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Master's Thesis in Environmental Toxicology
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”- og måker - jeg har sagt det før, og jeg sier det igjen:

Jeg hater måker! De skriker! Og oppfører seg som dyr, og jeg tenker:
Sånn er livet også ganske ofte -
som en våt morgen med dyr som skriker og drypper,
og det er skorpe på leverposteien,
og skorpe både på det ene og det andre.
Sånn er livet. – Og sånn er det å være i båt også -
særlig om morgenen - noen ganger.

Men noen andre ganger - eller for å si det på en annen måte -
noen ganger - i båt om morgenen - og i livet også - forsåvidt –

Noen ganger er det all right”

Odd Børretzen

Front page: Glaucous gull (*Larus hyperboreus*). Photo: Anja J. Haugerud.

ACKNOWLEDGEMENTS

"How did you catch the gulls?" has been one of the most frequent questions I have been asked during these last two years as a master student in biology at the Norwegian University of Science and Technology (NTNU). With an automatically triggered nest trap, has been my answer. In many ways, being trapped has also been one of the most frequent feelings I have had in these two years. My mind has been fixed upon my twenty eight glaucous gulls from Kongsfjorden. I have cherished and honored them for better and for worse these two years – and now it is time to let them fly off into the future. I do not know what will come – but I do know it has been a wonderful time getting to know both the gulls and myself better.

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My wonderful family deserves my thanks for encouraging me in all I do and keeping my spirits up. Thanks to my roommates for all the gourmet – and not so gourmet dinners, accompanied by Jane Austen's handsome Mr. Darcy and Mrs. Bennett's words of wisdom. Thanks to my fellow students and good friends at NTNU, especially for a lovely Byssus time! Thank you for supporting me, Olav – you are my very own Mr. Darcy.

Trondheim, 5th December 2011

Anja Johansen Haugerud

ABSTRACT

The glaucous gull (*Larus hyperboreus*) is one of the largest flying top predators in the Arctic and is exposed to rather high levels of different persistent organohalogenated contaminants (OHCs). The present study investigated whether the serum thyroid hormone (TH) levels in female glaucous gulls breeding in Kongsfjorden (79°N) may be affected by the various OHCs. A slight decrease in traditional OHCs, like polychlorinated biphenyls (PCBs) and some pesticides have been reported over the last decade, while increasing levels of new and emerging compounds like polybrominated diphenyl ethers (PBDEs) and perfluoroalkyl substances (PFASs) are detected in Arctic marine and terrestrial wildlife. Although further production of some possible endocrine disruptive chemicals have been banned by many nations, the chemicals persist, move throughout the biosphere and are potentially harmful to exposed wildlife and biota.

After 35 years of contaminant research, the Svalbard glaucous gull function as a bioindicator species. Previous assessments have concluded that OHC levels were high enough to be of concern for a number of physiological effects, among them endocrine alterations. However, this is to my knowledge the first biomarker study on levels of THs in breeding glaucous gulls from Kongsfjorden. Plasma from 19 nesting female glaucous gulls was analysed for PCBs, organochlorinated pesticides (OCPs) (such as dichlorodiphenyltrichloroethane [DDT] and chlordanes) and their metabolites, PBDEs and PFASs. Total and free ranging THs were quantified using radioimmuno assay (RIA). Multivariate statistics were used to investigate the relationships between the TH levels and the explanatory variables.

The PCBs represented the most pervasive compound group in the present study, as 61.3 % of the total contaminant burden in female glaucous gulls. The other contaminant groups contributed as follows: ΣDDTs (17.5 %) > ΣPFASs (14.8 %) > HCB (2.9 %) > ΣCHLs (2.7 %) > ΣPBDE (0.8 %) > ΣHCH (0.1 %). Several of the PFASs correlated significantly with the THs. Significant correlations were found between several of the long-chain Perfluoroalkyl carboxylates (PFCAs) and THs. Surprisingly, even though the PFCAs represented only 2.8 % of the total contaminant load in the present study, the PFCAs represented the most important variables explaining the variation in THs.

SAMMENDRAG

Polarmåker (*Larus hyperboreus*) er en av de største flyvende toppredatorene i arktiske strøk og har vist seg å ha høye nivåer av ulike klasser persistente organiske miljøgifter (POPs). Målet med dette studiet var å undersøke om nivåer av thyroidhormoner (TH) i hekkende polarmåkehunner fra Kongsfjorden (79^0N) på noen måte var påvirket av de ulike POPene. Noen av de mer tradisjonelle miljøgiftene, som utfasede PCBer og noen pesticider, har vist seg å minke i konsentrasjon det siste tiåret. Derimot måles det nå høyere nivåer av nyere miljøgifter som bromerte flammehemmere og perfluoroalkylerte komponenter (PFASs), både i marine og terrestriske arktiske økosystemer. På tross av utfasing av et utvalg kjemikalier, viser de seg å være resistente mot nedbryting, de forflyttes i biosfæren og er potensielt giftig for biologiske systemer.

Siden 1972 har polarmåker vært gjenstand for et stort antall studier, hovedsakelig på Bjørnøya. Arten har derfor status som bioindikator etter 35 års forskning. Likevel er dette studiet etter min kjennskap det første fra Kongsfjorden som undersøker nivåer av THer hvor effekter kan kobles til nivåer av POPs. Tidligere studier fra Kongsfjorden har vist høye nivåer av POPs i biota. Plasmaprøver fra 19 rugende polarmåkehunner ble analysert for polyklorerte bifenyler (PCBs), organoklorerte pesticider (diklordifenyltrikloretan [DDT], chlordaner) og deres metabolitter, bromerte flammehemmere (BFRs) og perfluorerte stoffer (PFASs). Total og fri fraksjon av thyroidhormoner (THs) ble analysert ved bruk av radioimmunoassay (RIA).

Det ble i dette studiet detektert 22 PCB kongenere, noe som utgjorde hele 61.3 % av miljøgiftfraksjonen. De andre miljøgiftgruppene utgjorde henholdsvis: ΣDDTs (17.5 %) > ΣPFASs (14.8 %) > HCB (2.9 %) > ΣCHLs (2.7 %) > ΣPBDE (0.8 %) > ΣHCH (0.1 %). Positive, signifikante korrelasjoner ble funnet mellom THs og flere av PFASs. Det var de langkjedede Perfluoroalkyl carboxylatene (PFCAs) som forklarte mest av den observerte TT3 variasjonen, mens TT4:TT3 ratioen var negativt korrelert med de samme PFCAene. Overraskende var det denne minste fraksjonen fluorerte forbindelser, som utgjorde kun 2.8 % av den totale miljøgiftmengden, som viste seg å være mest potent i forhold til observerte effekter i denne studien.

ABBREVIATIONS

AMAP	Arctic Monitoring and Assessment Program
BCI	Body condition index
BFR	Brominated flame retardant
CHL	Chlordane
cpm	Counts per minute
CV	Coefficient of variation
CV-ANOVA	Cross validated analysis of variance
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyltrichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EDC	Endocrine disrupting chemicals
EI	Electron impact
FT3	Free triiodothyronine
FT4	Free thyroxine
FTS	Fluorotelomer sulfonate
GC	Gas chromatograph
HCB	Hexachlorobenzene
ID	Identification number
IDs	Iodothyronine deiodinases
LC	Liquid chromatograph
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MRM	Multiple reaction monitoring
MS	Mass spectrometer
N	Number of observations
NILU	Norwegian Institute for Air Research
NPI	Norwegian Polar Institute
NTNU	Norwegian University of Science and Technology
OC	Organochlorine
OCN	Octachloronaphtalene
OHC	Organohalogenated contaminant
OCP	Organochlorinated pesticides
OPLS	Orthogonal projection to latent structures
p	Probability of rejecting the hypothesis
PBDE	Polybrominated diphenyl ethers
PC	Principal Component
PCA	Principal Component analysis
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction

PFAS	Perfluoroalkyl and polyfluoroalkyl substances
PFBA	Perfluorobutanoate
PFBS	Perfluorobutane sulfonate
PFCA	Perfluoroalkyl carboxylates
PFDcA	Perfluorodecanoate
PFDcS	Perfluorodecane sulfonate
PFDoA	Perfluorododecanoate
PFHpS	Perfluoroheptane sulfonate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoate
PFOA	Perfluorooctanoate
PFOS	Perfluorooctane sulfonate
PFPA	Perfluoropentanoate
PFSA	Perfluoroalkyl sulfonates
PFTeA	Perfluorotetradecanoate
PFTriA	Perfluorotridecanoate
PLS	Projection to latent structures
POP	Persistent organic pollutant
Q^2	Goodness of prediction coefficient
Qstd	Quantification standard
r	Pearson correlation coefficient
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RIA	Radioimmuno assay
R^2X	Explained variance
R^2Y	Goodness of fit, correlation coefficient
RPM	rounds per minute
SD	Standard deviation
SIM	Selective ion monitoring
SPE	Solid phase extraction
Spl	Sample
SRM	Certified reference material
SULTs	Sulfotransferases
TH	Thyroid hormone
T3	triiodothyronine
T4	thyroxine
TR	Thyroid receptor
TSH	Thyroid stimulating hormone
TTR	Transthyretin
UGTs	UDP-glucuronocyltransferases
UHP	Ultra high pressure (gas chromatograph)
UV	Unit variance
VIP	Variable importance plot
ww	Wet weight

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1 INTRODUCTION

Despite the fact that direct use of organohalogenated contaminants (OHCs) in the Arctic has been diminutive, persistent organic pollutants (POPs) biomagnify in the Arctic marine food web. Due to the intrusive long-range transportation of chlorinated and brominated POPs and perfluoroalkyl substances (PFASs) through air and ocean currents, these pollutants are deposited in the pristine Arctic. As a result of their physicochemical properties, POPs and PFASs are resistant to biodegradation and will therefore accumulate in wildlife (de Wit et al., 2004). Thus, high levels of POPs have been detected in the glaucous gull, an Arctic top predator and scavenger, as well as in most other compartments of the Arctic environment (Verreault et al., 2010). Fluctuating external conditions expose Arctic wildlife to varying levels of natural stress (e.g. temperature, precipitation, food availability, sea-ice conditions) and – on top of this – the combined effects from POPs or other anthropogenic stressors (Bustnes et al., 2008; Moe et al., 2009).

A decreasing trend of traditional POPs the last decade results from the restrictions in production and use of industrial chemicals and pesticides from the 1970s up to now (Braune et al., 2005; Bustnes et al., 2010). The Stockholm Convention banned the "the dirty dozen" in 2001 and the convention was put into effect in 2004. This provided awareness on production and use of certain POPs (Stockholm Convention, 2011a), and proceeded with the European Community regulation on chemicals and their safe use, called Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), which was established in 2007 (REACH, 2011). However, the increasing levels of emerging POPs, such as brominated flame retardants (BFRs) (de Wit et al., 2010) and PFAS, detected in Arctic biota (Dietz et al., 2008), compared to the "classic" organochlorinated anthropogenic pollutants are of concern. New and emerging chemicals tend to have different physicochemical properties, such as the combined hydrophobic and oleophobic nature of PFASs (Houde et al., 2006). Laboratory exposure studies assessing effects from PFASs report toxic endpoints, such as potent peroxisome proliferators or tumor promoters (Lau et al., 2007; Martin et al., 2007). These chemicals have no known route of abiotic or biotic degradation in the environment either (Martin et al., 2003). These concerning findings garnered the attention of national and

international environmental protection agencies (Martin et al., 2004) and demonstrated the need for regulations on production and use (Jensen and Leffers, 2008). Climate change might elicit the bioavailability and toxicity of the OHCs (Schiedek et al., 2007), in addition to an increasing amount of stress caused in wildlife when adapting to environmental alterations (Jenssen, 2006).

OHCs in the Arctic include industrial chemicals like polychlorinated biphenyls (PCBs), bi-products such as hexachlorobenzen (HCB), organochlorine pesticides (chlordanes, dichlorodiphenyltrichloroethane [DDT]) in addition to some more novel compounds, such as brominated flame retardants (BFRs) (e.g. polybrominated diphenyl ethers [PBDEs]) and perfluoro and polyfluoroalkyl substances (PFASs) (de Wit et al., 2004).

BFRs are widely used as fireproofing compounds in electrical equipment, furniture and building materials, like polyurethane foam. They are a proof of that not all contaminants are decreasing in the environment (de Wit et al., 2004). Due to their structural and chemical similarities to PCBs, the apprehensive expectations on negative health effects are equal. Despite the ban of production and use of e.g. *penta*- and *octa*-BDEs, increasing levels are detected in the environment, though low compared to PCBs (de Wit et al., 2004). A similar trend is expected in the time to come due to the rapid development of PBDE replacements (de Wit et al., 2010).

PFASs have been in use for more than 50 years in industrial and commercial products like lubricants, soil and stain repellents, paper coating, drugs, pesticides and fire-fighting foams (Houde et al., 2006; Buck et al., 2011). Their strong carbon-fluorine bond and their oil and water repellence due to their amphipatic characters are of great importance considering the chemical and thermal stability of these compounds in nature (Key et al., 1997; Houde et al., 2006). There are two major compound groups of PFASs detected in the environment – perfluoroalkyl carboxylate (PFCA) and perfluoroalkyl sulfonates (PFSA). The prevailing compounds found in biota are the PFCA Perfluorooctanoate (PFOA) and Perfluorooctan sulfonate (PFOS) (AMAP, 2009). Increasing amounts of PFASs have been detected in the environment over the last decades (Verreault et al., 2007; Miljeteig and Gabrielsen, 2010).

There is a growing body of evidence with respect to OHC-mediated responses in Arctic wildlife (Letcher et al., 2010). Complex mixtures of known and unknown contaminants

are assumed to represent the nature of effects from OHCs. The growing number of persistent OHCs is of great concern, as well as their metabolic and degradation products (Letcher et al., 2010). New and emerging OHCs, such as PFASs, are of great concern since there are no known biotic or abiotic degradation pathways for these chemicals and they display great bioaccumulation potential when the perfluorinated chain exceed 6-7 carbons (Martin et al., 2004).

Thyroid hormones (THs) play a key role for normal development and physiological function in vertebrates. Normal TH function is dependent on a range of factors, like iodine uptake, TH synthesis, transport, deiodination and TH nuclear receptor binding (Yen and Chin, 1994). In avian species the THs control the thermoregulation, body weight, growth, lipid metabolism, reproduction, moulting and secondary sex characteristics (similar to the function in mammals) (Merryman and Buckles, 1998a; Merryman and Buckles, 1998b; McNabb, 2000). The hypothalamic-pituitary-thyroid axis (HPT-axis) controls the production and release of thyroxine (T4) from the thyroid glands. When the pituitary gland is stimulated by the hypothalamus, it releases thyroid-stimulating-hormone (TSH) which stimulates the thyroid glands. In birds, the serum binding proteins albumin and transthyretin (TTR) transport and distribute T4, which is converted to the more potent and metabolic active triiodothyronine (T3) by peripheral deiodinase enzymes (McNabb, 2000; Ucan-Marin et al., 2010). The fact that TH receptors are present in the nuclei in most cells of the body, demonstrate the widespread effects of THs (McNabb, 2000; Widmaier et al., 2006). Several other factors influence regular thyroid function, including food availability and composition, dietary iodine levels, seasonality, age and time of day (McNabb, 2000). Diurnal patterns in birds demonstrate that plasma T4 peak during the dark period and T3 rise to their highest levels during light period (McNabb, 2000).

The glaucous gull (*Larus hyperboreus*) is one of the largest avian predators with a circumpolar breeding range (Gilchrist, 2001). As an apex predator and opportunistic scavenger, the diet varies from pelagic and marine invertebrates, fish, eggs, chicks or adults of other seabirds, to carrion or human refuse (Løvenskiold, 1964; Anker-Nilssen et al., 2000). The feeding ecology of the glaucous gull is important for the distribution of OHCs within populations (Bustnes et al., 2000). Since glaucous gulls occupy an apex position in the Arctic marine food web and their ability to biotransform contaminants

seem to be restricted (Henriksen et al., 2000), the species is vulnerable for bioaccumulation and biomagnification of OHCs (Bustnes et al., 2000).

Contaminant levels and patterns have been reported in glaucous gulls since the first survey on anthropogenic contaminants in Arctic wildlife in 1972 (Bourne and Bogan, 1972). Since then, high levels of a range of environmental pollutants like PCBs and pesticides have been suggested to cause several biological and ecological responses and effects in this top predator (extensively reviewed by Gabrielsen, (2007) and Verreault et al., (2010)). Previous studies have assessed that high blood levels of OHCs may contribute to behavioral, developmental and reproductive stress in glaucous gulls (Bustnes et al., 2003a; Bustnes et al., 2003b; Verreault et al., 2004; Bustnes et al., 2005). Endocrine disruptive effects are among them, due to alterations in circulating THs (Verreault et al., 2010).

Several of the OHCs detected in glaucous gulls during the last decades have been toxic evaluated as possible endocrine disrupters, such as BFRs and PCBs (reviewed by Boas et al., 2006). Therefore, it is crucial to monitor the POPs of environmental concern and include the recently identified POPs. Further, this biomarker effect study may contribute to establish possible links between contaminant exposure and effects. Combined effects from OHCs and other anthropogenic or natural stressors in glaucous gulls need to be established. Filling these important knowledge gaps may contribute to strengthen the legislation regarding new, emerging POPs. The possible harmful effects from contaminant exposure, novel POPs reaching the Arctic and the indications that climate change can impact the contaminants' toxicity and bioavailability, strengthen the need for continuous monitoring of POPs in Arctic wildlife.

Aim of study

The aim of the present study was to investigate whether a selection of OHCs disrupt levels of THs in female glaucous gulls breeding in Kongsfjorden. It is hypothesized that high levels of OHCs can be detected in this avian top predator, including the legacy POPs, such as PCBs, DDTs and chlorinated pesticides in addition to emerging OHCs, such as BFRs and PFASs. Negative effects from exposure to these recently identified POP candidates are expected. Therefore, the aim was to investigate possible endocrine disruptive effects elicited on circulating THs, either from single classes of contaminants or from their combined effects.

2 MATERIALS AND METHODS

2.1. Study area

Blood samples were collected from breeding glaucous gulls in Kongsfjorden (Figure 1) in the vicinity of Ny-Ålesund (79° N, 12-13°E), on the northwest coast of Spitsbergen in the Norwegian Arctic. The sampling took place between the 8th and 19th of June 2010. A survey during the field work established that there were approximately 40 breeding pairs of glaucous gulls in the Kongsfjorden area this season. Continuous daylight and fluctuating external conditions such as temperature, precipitation and food availability, characterize the breeding conditions in Kongsfjorden (Hop et al., 2002).

Kongsfjorden has the last years been influenced by Atlantic water masses via the West Spitsbergen current (Hop et al., 2006), which may contribute to transport of the various contaminants detected in air, water and biota far away from general anthropogenic production and use (de Wit et al., 2004).

Figure 1 The study area Kongsfjorden, on northwestern Spitsbergen, Svalbard. The sampling locations of breading glaucous gulls (*Larus hyperboreus*) were Storholmen, Prins Heinrichøya, Mietheholmen, Blomstrandhalvøya, Breøyane and Guissezholmen. Map: Oddveig Øien Ørvoll (NPI).

2.2 Field sampling

Breeding glaucous gulls (females, N=19) were captured with an automatically triggered nest trap (Figure 3) between the 8th and 19th of June 2010. The first chick hatched 18th of June. The catchment was therefore performed during the second half of the incubation period. A snare was placed on the edge of the nest bowl (Figure 2) and released using a radio transmitter when the birds entered the nest. Biometric data was recorded for all birds: body mass (± 10 g), wing length (± 1 cm), head- and bill length, gonys and culmen (± 0.1 mm). All individuals were ringed with numbered steel rings from the Norwegian Ringing Centre, Stavanger museum.

Figure 2 The snare and wooden eggs placed in the nest bowl.

Figure 3 The trap installed and camouflaged.

Blood samples of a total of 10 mL were taken from the brachial veins on the inside of the wings – 3 mL blood for serum and 7 mL for plasma. A 5 mL syringe and a heparinized (5000 IE/mL LEO Pharma AS) 10 mL syringe (BD Plastipak) were used, respectively, with 23G needles (BD Microlance). The samples were kept cool and dark in the field and centrifuged (7000 rpm, 7 min) and frozen upon return to the lab facilities in Ny-Ålesund, no more than eight hours after sampling. The serum samples for TH analysis were frozen in two 1.2 mL cryogenic tubes (Nalge© Company, USA), the plasma sample in a 5 mL cryogenic tube and the red blood cells for sex determination in eppendorf tubes, at -20 ^{0}C until time for analysis. Since all the birds sampled were breeding, thus sexually mature, the age of the birds were assumed to be five years old or more (Gilchrist, 2001).

A body condition index (BCI) was calculated for the birds as a condition factor using principal component analysis to obtain a single measure of size. A principal component for the total head length (including skull and bill) and wing length obtained a single size

measure (Jolicoeur and Mosimann, 1960). A linear regression of standardized body mass residuals (mean=0, SD=1) and the standardized size measure were used to calculate the BCI.

The approval for this project was given by the Governor of Svalbard (reference number 2010/00093-16), and all sampling and bird-handling were in accordance to current regulations of the Norwegian Animal Welfare Act.

