with little specificity for T_4 or T_3 (McNabb 2000; Merryman and Buckles 1998a). As yet, exceedingly few studies have been performed on competition between halogenated organic contaminants, especially compounds such as HCB and oxychlordane, and THs for binding sites on TTR and albumin in avian species. Other possible mechanisms for contaminant-induced modulation of thyroid functions and T_4 turnover have been reported in diverse reviews (e.g., Leatherland 2000; McNabb and Fox 2003; Peakall 1992).

Conclusions

In this study, we report an association between high blood levels of halogenated organic contaminants and alteration of circulating TH levels in glaucous gulls breeding at Bear Island. Because THs play an important role in initiating/regulating development, reproduction, and behavior, and because certain contaminants may decrease directly or indirectly the levels of THs through diverse mechanisms, high levels of contaminants may contribute to distortion of these physiologic functions in this species. Moreover, we suggest that alteration of TH levels in glaucous gulls, beyond the limits of homeostatic compensation, may lead to decreased basal metabolic rate, which in turn may alter lipid metabolism and sensitivity to cold temperature. The death of breeding glaucous gulls observed early in the chick period at Bear Island (Bakken V and Strøm H, personal communication) could be attributed to these adverse effects and to stressors associated to high-energy investment in parent birds during the incubation period.

The associations reported in this study showed that contaminant-mediated thyrotoxicity may affect male glaucous gulls more particularly, indicating a possible sex-specific response of thyroid functions to the action of halogenated organic contaminants. There is as yet no clear evidence that irreversible physiologic effects, normally linked to hypothyroidism during avian development, manifest in the glaucous gull, and further studies are required. Presumable exposure to current blood levels of OCs or other type of contaminants may possibly be insufficient to overwhelm the mechanisms of TH homeostasis in this species.

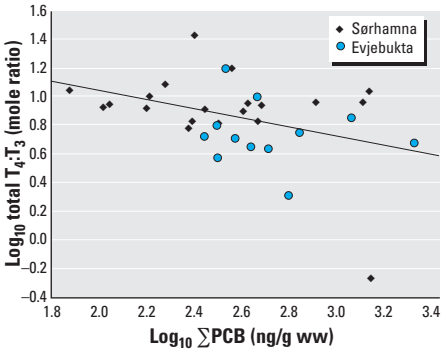

Figure 2. Relationship between the total T_4:T_3 ratio (log_{10} mole ratio) and blood levels of ΣPCB (log_{10} ng/g ww), corrected for extractable plasma fat (%) and day of capture, for male glaucous gulls (n = 32) breeding in two colonies (Evjebukta and Sørhamna) at Bear Island. r = –0.40; p = 0.031.

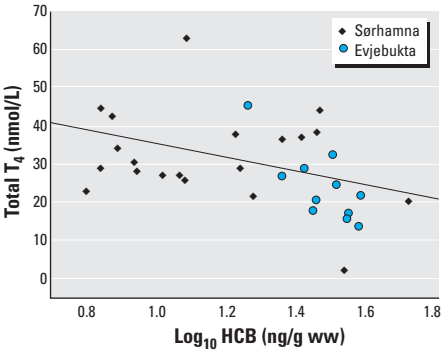

Figure 3. Relationship between plasma levels of total T_4 (nmol/L) and blood levels of HCB (log_{10} ng/g ww), corrected for extractable plasma fat (%) and day of capture, for male glaucous gulls (n = 32) breeding in two colonies (Evjebukta and Sørhamna) at Bear Island. r = –0.37; p = 0.043.

References

Andersen M, Lie E, Derocher AE, Belikov SE, Bernhoft A, Boltunov AN, et al. 2001. Geographic variations of PCB congeners in polar bears (*Ursus maritimus*) from Svalbard east to the Chukchi Sea. Polar Biol 24:231–238.

Astier H. 1980. Thyroid gland in birds: structure and function. In: Avian Endocrinology (Epple A, Stetson M, eds). New York:Academic Press, 167–189.

Ballschmiter K, Zell M. 1980. Analysis of polychlorinated biphenyls (PCBs) by glass capillary gas chromatography. Fresenius Z Anal Chem 302:20–31.

Bargar TA, Scott GI, Cobb GP. 2001. Maternal transfer of contaminants: case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white leghorn chickens (*Gallus domesticus*). Environ Toxicol Chem 20:61–67.

Barron MG, Galbraith H, Beltman D. 1995. Comparative reproductive and developmental toxicology of PCBs in birds. Comp Biochem Physiol 112:1–14.

Bernhoft A, Skaare JU, Wiig Ø. 1997. Organochlorines in polar bears (*Ursus maritimus*) at Svalbard. Environ Pollut 95:159–175.

Borgå K, Gabrielsen GW, Skaare JU. 2001. Biomagnification of organochlorines along a Barents Sea food chain. Environ Pollut 113:187–198.

