Sveriges lantbruksuniversitet
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Perfluoroalkyl substances in Arctic birds

- A comparison between glaucous gulls and black guillemots from Svalbard

Siri Axelson

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Perfluoroalkyl substances in Arctic birds – a comparison between glaucous gull and black guillemot from Svalbard

Perfluoroalkylerade ämnen i arktiska fåglar – en jämförelse mellan vittrut och tobisgrissla från Svalbard

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These months have been challenging and very intensive but also a lot of fun. I have gained a lot of knowledge and experience, and I hope that I can give you something in return with this thesis. Enjoy!

Abstract

Perfluoroalkyl substances (PFASs) are ubiquitous in the environment today and they have been detected even in remote areas such as the Arctic. PFASs can be transported by the atmosphere and ocean currents but the transport mechanism is not fully understood. PFASs may be harmful to organisms due to their persistence in the environment, bio-accumulation potential and toxicity. Studies have shown that PFASs can cause adverse effects on the metabolism as well as the endocrine- and reproduction systems in organisms. In this study, PFASs were investigated in glaucous gull (*Larus hyperboreus)* (*n*=5) and black guillemot (*Cepphus grylle)* (*n*=4) from Svalbard. In glaucous gull, the mean ∑PFAS concentrations were 147 ng g^{-1} in liver and 15 ng g^{-1} in muscle. In black guillemot, the mean ∑PFAS concentrations were 36 ng g^{-1} in liver and 2.5 ng g^{-1} in muscle. Perfluorooctane sulfonate (PFOS) was the most abundant compound, constituting in average 72 % of the ∑PFASs. The mean PFOS concentration was more than four times higher in glaucous gull than in black guillemot. This can be explained by the glaucous gull's high trophic level, migration patterns to more industrialized areas, and omnivorous feeding patterns. The concentration levels were higher in liver than in muscle tissue for both species, which complies with other similar studies. This might be due to the fact that PFAS typically accumulate in protein-rich tissues with high blood content, and that the liver has a detoxifying function and takes care of the contaminants in the body.

As PFASs are produced in industrial areas far away from Svalbard, the detected concentration levels in the studied species give reason to further investigate the fate and transport of PFASs, as well as their effects on wildlife in the Arctic region.

Sammanfattning

Perfluouroalkylerade ämnen (PFASs) är en grupp föreningar varav många är allmänt förekommande i miljön idag. Dessa ämnen har upptäckts även i avlägsna områden såsom Arktis. Mekanismerna för transporten av PFASs till Arktis är inte helt fastställda, men troligtvis transporteras de via luft och havsströmmar. PFASs kan vara skadliga för organismer och för miljön då de är persistenta, bioackumulerande och har påvisats ha negativa effekter på organismer.

I denna studie undersöktes koncentrationer av PFASs i vittrut (*Larus hyperboreus)* (*n*=5) och tobisgrissla (*Cepphus grylle)* (*n*=4) från Svalbard. I vittrut var de genomsnittliga ∑PFAS koncentrationerna 147 ng g^{-1} i lever och 15 ng g^{-1} i muskel. I tobisgrissla var de genomsnittliga ∑PFAS koncentrationerna 36 ng g^{-1} och 2.5 ng g^{-1}. PFOS var den dominerande föreningen i denna studie; den utgjorde i genomsnitt 72 % av ∑PFASs. Koncentrationsnivåerna av PFOS var mer än fyra gånger så höga i vittrut än i tobisgrissla. Detta kan förklaras av dess höga trofiska nivå, dess migration till mer industrialiserade områden, samt dess opportunistiska matvanor. De genomsnittliga koncentrationerna av PFASs var nämnvärt högre i lever- än i muskelvävnad i båda arterna, vilket stämmer överens med liknande studier. Detta beror troligen på att PFASs i huvudsak ackumuleras i proteinrika vävnader med högt blodinnehåll, samt att levern är ett renande organ som bland annat tar hand om gifter och främmande ämnen.

PFASs tillverkas huvudsakligen i industriella områden långt bort från Svalbard. Ändå påvisades de i relativt höga halter i de undersökta fåglarna. Detta ger skäl till att fortsätta forska inom området och ta reda på mer om PFASs transportvägar och effekter på miljön och organismer.

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Abbreviations

ECF	electrochemical fluorination
FOSA	perfluorooctanesulfonamide
FOSAA	perfluorooctanesulfonamidoacetic acid
FOSE	perfluorosulfonamide ethanol
HPLC	high performance liquid chromatography
ISTD	internal standard
MDL	method detection level
MS	mass spectrometry
N-EtFOSA	N-ethylperfluorooctanesulfonamide
N-EtFOSAA	N-ethylperfluorooctanesulfonamidoacetic acid
N-EtFOSE	N-ethylperfluorooctanesulfonamido-ethanol
N-MeFOSA	N-methylperfluorooctansulfonamide
N-MeFOSAA	N-methylperfluorooctanesulfonamidoacetic acid
N-MeFOSE	N-methylperfluorooctanesulfonamido-ethanol
PFBA	perfluorobutanoate
PFBS	perfluorobutane sulfonate
PFCAs	perfluorocarboxylates
PFDA	perfluorodecanoate
PFDoDA	perfluorododecanoate
PFDS	perfluorodecane sulfonate
PFHxA	perfluorohexanoate
PFHxDA	perfluorohexadecanoate
PFHxS	perfluorohexane sulfonate
PFHpA	perfluoroheptanoate
PFNA	perfluorononanoate
PFOA	perfluorooctanoate
PFOcDA	perfluorooctadecanoate
POP	persistent organic pollutant

PFOS	perfluorooctane sulfonate
PFSAs	perfluorosulfonates
PFPeA	perfluoropentanoate
PFTeDA	perfluorotetradecanoate
PFTriDA	perfluorotridecanoate
PFUnDA	perfluoroundecanoate
RSTD	recovery standard
USB	ultrasonic bath
6:2 FTS	6:2 fluorotelomer sulfonate