2.3 Contaminant analysis in glaucous gull plasma

The plasma samples were analysed for several OHCs: PCBs, DDTs, chlorinated pesticides, PBDEs and PFASs. In addition to contaminant analysis, an enzymatic lipid determination was used to classify the lipid classes present, such as free and total cholesterol, triglycerides and phospholipids as well as to determine the total lipid content. DNA, in the form of red blood cells, was used for sex determination, and serum samples were analysed for TH levels.

The OHC analysis was funded by the Norwegian Polar Institute (NPI) and carried out in the laboratories at The Norwegian Institute for Air research (NILU) in Tromsø. The sex determination and TH analysis was funded by and carried out at the Department of Biology, NTNU (Trondheim, Norway).

2.3.1 Analysis of organochlorinated and brominated compounds: chlorinated pesticides, PCBs and PBDEs

The glaucous gull plasma samples were quantified for the chlorinated pesticides, PCBs and PBDEs presented in Table 1. The extraction method used has been previously described by Herzke et al. (2003).

Table 1 The organochlorinated and brominated contaminants and chlorinated pesticides analysed in plasma from glaucous gull (*Larus hyperboreus*) breeding in Kongsfjorden in 2010.

Organochlorinated and brominated contaminants		
Group	**Acronym**	**Analyte**
CHLs	*trans*-Chlordane	*trans*-chlordane
	cis-Chlordane	*cis*-chlordane
	*oxy*chlordane	*oxy*-chlordane
	trans-Nonachlor	*trans*-Nonachlor
	cis-Nonachlor	*cis*-Nonachlor
	Heptachlor	3,4,5,6,7,8,8α-heptachlorodicyclopentadiene
	Heptachlor Epoxide	Heptachlor Epoxide
	Mirex	1,1α,2,2,3,3α,4,5,5,5α,5β,6-dodecachlorooctahydro-1*H*-1,3,4-(methanetriyl)cyclobuta[*cd*]pentalene
HCB	HCB	Hexachlorobenzene
HCH	α-HCH	1α,2α,3β,4α,5β,6β-hexachlorocyclohexane
	β-HCH	1α,2β,3α,4β,5α,6β-hexachlorocyclohexane
	γ-HCH	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
DDTs	*o,p,'*-DDT	*o,p,'*-dichloro-α,α-diphenyl-β,β,β-trichloroethane
	p,p,'-DDT	*p,p,'*-dichloro-α,α-diphenyl-β,β,β-trichloroethane
	o,p,'-DDE	*o,p,'*-dichloro-diphenyl-dichloroethylene
	p,p,'-DDE	*p,p,'*-dichloro-diphenyl-dichloroethylene
	o,p,'-DDD	*o,p,'*-dichloro-diphenyl-dichloroethane
	p,p,'-DDD	*p,p,'*-dichloro-diphenyl-dichloroethane
PCBs[1]	PCB-28	2,4,4'-trichlorobiphenyl
	PCB-47	2,2',4,4'-Tetrachlorobiphenyl
	PCB-52	2,2',5,5'-tetrachlorobiphenyl
	PCB-99	2,2',4,4',5-Pentachlorobiphenyl
	PCB-101	2,2',4,5,5'-pentachlorobiphenyl
	PCB-105	2,3,3',4,4'-pentachlorobiphenyl
	PCB-118	2,3',4,4',5-pentachlorobiphenyl
	PCB-123	2',3,4,4',5-Pentachlorobiphenyl
	PCB-128	2,2',3,3',4,4'-hexachlorobiphenyl
	PCB-138	2,2',3,4,4',5'-hexachlorobiphenyl
	PCB-141	2,2',3,4,5,5'-Hexachlorobiphenyl
	PCB-149	2,2',3,4',5',6-Hexachlorobiphenyl
	PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl
	PCB-156	2,3,3',4,4',5-Hexachlorobiphenyl
	PCB-157	2,3,3',4,4',5'-Hexachlorobiphenyl
	PCB-167	2,3',4,4',5,5'-Hexachlorobiphenyl
	PCB-170	2,2',3,3',4,4',5-heptachlorobiphenyl

[1] PCB congeners numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

	PCB-180	2,2',3,4,4',5,5'-heptachlorobiphenyl
	PCB-183	2,2',3,4,4',5',6-Heptachlorobiphenyl
	PCB-187	2,2',3,4',5,5',6-heptachlorobiphenyl
	PCB-189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
	PCB-194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
PBDEs[2]	BDE-28	2',4,4'-Tribromodiphenyl ether
	BDE-47	2,2',4,4'-Tetrabromodiphenyl ether
	BDE-66	2',3',4,4'-Tetrabromodiphenyl ether
	BDE-85	2,2',3',4,4'-Pentabromodiphenyl ether
	BDE-99	2,2',4,4',5'-Pentabromodiphenyl ether
	BDE-100	2,2',4,4',6'-Pentabromodiphenyl ether
	BDE-138	2,2',3',4,4',5'-Hexabromodiphenyl ether
	BDE-153	2,2',4,4',5,5'-Hexabromobiphenyl ether
	BDE-154	2,2',4,4',5,6'-Hexabromobiphenyl ether
	BDE-183	2,2',3',4,4',5',6'-Heptabromodiphenyl ether
	BDE-209	Decabromodiphenyl ether

[2] PBDE congeners numbered by the IUPAC system (International Union of Pure and Applied Chemistry).

Extraction

One mL plasma (homogenized by mixing) (equal to 1 g plasma with a CV%=1.6) was spiked with 100 µL of internal standard (25 pg/µL) containing mass-labeled OHCs (Appendix A). Two mL of deionized water – saturated with ammonium sulfate, and 2 mL of ethanol was added and vortexed to denature proteins. Extraction was conducted twice using 6 mL *n*-hexane as solvent. The extract was then up-concentration to 200 µL using RapidVap (Vacuum Evaporation System Model 7900001, Kansas city, MO, US). Lipid content was determined by enzymatic determination conducted by UniLab AS (Tromsø, Norway).

Clean-up

The extracted plasma samples were cleaned-up with activated florisil (magnesium silica, heated for 8 hours at 450^{o}C) to remove co-extracted biological matrix (i.e.proteins). Once cool, florisil was packed into individual solid phase extraction (SPE) cartridges (0.15 – 0.25 mm, Merck, Darmstadt, Germany) with 1 g (± 0.02 g). Concentrated plasma extracts were loaded onto florisil-packed SPE cartridges using a RapidTrace SPE Work Station (Caliper Life Science, Hopkinton, USA) and eluted with 9:1 *n*-hexane: Dichloromethane (DCM). To help prevent volatilization of the more volatile compounds, *iso*-octane was added to all samples after florisil clean-up as a keeper solvent to prevent evaporation. Samples were then concentrated to 200 µL, followed by gentle stream of N_2. Recovery standard (20 µL) octachloronaphtalene (OCN) 200 pg/µL was added prior to instrumental quantification on GC-MS. For further details on the extraction and clean-up, see Herzke et al. (2003).

Instrumental analysis

The organochlorine and brominated contaminants investigated within this study were analysed by Gas chromatograph – mass spectrometry (GC-MS). This instrumentation allows for the separation of target compounds based on differences in their volatility and interactions with the column stationary phase (Harris, 2010). Once separated, compounds elute from the column where they are ionized within the mass spectrometer and detected based on their mass/charge ratio (Harris, 2010). Several contaminant classes were analysed in this analysis, each using different instrumental parameters. The analysis details for the respective contaminant classes are given below.

Organochlorine pesticides and metabolites

Analysis was carried out using an Agilent 7890A gas chromatograph equipped with a 5975c mass spectrometer (Agilent Technologies, Böblingen, Germany). A 30 m DB5-MS column (0.25 mm id and 0.25 film thickness; J&W, Folsom, USA) was used for separation with helium as carrier gas at a flow rate of 1mL/min. A sample volume of 1 µL was injected in splitless mode using a split/splitless injector. The GC temperature program incorporated an initial temperature of 70^{o}C with a hold time of 2 min, increased by 15 ^{o}C/min to 180 ^{o}C, followed by a ramp of 5 ^{o}C/min to 280 ^{o}C and a 30 ^{o}C/min temperature ramp to 320 ^{o}C and held for 5 min. Electron capture negative ionization mode using methane as a reagent gas was used with the mass spectrometer being operated in single ion monitoring (SIM) mode for analyte detection.

DDT and respective metabolites

First, 1 µL of sample was injected into an Agilent 7890A gas chromatograph (Agilent Technologies, Böblingen, Germany) equipped with a triple quadrupole mass spectrometer, Quattro Micro GC (Waters Corporation, Manchester, UK). Separation was performed on a 30 m (+10 m guard) ZB5 column (0.25 mm id and 0.25 µm film thickness; Phenomenex, USA) with a programmable temperature vaporizing (PTV) injector operated in splitless mode. Initial temperature of PTV injector was 60 ^{o}C, followed by a temperature ramp of 300 ^{o}C/min to 250 ^{o}C and held for 5 min during injection of the sample. The injected sample was vaporized and swept onto the column using helium as the carrier gas at 1.0 mL/min. The oven temperature program used for separation of compounds was as follows: Initial oven temperature of 60 ^{o}C held for 3 min, followed by a 40 ^{o}C/min temperature ramp to 250 ^{o}C held for 7 minutes. A second temperature ramp followed of 20 ^{o}C/min to 300 ^{o}C held for 5 min.

Detection of DDT and its respective metabolites was done by using Multiple reaction monitoring (MRM) where the fragmentation transition of the parent ion to a daughter ion is monitored. Source temperature was set at 250 ^{o}C in positive electron impact mode (EI) using argon as a collision gas at a pressure of approximately 3.2×10^{-3} mbar. Dwell times for ion transition were 0.2 seconds. Information regarding specific transitions has been previously published in the literature (Pitarch et al., 2007).

PCBs

The analysis was similar to that for DDTs. PCBs were analysed by injecting 1 µL sample sample into an Agilent 7890A gas chromatograph (Agilent Technologies, Böblingen, Germany) equipped with a triple quadrupole mass spectrometer, Quattro Micro GC (Waters Corporation, Manchester, UK). Separation was performed on a 30 m DB5-MS column (0.25 mm id and 0.25 µm film thickness, J&W, Folsom, USA) with a split-splitless injector operated in splitless mode and heated to 250 ^{o}C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min and compounds were separated using the following oven temperature program: initial oven temperature was held at 70 ^{o}C (3 min hold), ramped at 15 ^{o}C/min to 180 ^{o}C, followed by a second temperature ramp of 5 ^{o}C/min until a final temperature of 280 ^{o}C (6 min hold). Source temperature was set at 250 ^{o}C in EI mode with MRM detection using argon as a collision gas at a pressure of approximately 3.2×10^{-3} mbar. Dwell times for ion transition ranged from 0.05 to 0.2 seconds. Information regarding specific transitions has been previously published in the literature (Pitarch et al., 2007).

Brominated flame retardants

The chromatographic analysis was performed on an Agilent 5890N gas chromatograph equipped with a Waters Autospec-V Ultima high-resolution mass spectrometer (Agilent Technologies, Böblingen, Germany). A sample volume of 1 µL was injected at 300 ^{o}C using a split-splitless injector in splitless mode with helium as a carrier gas using the following pressure program for separation: Initial pressure was held at 140 kPa for 2 min, followed by a rapid decrease to 60 kPa and held for 1.2 min. Pressure was then increased at 5.7 kPa/min to 117 kPa, followed by a second pressure increase of 2.7 kPa/min to 156 kPa and held for 5 min. A DB-HT5 column (15 m × 0.25 mm ID × 0.1 µm film thickness, J&W, Folsom, USA) was used for separation of targeted compounds using the following oven temperature program: 1) Initial oven temperature of 100 ^{o}C was held for 2.5 min. 2) Temperature increase of 13.2 ^{o}C/min to 220 ^{o}C. 3) Second temperature increase of 13.2 ^{o}C/min to 280 ^{o}C. 4) Final temperature increase of 50 ^{o}C/min to 320 ^{o}C and held for 5 min. The mass spectrometer was used in EI mode and targeted compounds were detected using SIM.

Quantification

Quantification of the compounds was done by running a quantification standard (Qstd) with a known concentration (Conc) of ^{12}C and ^{13}C together with the samples and

comparing their responses (peak areas) from the chromatograph. The ratio between ^{13}C and ^{12}C in the Qstd formed the basis of a relative response factor (RRF) (Equation 1), which was used to calculate the unknown concentrations of ^{12}C in the samples (spl) analysed based on the amount ^{13}C added (Equation 2). The sample amount lost during processing, indicated by the difference between calculated ^{13}C and added ^{13}C (Equation 3), were used to estimate the sample recovery (%).

$$RRF \times \frac{Amount_{C13-Qstd}}{Area_{C13-Qstd}} = \frac{Amount_{C12-Qstd}}{Area_{C12-Qstd}} \qquad (Equation\ 1)$$

$$Amount_{C12-Spl} = \frac{Area_{C12-spl} \times Amount_{C13-spl}}{RRF \times Area_{C13-spl}} \qquad (Equation\ 2)$$

$$Recovery\ (\%) = \frac{Amount_{C13-calc}}{Amount_{C13-added}} \times 100\% \qquad (Equation\ 3)$$

Quality assurance and method validation

Prior to the analysis, all glassware was burned and rinsed with *n*-hexane. Merck (Germany) supplied the solvents used and AGA (Oslo, Norway) supplied the nitrogen gas (N_2, 99 % purity) used for extract evaporation.

To quality assure and test the reproducibility and precision of the method, one blank and one standard reference material (SRM) (SRM 1589 Human Serum, National institute of standards and technology (NIST), MD, USA) were extracted for every tenth sample. Blank samples were below the detection limit and with no considerable contamination for most samples. If any compounds were detected in the blank samples, the limit of detection (LOD) (Appendix B) was set as three times the blank. Otherwise, the LOD was set to three times the instrument noise.

The recovery standard (OCN) was added prior to the instrumental analysis both as a quantification standard (volume correction) and as a test of the instrumental performance (recovery ability). The recoveries of the different internal standards were monitored in each sample, and the percent recoveries were above levels for approval (Appendix C). The laboratory regularly attends interlaboratory studies.

2.3.2 Analysis of perfluoroalkyl substances (PFASs)

The plasma samples were analysed for 19 PFASs, in total 11 PFCAs, five PFSAs PFOSA and three fluorotelomer sulfonates (FTS) (Table 2). The extraction method used has been previously described by Rylander et al. (2009) with some slight modifications. The samples were extracted using methanol (MeOH) for liquid-liquid extraction (LLE), clean-up procedure by means of Envi-Carb® and analysis using ultrahigh pressure liquid chromatography – mass spectrometry (UHPLC-MS/MS). Terminology and classification of the analysed PFASs are presented in Table 2 (Buck et al., 2011). Only one-way plastic equipment and glassware was used for sample preparation.

Table 2 PFASs analysed in glaucous gull (*Larus hyperboreus*) plasma samples, denoting acronyms, analytes, chemical formulas and number of carbons.

Perfluoroalkyl and Polyfluoroalkyl substances (PFASs): acronyms, analytes and chemical formulas				
	Acronym	**Analyte**	**Chemical formula**	**No. of Carbons**
PFCA		Perfluoroalkyl carboxylates	$C_nF_{2n+1}COOH$	
	PFBA	Perfluorobutanoate	$CF_3(CF_2)_2COOH$	4
	PFPA	Perfluoropentanoate	$CF_3(CF_2)_3COOH$	5
	PFHxA	Perfluorohexanoate	$CF_3(CF_2)_4COOH$	6
	PFHpA	Perfluoroheptanoate	$CF_3(CF_2)_5COOH$	7
	PFOA	Perfluorooctanoate	$CF_3(CF_2)_6COOH$	8
	PFNA	Perfluorononanoate	$CF_3(CF_2)_7COOH$	9
	PFDcA	Perfluorodecanoate	$CF_3(CF_2)_8COOH$	10
	PFUnA	Perfluoroundecanoate	$CF_3(CF_2)_9COOH$	11
	PFDoA	Perfluorododecanoate	$CF_3(CF_2)_{10}COOH$	12
	PFTriA	Perfluorotridecanoate	$CF_3(CF_2)_{11}COOH$	13
	PFTeA	Perfluorotetradecanoate	$CF_3(CF_2)_{12}COOH$	14
PFSA		Perfluoroalkyl sulfonates	$C_nF_{2n+1}SO_3H$	
	PFBS	Perfluorobutane sulfonate	$CF_3(CF_2)_3SO_3H$	4
	PFHxS	Perfluorohexane sulfonate	$CF_3(CF_2)_5SO_3H$	6
	PFHpS	Perfluoroheptane sulfonate	$CF_3(CF_2)_6SO_3H$	7
	PFOS	Perfluorooctane sulfonate	$CF_3(CF_2)_7SO_3H$	8
	PFDcS	Perfluorodecane sulfonate	$CF_3(CF_2)_9SO_3H$	10
Other PFASs	4:2 FTS	4:2 fluorotelomer sulfonate	$C_4F_9CH_2CH_2SO_3^-$	4
	6:2 FTS	6:2 fluorotelomer sulfonate	$C_6F_{13}CH_2CH_2SO_3^-$	6
	8:2 FTS	8:2 fluorotelomer sulfonate	$C_8F_{17}CH_2CH_2SO_3^-$	8

Extraction

The plasma sample was homogenized by mixing and a volume of 200 µL was spiked with 20 µL internal standard. The standard consists of a mixture of ^{13}C analogues of PFASs (0.1 ng/µL) (Appendix D). Finally, 1 mL MeOH was added before 3 x 10 minutes extraction in the ultrasonic bath (Branson 2210).

Clean-up

The methanol supernatant was transferred to eppendorf centrifuge tubes containing 25 mg ENVI-Carb graphitized carbon adsorbent (Supelclean ENVI-Carb® 120/400, Supelco, Bellefonte, PA, USA) and 50 µL glacial acetic acid. This clean-up will assure the removal of matrix, e.g. proteins, in the samples prior to UHPLC-MS/MS analysis. After centrifugation (10 000 rpm, 10 min), 500 µL of the supernatant was transferred to a glass vial. As a control parameter for the clean-up precision, 20 µL of recovery standard was added (branched PFDcA 0.1 ng/µL in MeOH), vortexed and kept in the fridge at approximately 3 ^{o}C until analysis.

Instrumental analysis

An aliquot of 100 µL extract was transferred to an autosampler vial with insert and diluted with the same amount of 2 mM aqueous NH_4OAc. PFASs were analysed by ultra-high pressure liquid chromatography triple-quadrupole mass spectrometry (UHPLC-MS/MS). Analysis was performed on a Thermo Scientific quaternary Accela 1250 pump with a PAL Sample Manager coupled to a Thermo Scientific Vantage MS/MS (Vantage TSQ). An injection volume of 10 µL was used for sample separation on a Waters Acquity UPLC HSS 3T column (2.1 ×100 mm, 1.8 µm) equipped with a Waters Van guard HSS T3 guard column (2.1 × 5 mm, 1.8 µm). In order to separate the PFCA contamination leaching out from the pump and the degasser, a Waters XBridge C18 column (2.1 × 50 mm, 5 µm) was installed after the pump and before the injector.

Separation was achieved using 2 mM NH_4OAc in 90:10 MeOH:water (A) and 2 mM methanolic NH_4OAc (B) as the mobile phases. Details about the analytical conditions are given in Hanssen et al. (2011) together with parent ions, monitored transitions and conditions on S-linse and collision energy.

Quantification

The chromatograms were quantified for PFASs using the LCQuan software (version 2.6, Thermo Scientific). Quantification was done with the internal standard method with

isotope labeled PFASs. An eight point calibration curve with a concentration range from 0.02 pg/µL to 10 pg/µL was used for quantification of PFASs.

Quality assurance and method validation

During analysis, solvent injections were done regularly in order to monitor instrument background and carry-over effects. To test cross-contamination as well as reproducibility and precision during the analysis, one blank sample and one sample certified reference material (SRM; 1589 human serum, National institute of standards and technology (NIST), MD, USA) were included for every tenth sample. The LOD was set to three times the instrument noise. If any compounds were detected in the blank samples, the limit of detection (LOD; Table E.1) was set as three times the median of the blank samples. Recoveries of the different internal standards were monitored in each sample, and the percent recoveries were above levels for approval (Table F.1).

The contaminant results are denoted in ng/g wet weight, since this exposure concentration is thought to be most relevant for effect studies where several classes of OHCs are included, such as in the present study (Braathen et al., 2004).

The laboratory regularly participates in interlaboratory testing.

2.4 Lipid analysis

The plasma lipid content was analysed at UniLab AS in Tromsø using enzymatic determination. The total lipid (TL) content was calculated from the amounts of free cholesterol (FC), total cholesterol (TC), triglycerides (TG) and phospholipids (PL) using equation 4 (Akins et al., 1989). The enzymatic determination is considered more accurate than gravimetric lipid determination (Covaci et al., 2006). The results in mg/dl were converted to lipid percentage per wet weight (ww) sample.

$$TL = 1.677 \times (TC - FC) + FC + TG + PL \quad \text{(Equation 4)}$$

Quality assurance and method validation

UniLab AS notified that the results and validations met the requirements set by the laboratory, although data on the variation and precision is not further presented here.