Bourne WRP, Bogan JA. 1972. Polychlorinated biphenyls in North Atlantic seabirds. Mar Pollut Bull 3:171–175.

Brevik EM. 1978. Gas chromatographic method for the determination of organochlorine pesticides in human milk. Bull Environ Contam Toxicol 19:281–286.

Brouwer A. 1989. Inhibition of thyroid hormone transport in plasma of rats by polychlorinated biphenyls. Arch Toxicol 13(suppl):440–445.

Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, et al. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequence for animal and human health. Toxicol Ind Health 14:59–83.

Brouwer A, van den Berg KJ. 1986. Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol Appl Pharmacol 85:301–312.

Brouwer A, van den Berg KJ, Blaner WS, Goodman DS. 1986.

Transthyretin (prealbumine) binding of PCBs, a model for the mechanism of interference with vitamine A and thyroid hormone metabolism. Chemosphere 15:1699–1706.

Burger J, Kannan K, Giesy JP, Gochfeld M. 2002. Effects of environmental pollutants on avian behaviour. In: Behavioural Ecotoxicology (Dell'Omo G, ed). Chichester, UK:John Wiley and Sons, 337–376.

Bustnes JO, Bakken V, Skaare JU, Erikstad KE. 2003a. Age and accumulation of persistent organochlorines: a study of Arctic-breeding glaucous gulls (*Larus hyperboreus*). Environ Toxicol Chem 22:2173–2179.

Bustnes JO, Erikstad KE, Bakken V, Mehlum F, Skaare JU. 2000. Feeding ecology and the concentration of organochlorines in glaucous gulls. Ecotoxicology 9:175–182.

———. 2001a. Patterns of incubation and nest-site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. J Appl Ecol 38:791–801.

———. 2001b. Whole blood concentrations of organochlorines as a dose metric for studies of the glaucous gulls (*Larus hyperboreus*). Environ Toxicol Chem 2:1046–1052.

———. 2003b. Ecological effects of organochlorine pollutants in the Arctic: a study of the glaucous gull. Ecol Appl 13:504–515.

Bustnes JO, Folstad I, Erikstad KE, Fjeld M, Miland ØO, Skaare JU. 2002. Blood concentration of organochlorine pollutants and wing feather asymmetry in glaucous gulls. Funct Ecol 16:617–622.

Colborn T, Clement C, eds. 1992. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton, NJ:Princeton Scientific Publishing.

Coulson JC, Thomas CS, Butterfield JEL, Duncan N, Monaghan P, Shedden C. 1983. The use of head and bill length to sex live gulls *Laridae*. Ibis 125:549–557.

Damstra T, Barlow S, Bergman Å, Kavlock R, van der Kraak G, eds. 2002. Global assessment of the state-of-the-science of endocrine disruptors. Geneva:World Health Organization, International Programme on Chemical Safety.

Davidson TF, Flack IH, Butler EJ. 1978. The binding of thyroxine and tri-iodothyronine to plasma proteins in the chicken at the physiological pH. Res Vet Sci 25:280–283.

Dawson A. 2000. Mechanisms of endocrine disruption with particular reference to occurrence in avian wildlife species: a review. Ecotoxicology 9:59–69.

Fox GA. 1993. What have biomarkers told us about the effects of contaminants on the health of fish-eating birds in the Great Lakes? The theory and a literature review. J Great Lakes Res 19:722–736.

Gabrielsen GW, Skaare JU, Polder A, Bakken V. 1995. Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. Sci Total Environ 160/161:337–346.

Gilchrist HG. 2001. Glaucous gull, *Larus hyperboreus*. In: The Birds of North America (Poole A, Gill F, eds). Philadelphia:Birds of North America, 1–31.

Green AJ. 2001. Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82:1473–1483.

Hair JF, Anderson RE, Tatham RL, Black WC. 1998. Multivariate Data Analysis. 5th ed. Upper Saddle River, NJ:Prentice Hall, 87–138.

Hebert CE, Keenleyside KA. 1995. To normalize or not to normalize? Fat is the question. Environ Toxicol Chem 14:801–807.

Henriksen EO, Gabrielsen GW, Skaare JU. 1998. Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gulls. Chemosphere 37:2627–2643.

Henriksen EO, Gabrielsen GW, Trudeau S, Wolkers J, Sagerup K, Skaare JU. 2000. Organochlorines and possible biochemical effects in glaucous gulls (*Larus hyperboreus*) from Bjørnøya, the Barents Sea. Arch Environ Contam Toxicol 38:234–243.

Ingebrigtsen K, Skaare JU, Teigen SW. 1984. Organochlorine residues in two Norwegian puffin (*Fratercula arctica*) colonies. J Toxicol Environ Health 14:813–828.

Lans MC, Spiertz C, Brouwer A, Koeman JH. 1994. Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. Eur J Pharmacol, Environ Toxicol Pharmacol 270:129–136.