1. Introduction

1.1 Perfluoroalkyl substances in the environment

A large amount of man-made chemicals have emerged during the last fifty years (Briggs, 2003). Many of these chemicals are prevalent in the environment today, in industrial regions as well as remote areas such as the Arctic (Giesy & Kannan, 2001). Perfluoroalkyl substances (PFASs) comprise an array of organic fluorinated compounds. They are considered as persistent organic pollutants (POPs) due to their persistence, bioaccumulation potential, long-range transport and potential adverse effects in the environment (Dietz et al, 2008). However, PFASs are different to classical POPs. POPs normally accumulate in lipid-rich tissues in organisms. In contrast, PFASs bind to blood protein and accumulate in liver or other protein-rich tissues. Additionally, the transport pathway for most PFASs is different from many classical POPs. POPs are generally semi-volatile and can travel long distances through the atmosphere (Butt et al, 2010). PFASs can be transported directly by ocean currents as well as indirectly by atmospheric long range transport. It has been suggested that volatile precursors of PFASs undergo long range atmospheric transport and then degrade to PFASs in the Arctic Regions (Muir & de Wit, 2010). Many of these precursors have been produced in a large amount, and are primarily used for industrial processes and for the synthesis of other fluorochemical products (3M Company, 1999). PFASs have been manufactured and used globally for more than 50 years (Butt et al, 2010). Due to the extensive use of PFASs, some of the compounds are distributed worldwide in humans as well as in animals and water (Giesy & Kannan, 2001; Olsen et al, 2007; Yamashita et al 2005).

1.2 Properties and structure

PFASs are amphipathic; they consist of both hydrophobic and hydrophilic groups. Furthermore, they have very high surface activity and thermal stability (Yamashita et al, 2005). This makes them suitable for a large variety of applications. The chemical structure of PFASs renders their unique properties. PFASs consist of a carbon chain where all carbon-hydrogen (C–H) bonds have been replaced by carbon-fluorine (C–F) bonds. The carbon-fluorine bond is one of the strongest bonds known in organic chemistry, due to its high electronegativity. This strong bond also implies that PFASs are persistent compounds (Lau et al, 2007). Consequently, they have a low biodegradation capacity, and are not likely to undergo metabolism in biota (Butt et al, 2010; Yamashita et al, 2005). For example, the average half-life in humans is 5.4 years for perfluorooctane sulfonate (PFOS) and 3.8 years for perfluorooctanoate (PFOA) (Olsen et al, 2007). PFASs consist of a wide variety of different groups, classified by the functional group. Some examples are perfluorocarboxylates (PFCAs), perfluorosulfonates (PFSAs), perfluorosulfonamides (FOSAs), perfluorosulfonamide ethanols (FOSEs), fluorotelomer sulfonates (FTSAs) and perfluorosulfonamidoacetates (FOSAAs). PFOS and PFOA are the most investigated compounds of the PFASs (Butt et al, 2010; Houde et al, 2011) and their chemical structures are presented in **Figure 1**.

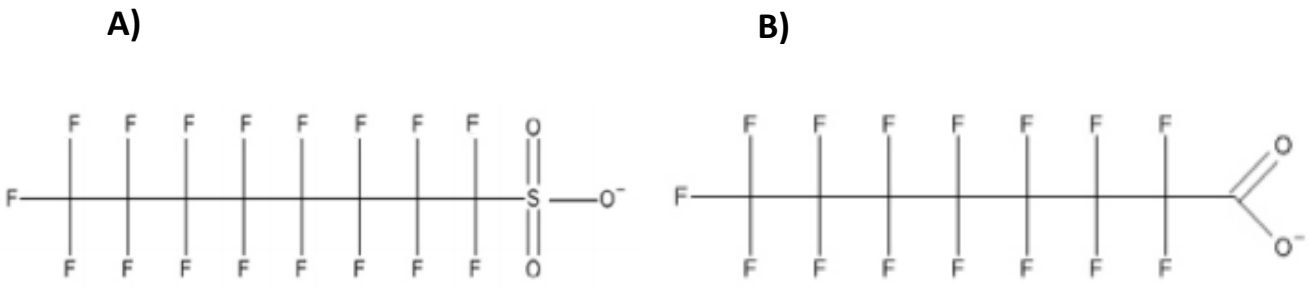

Figure 1. Chemical structure of A) PFOS and B) PFOA. Figure from Borg & Håkansson (2012).

1.3 Manufacture and usage

Some PFASs can occur naturally in the environment. However this is very rare and PFASs are primarily seen as anthropogenic chemicals (Lau et al, 2007). There are two main techniques for manufacturing of PFASs; telomerisation and electrochemical fluorination (ECF). ECF has been going on since the 1950's and telomerisation since the 1970's (Butt et al, 2010). PFOS is exclusively manufactured via ECF (Buck et al, 2011). However, the major manufacturers of PFOS voluntarily started to phase out the production in 2001 (OECD, 2002). Since then, the PFOS production has shifted to China and more short-chained PFASs have been produced to a larger extent as substitutes (Möller et al, 2010).

PFASs are suitable for a wide range of products due to their lipophobic and hydrophobic properties (Buck et al, 2011). For instance, PFASs are being used as surface coatings for paper, furniture and textiles. Furthermore, they are used in fire-fighting foams, paints, pesticides, waxes, shampoos, and photographic film. (Yamashita et al, 2005; Butt et al, 2010; Renner, 2001; Jensen & Leffers, 2008). In biota, PFOS is the most abundant compound of the PFASs. It is generally found in the highest concentration levels in biota and has been detected in many types of wildlife (Butt et al, 2010). In 2009, PFOS was added to the Stockholm Convention list of banned POPs. However, some exceptions are allowed. For instance, PFOS is still being used and produced for fire-fighting foams (Muir & De Wit, 2010)

1.4 Long range transport of PFASs to the Arctic

PFASs have been detected globally, even in the Arctic environment. Since there are few local point sources in the Arctic, PFASs are believed to originate from lower latitudes (Burkow & Kallenborn, 2000). There is limited knowledge about the transport of PFASs to these regions. However, studies have shown that there are two probable transport pathways: direct transport via ocean currents and indirect transport via precursors that undergo atmospheric long range transport. For instance, fluorotelomer alcohols (FTOHs) are common precursors that degrade to PFCAs. FTOHs have been produced in a large amount, especially in North America, and are mainly used as additives in industrial processes (Ellis et al, 2004). In addition, perfluorooctyl sulfonyl is a likely precursor of PFOS. It has been used as industrial raw material and as an intermediate in the production of other chemicals (3M Company, 1999).

Atmospheric transport is considered as the main transport pathway of POPs (and accordingly, PFASs) to the Arctic environment. They evaporate in the warmer regions, travel in the atmosphere and deposit in the northern regions. This phenomenon is called global fractionation or global distillation and is demonstrated in **Figure 2** (Wania & Mackay, 1996).