2.5 Sex determination

The sex of the birds was determined by analysis of sex chromosomes using a chelex extraction polymerase chain reaction (PCR) and gel electrophoresis at NTNU (Trondheim, Norway). The method used was established by Griffiths et al. (1998). The method is well established for glaucous gulls and is based on the detection of the female specific W-chromosome. When the PCR product is visualized in agarose gel, the heterogametic female (ZW) appear as two bands and the homogametic male (ZZ) as and one band (Figure 4).

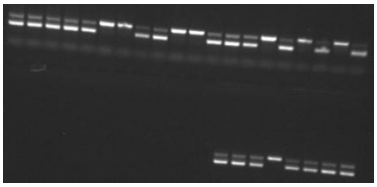

Figure 4 The PCR products visualised in agarose gel on UV light. The heterogametic female (ZW) appeared as two bands (N=20) and the homogametic male (ZZ) as and one band (N=8).

A droplet of blood (3 µL) was added to an eppendorf tube containing 200 µL 5 % chelex solution, followed by incubation (20 min, 56 ^{o}C), vortex, second incubation (8 min, 96 ^{o}C), vortex and centrifugation (3 min, 12 000 rpm). 20 µL supernatant from each individual was transferred to new tubes. 8µL of PCR stock solution (made of 1.75 µL Taq, 68.25 µL H_2O, 35 µL 10x, 70 µL Q, 21 µL MgCl, 14 µL Mix and 35 µL primer I (2550) and 35 µL primer II (2718)) were transferred to PCR wells. 2 µL of the DNA was added to each well before PCR (30 cycles with thermal profile 94 ^{o}C : 51 ^{o}C : 70 ^{o}C) (Stratagene MX3000).

The PCR product was then separated on 1 % agarose gel (made of 59 mL dest H_2O, 1.2 mL TAE pH buffer, 0.6 g agarose and 6 µL SYBR® Safe DNA gel stain) added running buffer (686 mL H_2O, 14 mL TAE buffer). The PCR wells were loaded with 10 µL sample before a ladder (10µL ladder, 10 µL gel loading buffer and 80 µL dest H_2O) was added and the gel was run (75 V, 45 min). The PCR product was visualised using UV light and the ZW and ZZ bands registered (Figure 4).

Quality assurance and method validation using PCR

The method was quality assured by comparing the results from the PCR analysis with biometric measurements obtained in the field. The PCR results matched the determination based upon total head length for gull species, as recommended by

Coulson et al. (1983). None of the bands were vague, so the uncertainty was insignificant.

2.6 Determination of thyroid hormone levels in glaucous gull serum

The thyroid hormone analysis was carried out using radioimmunoassay (RIA) Coat-A-Count® TT4, FT4, TT3 and FT3 kits (Siemens medical solution, Diagnostics, Los Angeles, CA, USA) at NTNU (Trondheim, Norway). The kit is a solid-phase ^{125}I radioimmunoassay designed to quantitatively measure the TH levels in serum to assess the thyroid status. Briefly, the method is based on competitive binding between sample THs (T3, T4) and radioactive ^{125}I labeled THs (T3*, T4*) in pre-coated antibody tubes. A calibration curve of known concentrations makes quantification of bound ^{125}I labeled THs possible (Sigmond et al., 1992). The RIA is of the most sensitive and accurate techniques for this purpose and have been in common use for analyzing avian THs since late 1970s (McNabb, 2000).

Serum samples were thawed upon analysis and added to the coated tubes. Four fractions of thyroid hormones were analysed; the total and free (unbound) thyroxin (TT_4 and FT_4) and total and free triiodothyronine (TT_3 and FT_3) and the detection limits were 309 nmol/L, 129 pmol/L, 9.22 nmol/L and 65 pmol/L, respectively. The kits procedures were followed and the method is well established and can be extrapolated to birds (McNabb, 2000; Verreault et al., 2004).

The bound radioactive TH antigen fraction was counted using a gamma scintillation counter (Cobra Auto Gamma, model 5003, Packard Instrument Company, Dowers grove, IL, USA). A calibration curve made of known concentrations and the gamma software were used to calculate the TH concentrations in the samples.

The results were obtained for all glaucous gulls – except for one female (number 13), which were omitted from further presentation and statistics.

Quality assurance and method validation using RIA

The repeatability and inter- and intra assay precision was tested for each kit by running duplicates of SRM human serum controls (Immunoassay Plus Control level 2, Biorad Laboratories, CA, USA), the laboratories own quality control bovine (*Bos primigenius*) plasma samples and triplicates (for TT4 and FT4) and duplicates (TT3 and FT3) of

glaucous gull serum. Readings with coefficient of variation (CV) >15 % were not approved and reruns performed. According to the laboratories control routines, a range limit of 15 % was set. Thus, one female bird was removed from further analysis, resulting in a total of 19 female glaucous gulls presented in this study and included in statistics.

The blank samples were below LOD, and the range of the results and CV% met the requirements established by the laboratory. The CV% values of the reference material were below 15 % for both the intra- and inter-assay precision tests for TT4 (<4.5 %), FT4 (<12.8 %), TT3 (<6.3 %) and FT3 (<13.9 %). For TT4 sample triplicates, CV% ranged from 0.8 – 13.7 %. For FT4 sample triplicates, CV% ranged from 1.9-14.6 %. For TT3 sample replicates, CV% ranged from 0.1-7.8 %. For FT3 sample replicates, CV% ranged from 0.2-26.3 %. Due to the low concentration range of FT3, the CV% exceeded 15 % for four of the FT3 samples (15.9-26.3 %). This might be a result of the FT3 were run in duplicates instead of triplicates. The concentrations in these individuals did not differ considerably from the other samples, a correlation test revealed these samples correlated with the TT3 values (p<0.001, r=0.858) as for the other samples. When calculating the relative error the two replicates did not differ more than ±19 % to the mean. Values in the lower part of the standard curve are important to include in the data set, despite that CV% ≥15. The difference between the two replicates with CV%=26.3 constitute of only/amount to 0.46 pmol, which result in the bigger CV% for low FT3 concentrations. Therefore, the mean concentration was considered acceptable to use and TH results from 19 females were included in the multivariate data analysis.

2.7 Statistics

Software

SIMCA P12+ (Version 12.0.0, Umetrics, Umeå, Sweden) and SPSS Statistical software (Version 19.0 for Windows, IBM, SPSS Inc., Chicago, IL) were used for the multivariate data analysis. Calculations were performed using Microsoft Excel for Windows 2010. Statistical significance was set at $p \leq 0.05$, with a borderline significance p=0.5–0.75.

Normal distribution

Data were analysed for normality distribution using Shapiro-Wilk's test (n≤50) in SPSS 19. Variables that were not normally distributed were $\log_{10}$ transformed. This applied

for the following variables: Capture date, number of eggs, TT3, β-HCH, *oxy*chlordane, *trans*-nonachlor, mirex, *p,p'*-DDT, *p,p'*-DDE, PCB congeners (PCB)-28/31, -47, -52, -74, -99, -105, -128, -138, -149, -153, -156, -167, -170, -180, -183, -187, -189, -194, each and all of the PFASs and PBDEs (except BDE-153), and all the contaminant sums (Σ). All variables were normally distributed either with no transformation or log10, except for the PFASs. Due to some extreme outliers according to the SPSS software description among the PFSAs PFOS (min-max 3.5 – 429.8 ng/g ww) and PFHxS (0.3 – 27 ng/g ww), these were not normally distributed before the two outliers were excluded. The current extreme outliers achieved the criteria for extreme outliers in SPSS and all regressions were tested both with and without the two extreme outlying samples.

Detected compounds were analysed in statistics as individual compounds and congeners, in addition to summed concentrations, indicated with the symbol Σ. This denotes for ΣCHLs[3], ΣHCH[4], ΣDDTs[5], ΣPCBs[6], Σdl-PCBs[7], ΣPBDEs[8] and ΣPFASs[9].

Statistical treatment of values <Limit of detection (LOD)

All compounds detected in more than 70 % of samples (70 % > LOD) were included in the statistical analysis. Samples <LOD were set to ½LOD for further use in statistics. Limit of quantification (LOQ) was set to 3xLOD. Sample concentrations <LOQ were set to ½LOQ. Individual LODs for OCP/PCB/PBDE are presented in Table B.1, and for PFASs in Table E.2 in Appendix.

Based upon the above criteria, statistical exclusion applied for the following compounds: the pesticides α-HCH, γ-HCH, Heptachlor, *cis*-chlordane, *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, the PBDE congeners (IUPAC numbers) BDE-85, -138, -183 and -209, and the PFASs PFBA, PFPA, PFHpA, PFHxA, PFOA, PFBS, PFDcS, 4:2 FTS, 6:2 FTS, 8:2 FTS and PFOSA.

[3] ΣCHLs include *trans*-Chlordane, *oxy*chlordane, *trans-n*onachlor, *cis*-nonachlor and mirex.

[4] ΣHCH include β-HCH.

[5] ΣDDTs include *o,p'*-DDT and *o,p'-DDE.*

[6] ΣPCBs include the 22 PCB congeners (PCB)-28/31, -47, -52, -66, -74, -99, -101, -105, -118, -128, -138, -149, -153, -156, -157, -167, -170, -180, -183, -187, -189 and -194.

[7] Σdl-PCBs include the six congeners (PCB)-105, -118, -156, -157, -167 and -189.

[8] ΣPBDEs include the congeners (BDE)-28, -47, -66, -99, -100, -153 and -154.

[9] ΣPFASs include PFNA, PFDcA, PFUnA, PFDoA, PFTria, PFTeA PFHxS and PFOS.

The lipid percent was calculated and used in the data analysis. Results were obtained from all analysis (contaminants and THs most importantly) for the 19 females included in the statistics. Total head length was included as a biometric variable, and the BCI was included as a condition factor.

Principal component analysis (PCA)

In Simca P12+, Principal component analysis (PCA) was applied to explore the similarities and differences between observations and identify the variables that made samples different. In a PCA plot, the variables included are transformed to a dataset of uncorrelated variables orthogonally projected on each other (Eriksson et al., 2006). The variables are unit variance (UV) scaled, so that their contribution to the final model is equal, independent of their absolute values. In addition, the variables are mean centered (the mean value is subtracted) to increase the models interpretability (Eriksson et al., 2006). A critical validation of the PCA is essential. This is performed with respect to explained variance of each of the principal components and the models goodness of prediction. Significant PCs have eigenvalues>1. BCI or Σ was not included in the PCA to avoid strong covariations.

Pearson correlation tests

A Pearson correlation test was used to further investigate and confirm or invalidate the indications from the PCA plot. A significance level of $p<0.05$ and a borderline significance of $p=0.05$-0.075 was set when investigating the relationships between the THs and explanatory variables in SPSS. Correlations are expressed using the Pearson correlation coefficient r.

OPLS – Orthogonal projection to latent structures regression

When the explanatory variables show a high degree of multicolinearity, like in the present dataset, orthogonal projections to latent structures (OPLS) regression can be done using Simca P12+. This is a statistical tool designed to perform multiple regression when the number of observations are somehow limited and there is a high degree of co-linearity, such as in the present dataset. This multiple regression method can assess the relationships (positive or negative) between the THs and the most important predictor variables. Further, it may identify combined effects from OHCs on THs. Therefore, the OPLS complements the statistical series and may detect relationships when several explanatory variables affect the model, not displayed by a PCA or bivariate test. The

indications from the PCA and correlation test were used to select a group of explanatory variables included in the OPLS, to see if significant OPLS regression models could be obtained for any of the THs.

According to Trygg and Wold (2002), the OPLS and PLS is a better tool than multiple regression when handling data with a high degree of colinearity and noise in *X* and *Y* matrix. What separates the OPLS from the precursor PLS, is that an OPLS separates the variation in predictor variable *X* into variation which is correlating and non-correlating (orthogonal [90^0]) with the variation in response variable *Y*. An OPLS is critical validated with respect to R^2X (explained variance) and R^2Y (goodness of fit), and a high goodness of prediction (Q^2) is essential. R^2Y >0.7 and a Q^2>0.4 denote highly significant models when analyzing biological data (Lundstedt et al., 1998). "Variable importance in projection" (VIP) plots denote the importance of each *X* variable in the predicted model and rank them in their explanatory power of *Y.* Combined with Coefficient plots with jack-knifed confidence intervals, one can identify both the important and significant model predictors. VIP values >1 are the most relevant variables explaining the *Y*. An "Analysis of variance of the cross-validated residuals" (CV-ANOVA) test the significance of the OPLS regression.

For the THs where significant OPLS models could not be obtained, a multiple linear regression was performed between the THs and the most correlated variables. This was performed in the default enter method in SPSS to examine the possible multicolinearity. The aim for the OPLS and multiple regressions was to investigate the combined effects from POPs on THs.

3 RESULTS

3.1 Biometric characteristics

Biometric characteristics are presented as mean ± SD, minimum and maximum levels (Table 3). Individual biometric characteristics are presented in Table G.1.

Table 3 Mean ± standard deviation (SD), median, minimum and maximum of biometric variables measured in female glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard.

	Females (N=19)		
	Mean ± SD	**Median**	**Min - Max**
Body mass (g)	1458 ± 81.0	1445.0	1300 – 1585
Total head length (mm)	137.08 ± 3.21	137.4	132.9 – 146
Bill (mm)	57.90 ± 2.00	57.7	55.1 – 61.4
Gonis (mm)	21.20 ± 0.80	21.1	19.7 – 23.1
Wing lenght (mm)	458.00 ± 9.00	460.0	442 – 473
Body Condition Index	0 ± 1	0.12	(-1.3) - 1.4
Plasma Lipid content (mg/dL)	1394.57 ± 282.31	1358.92	952.9 - 1904.5

3.2 Contaminant levels and pattern

The contribution of each of the contaminant groups to the mean OHC burden (ng/g ww) in female glaucous gulls are presented in Figure 5. The mean ± SD, minimum and maximum OHC concentrations are presented in Table 4.

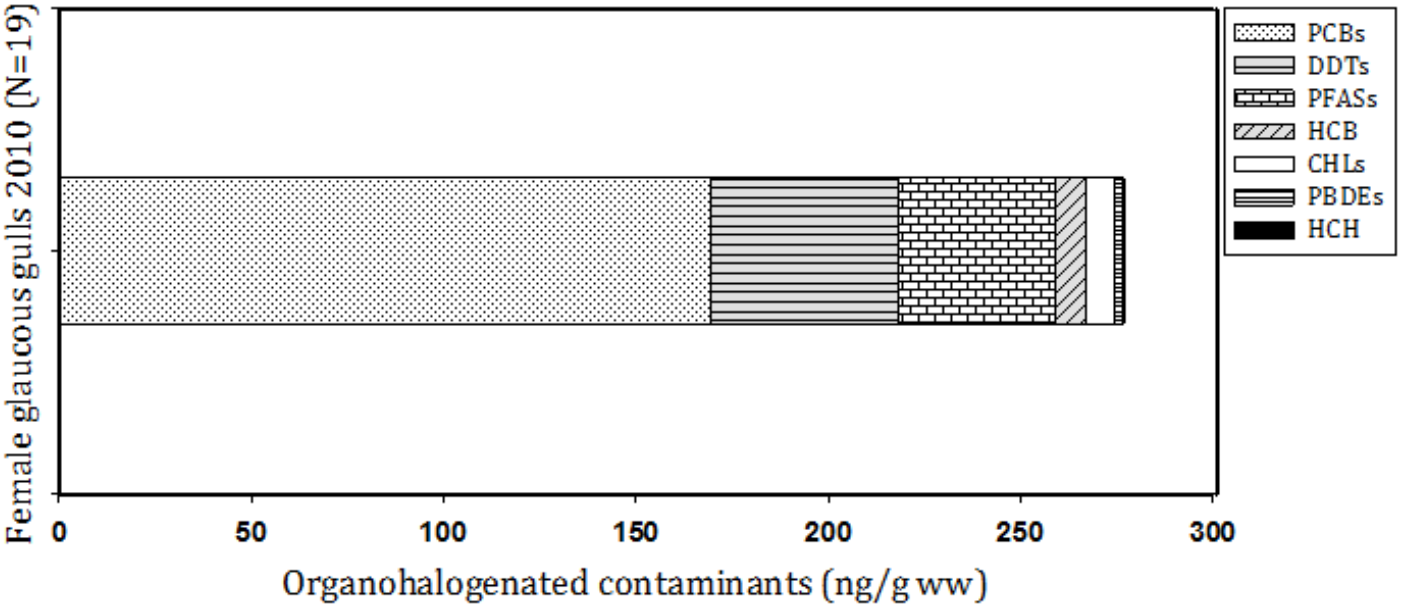

Figure 5 Mean concentrations (ng/g ww) of organohalogenated contaminants (OHC) detected in female glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden 2010. The contribution of the groups range from ΣPCB (61.3 %) to HCH (0.1 %) (the latter barely visible as a black line to the far right).

The percent contribution of each contaminant group to the total concentration was as follows: ΣPCB (61.3 %) > ΣDDTs (17.5 %) > ΣPFSAs (12.0 %) > HCB (2.9 %) >ΣPFCA (2.8 %) > ΣCHLs (2.7 %) > ΣPBDE (0.8 %) > β-HCH (0.13 %).

The PCBs and OCPs were the prevailing compound groups measured in female glaucous gull plasma in the present study. ΣPCBs were 4 times higher than ΣDDTs and 5 times higher than the ΣPFSAs. The most abundant PCBs were PCB-153 (32.1 %), PCB-180 (18.4 %) and PCB-138 (15.6 %), which constitutes 66 % of the total PCB fraction. The dioxin-like PCB congeners[10] (Σdl-PCBs) constitute 11.27 % of the total PCB fraction, and 9.3 % of the total OHC burden in these birds. PCB-118 and PCB-156 represent 38.5 %

[10] Dioxin-like PCB congeners include (PCB)-105, -118, -156, -157, -167, -189.

and 36.5 % of the Σdl-PCBs, respectively. The ΣDDTs was the second largest OHC group, whereas the metabolite *p,p'*-DDE made up 99.5 % of the DDT group (Table 4 below). The levels of HCB, ΣPFCA, ΣCHL, ΣPBDE and HCH all contributed <2.9 % to the total contaminant burden which were low compared to the other groups of OHCs.

The ΣPFASs[11] represented the third most abundant contaminant group in the present study. They constituted 14.8 % of the compound burden in female glaucous gulls. Mean PFAS plasma concentrations ± SD, median, minimum and maximum are presented in Table 4. Of the 15 PFASs analysed in the plasma, 11 were detected and eight of these analytes had 70 % >LOD. PFOS, PFUnA, PFHxS, PFTriA, PFNA, PFDcA, PFDoA, PFTeA, PFOA, PFBS, PFDcS were detected in all individuals. The short chain PFCAs (PFBA, PFPA, PFHxA and PFHpA) were not detected in any of the glaucous gull samples. The eight PFAS with 70 % >LOD contributed to ΣPFASs as follows: PFOS (76.3 %) > PFUnA (6.04 %) > PFHxS (5.2 %) > PFTriA (4.3 %) > PFNA (3.8 %) > PFDcA (2.0 %) > PFDoA (1.5 %) > PFTeA (0.95 %). The highest PFAS concentration measured in all samples was PFOS, followed by PFHxS. These two compounds constitute 81.5 % of the total PFAS burden in glaucous gulls from the present study. Even though the levels of the two PFSAs were in general higher than the PFCA concentrations, the PFCAs showed greater potency regarding effects on THs.

ΣPBDE and ΣHCH was the smallest OHC fractions and represent < 1 % of the total contaminant burden in the female glaucous gulls in the present study. The individual PBDE congeners contributed to ΣPBDE as follows: BDE-47 (63 %) > BDE-100 (12.6 %) > BDE-153 (8.4 %) > BDE-99 (8.1 %) > BDE-154 (6.6 %) > BDE-28 (0.89 %) > BDE-66 (0.45 %). β-HCH was the only HCH detected.

[11] ΣPFASs include both PFCAs and PFSAs.

Table 4 Mean ± standard deviation (SD), median, minimum and maximum concentrations (ng/g ww) of individual organochlorinated and brominated contaminants and polyfluoroalkyl substances in female glaucous gulls (*Larus hyperboreus*) from Kongsfjorden in 2010. Included are the sums denoted with Σ. N denotes the number of observations per variable.