Leatherland JF. 2000. Contaminant-altered thyroid function in wildlife. In: Environmental Endocrine Disrupters (Guillette L, Crain DA, eds). New York:Taylor & Francis, 155–181.

Letcher RJ, Klasson-Wehler E, Bergman Å. 2000. Methyl sulfone and hydroxylated metabolites of polychlorinated biphenyls. In: Handbook of Environmental Chemistry—New Types of Persistent Halogenated Compounds (Paasivita J, ed). Heidelberg:Springer-Verlag, 315–360.

Løvenskiold HL. 1964. Avifauna svalbardensis [in Norwegian]. Norsk Polarinstitutt Skrifter 129:1–460.

McNabb A. 1992. Thyroid hormones: production, storage, and release by the thyroid gland. In: Thyroid Hormones (McNabb A, ed). Englewood Cliffs, NJ:Prentice Hall, 21–48.

———. 2000. Thyroids. In: Sturkie's Avian Physiology (Whittow GC, ed). 5th ed. London:Academic Press, 461–471.

McNabb A, Fox GA. 2003. Avian thyroid development in chemically contaminated environments: is there evidence of alterations in thyroid functions and development? Evol Dev 5:76–82.

Mehlum F, Bakken V. 2000. Seabirds in Svalbard (Norway): status, recent change and management. In: The Status of Marine Birds Breeding in the Barents Sea Region (Nilssen T, Bakken V, Strøm H, Golovkin A, Bianki V, Tatarinkova I, eds). Rapport 113. Tromsø:Norsk Polarinstitutt, 94–96.

Merryman JI, Buckles EL. 1998a. The avian thyroid gland. Part one: a review of the anatomy and physiology. J Avian Med Surg 12:234–237.

———.1998b. The avian thyroid gland. Part two: a review of function and pathophysiology. J Avian Med Surg 12:238–242.

Moccia RD, Fox GA, Britton A. 1986. A quantitative assessment of thyroid histopathology of herring gulls (*Larus argentatus*) from the Great Lakes and a hypothesis on the causal role of environment contaminants. J Wildlife Dis 22:60–70.

Norstrom RJ, Clark TP, Jeffrey DA, Won HT, Gilman AP. 1986. Dynamics of organochlorine compounds in herring gulls (*Larus argentatus*). I. Distribution and clearance of [^{14}C]DDE in free-living herring gulls (*Larus argentatus*). Environ Toxicol Chem 5:41–48.

NRC (National Research Council). 1999. Effects on reproduction and development. In: Hormonally Active Agents in the Environment. Washington, DC:National Academy Press, 119–170.

Peakall D. 1992. Thyroid function, retinols, haem and regulatory enzymes. In: Animal Biomarker as Pollution Indicators (Depledge MH, Sanders B, eds). London:Chapman & Hall, 108–117.

Pisarev DLKD, Molina MDCRD, Viale LCSMD. 1990. Thyroid function and thyroid metabolism in hexachlorobenzene-induced porphyria. Biochem Pharmacol 39:817–825.

Refetoff S, Robin NI, Fang VS. 1970. Parameters of thyroid functions in serum of 16 selected vertebrate species: a study of PBI, serum T_4, free T_4, and the pattern of T_4 and T_3 binding to serum proteins. Endocrinology 86:793–805.

Rolland RM. 2000. A review of chemically-induced alterations in thyroid and vitamine A status from field studies of wildlife and fish. J Wildlife Dis 36:615–635.

Rothman KJ. 1990. No adjustments are needed for multiple comparisons. Epidemiology 1:43–46.

Sæther BE, Lorentsen SH, Tveraa T, Andersen R, Pedersen HC. 1997. Size-dependant variation in reproductive success of a long-lived seabird, the Antarctic petrel (*Thalassoica antarctica*). Auk 114:333–340.

Sagerup K, Henriksen EO, Skaare JU, Gabrielsen GW. 2002. Intraspecific variation in trophic feeding levels and organochlorine concentrations in glaucous gulls (*Larus hyperboreus*) from Bjørnøya, the Barents Sea. Ecotoxicology 11:119–125.

Savinova TN, Polder A, Gabrielsen GW, Skaare JU. 1995. Chlorinated hydrocarbons in seabirds from the Barents Sea area. Sci Total Environ 160/161:497–504.

StatSoft. 2002. Statistica Electronic Manual. Version 6. Tulsa, OK:StatSoft, Inc.

Tanabe S. 2002. Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. Mar Pollut Bull 45:69–77.

van den Berg KJ, van Raaij JAGM, Bragt PC, Notten WRF. 1991. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels *in vivo*. Arch Toxicol 65:15–19.

Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, et al. 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol 30:71–133.

Zar JH. 1999. Biostatistical Analysis, 4th ed. Englewood Cliffs, NJ:Prentice Hall, 65–90.