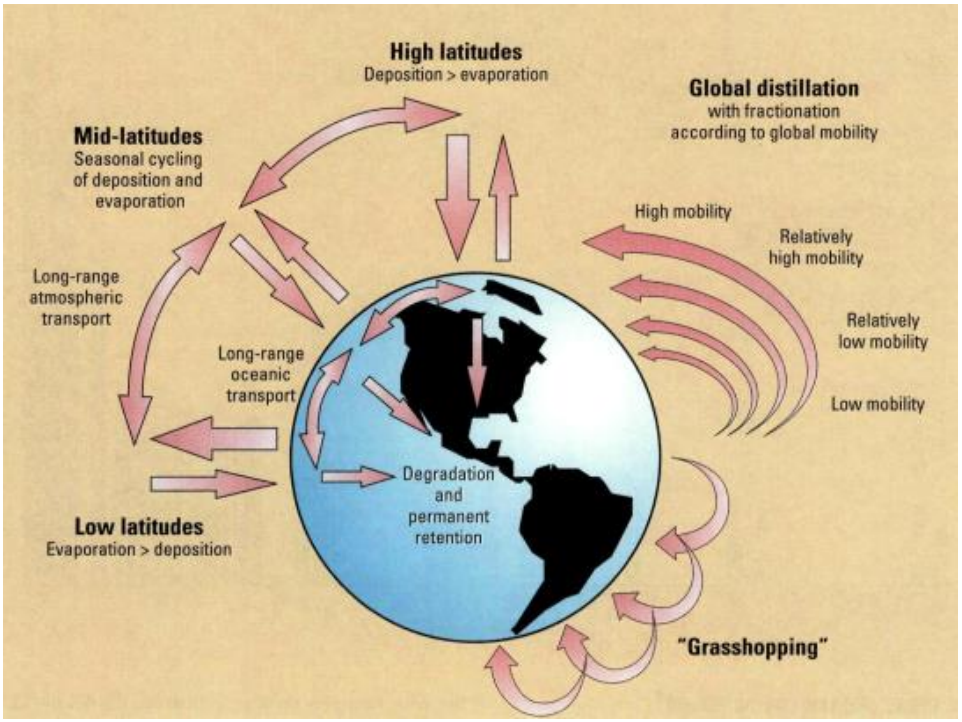

Figure 2. Global atmospheric transport of POPs. Picture from Wania & Mackay (1996).

1.5 Birds in the Arctic

The terrestrial animal life on Svalbard is very limited due to the harsh climate. However, the birdlife is rich, especially during the summer (Kavli institute for systems neuroscience, 2014). Black guillemots are sedentary and can be found on Svalbard all year round (Borgå et al, 2007). Hence, the levels of PFASs in black guillemots will reflect local exposure at Svalbard. In contrast, glaucous gulls migrate during the winter, for example to Iceland and the northern parts of Norway (unpublished data in Gabrielsen et al, 1995). The glaucous gull is an omnivore. Its diet includes fish and crabs, but also eggs and chicks of other bird species. Moreover, they occasionally scavenge on carcasses and may even eat garbage (Sagerup et al, 2002). The black guillemot mostly feed on benthic prey such as crustaceans and benthic fish species (Martin et al, 2004). Both species are seabirds and part of the Arctic marine food web (Borgå et al, 2007).

1.6 Exposure and effects of PFASs

Several studies have shown that PFASs typically bio-magnify in the marine food chain due to their persistence and low biodegradation (Haukås et al, 2007; Tomy et al, 2004). For sea birds, the main exposure of PFASs is via their food intake. In sea birds, the detoxifying rates are relatively low, and consequently they have a high accumulation potential (Gabrielsen et al, 1995).

PFASs are potentially hazardous to organisms. Studies have shown that some of these compounds can cause peroxisome proliferation, which is a formation of cancer (Berthiaume & Wallace, 2002; Hu et al, 2002). Other studies have shown adverse effects on the lipid metabolism and reproduction of some organisms, for instance a study on rats conducted by Lau et al (2003). Furthermore, some PFASs are suspected to be endocrine disruptors (Jensen & Leffers, 2008).

In general, the toxicity of PFASs increases with the chain-length (Jensen & Leffers, 2008). However, the knowledge about the effects of PFASs on organisms and the environment is limited. In many cases, the studies include laboratory experiments with high, short-term exposure of the pollutants (Lau et al, 2007). Nevertheless, this does not simulate realistic conditions for the exposure of organisms (Fisk et al, 2005).

1.7 Aim

This study was a collaboration between the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, and the University Center of Svalbard (UNIS), Longyearbyen, Svalbard.

The aim of this study is to compare the concentration levels and composition profile of PFASs in the two bird species glaucous gull (*Larus hyperboreus*) and black guillemot (*Cepphus grylle*) from Svalbard.

2. Material and methods

2.1 Sample collections

Four individuals of each species (i.e. glaucous gulls and black guillemots) were collected in June 2007. The black guillemots were shot nearby Diabas in Isfjorden and the glaucous gulls were shot at a dumpsite in Adventfjorden, Longyearbyen. In addition, one glaucous gull was collected nearby Hopen in May 2008/2009 (see **Figure 3)**.

Coordinates for the sampling sites

Location	Latitude	Longitude
Longyearbyen	78°13’20”	15°37’53”
Diabas	78°22'74"	16°9'43"
Hopen	76°35'25"	25°15'07"

Figure 3. Map of Svalbard. The red dots represent the sampling sites (Source: Google Maps 2014, Google©).

The birds were dissected immediately after the sampling and have been stored in the freezer afterwards. Liver and muscle samples were collected from all bird samples for the analysis of PFASs. The stomach content of glaucous gulls contained remains of plastic bags and other non-biodegradable units. Unfortunately there was no information available about the age or sex of the individuals. All samples are presented in **Table 1.**

Table 1. The samples are listed according to species, body tissue, location and time of sampling.