		Female glaucous gulls (N=19)			
	Analyte (ng/g ww)	**N**	**Mean ± SD**	**Median**	**Min - max**
	Lipid%	19	1.39 ± 0.28	1.36	0.95 - 1.90
CHLs	*trans*-Chlordane	19	0.45 ± 0.23	0.41	0.16 - 1.08
	oxy-Chlordane	19	1.88 ± 2.03	0.99	0.05 - 8.49
	trans-Nonachlor	19	0.96 ± 0.77	0.93	0.17 - 3.48
	cis-Nonachlor	19	0.84 ± 0.47	0.83	0.23 - 2.05
	Mirex	19	3.22 ± 2.50	2.17	0.78 - 8.90
	ΣCHLs[a]	19	7.35 ± 5.30	6.09	1.76 - 23.99
DDTs	*p,p'*-DDT	14	0.30 ± 0.31	0.23	0.06 - 1.32
	p,p'-DDE	19	48.13 ± 30.60	48.91	13.769 - 133.012
	ΣDDTs[b]	19	48.35 ± 30.82	49.23	13.84 - 134.33
HCB	HCB	19	8.09 ± 3.35	7.74	3.83 - 16.64
HCHs	β-HCH	18	0.39 ± 0.20	0.33	0.19 - 0.94
PCBs	PCB-28/31	19	0.27 ± 0.12	0.29	0.15 - 0.63
	PCB-47	19	0.57 ± 0.29	0.49	0.24 - 1.43
	PCB-52	16	0.44 ± 0.39	0.35	0.04 - 1.36
	PCB-66	19	1.12 ± 0.58	1.19	0.46 - 2.86
	PCB-74	19	1.06 ± 0.56	0.99	0.40 - 2.71
	PCB-99	19	6.72 ± 4.55	6.69	2.01 - 21.27
	PCB-101	16	0.94 ± 0.80	0.71	0.05 - 2.87
	PCB-105	19	3.30 ± 1.97	2.54	0.92 - 8.57
	PCB-118	19	12.29 ± 7.60	9.77	3.31 - 30.79
	PCB-128	19	3.30 ± 2.03	2.63	0.94 - 8.96
	PCB-138	19	26.48 ± 16.70	24.44	7.89 - 74.82
	PCB-149	18	0.53 ± 0.33	0.45	0.22 - 1.61
	PCB-153	19	54.36 ± 50.06	38.31	13.19 - 228.51
	PCB-156	19	2.51 ± 1.96	1.79	0.57 - 7.99
	PCB-157	13	0.79 ± 0.52	0.67	0.27 - 2.11
	PCB-167	19	1.37 ± 0.92	1.03	0.36 - 3.16
	PCB-170	19	10.35 ± 14.470	5.538	1.570- 66.283
	PCB-180	19	31.05 ± 37.07	16.42	5.88 - 166.35
	PCB-183	19	3.73 ± 2.79	2.78	0.97 - 11.58
	PCB-187	19	3.99 ± 2.30	3.25	1.52 - 9.95
	PCB-189	17	0.52 ± 0.68	0.26	0.08 - 2.97

	PCB-194	19	4.39 ± 5.82	2.02	0.84 - 26.10
	ΣPCB[c]	19	169.61 ± 148.50	122.76	42.76 - 682.90
	Σdl-PCB[d]	19	19.11 ± 12.60	13.99	4.79 - 52.44
PBDEs	BDE-28	19	0.02 ± 0.01	0.01	0.005 -0.05
	BDE-47	19	1.36 ± 0.71	1.36	0.51 - 3.91
	BDE-66	19	0.01 ± 0.009	0.007	0.001 - 0.04
	BDE-99	19	0.17 ± 0.11	0.15	0.05 - 0.52
	BDE-100	19	0.27 ± 0.13	0.27	0.10 - 0.73
	BDE-153	19	0.18 ± 0.11	0.18	0.03- 0.35
	BDE-154	19	0.14 ± 0.73	0.13	0.05 - 0.38
	ΣPBDE[e]	19	2.16 ± 1.09	2.14	0.79 - 5.97
PFCA	PFNA	19	1.54 ± 0.80	1.33	0.63 - 3.79
	PFDcA	19	0.82 ± 0.66	0.61	0.42 - 3.39
	PFUnA	19	2.47 ± 3.26	1.58	1.01 - 15.67
	PFDoA	19	0.61 ± 0.69	0.44	0.30 - 3.43
PFASs	PFTriA	19	1.76 ± 2.47	1.04	0.57 - 11.73
	PFTeA	19	0.38 ± 0.54	0.22	0.16 – 2.53
	ΣPFCA	19	7.58 ± 8.19	5.31	3.25 - 40.54
PFSA	PFHxS	19	2.10 ± 606.40	0.65	0.34 - 27.12
	PFOS	19	31.12 ± 96.78	6.66	3.45 - 429.81
	ΣPFSA	19	33.23 ± 102.84	7.20	4.02 – 456.93
	ΣPFASs[f]	19	40.80 ± 102.62	12.93	7.81 - 460.18

[a] ΣCHLs include *trans*-Chlordane, ocychlordane, *trans*-Nonachlor, *cis*-Nonachlor and mirex.

[b] ΣDDTs include *o,p'*-DDT and *o,p'-DDE.*

[c] ΣPCBs include the 22 PCB congeners (PCB)-28/31, -47, -52, -66, -74, -99, -101, -105, -118, -128, -138, -149, -153, -156, -157, -167, -170, -180, -183, -187, -189 and -194.

[d] Σdl-PCBs include the six congeners (PCB)-105, -118, -156, -157, -167 and -189.

[e] ΣPBDEs include the congeners (BDE)-28, -47, -66, -99, -100, -153 and -154.

[f] ΣPFASs include PFNA, PFDcA, PFUnA, PFDoA, PFTria, PFTeA PFHxS and PFOS.

3.3 Thyroid hormone levels

The serum thyroid hormone levels in female glaucous gulls are presented in Table 5. Levels of T4 dominated over levels of T3, both for the bound and free fraction (Table 5). Individual thyroid hormone levels are presented in Table H.1.

Table 5 Mean concentrations of total (nmol/L) and free (pmol/L) T3 and T4 and TT4:TT3 mole ratios ± standard deviation (SD), median, minimum and maximum, measured in serum from female glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden, Svalbard.

	Females (N=19)		
	Mean ± SD	**Median**	**Min - max**
TT3[a] (nmol/L)	1.81 ± 0.97	1.55	0.89 - 4.37
FT3[b] (pmol/L)	2.73 ± 1.39	2.23	0.86 - 6.14
TT4[c] (nmol/L)	22.73 ± 8.55	23.46	8.91 - 39.14
FT4[d] (pmol/L)	21.58 ± 9.20	21.76	7.15 - 43.11
Total T4:T3	15.05 ± 8.28	12.63	4.96 - 31.16

[a] total triiodothyronine
[b] free triiodothyronine
[c] total thyroxine
[d] free thyroxine

3.4 Relationships between biometric characteristics, TH levels and OHCs

3.4.1 Principal component analysis and Pearson bivariate correlation test

A principal component analysis (PCA) investigated the relationship between biometric characteristics, individual contaminant levels and THs. Six significant principal components (PCs) resulted from the analysis (eigenvalues>1). The two first PCs explained 50.7 % and 23.1 % of the variation, respectively (Figure 6). The TH variables were mainly explained by PC1. Certain relationships indicated in the PCA plot was identified and supported by a significant Pearson correlation test (Table 6 and 7).

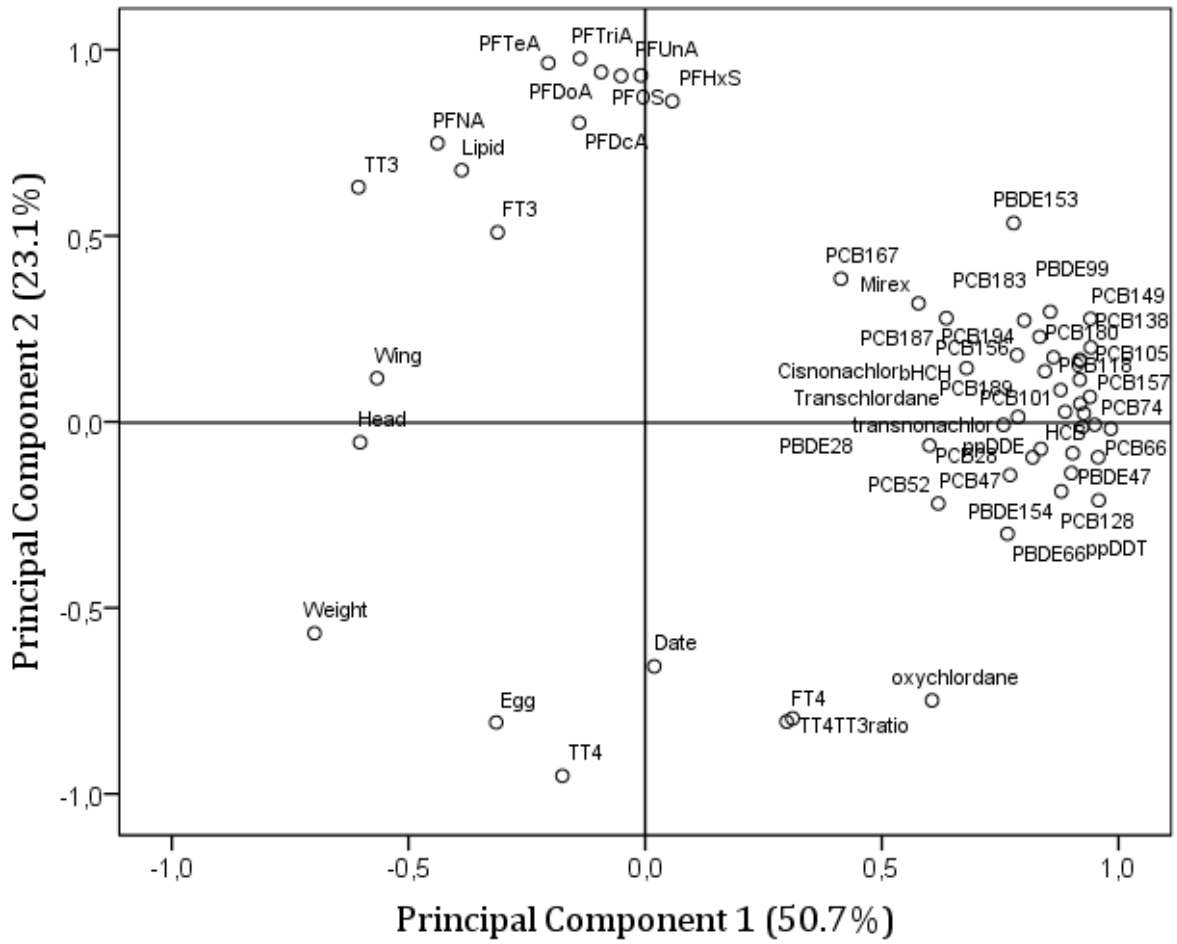

Figure 6 Principal component analysis plot with thyroid hormone variables and explanatory variables for female glaucous gull (*Larus hyperboreus*) from Kongsfjorden, Svalbard. The first two principal components explained 73.8 % of the variation. Date denotes for date of capture. Egg denotes for the number of eggs in the clutch. The other abbreviations are explained in the text.

Along PC1 in the PCA plot (Figure 6), the PFASs grouped (PC≈0.9**;** Individual PCA loadings in Table J.1) together with TT3, FT3 and lipid percent. Significant positive bivariate correlations were found for TT3 and PFDcA, PFUnA, PFTriA, PFTeA, PFOS and lipid percent. Lipid percent correlated positive with PFTriA, PFTeA, PFHxS and PFOS. TT4, FT4, TT4:TT3 ratio, date of capture and number of eggs, grouped on the opposite side of PC1 (PC≈0.9).

Along PC2, the chlorinated and brominated contaminants clustered on one side (PC≈0.9), and displayed a distinct separation from the PFASs. The head and wing length grouped on the negative side of PC2 (PC ≈ -0.2). A significant inverse relationship was found between head and PCB-167 and between wing length and β-HCH, HCB, PCB-47 and PCB-187 (Table 7).

The PCA indicated correlations between the THs (Figure 6). This was further supported by bivariate correlations. TT3 was positively linked with FT3 (p<0.001) and inversely correlated with TT4:TT3 (p<0.002). TT4 was positively correlated with FT4, FT3 and TT4:TT3 (p≤0.015). Furthermore, FT4 was positively correlated with TT4:TT3. The BCI did not explain the variation in TH levels or TT4:TT3 ratio (p<0.001). None of the biometric variables were significantly correlated with THs. However, a significant positive correlation was found between the extractable plasma lipid percent and TT3 (Table 6). In contrast, lipid percent was negatively correlated with TT4:TT3 (Table 6).

Neither the body weight, number of eggs nor date of capture was well explained by the PCA plot. However, some negative relationships were supported by the bivariate analysis (Table 7). The body weight was inversely correlated with *trans*-nonachlor, (PCB)-118, -157, -167, -170, -189 and -194 (Table 7). The BCI was inversely correlated with PCB-189.

Relationships between the different OHCs

The contaminant variables were better explained by the PCA plot (Figure 6). Along PC1, the PFASs grouped (PC1=0.7 – 1.0). The six individual PFCAs were not correlated with the two individual PFSAs or ΣPFASs. Positive correlations between the individual PFCAs were observed both in the PCA, and further confirmed by the correlation analysis (p=<0.043). The two PFSAs (PFHxS and PFOS) were correlated, both with each other and the ΣPFASs. Since PC1 and PC2 are orthogonal projected on each other, the PCA

indicate no correlations between the PFASs and the chlorinated and brominated contaminants. This was supported by the bivariate correlation test, as no significant test values were found. The chlorinated and brominated compounds (CHLs, DDTs, HCB, PCBs and PBDEs) grouped along PC2 (Figure 6). The bivariate correlation test supported that most of these POP groups were significantly correlated with one another. The 22 individual PCB congeners (except for PCB-52) were significantly correlated with each other, with ΣPCBs ($p \leq 0.009$) and Σdl-PCBs ($p \leq 0.025$). Chlorinated pesticides and PBDEs were all positively correlated with each other and with ΣPCBs, Σdl-PCBs and ΣPBDEs ($p \leq 0.001$).

Relationship between THs and the explanatory variables

The PCA plot indicated a positive relationship between the PFASs and the TT3 and FT3, while a negative relationship with TT4, FT4 and TT4:TT3. Statistically significant bivariate correlations between several of the PFASs and TT3 and TT4:TT3 confirmed this (Table 6). The Pearson correlation coefficient *r* is denoted in brackets. TT3 was positively correlated with PFDcA, PFUnA, PFTriA, PFTeA and PFOS. TT4:TT3 was significantly inversely correlated with PFTeA ($p=0.006$) and PFTriA ($p=0.019$).

Table 6 Pearson *p*-values with *r*-values in brackets between thyroid hormones, lipid% and perfluoroalkyl substances in plasma from breeding female glaucous gull (*Larus hyperboreus*) from Kongsfjorden, Svalbard. Borderline significance in *italic* when $p > 0.05 \leq 0.075$.

Variables	TT3	FT3	TT4	FT4	Total T4:T3	Lipid%
PFNA	-	-	-	-	-	-
PFDcA	*0.067 (0.429)*	-	*0.058 (0.454)*	-	-	-
PFUnA	0.048 (0.459)	-	-	-	-	-
PFDoA	-	-	-	-	-	-
PFTriA	0.006 (0.623)	-	-	-	0.011 (- 0.567)	0.008 (0.603)
PFTeA	0.019 (0.526)	-	-	-	0.006 (- 0.609)	0.002 (0.672)
PFHxS	-	-	-	-	-	0.024 (0.544)
PFOS	*0.067 (0.455)*	-	-	-	-	0.003 (0.675)
Lipid%	0.004 (0.631)	-	-	-	0.002 (- 0.664)	-

- : Denotes Pearson correlation coefficients *p>0.075*, no significance detected.

The inverse relationships between TT4 and FT4, and several of the PFASs suggested by the PCA, were not confirmed by the Pearson correlation test. However, a positive borderline correlation between TT4 and PFDcA (p=0.058) was found.

Although the relationships between THs and the chlorinated or brominated compounds were not well explained by the in the PCA plot (Figure 6), certain relationships were supported by the bivariate analysis (Table 7).

Further, significant relationships were found between TT4, FT4 and the biometric variables (Table 7). TT4 and FT4 were also significantly associated with a selection of the organochlorinated and brominated compounds (Table 7). TT4 displayed negative correlations with BDE-99 and BDE-153. Positive correlations were found between FT4 and PCB-52 and an inverse relationship between FT4 and PCB-167.

Table 7 Pearson *p*-values and *r*-values in brackets for the significant Pearson correlations between biometric variables, thyroid hormones and organochlorinated and brominated contaminants in plasma from breeding female glaucous gull (*Larus hyperboreus*) from Kongsfjorden, Svalbard. Borderline significance in *italic* when $p > 0.05 \leq 0.075$.

	Weight	Head	Wing	BCI	TT4	FT4
***Trans*-nonachlor**	*0.062 (-0.436)*	-	-	-	-	-
β-HCH	-	-	0.035 (-0.499)	-	-	-
HCB	-	-	0.019 (-0.530)	-	-	-
PCB-47	-	-	*0.066 (-0.430)*	-	-	-
PCB-52	-	-	-	-	-	0.036 (0.512)
PCB-118	*0.058 (-0.443)*	-	-	-	-	-
PCB-157	0.040 (-0.575)	-	-	-	-	-
PCB-167	0.041 (-0.652)	0.019 (-0.720)	-	-	-	0.041 (-0.653)
PCB-170	0.047 (-0.450)	-	-	-	-	-
PCB-180	-	-	-	-	-	-
PCB-187	-	-	0.038 (-0.466)	-	-	-
PCB-189	0.020 (-0.557)	-	-	0.038 (-0.507)	-	-
PCB-194	0.034 (-0.475)	-	-	-	-	-
BDE-99	-	-	-	-	*0.075 (-0.418)*	-
BDE-153	-	-	-	-	*0.053 (-0.450)*	-

The linear relationship between TT3 and the PFTriA are presented graphically in Figure 7, and between TT3 and PFTeA in Figure 8. The relationship between TT4:TT3 and PFTriA are presented graphically in Figure 9, and between TT4:TT3 and PFTeA in Figure 10.

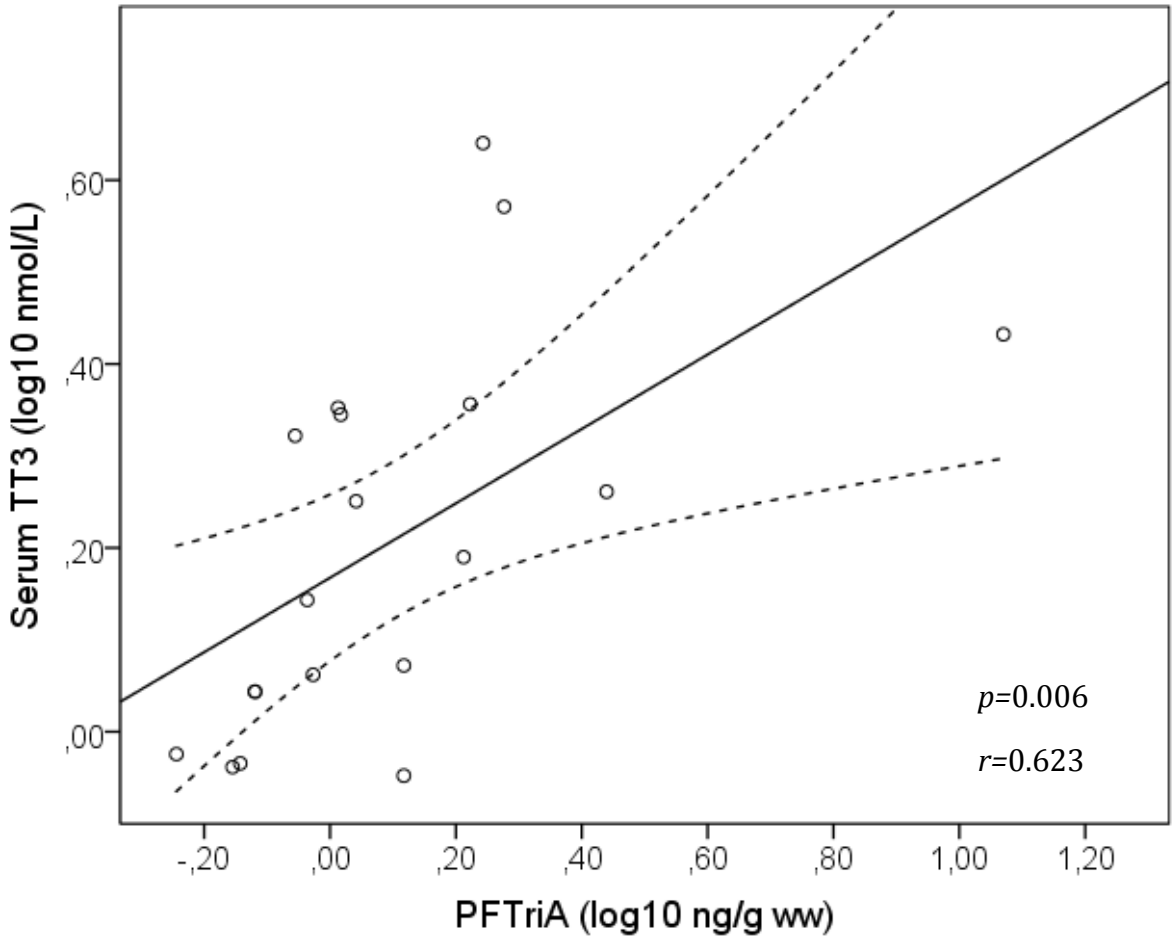

FIGURE 7 The linear relationship (± 95 % confidence interval) between total triiodothyronine (TT3) serum concentrations (log_{10} nmol/L) and Perfluorotridecanoate (PFTriA) plasma concentration (log_{10} ng/g ww) for female glaucous gulls (*Larus hyperboreus*) captured during the incubation period of 2010 in Kongsfjorden. R^2=0.388, F-value=10.15, slope=3.19 (t-value). The Pearson correlations *p* and *r*-values for transformed data are shown in the plot.

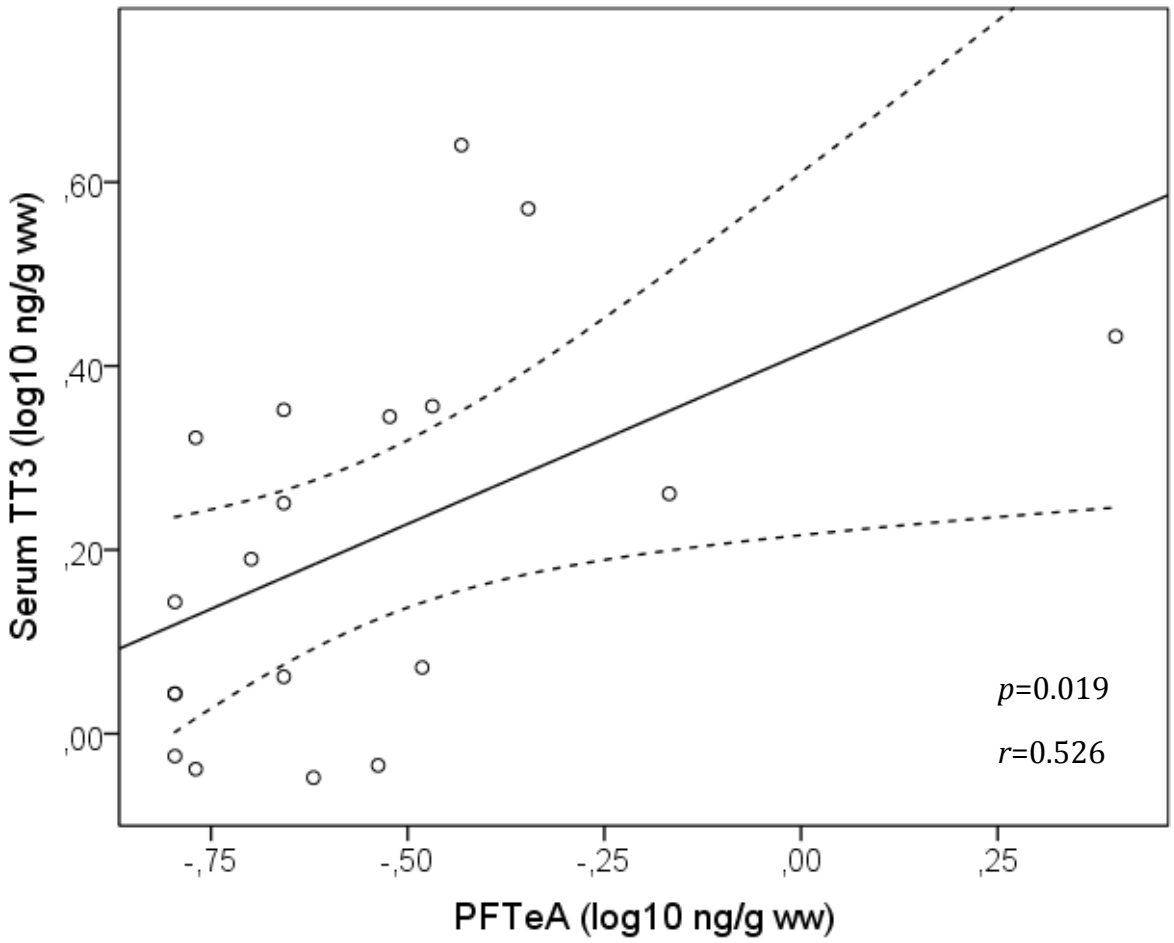

FIGURE 8 The linear relationship (±95 % confidence interval) between total triiodothyronine (TT3) serum concentrations (log_{10} nmol/L) and perfluorotetradecanoate (PFTeA) plasma concentration (log_{10} ng/g ww) in female glaucous gulls (*Larus hyperboreus*) captured during the incubation period of 2010 in Kongsfjorden. R^2=0.276, F-value=6.11, slope=2.47 (t-value). The Pearson correlation *p* and *r*-values for transformed data are shown in the plot.

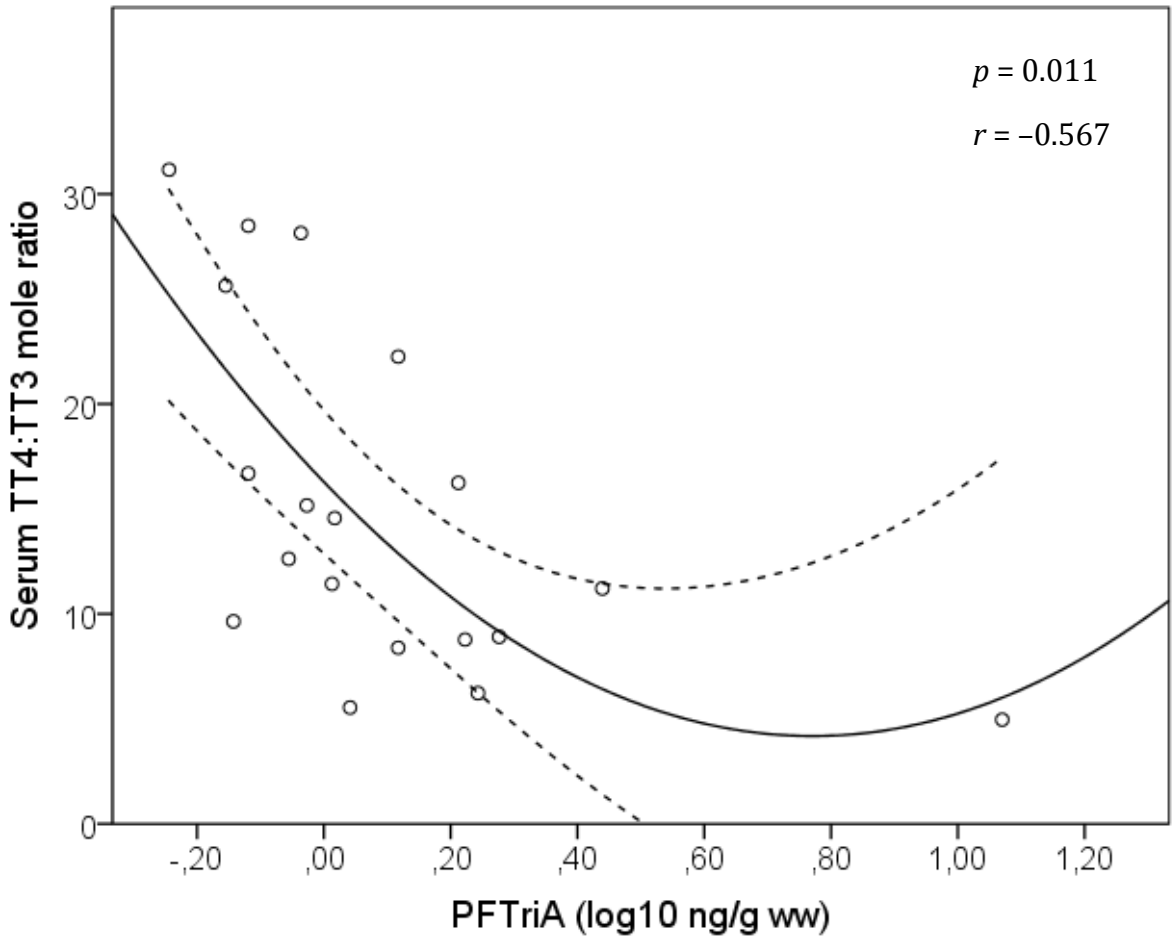

FIGURE 9 The quadratic relationship (±95 % confidence interval) between total thyroxine to total triiodothyronine (TT4:TT3) serum molar ratio and perfluorotridecanoate (PFTriA) plasma concentration (ng/g ww) for female glaucous gulls (*Larus hyperboreus*) captured during the incubation period of 2010 in Kongsfjorden. R^2=0.429, F-value=8.05, slope= –2.84 [t-value] and Pearson correlation *p* and *r*-values in the plot.

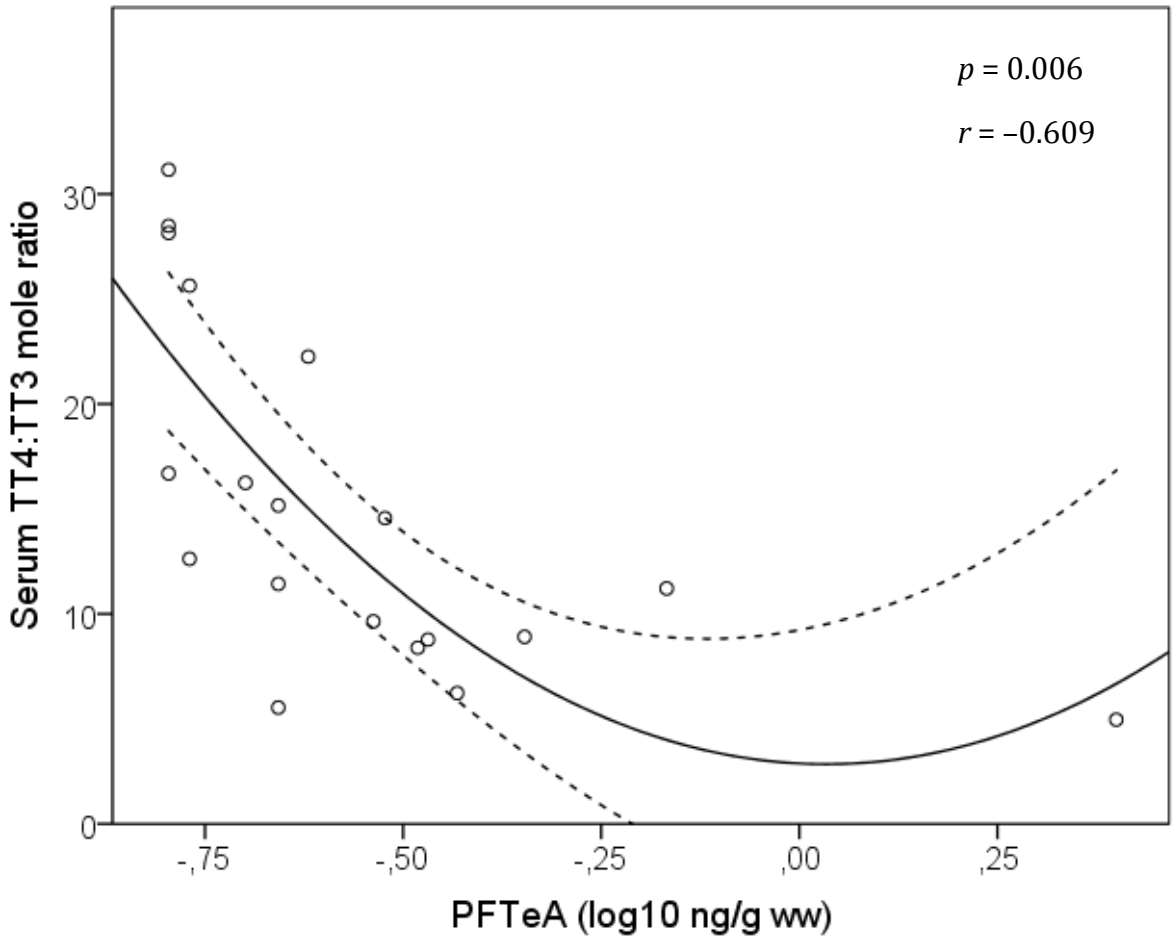

FIGURE 10 The quadratic relationship (±95 % confidence interval) between total thyroxine to total triiodothyronine (TT4:TT3) serum molar ratio and perfluorotetradecanoate (PFTeA) plasma concentration (ng/g ww) for female glaucous gulls (*Larus hyperboreus*) captured during the incubation period of 2010 in Kongsfjorden. R^2=0.371, F-value=10.03, slope= –3.17 [t-value] and Pearson correlation *p* and *r*-values in the plot.

3.4.2 OPLS – Orthogonal projections to latent structures

The OPLS predicted a significant variation in TT3 (Figure 11 and 12) and TT4:TT3 ratio (Figure 13 and 14). These multiple regression results further confirm the variation predicted in the PCA plot and Pearson correlation test and complements the statistical series.

The PCA and Pearson correlations were used to select the *X* variables included in the OPLS. The less important *X* variables were then removed stepwise, resulting in significant OPLS models for TT3 and TT4:TT3. The OPLS is a strong statistical tools, and need strong data with clear correlations to become significant. The significant models obtained for TT3 (Figure 11 and 12) and TT4:TT3 ratio (Figure 13 and 14) can therefore be interpreted as models with clear correlations. The OPLS is a multiple regression analysis, including both the positive and negative explanatory variables. Further, it assessed the combined effects from several of the OHCs on circulating THs.

The *Y*= TT3 model resulted in a CV-ANOVA; p=0.052 (R^2X=0.36, R^2Y=0.34 and Q^2=0.31). The information from the variable importance plot (VIP) (Figure 11) is complemented with a Coefficient plot (Figure 12). These two plots summarize the overall contribution from each *X* variable indicating which are correlated with *Y,* and points out whether the relationship (between *Y* and the *X* variables) is positive or negative. The OPLS model describing the variation in TT3 estimated the importance of each *X* variable (Figure 11) to VIP>0.5 for (in ascending order) PCB-170, PCB-194, PCB-189, PFNA, BDE-66, PCB-52, PFDoA, total head length, PCB-101 and PFUnA. The variables with VIP>1.0 denotes for (in ascending order) *p,p'*-DDT, PFTeA, PFTriA, PFDcA and lipid percent, and these variables are considered the most important for the variation in TT3. Lipid percent was the most important variable in this OPLS (Figure 11).

The Coefficient plot estimated the relationship directions (positive or negative) between TT3 and the *X* variables (Figure 12). The variables with jack-knife intervals not crossing the 0-line are considered the ones with highest reliability. This denoted for *p,p'*-DDT, PCB-101, PCB-52, PBDE-66, PFNA, PFTeA, PFTriA, PFDcA and lipid percent.

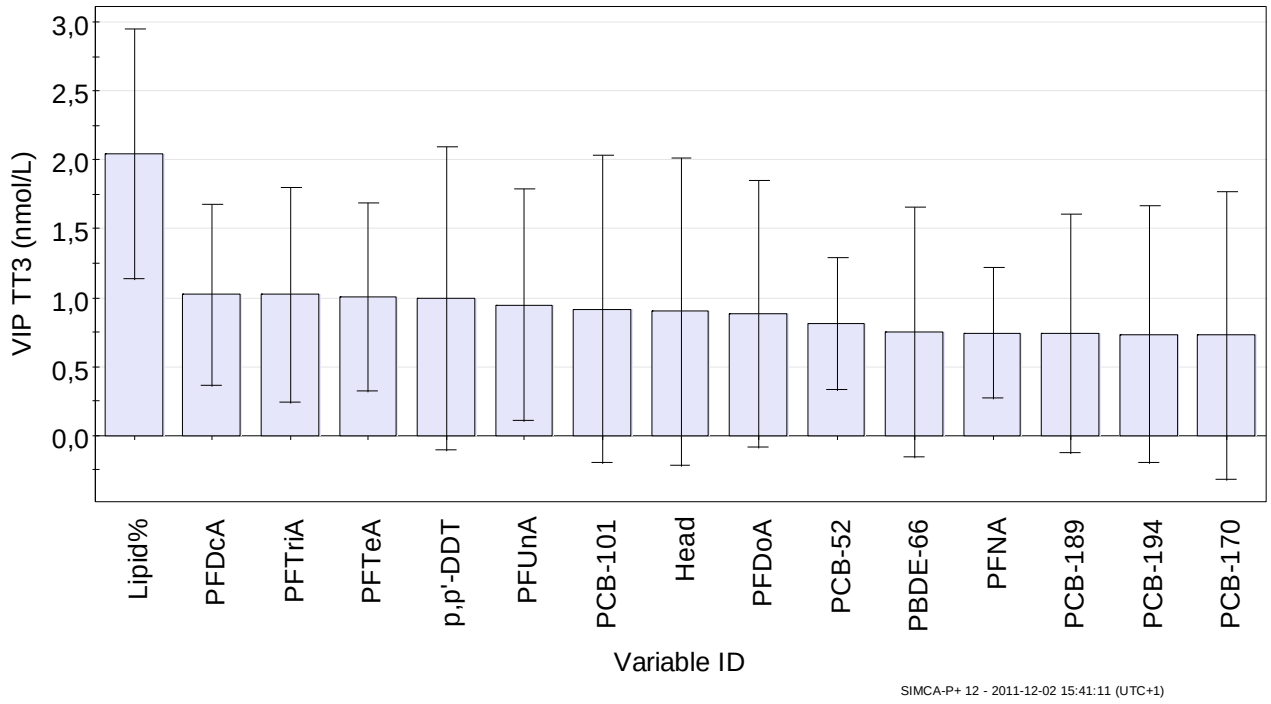

Figure 11 OPLS plot showing important variables explaining the TT3 levels in female glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard. The variables with VIP value > 1 and with jack-knife intervals not crossing the 0-line are considered the ones with higher reliability. Abbreviations explained in the text.

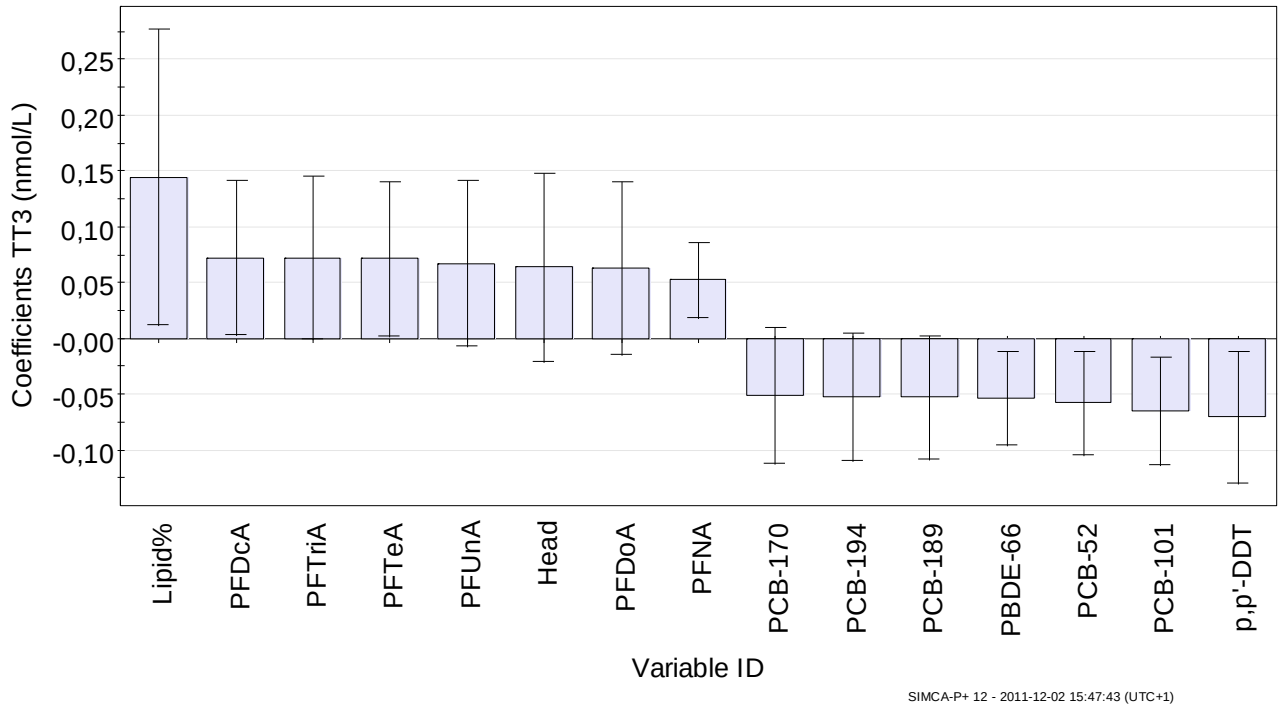

Figure 12 OPLS coefficient plot explaining the direction of influence for predictor variables explaining TT3 levels in female glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard. The variables with jack-knife intervals not crossing the 0-line are considered the ones with higher importance. Abbreviations explained in the text.

The *Y*=TT4:TT3 OPLS resulted in a significant CV-ANOVA; p=0.030 (R^2X=0.30, R^2Y=0.54 and Q^2=0.36) including the following *X* variables with VIP>1.0 in descending order: lipid% > PCB-52 > BDE-66 > PFTeA > PFTriA > BDE-28 > PFDoA > PCB-74 > mirex (Figure 13). The Coefficient plot estimated the relationship directions (positive or negative) between TT4:TT3 and the *X* variables (Figure 14).

Weaker, non-significant models were obtained for the other *Y* variables TT4, FT4 and FT3 and are consequently not included here. Consequently, the OPLS implies that the correlations for these response variables were not as strong as for TT3 and TT4:TT3.