Sample ID	Tissue	Location	Time
Glaucous gull			
G1	Liver	Longyearbyen	June-07
G2	Liver	Longyearbyen	June-07
G3	Liver	Longyearbyen	June-07
G4	Liver	Longyearbyen	June-07
G5	Liver	Hopen	May-08/09
G6	Muscle	Longyearbyen	June-07
G7	Muscle	Longyearbyen	June-07
G8	Muscle	Longyearbyen	June-07
G9	Muscle	Longyearbyen	June-07
G10	Muscle	Hopen	May-08/09
Black guillemot			
B1	Liver	Diabas	June-07
B2	Liver	Diabas	June-07
B3	Liver	Diabas	June-07
B4	Liver	Diabas	June-07
B5	Muscle	Diabas	June-07
B6	Muscle	Diabas	June-07
B7	Muscle	Diabas	June-07
B8	Muscle	Diabas	June-07

2.2 Chemicals

In this study, 26 PFASs were investigated. The PFASs include C_4–C_{14}, C_{16}, C_{18} PFCAs (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxDA, PFOcDA), C_4, C_6, C_8, C_{10} PFSAs (i.e. PFBS, PFHxS, PFOS, PFDS,) FOSAs (i.e. FOSA, N-MeFOSA, N-EtFOSA), FOSEs (i.e. N-MeFOSE, N-EtFOSE) and FOSAAs (i.e. FOSAA, N-MeFOSAA, N-EtFOSAA) and 6:2 FTSA (see **Table 2**).

All PFASs used for the analytical method were purchased from Wellington laboratories (Guelph, Ontario, Canada) with a purity of >98 %. In addition, four ISTDs were used (i.e. $^{13}C_4$ PFBA, $^{13}C_4$ PFOA, $^{13}C_5$ PFNA, and $^{13}C_2$ PFDoDA, c=0.1 ng μL^{-1}) with a purity of >98 % purchased from Sigma-Aldrich (Norway AS) or Wellington Laboratories Inc. (Guelph, Ontario, Canada). The recovery standard (RSTD) was $^{13}C_8$ PFOA with a purity of >98 % purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). For the clean-up, Superclean ENVI-carb 120/400 (Supelco 57210-U) (Supelco, PN, USA or Bellefonte, USA) was used together with glacial acetic acid from Merck, Germany.

Table 2. All PFASs investigated in this study, organised by subgroups.

Per- and polyfluoroalkyl substances	
PFBS	perfluorobutane sulfonate
PFHxS	perfluorohexane sulfonate
PFOS	perfluorooctane sulfonate
PFDS	perfluorodecane sulfonate
PFBA	perfluorobutanoate
PFPeA	perfluoropentanoate
PFHxA	perfluorohexanoate
PFHpA	perfluoroheptanoate
PFOA	perfluorooctanoate
PFNA	perfluorononanoate
PFDA	perfluorodecanoate
PFUnDA	perfluoroundecanoate
PFDoDA	perfluorododecanoate
PFTriDA	perfluorotridecanoate
PFTeDA	perfluorotetradecanoate
PFHxDA	perfluorohexadecanoate
PFOcDA	perfluorooctadecanoate
FOSA	perfluorooctanesulfonamide
N-MeFOSA	*N*-methylperfluorooctansulfonamide
N-EtFOSA	*N*-ethylperfluorooctanesulfonamide
N-MeFOSE	*N*-methylperfluorooctanesulfonamido-ethanol
N-EtFOSE	*N*-ethylperfluorooctanesulfonamido-ethanol
FOSAA	perfluorooctanesulfonamidoacetic acid
N-MeFOSAA	*N*-methylperfluorooctanesulfonamidoacetic acid
N-EtFOSAA	*N*-ethylperfluorooctanesulfonamidoacetic acid
6:2 FTSA	6:2 fluorotelomer sulfonate

2.3 Sample extraction

The sample extraction was conducted at UNIS. For the sample extraction, the Powley method was used with a few modifications (Powley et al, 2005). Briefly, initially, the biota sample was homogenised. Then, 1 g of the sample was weighed and put into a 45 mL polypropylene tube and spiked with 20 µL ISTD (c=0.1 ng μL^{-1}) . 8 mL of methanol was added, and the tube was capped and mixed by using vortex. The tube was put into an ultrasonic bath three times for 10 minutes, and mixed with vortex in between the baths. Then the tube was centrifuged for 5 minutes at 2000 rpm for sedimentation. The supernatant was transferred to Turbovap and evaporated until approximately 1 mL of the solution remained. In the following, a 1.7 mL Eppendorf centrifuge tube was prepared with 25 mg ENVI-Carb and 50 µL glacial acetic acid. Then the supernatant extract from the Turbovap was transferred to the Eppendorf centrifuge tube. It was capped and mixed thoroughly with vortex. The Eppendorf tube was centrifuged for 10 minutes at 10000 rpm. The supernatant solution was transferred into a vial and stored in the freezer until the transport to Sweden.

All biota samples were extracted in duplicates. In general, four replicates were prepared at the same time. Three lab blanks were also prepared. The lab blank extraction was carried out as the natural samples but with no biota in the sample. After the transport to Sweden, the volume of the samples were adjusted to exactly 1 mL and 10 µL of the RSTD was added (c=0.2 ng μL^{-1}). Then the vials were centrifuged for 6 minutes at 5000 rpm.

2.4 Instrumental analysis

The instrumental analysis was conducted in the POPs-lab at the Department of Aquatic Sciences and Assessment at SLU. All PFASs extracts were analysed using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) according to a method described by Ahrens et al (2009a).

3. Results

3.1 Quality control (QC)

The method detection limit (MDL) was calculated by multiplying 3 x standard deviation of the blank with the mean blank concentration. For the blanks with no detected PFASs the MDL was set to the lowest calibration standard level (i.e. 0.05 ng mL^{-1}). Contamination of the samples can occur during sampling, sample preparation or instrumental analysis. Three of the 26 compounds (i.e. PFOS, FOSA and EtFOSA) were found in one or more of the blank samples with low concentrations ranging from 0.008 to 0.02 ng g^{-1} (see **Table 3**). The MDL ranged between 0.06 and 0.11 ng g^{-1}.

All samples were extracted in duplicates. The PFAS concentrations of each replicate are listed in **Table A1** in the Appendix. In average, the mean percentage standard deviation was ~24 %.

The standard deviation was higher for the compounds detected in lower levels, for example PFHxS (~60 %) and MeFOSAA (~46 %).

Table 3. Mean concentration levels of the blank samples and method detection limits (MDL) for each compound.[a]

Compounds	Blanks (ng g^{-1})	MDL (ng g^{-1})
PFBS	nd	0.05
PFHxS	nd	0.05
PFOS	0.02	0.16
PFDS	nd	0.05
PFBA	nd	0.05
PFPeA	nd	0.05
PFHxA	nd	0.05
PFHpA	nd	0.05
PFOA	nd	0.05
PFNA	nd	0.05
PFDA	nd	0.05
PFUnDA	nd	0.05
PFDoDA	nd	0.05
PFTriDA	nd	0.05
PFTeDA	nd	0.05
PFHxDA	nd	0.05
PFHxDA	nd	0.05
PFOcDA	nd	0.05
FOSA	0.008	0.06
MeFOSA	nd	0.05
EtFOSA	0.02	0.11
MeFOSE	nd	0.05
EtFOSE	nd	0.05
FOSAA	nd	0.05
MeFOSAA	nd	0.05
EtFOSAA	nd	0.05
6:2 FTS	nd	0.05

[a] nd = not detectable

Quantification of PFASs in the samples was enabled by using internal calibration method. The recovery was calculated for each sample by dividing the area of the RSTD with the mean calibration area (from the calibration curves), and multiplying by 100. Since the analysed PFASs have different chain length, four different ISTDs were monitored. The recoveries of the ISTD in the samples were in average 96 ± 34% for $^{13}C_4$ PFBA, 70 ± 18% for $^{13}C_4$ PFOA, 86 ± 37% for $^{13}C_5$ PFNA, and 80 ± 53% for $^{13}C_2$ PFDoDA (see **Table 4**).

Table 4. Recoveries of the ISTDs in the biota samples (*n*=9) presented as percentage.

(%)	$^{13}C_4$ PFBA	$^{13}C_4$ PFOA	$^{13}C_5$ PFNA	$^{13}C_2$ PFDoDA
mean	96	70	86	80
SD	34	18	37	53

3.1 Composition profile

The relative distribution of the different PFASs is presented in **Figure 4**. The most abundant compound in this study was PFOS. It constituted in average ~72 % of the ∑PFASs; ~75 % in glaucous gull and ~69 % in black guillemot. Regarding tissue distribution, PFOS constituted in average ~86 % and ~57 % in liver and muscle respectively.

After PFOS, PFUnDA was the next abundant compound. It constituted in average 16 % of the∑PFASs. With regard to the subgroups, the PFSAs were dominating. In average, the PFSAs comprised ~72 % of the ∑PFASs (mainly PFOS, constituting 99,6% of the ΣPFSAs). The ∑PFCAs and the ∑FOSAAs comprised ~28 % and ~0.5 % respectively.

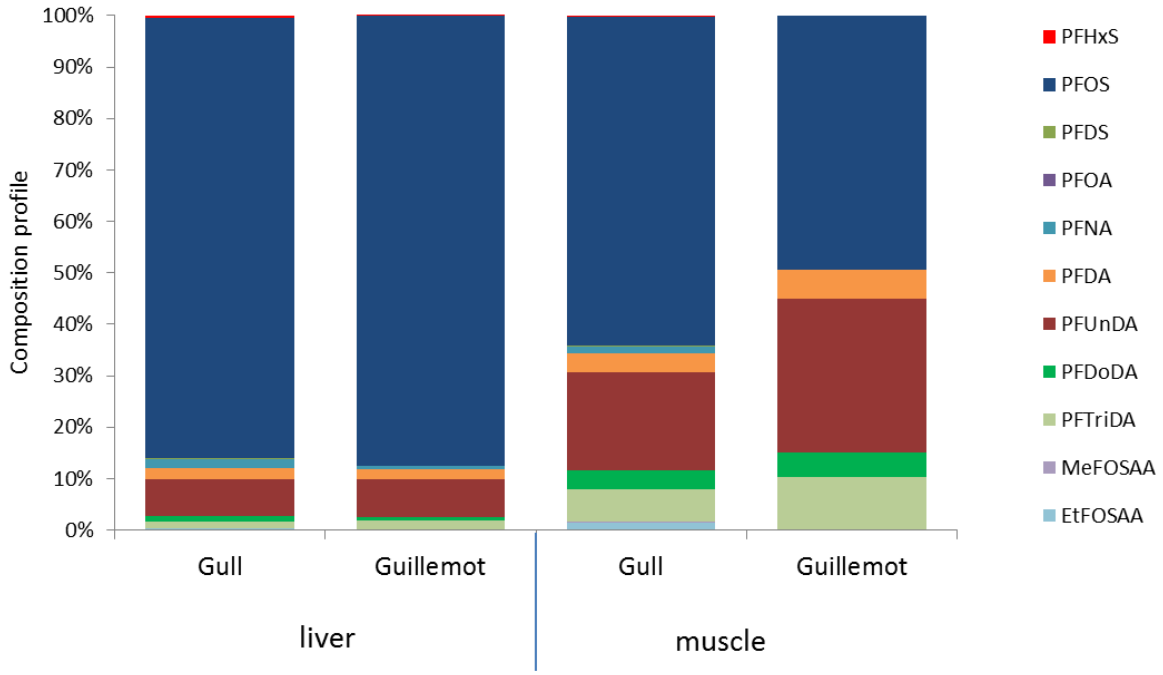

Figure 4. PFAS distribution in the different birds and tissues (%). The values are based on the median concentrations levels.

3.2 Concentration levels

Of the 26 PFASs that were analysed in liver and muscle samples from the two species, 11 compounds were detected. The PFASs that were not detected were PFBS, PFBA, PFPeA, PFHpA, PFTeDA, PFHxDA, PFOcDA, FOSA, MeFOSA, EtFOSA, MeFOSE, EtFOSE, FOSAA and 6:2 FTS, and will not be presented in the following. PFOS, PFNA, PFDA, PFUnDA, PFDoDA and PFTriDA were found in all biota samples. Some compounds were only detected in glaucous gull but not in black guillemot (i.e. PFDS, PFOA and PFNA and MeFOSAA). The PFAS concentrations in the two species are compiled in **Table 5** and **Table 6**. In addition, the PFAS concentration levels in the different species and body tissues are presented in **Figure 5** and **Figure 6**. In glaucous gull, the ΣPFAS concentrations in the individual samples ranged from 87.4 to 187 ng g^{-1} wet weight (ww) in liver (in average 147 ng g^{-1} ww) and from 7.2 to 30.1 ng g^{-1} ww in muscle (in average 15.5 ng g^{-1} ww). In black guillemot, ΣPFASs concentrations ranged from 8.07 to 75.5 ng g^{-1} ww in liver (in average 36.2 ng g^{-1} ww) and 1.61 to 3.82 ng g^{-1} in muscle (in average 2.45 ng g^{-1} ww).