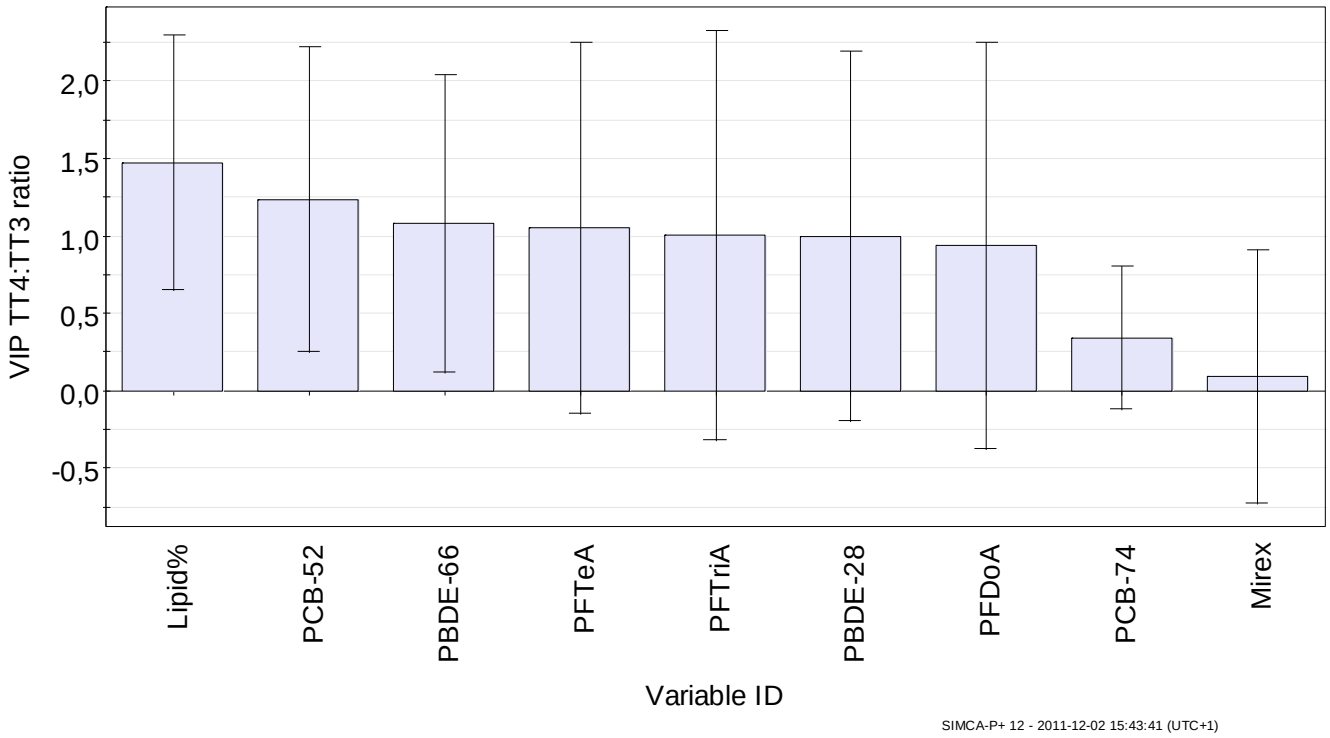

Figure 13 Important variables explaining the TT4:TT3 ratio in female glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard. The variables with VIP value > 1 and with jack-knife intervals not crossing the 0-line are considered the ones with higher reliability.

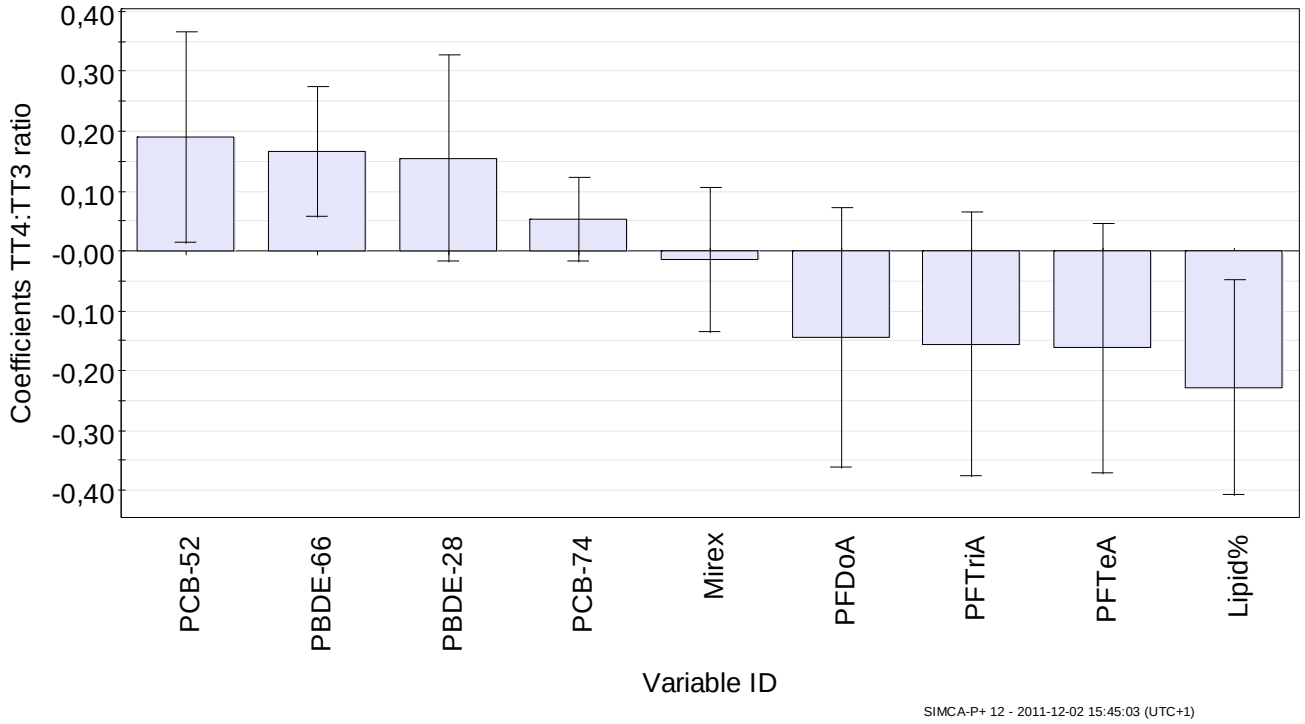

Figure 14 OPLS coefficient plot explaining the direction of influence for predictor variables explaining TT4:TT3 ratio in female glaucous gulls (*Larus hyperboreus*) from Kongsfjorden, Svalbard. The variables with jack-knife intervals not crossing the 0-line are considered the ones with higher importance. Abbreviations explained in the text.

4 DISCUSSION

4.1 Prevalence of organohalogenated contaminants (OHCs)

The present study reported the plasma OHC levels in female glaucous gulls breeding in Kongsfjorden 2010 and assessed a possible link between several of the OHCs and serum thyroid hormone levels. PCBs and OCPs were the prevailing compound groups in glaucous gull plasma (Table 4). The present study aimed to identify the effects of single classes of contaminants, in addition to any combined effects. Surprisingly – the long-chain PFCAs constituted of less than 2.8 % of the total POP load in these female glaucous gulls, but were among the most important explanatory variables regarding the variation in THs.

It is challenging to elucidate effects from OHCs in wildlife. The sample size used in the present study, represent a small selection of breeding glaucous gull from the population in Kongsfjorden. In addition, the present study focus on effect assessment in female glaucous gulls, due to the small male sample size (N=8) in 2010. Therefore, it is not appropriate to report the differences in levels or effects between sexes in the present study. There is limited knowledge about the individual life history and general physiological conditions of these randomly captured animals. The period the study was conducted represents a short time window of the glaucous gull life cycle; in addition there are logistical constraints associated with the study area and season. Arctic biota is exposed to a complex mixture of anthropogenic contaminants, of which the quantitatively and qualitatively composition is not fully elucidated (de Wit et al., 2004). For instance, the OHCs analysed in the present study represents a selection of compounds within a selection of compound groups. For this reason, it is not possible to account for the possible impacts or interactions from non-analysed contaminants in the present study. Furthermore, the contaminants in the present study showed a high degree of covariation. Such strong intercorrelations in a dataset can complicate the multivariate data analysis when elucidating responses (Trygg and Wold, 2002). Multivariate data analysis methods, such as the OPLS, are developed to better handle the challenges regarding intercorrelations between variables. However, there are still some uncertainties associated with the interpretation of data. For instance, the non-analysed

compounds may contribute to the observed indications of effects on THs, while others merely covary with the compound explaining the variation. Nonetheless, based on this one-year study from 2010, it is not proper to conclude on a time trend for glaucous gulls in Kongsfjorden. It is merely possible to compare the geographical relationships, in time and space, with studies on glaucous gulls from other localities, such as Bjørnøya. When doing so, it is essential to allow for differences in tissue, analysis procedures, data handling, sample size and study area. Also note that dissimilarities between sexes, species, lipid content and time of sampling do take place. In elucidation of these factors, comparisons are discussed using wet weight basis and focusing on female glaucous gulls. However, the sampling of data to the present study was conducted during a short time period (the second half of the incubation period), and is therefore considered to be a standardised data set.

4.2 Contaminant levels and pattern

PCBs and OCPs were the prevailing compound groups measured in plasma from the female glaucous gull in the present study colony. This pattern reflect the general pattern of OHCs measured in Arctic seabird tissue (Letcher et al., 2010) and glaucous gull plasma (Verreault et al., 2006). However, levels of ΣPFASs constituted of only 2.7 % less than levels of ΣDDTs. Thus, high levels of ΣPFASs were reported in the present study.

The OHC concentrations detected in female glaucous gulls from the present study were compared to previous levels measured in glaucous gulls from Bjørnøya (Verreault et al., 2005a; Verreault et al., 2005b). The levels detected in the present study were found to be similar to or lower than levels reported in glaucous gull plasma sampled at Bjørnøya in 2004 (Verreault et al., 2006). Data from a one year study like the present is not sufficient material to draw conclusions considering a time trend in glaucous gulls from Kongsfjorden. However, the numerous studies on glaucous gulls from Bjørnøya show decreasing plasma levels of POPs (Bustnes et al., 2010). It might be that future studies continue to show decreasing levels of some POPs, compared in time or space. Therefore, the levels in glaucous gulls from Kongsfjorden in the present study may possibly reflect the overall trend that some of the legacy chlorinated POPs are declining in Arctic marine wildlife species (Braune et al., 2005; Bustnes et al., 2010; De Laender et al., 2011), including the glaucous gull (Verreault et al., 2010). It is noteworthy this exclusively

apply for the POPs faced out of production and use, like PCBs, *oxy*chlordane, HCB and PFOS (Stockholm Convention, 2011a). Nevertheless, the present study confirm the persistency of these legacy POPs in biota (de Wit et al., 2004), and display evidence for a continuous slow decline in levels of POPs in the decades to come.

The major compound group detected in the present study of female glaucous gulls was PCBs. The PCB blood concentrations in the present study ranged from 42 – 683 ng/g ww (Table 4), compared to 52 – 1079 ng/g ww in a study from Bjørnøya in 2001 (Bustnes et al., 2001). Compared to other seabirds in the Arctic, the levels reported in these two studies may reflect the glaucous gulls' trophic position and feeding habits (Wold et al., 2011). However, the somewhat lower levels detected in Kongsfjorden may indicate a difference in feeding habits and choice of pray between the two locations. This may be explained by findings indicating that the glaucous gull diet at Bjørnøya in larger part consist of eggs and chicks from other seabirds (Bustnes et al., 2000). The present study fall into the line of temporal trends suggesting that the concentrations of legacy POPs only will decrease slowly in the years to come.

The ΣPBDE was the smallest compound group detected in the female glaucous gull plasma, only followed by β-HCH (Figure 5). The levels of PBDEs were lower for all congeners in the females from the present study, compared to a former survey on plasma concentrations in glaucous gulls sampled at Bjørnøya in the Norwegian Arctic (Verreault et al., 2005a). BDE-47 was the main contributor to ΣPBDE in both studies. However, the levels of BDE-47 were sevenfold lower in the present study (1.6 ng/g ww) compared to what Verreault et al. reported in 2005. However, the pattern of congeners was the same, as BDE-47 was followed by BDE-99, -100 and 153. This PBDE pattern is seen in glaucous gulls as well as other Arctic species like the polar cod (*Boreogadus saida*) and black guillemot (*Cepphus grylle*) (Haukås et al., 2007). One of the concerns regarding PBDEs is the possible toxicological potential as endocrine disrupters detected in the present study, in spite of the concentrations being 1/10 of the PCBs and OCPs concentrations. In addition, there is a rapid replacement of existing compounds by new brominated contaminants. The ban of one hazardous chemical may be followed by the development and release of new potential toxic congeners (de Wit et al., 2010).

The different physicochemical properties of the OHCs contribute to the correlations between POPs indicated both by the PCA (Figure 6) and the bivariate analysis in the present study. These patterns of inter-correlation are consistent with earlier studies of species in the Barents Sea food web, including glaucous gulls (Haukås et al., 2007). Organochlorinated and brominated POPs possess similar physicochemical properties, thus differing from PFASs. The proteinophilic nature of PFAS compared to the more lipophilic organochlorinated and brominated POPs (de Wit et al., 2004; Ahrens et al., 2009), result in differences in physiological partitioning. The PFASs are rather partitioned into blood and liver due to their protein affinity, as the lipophilic OHCs partition into the lipid rich compartments. Therefore, the correlation between PCBs, OCPs and BFRs, and them not correlating with the PFASs as observed in the present study, was as expected.

The levels of PFAS have previously not been assessed in glaucous gulls from Kongsfjorden. High levels of 11 PFASs were detected in the present study. Widespread distributions of PFSAs and PFCAs have been reported in several trophic levels in the Arctic (Giesy and Kannan, 2001). The present study support that glaucous gulls occupy an apex position in Kongsfjorden and may be vulnerable for bioaccumulation of PFASs. Compared to levels in polar bears, another top predator in the Arctic, the pattern of PFASs found in female glaucous gulls from Kongsfjorden in the present study are consistent with PFASs detected in polar bears (Bentzen et al., 2008). Thus, PFOS were the dominating fluorinated compound in both the present study and the one reported by Bentzen et al., (2008). The present findings of prevailing PFASs levels are in line with studies from the Norwegian and Russian Arctic, such as in ivory gulls (*Pagophila eburnea*) (Miljeteig et al., 2009) and Brünnich guillemot (*Uria lomvia*) eggs from Kongsfjorden (Miljeteig and Gabrielsen, 2010). In addition to glaucous gulls and other seabirds and mammals from the Canadian Arctic (Tomy et al., 2004a).

PFOS continue to be the predominant PFAS detected in fish, reptiles and marine mammals worldwide (Houde et al., 2011), despite the voluntary phase out of PFOS from production and use in the Western countries in 2002, followed by ban within the EU in 2008. PFOS was the most pervasive fluorinated compound detected in the present study. However, the mean plasma concentrations of PFOS were four times higher in mean plasma levels in glaucous gulls sampled at Bjørnøya in 2004 (Verreault et al., 2005b).

The ΣPFCAs was 13 times higher in the study by Verreault et al. (2005b), compared to the present study. There seem to be an indication of geographical difference (Verreault et al., 2005b), or a temporal decline of PFOS. Temporal trends of PFOS demonstrated a weak decrease in seabird eggs of herring gulls and Brünnich's guillemot from Northern Norway and Svalbard sampled from 1983 to 2003 (Helgason et al., 2011). A slight decrease was also reported in Brünnich's guillemots from Kongsfjorden (Miljeteig and Gabrielsen, 2010) and ringed seals (*Phoca hispida*) from the Canadian Arctic (Butt et al., 2007). However, the temporal trends pointed out by Helgason et al. (2012) and Miljeteig and Gabrielsen (2010), indicate increasing levels of other PFASs, such as PFTriA and PFUnA. The slight decrease in PFOS, which is also reflected in industrialized regions (Kato et al., 2011), may be explained by the 3M company ordered phase out of Perfluorooctansulfonyl fluoride (POSF) chemistry (including PFOS), as their production ended in 2002 (3M Company, 2000). In addition, PFOS was listed in annex B of the Stockholm Convention in 2009 (Stockholm Convention, 2011b), resulting in a restriction of use only permitted in specific situations. However, the continuous emissions of PFOS from Chinese industry are of concern, as the slow decrease in nature may stagnate due to this (Butt et al., 2010).

PFOS and PFOA are the most known PFSA and PFCA, respectively (Jensen and Leffers, 2008). High levels of PFOS were detected in all samples of this study, in contrast to PFOA, which was only detected in lower levels and 42 % of the samples. A similar ratio is observed in previous studies on PFASs in glaucous gulls from Bjørnøya (Verreault et al., 2005b). Little is known about how and to what extent OHCs are biotransformed in Arctic seabirds (Helgason et al., 2010). However, studies demonstrate low activity of Cythochrome P450 enzymes in glaucous gull, suggesting the species have a low OHC metabolizing capacity (Henriksen et al., 2000). This may contribute to the accumulation of POPs in glaucous gulls (Henriksen et al., 2000). However, an *in vitro* study demonstrated that rat liver cells are able to biotransform one of the most important precursors in PFOS based products, perfluorooctansulfonamide (PFOSA), into PFOS (Xu et al., 2004). The study by Xu et al, (2004), in combination with the concurrent ratio differences observed between PFOS and PFOA in the present study, may indicate that glaucous gulls or organisms in their food chain are able to biotransform several precursor into PFOS (Tomy et al., 2004b). This may explain why PFOS bioaccumulate in

the environment. However, it is not appropriate to conclude from this as more studies on the glaucous gulls' ability to transform PFASs are needed.

In the present study, the PFCA profile was dominated by odd- and long-chain PFCAs, like PFUnA and PFTriA. Thus, the results show lower concentrations of even-length PFCAs with one less carbon than odd-length PFCAs. The same accumulation profile of PFCAs as in the present study was found in eggs from herring gulls (*Larus argentatus*), collected in northern Norway from 1983 – 2003 (Verreault et al., 2007). The profile is dominated by mean concentration of 4.2 and 2.8 ng/g ww, respectively, compared to 2.5 and 1.8 ng/g ww in the present study. The concentrations were somewhat lower in the present study, both for PFUnA and PFTriA, although one should be aware of the comparison between eggs and plasma in this example. This pattern is suggested to be explained by long-range transport of volatile fluorotelomer alcohols (FTOHs) into the Arctic (Martin et al., 2004). Two of the major sources to PFCAs in Arctic biota can be found as 8:2 FTOH, and 10:2 FTOH, which are found to degrade to PFCAs (Martin et al., 2004). 8:2 FTOH degrade to PFOA and PFNA, which is only detected in lower levels in all samples of this study. However, 10:2 FTOH degrade to PFDcA and PFUnA, which may contribute to the prevalent concentrations of PFUnA detected in the present study. Therefore, long-range transport may to a certain extent explain the deposition of PFASs in the Arctic. However, transportation alone do probably not account for the high levels of PFAS detected in the present study. Most likely, trophic magnification of PFASs along the food chain and exposure to PFAS in winter habitat may better explain the high levels detected in all the samples of the present study (Haukås et al., 2007; Kelly et al., 2009). Further studies are needed to fully elucidate the sources of exposure, in addition to the PFAS pattern observed in the glaucous gulls in the present study, in order to eliminate exposure and risk of effects.

4.3 Thyroid hormone levels

There is a lack of well-established baseline TH levels for glaucous gulls. However, according to McNabb (2000), many avian adults have plasma or serum T4 concentrations ranging from 6 – 19 nmol/L and T3 concentrations ranging from 0.7 – 1.5 nmol/L. In the present study the total T4 ranged from 8.9 – 39.1 nmol/L and the total T3 ranged from 0.89 – 4.37 nmol/L. Considering that several factors influence thyroid levels, from species differences, food availability, iodine access, season, age and

even time of day (McNabb, 2000) – it may not be appropriate to draw conclusions on TH levels being higher or lower in this study compared to the concentrations presented above. A more appropriate comparison can be done with two field studies from Bjørnøya in the Barents Sea, which reports TH levels in breeding glaucous gulls (Verreault et al., 2004; Verreault et al., 2006). The glaucous gulls in the two studies by verreault et al. (2004 and 2006) were sampled during the breeding seasons of 2001 and 2004, and both studies analysed THs using RIA kit from the same producer as in the current study (now known as Siemens medical solution, Diagnostics). The levels are compared in Table 8.

Table 8 A selection of thyroid hormone levels from female glaucous gulls (*Larus hyperboreus*) breeding on Bjørnøya in 2001 and 2004, as reported by Verreault et al. in 2004 and 2007, respectively, and breeding in Kongsfjorden in 2010 (the present study).

Sample year	N	TT3 (nmol/L)	FT3 (pmol/L)	TT4 (nmol/L)	FT4 (pmol/L)	TT4:TT3 ratio
2001	34	1.38 - 5.25	1.25 - 5.90	1.61 - 56.50	11.40 – 65.10	0.54 - 41.0
2004	12	0.90 - 5.10	0.80 - 6.70	13.90 - 31.0	16.70 – 34.30	–
2010	19	0.89 - 4.37	0.86 - 6.14	8.91 - 39.14	7.15 - 43.10	4.96 - 31.16

– : Not reported

Based on this, it can be established that TH levels in the present study agree with former studies on female glaucous gulls from Bjørnøya. The TH analysis is therefore considered to resemble earlier years and the RIA method may be considered as accurate.

4.4 Relationships between biometric variables and OHCs

In the present study, significant negative relationship between the biometric variables and mainly PCBs or BFRs were detected (Table 7). The wing length was inversely correlated with β-HCH, HCB, PCB-47 and PCB-187. The body mass was inversely correlated with *trans*-nonachlor and seven of the PCBs (Table 7).