PFOS was detected in the highest levels. In glaucous gull, the mean PFOS concentration was ~126 ng g^{-1} ww in liver and ~10 ng g^{-1} ww in muscle. In black guillemot, the mean PFOS concentration was ~31 ng g^{-1} ww in liver and ~1 ng g^{-1} ww in muscle.

Table 5. Concentrations (ng g^{-1} ww) for the detected PFASs in in glaucous gull (*n*=5). The results are denoted as mean, standard deviation (SD), minimum and maximum values.[a]

	Liver tissue					Muscle tissue				
Compound	**Mean**	**SD**	**Min**	**Max**		**Compound**	**Mean**	**SD**	**Min**	**Max**
PFHxS	0.59	0.60	0.14	1.62		PFHxS	0.04	0.07	nd	0.15
PFOS	126	33.4	72.4	163		PFOS	10.1	6.04	4.50	20.1
PFDS	0.36	0.16	0.13	0.50		PFDS	0.02	0.03	nd	0.08
PFOA	0.02	0.05	nd	0.12		PFOA	nd	nd	nd	nd
PFNA	2.40	0.47	1.93	3.15		PFNA	0.17	0.12	0.03	0.35
PFDA	3.16	0.51	2.61	3.78		PFDA	0.56	0.26	0.39	1.02
PFUnDA	10.2	2.64	7.78	13.7		PFUnDA	2.94	1.55	1.33	5.43
PFDoDA	1.59	0.57	0.84	2.33		PFDoDA	0.56	0.32	0.29	1.06
PFTriDA	1.78	0.60	1.29	2.49		PFTriDA	0.96	0.56	0.47	1.77
MeFOSAA	0.10	0.10	nd	0.24		MeFOSAA	0.03	0.04	nd	0.09
EtFOSAA	0.53	0.73	nd	1.81		EtFOSAA	0.16	0.27	nd	0.65
ΣPFASs	**147**	**36.72**	**87.4**	**187**		**ΣPFASs**	**15.5**	**8.67**	**7.22**	**30.1**

[a] nd = not detectable

Table 6. Concentrations (ng g^{-1} ww) for the detected PFASs in black guillemot (*n*=4). The results are denoted as mean, standard deviation (SD), minimum and maximum values.[a]

	Liver tissue					Muscle tissue				
Compound	**Mean**	**SD**	**Min**	**Max**	**Compound**	**Mean**	**SD**	**Min**	**Max**
PFHxS	0.04	0.08	nd	0.16	PFHxS	nd	nd	nd	nd
PFOS	31.2	29.8	6.36	68.1	PFOS	1.21	0.37	0.80	1.63
PFDS	nd	nd	nd	nd	PFDS	nd	nd	nd	nd
PFOA	nd	nd	nd	nd	PFOA	nd	nd	nd	nd
PFNA	0.22	0.21	nd	0.48	PFNA	nd	nd	nd	nd
PFDA	0.75	0.46	0.31	1.22	PFDA	0.14	0.07	0.08	0.23
PFUnDA	2.62	1.39	1.06	4.13	PFUnDA	0.73	0.42	0.36	1.29
PFDoDA	0.28	0.12	0.12	0.41	PFDoDA	0.12	0.06	0.07	0.20
PFTriDA	0.63	0.33	0.23	1.04	PFTriDA	0.25	0.16	0.12	0.47
MeFOSAA	nd	nd	nd	nd	MeFOSAA	nd	nd	nd	nd
EtFOSAA	0.01	0.03	nd	0.06	EtFOSAA	nd	nd	nd	nd
ΣPFASs	**36.2**	**31.4**	**8.07**	**75.5**	**ΣPFASs**	**2.45**	**1.05**	**1.61**	**3.82**

[a] nd = not detectable

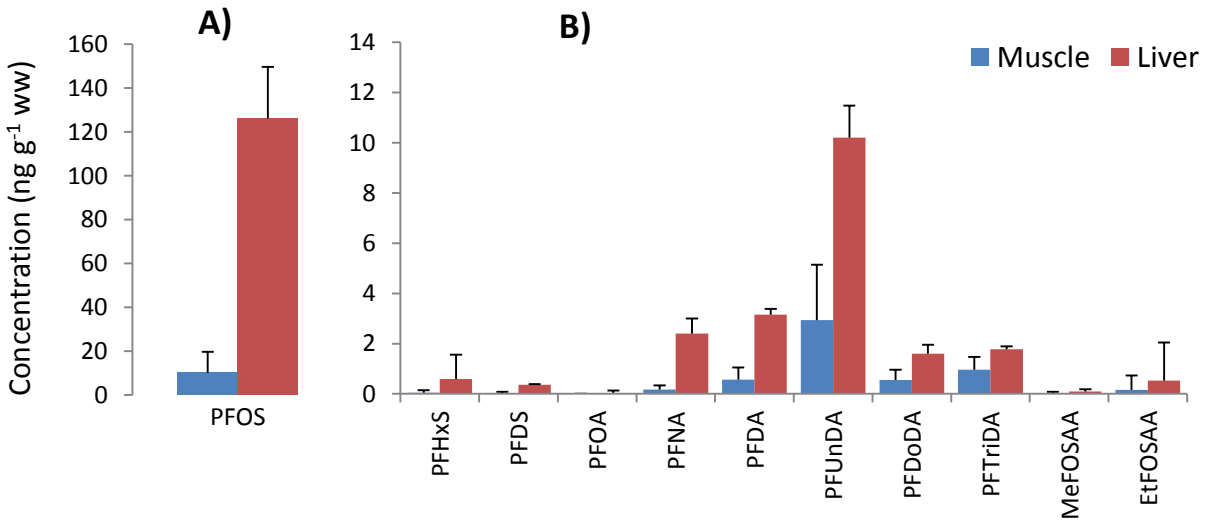

Figure 5. Concentration levels in glaucous gull in ng g^{-1} ww in A) PFOS and B) other PFASs. The levels for each compound are presented as mean (columns) and standard deviation (error bars).