Reporting effects on biometric variables were not considered as the main focuses of the present study. Since the relationships detected were weak, and the conditions may be random explained, it is not considered appropriate to conclude on any of these findings.

4.5 Relationships between OHCs and thyroid hormones.

In the present study, significant positive associations were found between blood levels of selected PFASs and levels of TT3, TT4 and TT4:TT3 ratio in female glaucous gulls breeding in Kongsfjorden (Table 6). Significant inverse correlations were found between TT4 and BDE-99 and BDE-153, in addition to FT4 and the dioxin-like PCB-167 (Table 7). These findings suggest an exposure effect on THs from the different OHCs.

The OPLS model displayed that the lipid percent was among the most important explanatory variables regarding TT3, along with several of the long-chain PFCAs, namely PFDcA, PFTriA, PFTeA and PFUnA. PCB-101 and the pesticide *p,p'*-DDT was the most important negative predictors of TT3 (Figure 11). These findings suggest that there might be combined effects from the various OHCs on THs.

The lipid percent being such a strong explanatory variable may indicate a relationship between TT3 and physiological conditions or feeding ecology. The lipid percent was a positive predictor for TT3, and a negative predictor for the TT4:TT3 ratio (Figure 12). This indicates a reduction in TT4, compared to TT3, regarding the ratio.

The positive relationship between TT3 and lipid percent observed in the present study, may indicate that the general physiological condition for the birds were good. Better fit birds may forage more or choose pray from different trophic levels than poorer fit birds. Thyroid hormone levels are affected by several physiological and external factors and may vary with body condition and nutritional state of the bird (McNabb, 2000). It may be that the birds with the higher levels of T3 are in better condition, hence having a better nutritional status. It is established that a reduction in body fat reserves mobilizes OHCs stored in body fat and increases levels in tissue and blood (Henriksen et al., 1998). This was supported by significant relationships between body mass and several of the PCBs found in the present study (Table 7). In addition, the incubation period possibly represent a period of energy deprivation for the female gulls due to egg production (Bustnes et al., 2001), followed by increased time spent on the nest (compared to foraging) during the ~27 days of incubation. Female glaucous gulls tend to incubate more than males, 54.4 % compared to 45.5 %, respectively (Bustnes et al., 2001). Therefore, it is challenging to use nutritional status as an explanation for the increased levels of THs found in the present study.

A study of the fatty acid composition in glaucous gulls, where the aim was to establish the species' trophic position compared to other seabirds in Kongsfjorden, displayed great individual differences between individual glaucous gulls (Wold et al., 2011). Some gulls specialise on zooplankton and fish, whereas others forage on seabird eggs and chicks. This can influence the OHC composition in their food, hence the composition in blood and tissue (Bustnes et al., 2000). Brünnich's guillemot and common eider (*Somateria mollissima*) eggs and hatchlings have been found to represent a similar OHC composition as the present study (Murvoll et al., 2007; Miljeteig and Gabrielsen, 2010). Seabird eggs and hatchlings are an important protein and lipid rich food source for glaucous gulls during the breeding season. The positive associations between TT3, lipid percent and several of the PFASs in the glaucous gulls in this study may be better explained by the increase of eggs and chicks to their diet during the nesting period in Kongsfjorden.

On the other hand, the high TT3 levels may represent a biomarker indicating PFAS-mediated endocrine alterations. However, linking responses observed in these glaucous gulls to a specific cause, is one of the major challenges when trying to understand the impacts of OHCs in wildlife, and is not easy to conclude upon (Letcher et al., 2010). In the present study, there are mainly implications of a positive relationship between TT3 and the long-chain PFCAs with ten or more carbons. None of the short chain PFCAs such as PFBA, PFPA or PFHxA were detected. In accordance with the present study, Nøst et al (2011) investigated possible correlations of OHCs and THs in black-legged kittiwake (*Rissa tridactyla*) and Northern fulmar (*Fulmarus glacialis*) chicks from Kongsfjorden in 2006. First, the PFAS profile detected in the study by Nøst et al. (2011) agree with the present study, reporting that the main PFASs detected in these seabirds were PFOS and PFUnA. Second, the correlations found by Nøst et al. (2011) imply that OHCs may disrupt the TH homeostasis in developing seabirds, as well as in adults. The present study implies the same. Furthermore, in accordance with the present study, Nøst et al. (2011) found positive correlations between TT4 and PFNA in both seabird species. This is also in accordance with findings in Northern fulmars from the Canadian Arctic (Braune et al., 2011)

However, it is challenging to elucidate the possible mechanisms of toxicity based on the present biomarker study or the study by Nøst et al. (2011). However, there are some

recent published studies on other species which may explain the observed results in the present study. After a two-generation PFNA exposure study on zebrafish (*Danio rerio*), Liu et al. (2011) reported significantly elevated plasma T3 levels in adults from both generations (F0 and F1) after 180 days of PFNA exposure. In addition, histological changes of the thyroid follicles, elevated TTR levels and decreased UDP-glucuronocyltransferases (UGTs) in the liver was reported by Liu et al. (2011). A down-regulation of UGTs could result in decreased TH elimination through the bile, hence increased TH blood levels (Zoeller et al., 2007). Liu et al. (2011) also reported reduced gene expression of the TH elimination enzymes UGTs. TH disrupting effects were even displayed in third generation F1 zebrafish larvae, as elevated gene expression related to TH synthesis and metabolism. The present study indicates increasing levels of TT3 with increasing levels of long-chain PFCAs. PFNA was included in the OPLS VIP plot as one of the more important variables explaining TT3 variation. However, neither TTR nor UGTs were subject of study in the present thesis. Nevertheless, it is feasible that the findings in zebrafish by Liu et al. (2011) could be extrapolated to the present study as a possible explanation for the correlations found between TT3 and long-chained PFCAs. However, aware of the between species differences, it is recommended that future studies focus on filling the knowledge gaps on the mechanisms of toxicity in avian species from the Arctic.

Significant positive relationships were found between TT3 and PFOS in glaucous gulls from the present study. Shi et al. (2009) explored the disruptive effects of PFOS on the HPT-axis in zebrafish larvae. They discovered that PFOS had the potential to disrupt TH function by interfering with the gene transcriptions that constitutes a part of the HPT-axis. Shi et al. (2009) reported among other factors, that corticotropin-releasing factor (CRF) and thyroid-stimulating hormones (TSH) were significantly up-regulated and down-regulated, respectively. TTR gene expression was down-regulated. They also found that the nuclear thyroid receptors (TR) α and β was up-regulated and down-regulated, respectively. What is especially interesting is that during the experimental exposure, the T4 content remained unchanged, whereas the T3 levels were significantly increased. Also here, it is important to express awareness of between-species disparity. However, the proposed effects of PFOS exerted on HPT-axis reported by Shi et al., may explain or partly explain the positive relationship between TT3 and PFOS obtained in this study. THs exert a wide range of effects on biological function and are important in

all vertebrates, either fish (Power et al., 2001), birds (McNabb, 2000) or humans (Widmaier et al., 2006). Furthermore, several physiological and external factors play a key role in TH function, such as iodine uptake, TH synthesis, deiodination and TH nuclear receptor binding (Yen and Chin, 1994; McNabb, 2007). Considering all these potential routes of toxicity and the numerous possible sites of actions regarding effects of OHCs on THs, it is a challenge to evaluate mechanisms of toxicity in the present study. Furthermore, the potential toxicity of PFASs still remains to be fully elucidated. However, establishing the mechanisms of toxicity in one species may contribute to understand the toxic potential of these contaminants in other species. Furthermore, knowing the mechanisms of toxicity might make it easier to evaluate the physiological and ecological consequences for glaucous gulls exposed to high levels of OHCs in the future.

The significant inverse relationships between TT4 and the BFRs BDE-153 and BDE-99, in addition to FT4 and PCB-167 found in the present study, are consistent with earlier findings in glaucous gulls (Verreault et al., 2006). PBDEs and dioxin-like PCBs are proposed endocrine disrupters by e.g. Boas et al. (2006), as their structural resemblance to THs may interfere with the HPT-axis. The general understanding of the interaction suggests that chlorinated and brominated contaminants may alter TH levels in gulls as well as humans, by competitive binding on TH carrier proteins, like albumin or TTR (Ucan-Marin et al., 2010). It has been suggested that T4 is more susceptible than T3 towards the disturbance of transport by TTR in gull species (Ucan-Marin et al., 2009). Several *in vivo* and *in vitro* studies have reported that organochlorinated and brominated contaminants may outdistance T4 for the binding sites on TTR plasma proteins (Meerts et al., 2000; Hakk and Letcher, 2003; Legler and Brouwer, 2003; Ucan-Marin et al., 2010). Based on this, the results in question support the hypothesis that T4 displaced from TTR binding sites may result in increased T4 excretion through urine or bile (Verreault et al., 2006).

Possible explanations for the decrease in TT4:TT3 ratio may be presented based on a decline in T4 relative to T3. In the present study, levels of T4 dominated over levels of T3, both for the bound and free fraction (Table 5). This is consistent with T4 being the major TH excreted from the thyroid gland, reflected by being the major plasma circulating TH (McNabb, 1992). Deiodinases in the peripheral tissue convert T4 to T3

(Yen and Chin, 1994). However, increasing plasma OHC concentrations are reported to disturb the production, conversion and distribution of THs. For instance, OHCs were found to disturb the conversion of T4 to T3 mediated by mono-deiodinase enzymes (Brouwer et al., 1998). In addition, OHCs were found to interfere directly with the thyroid gland and with several of the TH metabolizing enzymes converting T4 into T3. For instance, UGTs, iodothyronine deiodinases (IDs), and sulfotransferases (SULTs) in both brain and liver, in addition to the plasma transport system of THs. This proposes an explanation for the observed decline in TT4:TT3 ratios in the present study.

Further, Vongphachan et al. (2011) observed an up-regulation of type II deiodothyronine deiodinase (D2) mRNA in neuronal cell cultures from embryonic herring gulls (*Larus argentatus*) and chickens (*Gallus domesticus*) exposed to PFCAs. D2 enzymes convert T4 into the physiologically more active T3. If these *in vitro* results can be physiologically extrapolated, a consequence could be elevated T4 deiodination in D2 containing tissue like the brain, pituitairy gland or muscles, consequently increasing the T3 levels at the site of action (Kogai and Brent, 2005). Consequently, this may explain or partly explain the increased levels of circulating T3 detected in the present study.

Vongphachan et al. (2011) also reported that TTR mRNA expression was down-regulated after administration of PFHxS in concentrations as low as 0.1 μM. One of the major tasks for TTR is to transport T4 into the central nervous system prior to T3 formation (McNabb, 2007). A reduction in TTR could consequently lead to a TH deficiency in target tissue, possibly altering TH depending processes (Vongphachan et al., 2011). Consequently, this may explain or partly explain the decreased levels of circulating TT4 compared to TT3 detected in the present study.

Lipid percent, PCB-52 and BDE-66 were among the most important explanatory variables concerning the TT4:TT3 variation. One significant and one nearly significant decrease in TT4:TT3 ratios were found in two former studies on glaucous gulls, associated with specific organochlorine (OC) classes, like DDTs (Verreault et al., 2004; Verreault et al., 2006). Birds with higher OC exposure had lower levels of plasma T4 and TT4:TT3 ratios, concurrently elevated T3 levels. In the present study, the OPLS predicted significant explanations for TT3 levels (p=0.052), lipids and PFASs were positive explanatory variables, hence *p,p'*-DDT and PCB-101 were negative explanatory variables. These findings may indicate combined effects from the different classes of

contaminants. Diurnal patterns demonstrated in birds, indicate that plasma T4 peak during the dark period and T3 rise to their highest levels during light period (McNabb, 2000). This might be a physiological variation explaining the TH ratios observed in the present study. However, it is not elucidated what the consequence might be for the glaucous gulls if the positive correlations observed in the present study between TT3 and PFASs might have increased the T3 levels additionally. In long-term, a T4 deficiency may result in less conversion of T4 into T3. Such an alteration in TH function may result in behavioral, developmental or reproductive stress (Bustnes et al., 2003a; Bustnes et al., 2003b; Verreault et al., 2004; Bustnes et al., 2005).

Diurnal patterns in avians indicate that plasma T4 peak during the dark period and T3 rise to their highest levels during light period (McNabb, 2000). This might be a physiological variation explaining the TH ratios observed in the present study. However, it is not elucidated what the consequence might be for the glaucous gulls if the positive correlations between TT3 and PFASs observed in the present study cause an additional increase in TT3 levels. In the long-term, a T4 deficiency may result in less conversion of T4 into the physiologically active FT3. Such an alteration in TH function may result in behavioral, developmental or reproductive stress (Bustnes et al., 2003a; Bustnes et al., 2003b; Verreault et al., 2004; Bustnes et al., 2005).

Climate change is considered one of the major stressors to Arctic wildlife (Jenssen, 2006; Letcher et al., 2010). Alterations in climate been displayed through changes in levels and patterns of OHCs in for instance Hudson Bay polar bears (McKinney et al., 2009), explained by changes in sea-ice conditions and consequently a change in polar bear diet. McKinney et al. found that levels of CHLs and PCBs went from decreasing to increasing levels, and PBDEs increased faster than expected due to changes in feeding ecology. Feeding ecology is an important explanatory factor regarding OHC levels and pattern in glaucous gulls as well (Bustnes et al., 2000). It is therefore recommended to further investigate what climate change might cause of alterations in food webs in Kongsfjorden and elsewhere in the Arctic. It is also considered important to assess what climate change might do with bioavailability, toxicity or even biodegradation of OHCs in the Arctic (Letcher et al., 2010).

It is a challenge to detect changes in endocrine endpoints due to the general lack of baseline endocrine information in Arctic species. In addition, most of the toxicological

studies establishing effect thresholds have been assessed in non-Arctic animals. It is not established what additional stressors, such as climate change, combined with anthropogenic contaminants might result in. Therefore, it is tenuous to draw conclusions applicable in wild populations based on individual biomarker studies (Letcher et al., 2010). However, the growing body of evidence is favoring the hypothesis that environmental contaminant exposure, regarding both old and new emerging OHCs, may be linked to endocrine disruptive endpoints in Svalbard glaucous gulls.

5 Concluding Remarks and Further Work

The correlations in the present study between the endocrine endpoints and plasma levels of OHCs indicate an exposure effect on circulating thyroid hormones in female glaucous gulls from Kongsfjorden. The positive correlations between TT3 and the long-chain PFCAs may indicate a disturbance of TH homeostasis. Hence, potential biological hazardous effects may be related to these compounds. It is recommended that future studies evaluate the effect mechanisms behind these potential effects on circulating THs. Surprisingly; it was the minor levels of PFCAs that displayed the highest potency regarding effects on THs and explained more of the variation in TT3 than the higher concentrations of e.g. PCBs or PFOS. Based upon the indications of endocrine disruptive effects from PFASs in the present study, the lack of control on production and use are of concern.

High levels of OHCs were detected in female glaucous gulls from Kongsfjorden compared to other seabirds breeding in the area, such as common eiders and Brünnich guillemots (both birds and eggs) (Murvoll et al., 2007; Miljeteig and Gabrielsen, 2010). However, the levels were in general a little lower in this particular study of glaucous gulls, compared to former studies on glaucous gulls from Bjørnøya (Bustnes et al., 2010; Verreault et al., 2010). The results in question may indicate geographical differences in OHC exposure between glaucous gull populations or they may be in agreement with studies that show slowly decreasing levels of some legacy POPs (Braune et al., 2005; De Laender et al., 2011). The mechanisms behind the possible interactions between THs and OHCs, as observed in the present study, may be explained or partly explained by increased TH production, increased conversion of T4 into T3, increased excretion of T4 relative to T3 or inhibited metabolism of TH.

In elucidation of the indications that the Arctic and Kongsfjorden environment is changing due to alterations in climate, it is important to be a step ahead to assess what changes in environmental factors can cause of stress on wildlife. E.g. increased sea or air temperatures or reduced ice-cover, in addition to anthropogenic stressors as new hazardous chemicals (e.g. siloxane, PFCAs) may elicit new or expanding effects in biota.

Only future surveys may display the potential impacts from climate change on the bioavailability, degradation or effects of OHCs in Arctic wildlife (Letcher et al., 2010).

The rapid replacement of established hazardous chemicals (de Wit et al., 2010; Stockholm Convention, 2011b), point out the importance of regulations on production and use of chemicals by national and international environmental protection agencies.

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APPENDIX A: INTERNAL STANDARD POP I AND BROM I

Table A.1: Constituents in the internal standard including POP I and BROM I, added during contaminant analysis of PCBs, DDTs and chlorinated pesticides in glaucous gull (*Larus hyperboreus*) plasma samples.

Compound group	^{13}C Compounds
OCPs	^{13}C α-HCH (Hexachlorocyclohexane)
	^{13}C β-HCH
	^{13}C γ-HCH
	^{13}C HCB (hexachlorobenzene)
	^{13}C *trans*-chlordane
	^{13}C *cis*-chlordane
	^{13}C *trans*-nonachlor
	^{13}C *cis*-nonachlor
	^{13}C *trans*-chlordane
	^{13}C *oxy*chlordane
	^{13}C *p, p'- DDT*
	^{13}C *p,p'- DDE*
	^{13}C Mirex
PCBs	^{13}C PCB 28
	^{13}C PCB 52
	^{13}C PCB 101
	^{13}C PCB 105
	^{13}C PCB 118
	^{13}C PCB 138
	^{13}C PCB 153
	^{13}C PCB 167
	^{13}C PCB 180
PBDEs	^{13}C PBDE 28
	^{13}C PBDE 47
	^{13}C PBDE 99
	^{13}C PBDE 153
	^{13}C PBDE 183
	^{13}C PBDE 209

APPENDIX B: DETECTION LIMITS FOR OCPS, PCBS AND PBDES

Table B.1: Upper and lower detection limits for the OCP-, PCB- and PBDE analysis in glaucous gull (*Larus hyperboreus*) plasma. LOD was calculated as 3xBlank. Limit of quantification (LOQ) was calculated as 3xSignal/noise.

Detection limits			
Compound	**ng/mL**	**Compound**	**ng/mL**
o,p'- DDT	0.04 - 0.06	PCB-118	0.03 - 0.12
p,p'- DDT	0.04 - 0.06	PCB-128	0.05 - 0.18
o,p'- DDE	0.02 - 0.08	PCB-138	0.03 - 0.16
p,p'- DDE	0.01 - 0.13	PCB-149	0.03 - 0.14
o,p'- DDD	0.02 - 0.03	PCB-153	0.03 - 0.13
p,p'- DDD	0.02 -0.04	PCB-156	0.04 - 0.12
α-HCH	0.01 - 0.02	PCB-157	0.03 - 0.10
β-HCH	0.02 -0.08	PCB-167	0.04 - 0.12
γ-HCH	0.01- 0.02	PCB-170	0.06 - 0.20
HCB	0.4 - 0.9	PCB-180	0.06 - 0.22
Heptachlor	0.01 -0.03	PCB-183	0.06 - 0.17
cis-chlordane	0.01 - 0.05	PCB-187	0.05 - 0.19
trans-chlordane	0.01 -0.02	PCB-189	0.05 - 0.19
*oxy*chlordane	0.01- 0.02	PCB-194	0.05 - 0.19
cis-nonachlor	0.03 - 0.05	PBDE-28	0.001 -0.01
trans-nonachlor	0.01- 0.02	PBDE-47	0.01-0.02
Mirex	0.01- 0.02	PBDE-66	0.001 - 0.01
PCB-28	0.01 - 0.11	PBDE-85	0.001 -0.01
PCB-47	0.01 - 0.04	PBDE-99	0.01-0.05
PCB-52	0.01 - 0.06	PBDE-100	0.001 -0.01
PCB-66	0.01 - 0.04	PBDE-138	0.01-0.04
PCB-74	0.01 - 0.04	PBDE-153	0.01-0.05
PCB-99	0.01 - 0.10	PBDE-154	0.01-0.02
PCB-101	0.01 - 0.12	PBDE-183	0.01-0.02
PCB-105	0.03 - 0.12	PBDE-209	0.32 - 0.35

APPENDIX C: RECOVERIES OCPS, PCBS & PBDES

Table C.1: Measured recoveries (%) of the different internal standards added during the analysis of organochlorinated pesticides, PCBs and PBDEs in glaucous gull plasma (*Larus hyperboreus*). The %recoveries are presented as minimum and maximum.

Compound	Recovery (%)
13C *p,p'*-DDE	68 -91
13C *p,p'*-DDT	49 - 70
13C α-HCH	37 - 61
13C β-HCH	30 - 48
13C γ-HCH	38 - 60
13C HCB	31 - 99
13C *trans*-chlordane	46 -70
13C *trans*-nonachlor	43 - 66
13C PCB 28	42 - 61
13C PCB 52	45 - 70
13C PCB 101	46 - 73
13C PCB 118	43 - 69
13C PCB 138	47 - 70
13C PCB 153	51 - 71
13C PCB 167	48 - 70
13C PCB 180	50 - 70
13C PBDE 28	72 - 124
13C PBDE 47	63 -116
13C PBDE 99	66 - 133
13C PBDE 153	60 - 131
13C PBDE 183	56 - 142
13C PBDE 209	9 -94

APPENDIX D: INTERNAL STANDARD PFASS

Table D.1: Constituents in the internal standard added during contaminant analysis of PFASs in glaucous gull (*Larus hyperboreus*) plasma samples.

^{13}C PFASs
13C4 PFBA
13C5 PFPA
13C5 PFHxA
13C4 PFHpA
13C4 PFOA
13C5 PFNA
13C6 PFDcA
13C7 PFUnA
13C2 PFDoA
13C3 PFHxS
13C4 PFOS
13C8 PFOSA

APPENDIX E: DETECTION LIMITS FOR PFASS

Table E.1: Limits of detection (LOD) for the PFAS analysis in glaucous gull (*Larus hyperboreus*) plasma. LOD was calculated as 3xBlank. Limit of quantification (LOQ) was calculated as 3xSignal/noise.

Compound	Detection limits (ng/mL)
PFBA	0.301
PFPA	0.326
PFHxA	0.831
PFHpA	0.524
PFOA	0.485
PFNA	0.014
PFDcA	0.196
PFUnA	0.0003
PFDoA	0.132
PFTriA	0.027
PFTeA	0.157
PFBS	0.001
PFHxS	0.0001
PFOS lin	0.0001
PFDcS	0.001
4:2 FTS	0.004
6:2 FTS	0.016
8:2 FTS	0.394
PFOSA	0.056

APPENDIX F: RECOVERIES PFASS

Table F.1: Measured recoveries (%) of the different internal standards added during the per- and polyfluoroalkyl substance analysis, presented as mean ± SD, and minimum and maximum levels.

Compound	Mean ± SD	Min - Max
13C PFBA	47.9 ± 9.2	29-62
13C PFPA	50.7 ± 9.9	29-64
13C PFHXA	48.0 ± 9.1	28-65
13C PFHpA	49.9 ± 9.1	30-63
13C PFOA	49.3 ± 9.6	28-62
13C PFNA	55.7 ± 12.1	20-69
13C PFDA	53.1 ± 9.5	29-63
13C PFUNA	55.5 ± 9.6	27-65
13C PFDOA	53.5 ± 8.4	21-61
13C PFHXS	40.8 ± 7.6	24-56
13C PFOS	41.4 ± 6.9	26-51
13C PFOSA	46.4 ± 8.5	22-58

APPENDIX G: INDIVIDUAL BIOMETRIC MEASUREMENTS

Table G.1 Individual biometric measurements of female glaucous gulls (*Larus hyperboreus*) included in the present study. They were captured during the breeding season of 2010 in Kongsfjorden, Svalbard.

ID	Capture date	Body mass (g)	Head (mm)	Bill (mm)	Gonis (mm)	Wing (mm)	BCI
GG1-2010	08.06.2010	1355	133.8	55.3	20.5	455	-1.003
GG3-2010	09.06.2010	1380	138.9	60.6	20.9	463	-1.102
GG5-2010	09.06.2010	1400	135.1	57.9	20.9	460	-0.699
GG7-2010	10.06.2010	1480	139.1	57.8	21.1	466	-0.044
GG8-2010	10.06.2010	1410	136.0	57.5	21.2	444	0.123
GG9-2010	10.06.2010	1525	140.7	55.4	21.9	470	0.302
GG12-2010	12.06.2010	1585	146.0	61.4	23.1	462	1.329
GG16-2010	14.06.2010	1535	133.3	55.1	21.6	452	1.281
GG17-2010	14.06.2010	1535	139.6	61.4	23.0	473	0.297
GG18-2010	14.06.2010	1300	132.9	59.3	20.6	447	-1.297
GG19-2010	16.06.2010	1585	137.6	58.0	21.6	468	1.131
GG20-2010	16.06.2010	1445	137.7	58.4	20.7	468	-0.539
GG21-2010	18.06.2010	1440	137.4	57.6	21.2	444	0.469
GG22-2010	18.06.2010	1510	134.8	56.2	20.6	442	1.414
GG23-2010	19.06.2010	1345	138.8	60.6	22..0	451	-0.986
GG24-2010	19.06.2010	1505	133.0	57.7	19.7	457	0.703
GG26-2010	19.06.2010	1440	138.0	57	21.3	465	-0.468
GG27-2010	19.06.2010	1500	134.8	55.9	21.0	455	0.718
GG28-2010	19.06.2010	1420	137.0	56.4	20.8	460	-0.476

APPENDIX H: INDIVIDUAL THYROID HORMONE CONCENTRATIONS

Table H.1 Individual concentrations of thyroid hormones, total and free thyroxine and total and free thriiodothyronine in nmol/L and pmol/L, respectively, from female glaucous gulls (*Larus hyperboreus*) breeding in Kongsfjorden.

ID	TT4 (nmol/L)	FT4 (pmol/L)	TT3 (nmol/L)	FT3 (pmol/L)	TT4:TT3 molar ratio
GG1-2010	8.91	8.84	0.92	0.86	9.64
GG3-2010	13.43	13.23	2.71	3.18	4.96
GG5-2010	23.46	31.09	0.92	2.82	25.63
GG7-2010	20.45	18.45	1.82	2.19	11.21
GG8-2010	33.18	23.43	3.73	4.46	8.91
GG9-2010	25.16	31.47	1.55	2.80	16.24
GG12-2010	31.51	30.36	1.11	2.04	28.49
GG16-2010	17.49	16.75	1.15	1.81	15.16
GG17-2010	27.19	23.08	4.37	6.14	6.23
GG18-2010	19.94	24.90	0.90	2.16	22.25
GG19-2010	9.87	7.16	1.78	2.23	5.54
GG20-2010	18.45	13.89	1.11	1.09	16.69
GG21-2010	19.93	15.74	2.27	3.34	8.77
GG22-2010	9.90	11.15	1.18	1.23	8.39
GG23-2010	26.49	21.76	2.09	3.80	12.62
GG24-2010	29.45	26.56	0.95	1.56	31.16
GG26-2010	25.72	19.04	2.25	3.15	11.43
GG27-2010	39.14	43.12	1.39	2.02	28.14
GG28-2010	32.22	30.00	2.21	5.08	14.56

APPENDIX I: INDIVIDUAL OHC CONCENTRATIONS

Table I.1 Individual organohalogen contaminant concentrations (ng/g ww) measured in plasma from female glaucous gulls (*Larus hyperboreus*) included in the present study. They were captured during the breeding season of 2010 in Kongsfjorden, Svalbard.

ID	Lipids (mg/dl)	Lipid%	β-HCH	HCB	*trans*-chlordane	*oxy*chlordane	*trans*-nonachlor	*cis*-nonachlor	Mirex	*p,p'*-DDT	*p,p'*-DDE
1	1217.2	1.217	0.418	11.488	0.828	4.442	1.964	0.965	6.873	0.202	83.685
3	1667.8	1.668	0.433	9.482	0.585	0.270	1.560	1.126	4.032	0.313	48.911
5	1048.1	1.048	0.187	5.088	0.288	0.046	0.191	0.254	0.985	–	22.924
7	1624.2	1.624	0.344	7.530	0.355	0.896	0.412	0.846	7.780	0.212	72.785
8	1817.1	1.817	0.721	13.536	0.592	0.312	0.998	1.020	3.168	–	56.413
9	952.9	0.953	0.319	6.232	0.413	1.541	1.087	0.825	2.166	0.354	30.486
12	1524.0	1.524	0.549	10.352	0.539	3.212	0.927	1.383	1.905	0.264	70.059
16	1341.7	1.342	0.197	5.790	0.270	1.429	0.834	0.834	1.240	0.098	17.744
17	1904.5	1.905	0.277	6.546	0.369	3.403	0.659	0.539	2.280	–	32.083
18	1300.0	1.300	0.937	16.642	1.075	8.487	3.477	2.048	8.900	1.320	133.012
19	1658.6	1.659	0.192	4.288	0.183	0.690	0.395	0.226	1.386	0.128	16.982
20	1068.4	1.068	–	3.832	0.157	0.655	0.166	0.386	1.344	–	18.832
21	1485.3	1.485	0.562	10.419	0.361	2.268	0.451	0.688	4.244	0.226	67.333
22	1704.6	1.705	0.458	8.214	0.479	0.783	0.926	0.394	2.131	–	69.731
23	1138.6	1.139	0.226	4.969	0.228	0.653	0.340	0.582	0.830	0.071	13.769
24	1030.1	1.030	0.261	7.742	0.505	2.993	1.302	0.709	5.507	0.445	52.854
26	1374.4	1.374	0.289	8.764	0.517	2.260	1.112	1.090	4.523	0.318	56.624
27	1358.9	1.359	0.381	8.178	0.554	0.987	1.078	1.596	1.023	0.229	32.274
28	1280.4	1.280	0.225	4.610	0.217	0.390	0.440	0.521	0.775	0.061	17.924

– : Not detected.

Table I.2 Individual organohalogen contaminant concentrations (ng/g ww) measured in plasma from female glaucous gulls (*Larus hyperboreus*) included in the present study. They were captured during the breeding season of 2010 in Kongsfjorden, Svalbard.

ID	PCB-28/31	PCB-47	PCB-52	PCB-66	PCB-74	PCB-99	PCB-101	PCB-105	PCB-118	PCB-128	PCB-138	PCB-149
1	0.333	0.810	0.109	1.768	1.815	11.526	–	6.472	25.805	6.078	44.730	0.459
3	0.344	0.673	0.557	1.597	1.260	8.307	1.572	4.207	15.208	3.748	38.431	0.792
5	0.161	0.478	0.043	0.833	0.624	3.998	0.055	1.979	6.593	1.993	14.397	0.221
7	0.225	0.466	0.144	1.187	1.180	8.479	1.408	4.888	20.753	4.265	39.898	0.657
8	0.324	0.812	–	1.544	1.402	8.167	0.051	4.157	15.782	4.016	27.301	0.375
9	0.238	0.391	0.654	0.936	0.782	4.003	0.811	2.100	8.262	1.962	16.915	0.442
12	0.396	0.983	0.531	1.654	1.287	10.482	1.301	4.362	13.907	4.772	34.666	0.604
16	0.162	0.309	0.375	0.642	0.578	2.855	0.305	1.802	5.948	1.635	10.813	0.263
17	0.285	0.480	0.065	1.004	0.818	4.690	–	2.257	8.033	2.576	18.922	0.425
18	0.625	1.434	1.357	2.858	2.711	21.271	2.870	8.570	30.788	8.963	74.817	1.610
19	0.148	0.284	0.150	0.531	0.470	2.717	0.476	1.340	4.813	1.376	11.646	–
20	0.151	0.256	0.162	0.507	0.487	2.620	0.396	1.354	5.612	1.476	12.007	0.236
21	0.306	0.678	0.131	1.364	1.286	7.996	0.604	3.730	14.083	4.414	35.879	0.557
22	0.212	0.596	–	0.974	0.928	6.687	–	2.539	9.771	2.628	24.437	0.241
23	0.174	0.301	–	0.631	0.523	2.584	0.266	1.349	4.610	1.199	9.582	0.251
24	0.291	0.608	0.649	1.458	1.290	7.975	1.784	4.312	17.616	4.755	37.586	0.628
26	0.308	0.490	0.624	1.419	1.281	7.066	0.840	4.126	15.475	4.059	28.451	0.533
27	0.353	0.604	1.188	1.357	0.992	4.212	1.942	2.298	7.211	1.797	14.772	0.813
28	0.146	0.239	0.330	0.457	0.399	2.013	0.342	0.917	3.305	0.936	7.893	0.338

– : Not detected.

Table I.3 Individual organohalogen contaminant concentrations (ng/g ww) measured in plasma from female glaucous gulls (*Larus hyperboreus*) included in the present study. They were captured during the breeding season of 2010 in Kongsfjorden, Svalbard.

ID	PCB-153	PCB-156	PCB-157	PCB-167	PCB-170	PCB-180	PCB-183	PCB-187	PCB-189	PCB-194
1	102.246	5.611	1.285	2.900	18.895	60.590	7.573	5.390	0.899	8.706
3	65.305	3.037	0.706	1.996	10.447	32.936	4.953	5.957	0.388	4.455
5	21.549	1.044	0.312	0.572	2.851	8.280	1.420	2.637	0.128	0.925
7	91.517	4.806	1.157	2.513	15.975	54.239	6.536	3.932	0.734	8.130
8	54.769	2.588	0.666	1.420	8.697	27.654	3.913	4.533	0.439	3.721
9	31.618	1.785	–	1.032	5.924	20.088	2.659	3.249	0.261	2.797
12	44.675	1.907	0.543	1.095	5.538	15.939	2.783	5.191	0.157	1.683
16	19.690	1.081	0.305	0.623	2.629	8.613	1.265	1.666	0.172	1.251
17	34.392	1.563	–	0.930	4.516	14.901	2.325	2.898	0.228	1.825
18	228.514	7.998	2.113	3.164	66.283	166.351	11.578	9.950	2.967	26.104
19	21.553	1.063	–	0.590	3.482	11.200	1.595	1.601	0.166	1.484
20	21.540	1.126	0.270	0.577	3.689	10.820	1.675	1.720	–	1.585
21	61.165	2.573	0.645	1.654	10.176	33.632	5.369	7.831	0.413	4.515
22	38.311	1.439	–	0.826	4.969	16.423	3.267	6.243	0.250	2.017
23	16.465	0.796	–	0.434	3.055	8.138	1.206	1.521	0.207	0.987
24	82.902	4.295	1.061	2.807	15.555	55.962	6.346	3.806	0.817	7.464
26	63.674	3.332	0.908	1.953	9.866	31.025	4.188	2.767	0.480	3.923
27	19.724	1.009	0.321	0.589	2.475	7.319	1.303	3.026	0.079	0.843
28	13.188	0.572	–	0.361	1.570	5.877	0.974	1.936	–	0.967

–: Not detected.

Table I.4 Individual organohalogen contaminant concentrations (ng/g ww) measured in plasma from female glaucous gulls (*Larus hyperboreus*) included in the present study. They were captured during the breeding season of 2010 in Kongsfjorden, Svalbard.

ID	BDE-47	BDE-66	BDE-99	BDE-100	BDE-153	BDE-154	PFNA	PFDcA	PFUnA	PFDoA	PFTriA	PFTeA	PFHxS	PFOS
1	1.363	0.003	0.139	0.322	0.234	0.173	1.330	0.610	1.820	0.480	0.720	0.290	0.820	6.380
3	1.447	0.012	0.186	0.324	0.244	0.153	3.790	3.390	15.670	3.430	11.730	2.530	1.310	34.170
5	1.247	0.001	0.187	0.249	0.345	0.102	0.630	0.420	1.010	0.320	0.700	0.170	27.120	429.810
7	0.985	0.009	0.149	0.212	0.198	0.119	2.400	1.260	2.970	0.910	2.750	0.680	0.950	10.670
8	1.397	0.002	0.118	0.279	0.157	0.151	1.740	1.000	2.680	0.550	1.890	0.450	0.650	8.830
9	1.053	0.009	0.112	0.221	0.146	0.095	1.320	0.800	3.070	0.580	1.630	0.200	0.350	4.870
12	1.740	0.021	0.094	0.344	0.082	0.183	1.770	0.800	1.430	0.390	0.760	0.160	0.600	6.040
16	0.675	0.007	0.092	0.137	0.093	0.087	1.070	0.490	1.130	0.390	0.940	0.220	1.230	9.640
17	1.551	0.006	0.247	0.273	0.340	0.131	1.150	0.820	2.400	0.610	1.750	0.370	0.970	15.830
18	3.905	0.041	0.518	0.730	0.340	0.384	1.100	0.700	2.260	0.550	1.310	0.240	0.730	9.100
19	1.122	0.006	0.265	0.192	0.229	0.100	1.050	0.520	1.190	0.370	1.100	0.220	0.930	8.810
20	0.664	0.004	0.065	0.138	0.041	0.066	1.340	0.590	1.230	0.380	0.760	0.160	0.340	5.290
21	1.587	0.009	0.229	0.339	0.218	0.204	1.200	0.440	1.860	0.510	1.670	0.340	0.580	6.330
22	1.448	0.002	0.352	0.276	0.301	0.128	0.690	0.420	1.060	0.440	1.310	0.330	0.650	5.450
23	0.889	0.002	0.046	0.162	0.029	0.085	1.560	0.760	1.710	0.320	0.880	0.170	0.570	6.540
24	1.639	0.013	0.152	0.324	0.184	0.166	0.960	0.550	1.210	0.340	0.570	0.160	0.570	3.450
26	1.081	0.018	0.167	0.264	0.142	0.169	1.600	0.540	1.400	0.350	1.030	0.220	0.430	6.660
27	1.557	0.017	0.117	0.295	0.058	0.154	3.140	0.820	1.580	0.300	0.920	0.160	0.790	8.840
28	0.510	0.004	0.063	0.095	0.058	0.046	1.330	0.590	1.220	0.440	1.040	0.300	0.380	4.600

APPENDIX J: PCA LOADINGS

Table J.1: PCA loadings for the individual variables in the PCA analysis, resulting in six significant Primcipal Components.

	Principal Components					
	1 (50.7 %)	**2 (23.1 %)**	**3 (11.8 %)**	**4 (7.4 %)**	**5 (5.8 %)**	**6 (1.1 %)**
Weight	-0.766	-0.331	-0.007	0.366	0.197	-0.314
Head	-0.077	0.246	0.274	0.832	0.011	-0.332
Wing	-0.210	0.315	0.221	0.419	0.741	0.079
TT4 (nmol/L)	-0.178	-0.770	0.388	0.322	0.175	0.122
FT4 (pmol/L)	-0.027	-0.728	0.568	0.065	0.124	-0.036
TT3 (nmol/L)	-0.179	0.722	0.050	0.339	-0.236	0.501
FT3 (pmol/L)	0.146	0.557	-0.001	0.336	-0.500	0.494
Lipid%	-0.075	0.771	0.310	0.371	-0.209	-0.244
Trans-chlordane	0.870	-0.217	0.336	-0.237	0.011	-0.014
oxychlordane	0.489	-0.720	-0.373	0.041	-0.145	-0.222
transnonachlor	0.614	-0.248	0.371	-0.486	0.144	0.104
Cis-nonachlor	0.566	-0.306	0.624	-0.198	-0.081	-0.180
Mirex	0.753	0.311	-0.491	0.104	0.243	0.123
ppDDT	0.956	-0.202	0.071	-0.085	0.122	0.121
ppDDE	0.903	0.032	-0.110	0.387	0.009	-0.091
HCB	0.913	-0.113	0.136	-0.034	-0.313	-0.141
bHCH	0.804	0.008	0.275	0.185	-0.422	-0.234
PCB28	0.844	-0.240	0.433	0.124	-0.150	0.039
PCB47	0.854	-0.203	0.318	0.175	-0.178	-0.197
PCB52	0.327	-0.503	0.587	-0.382	0.216	0.173
PCB-66	0.955	-0.158	0.216	-0.049	-0.059	-0.059
PCB74	0.983	-0.071	0.040	0.149	-0.008	0.050
PCB99	0.951	0.042	-0.032	0.247	0.045	-0.161
PCB-101	0.763	-0.201	0.404	-0.228	0.289	-0.041
PCB-105	0.940	0.104	-0.126	0.218	0.178	-0.081
PCB-128	0.910	0.026	-0.248	0.280	0.076	-0.093
PCB-138	0.922	0.187	-0.154	0.253	0.105	-0.058
PCB-149	0.881	-0.037	0.379	-0.027	0.052	0.063
PCB-153	0.915	0.163	-0.320	0.057	0.175	-0.004
PCB-156	0.847	0.196	-0.385	0.052	0.297	0.057
PCB-157	0.899	-0.023	-0.264	-0.180	0.206	-0.065
PCB-170	0.919	0.135	-0.333	-0.043	0.148	0.003
PCB-180	0.881	0.165	-0.398	-0.030	0.183	0.034

PCB-183	0.857	0.248	-0.386	0.127	0.148	0.078
PCB-187	0.844	0.182	0.070	0.216	-0.337	-0.103
PCB-189	0.820	0.134	-0.489	-0.178	0.183	0.030
PCB-194	0.854	0.198	-0.431	-0.100	0.174	0.024
PBDE-28	0.650	-0.227	0.534	0.102	0.120	0.388
PBDE-47	0.886	-0.286	0.255	-0.006	-0.168	-0.050
PBDE-66	0.744	-0.441	0.388	0.012	0.073	-0.009
PBDE-99	0.894	0.109	-0.161	-0.231	-0.247	0.180
PBDE-100	0.923	-0.204	0.228	0.062	-0.171	0.045
PBDE-153	0.841	0.384	-0.268	-0.240	-0.083	0.050
PBDE-154	0.912	-0.270	0.096	0.083	-0.272	0.065
PFNA	-0.145	0.516	0.746	0.198	0.158	0.146
PFDcA	0.148	0.741	0.561	-0.006	0.314	-0.026
PFUnA	0.277	0.852	0.387	-0.071	0.052	0.111
PFDoA	0.226	0.922	0.229	-0.082	0.079	0.003
PFTriA	0.164	0.944	0.250	-0.062	-0.047	0.101
PFTeA	0.122	0.969	0.149	-0.055	0.050	0.093
PFHxS	-0.255	0.610	0.275	-0.580	0.057	-0.359
PFOS	0.025	0.810	0.485	-0.287	-0.059	-0.017
Egg	-0.176	-0.684	0.284	0.419	0.354	0.309
Date	-0.118	-0.721	-0.207	0.007	-0.208	0.606