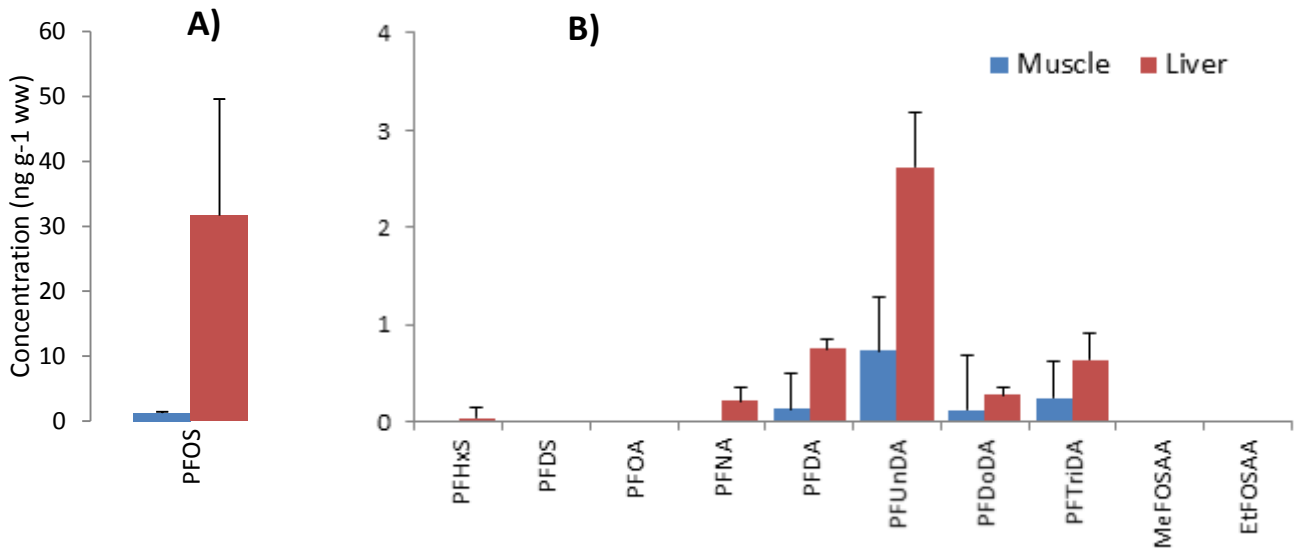

Figure 6. Concentration levels in black guillemot in ng g^{-1} ww in A) PFOS and B) other PFASs. The levels for each compound are presented as mean (columns) and standard deviation values (error bars).

In **Figure 7**, the concentration levels of PFASs in the different bird species and body tissues are presented. The liver samples had higher levels of PFASs in both glaucous gull and black guillemot. The glaucous gull had higher levels than black guillemot in both muscle and liver tissue. PFOS was found in the highest concentrations in all samples.

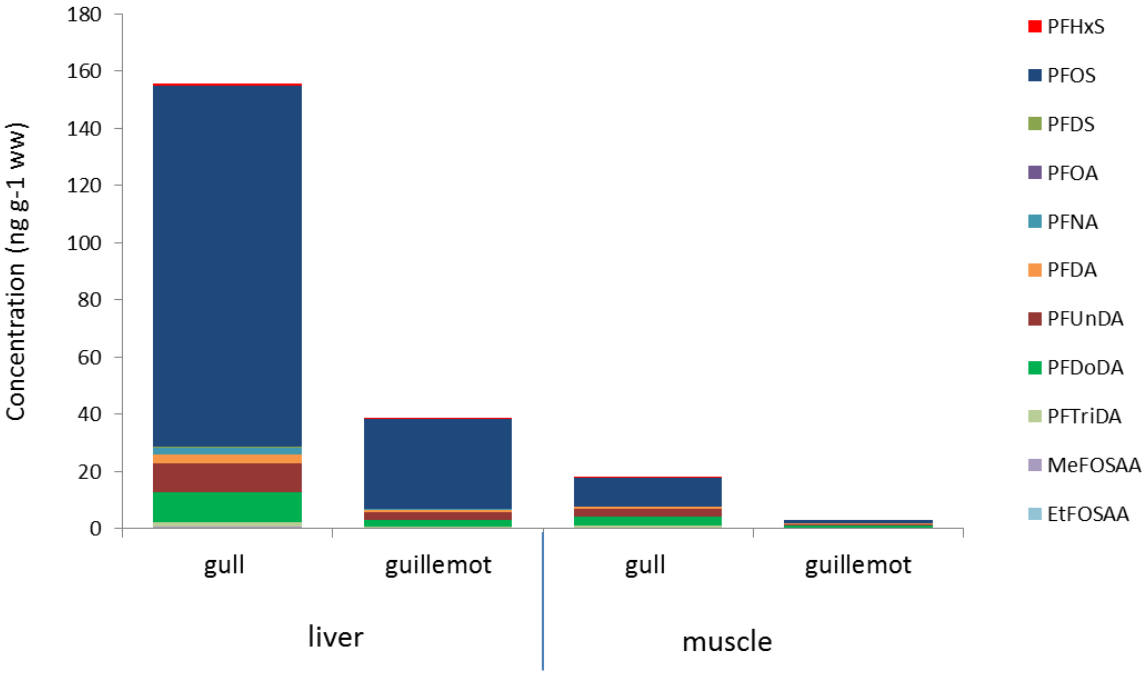

Figure 7. Concentration levels of PFASs in ng g^{-1} ww in both species and body tissues.

4. Discussion

4.1 Comparison of the composition profile

In this study, 11 of the 26 analysed PFASs were detected in liver and muscle samples from glaucous gull and black guillemot. The ΣPFSAs comprised ~72 % of the ∑PFASs, while the ∑PFCAs and the ∑FOSAAs comprised ~28 % and ~0.5 % respectively. PFOS was the predominant compound in both bird species and both body tissues. In average, it constituted ~72 % of the ΣPFAS concentration levels (99,6 % of the ∑PFSAs). This is consistent with other studies on biota, as PFOS generally constitutes the largest proportion of PFASs (Butt et al, 2010). In addition, a recent study on seabirds in the Barents Sea, east of Svalbard, showed that PFOS was the major component in both glaucous gull and black guillemot (Haukås et al, 2007). This is in accordance with the present study.

As stated by Butt et al (2010), seabirds generally have high proportions of long-chained PFCAs (C_{11}–C_{15}). In the present study, PFUnDA (C_{11}), PFDoDA (C_{12}) and PFTriDA (C_{13}) were detected in quite high proportions, in average covering 23 % of ∑PFASs altogether. This may be due to their high accumulation potential (Martin et al, 2003). The detected PFOA concentrations were relatively low, which is in line with other studies on PFASs in birds (Bossi et al, 2005; Kannan et al, 2002). In general, PFOA concentrations are relatively low in biota in the Arctic (Butt et al, 2010).

The composition profile differed between liver and muscle tissue. In liver, PFOS comprised 86 % of the ∑PFASs compared to 57 % in muscle. This can be explained by the tissue depending bioaccumulation of the different PFASs in biota (Ahrens et al, 2009b).

4.2 Comparison of the concentration levels

In the present study, the mean ΣPFASs in glaucous gull were 147 ng g^{-1} ww in liver and 15.5 ng g^{-1} ww in muscle. The mean ∑PFASs in black guillemot were 36.2 ng g^{-1} ww in liver and 2.45 ng g^{-1} ww in muscle. These results are in accordance with the results from a similar study on PFASs in glaucous gulls and black guillemots from the Arctic. Haukås et al (2007) reported mean ∑PFAS concentrations in liver ranging from 9.6-240 ng g^{-1} ww in glaucous gull and 0.3-46 ng g^{-1} ww in black guillemot. Along with the present study, the levels in glaucous gull were much higher than in black guillemot.

The mean PFOS concentration was approximately four times higher in glaucous gull than in black guillemot. One explanation is that PFASs are known to bio-magnify in the food chain and glaucous gull has a high trophic level compared to black guillemot (Borgå et al, 2007). There are more possible explanations to the higher PFAS levels in glaucous gulls. Guillemots are sedentary whereas glaucous gulls migrate to more industrial areas. Furthermore, glaucous gulls have very opportunistic feeding habits (Sagerup et al, 2002). The glaucous gulls in this study were collected at a dumpsite and contained remnants of plastic bags, which may explain the high levels of PFOS in some of the samples.

As regards the different body tissues, the PFAS concentration levels in this study were consistently higher in liver than in muscle tissues in both glaucous gull and black guillemot. This can be explained by the tendency of PFASs to accumulate in protein-rich tissues. However, a study on glaucous gulls conducted by Verreault et al (2005) showed that PFAS concentrations were higher in blood than in liver. This indicates that the concentration levels may depend on the blood content of the analysed tissue. Another plausible explanation for the high accumulation of PFASs in liver could be enterohepatic recirculation. Since PFASs are known not to metabolise in biota, it keeps circulating in the detoxifying system of the birds and hence, accumulate in the liver (Jones et al, 2013).

There are relatively few studies on PFASs in muscle tissue. However, a study of Belgium barn owls (Jasper et al, 2013) showed that the PFOS concentrations in muscle were two times lower than in liver tissue. This is in accordance with the present study.

4.3 Comparison of PFASs in birds from other areas in the world

In comparison with studies from other areas in the Arctic Regions, the black guillemot in this study had relatively high levels of PFASs, particularly PFOS (in average 31 and 1 ng g^{-1} ww in liver and muscle respectively). For instance, in a study conducted in the Canadian Arctic by Martin et al (2004), PFASs in black guillemots were not detected. However, the PFAS concentration levels in this study were in the same magnitude as in a study of black guillemots from Greenland (Bossi et al, 2005), with an average PFOS concentration of 10.8 ng g^{-1} ww. Furthermore, the mean ∑PFAS concentration in glaucous gull was higher in the present study than in a similar study from the Eastern Arctic (Tomy et al, 2004). This might be due to the plastic units that were found in the stomach content of the collected gulls.

In industrialized areas, PFASs are generally detected at higher levels than in Arctic areas (Jaspers et al, 2013; Kannan et al, 2002). In Korea and Japan, several bird species were investigated for PFASs in liver tissue, including common cormorant and four different gulls (Kannan et al, 2002). PFOS was the prominent compound; in Japan it was detected in 38 of 40 samples (mean PFOS concentrations ranged from 40 ng g^{-1} ww in sea gull to 390 ng g^{-1} ww in common cormorant).

4.4 Long range transport of PFASs to the Arctic

There are no known point sources of PFASs in the Arctic, and long range transport is believed to be the main reason for the detected compounds in this study. This supports the theory that more volatile substances travel through the atmosphere and degrade to PFASs (Butt et al, 2007). In addition, oceanic transport is also a potential pathway for PFASs (Butt et al, 2010). However, local contamination is also a possible source of contamination. PFASs are used in a large variety of products, which of many are present in Longyearbyen and in adjacent areas. Many outdoor clothes and impregnating agents contain PFASs, as well as skiing waxes (Buck et al, 2011). Therefore, contamination from the sampling area cannot be excluded. A recent

study on PFASs in ice cores, surface snow and surface water suggested that local sources are a contributing factor to the current levels (Kwok, 2013). However, local contamination is not likely to be a large contributing factor in this study. The most probable contribution to the high concentration levels must be PFASs from industrial areas that undergo long range transport via precursors.

4. 5 Outlook and future perspectives

On the subject of PFASs, it is important to be careful when handling and storing the samples to avoid contamination. In this study, several PFASs were detected in low levels in the lab blanks. This may be problematic because it indicates that contamination of the samples occurred in some steps during the extraction process. However, the concentration levels in the blanks were quite low and the PFAS concentrations in the samples could effectively be estimated. Generally, the recoveries in this study were high; on average it was 83 %. For some replicates (i.e. replicate 14 and 16, for $^{13}C_2$PFDoDA), the recovery was over 200 %. This might be due to incorrect spiking volumes of the ISTD or RSTD. Thus, the analysis of PFASs in biota samples from remote regions such as Svalbard has to be performed under clean conditions and quality assurance and quality control during the sample treatment is very important.

There was a high variation between the individuals in this study and the number of samples was too small to be statistically significant. Moreover, the age and sex of the birds in this study were unknown. These factors might have an influence on the concentration levels. The time schedule of this study was limited, however, for future studies a larger sample number should be analysed and more information about the age and sex of the investigated birds is needed.

The samples in this study are from 2007. Since then, it is uncertain whether the concentration levels in the sample area have increased or decreased. However, the phase-out of PFOS in 2001 (OECD 2002) may have contributed to lower levels of PFOS and higher proportions of other compounds today.

The fact that high concentrations of PFAS were detected in birds on Svalbard gives reason for further research within the subject. In specific, studies of temporal trends are needed to gain knowledge about the fate and effects of PFASs over time.

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