PERFLUORINATED ORGANIC COMPOUNDS COMPARED TO BROMINATED AND CHLORINATED ORGANIC POLLUTANTS IN EUROPEAN SHAG *(PHALACROCORAX ARISTOTELIS)* AND COMMON EIDER (*SOMATERIA MOLLISSIMA)* FROM NORWAY

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Introduction

Perfluorinated organic compounds (PFCs) are a group of chemicals that have attracted increasing attention from scientists, government agencies, and the general public in recent years. These chemicals are used in a variety of manufactured products such as liquid repellants for paper, packaging, textile, leather, adhesives, fire fighting foams and other industrial products.[1] The main two groups of PFCs, perfluoroalkylsulfonates and perfluoroalkylcarboxylates, were found in many environmental compartments including water, sediment and biota. PFCs have been found in several species of wildlife from various locations including some remote areas.[2–4]

Because of the stable carbon-fluorine bond, PFCs resist hydrolysis, photolysis and biodegradation[1]. They can repel both water and oils, because of the hydrophobe nature of the perfluorinated carbon chain and the lipophobe nature of the functional group at the end of the chain[4]. Perfluorinated compounds seem to have distribution patterns similar to those of persistent organohalogenated pollutants (POPs) with regard to global biospheric distribution, bioaccumulation and biomagnification. Unlike POPs, which accumulate in lipid-rich tissues, perfluorinated compounds bind to the serum albumin and are found in the protein fraction of blood, and in the liver[5]. Fluorochemicals such as perfluorooctane sulfonate (PFOS) were detected in tissues from a great diversity of aquatic bird species from Japan, Korea, Canada, the USA, the Northern Pacific region and Europe[2, 3, 10]. Until now, the only data describing the contamination of the marine wildlife with PFCs in Norway, are from glaucous gull and black guillemot from the Norwegian Arctic[8, 11]. No PFCs data are available from the Norwegian mainland.

The present study reports the results from the investigation of two seabird species, European shag *(Phalacrocorax aristotelis)* and common eider (*Somateria mollissima)* to assess the concentration of PFCs in comparison to brominated flame retardants (BFR) and as well as PCBs, DDTs and other organochlorines. The samples were collected at a remote bird colony (Island Sklinna, 65º12'N 11º00'E, ca 35 km from the Norwegian coast, Figure 1), a National seabird monitoring site since the early 1980s. Eggs and blood samples were taken from breeding shag females. Livers of juveniles were collected. Eggs from common eider were taken from the same site, one from each nest. The project was started in the spring of 2003 and the last field-season was completed in mid-July 2004.

Material and methods

The Norwegian Institute for Nature Research (NINA) provided a set of 28 liver samples, 37 egg samples and 18 plasma samples from European shag, additional to 23 egg samples from common eider, collected in 2003/4. From nests with approximately the same laying-date, one egg (the first laid, distinguishable by its darker color) from each nest was taken. The sampling of eggs took place early in the egg-lying season to ensure that the ages of the egg-lying females are approximately equal (old females lay early). The growth and survival of the remaining chicks was recorded. One nestling from each of 20 other complete clutches was collected after the

growth curves of all chicks in the nests had been established through repeated weighing throughout the season. Blood was drawn from the brachial vein of the breeding female from each shag nest and centrifuged.

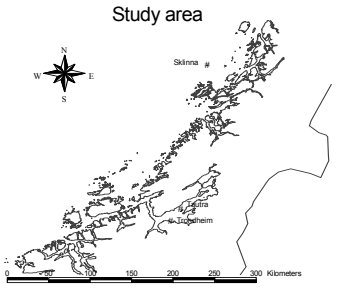

Figure 1: The study site Sklinna is located in the Norwegian sea off the Norwegian coast, ca 200 km north of Trondheim

The female was chosen, because she determines the levels in eggs, and to reduce biological variation caused by sex. Liver was collected from 3-week old chicks. All liver and blood samples were deep-frozen immediately after collection in liquid nitrogen, eggs were kept refrigerated until emptied, then deep-frozen at -21ºC. Samples were taken under permission from the environmental authorities.

Sample treatment

POPs: Tissue and egg samples were extracted and prepared as described previously[6]. Briefly, tissue samples were homogenised, subsequently dried in a 10 fold amount of dry sodium sulfate, and extracted. The amount of extractable organic material was determined gravimetrically. Lipid removal was performed on a gel permeation chromatography (GPC) system. An additional fractionation was carried out on a florisil column. Plasma samples were extracted twice with 10 ml n-hexane, the extract was concentrated and run over a florisil column. A recovery standard (octachloronaphthalene, 10 µL of a 1 ng/µL solution in isooctane) was added to all samples prior to quantification. For quantitation the internal standard method was used. ^{13}C-labeled compounds were used, representing each group of analytes.

PFCs: Prior to analysis samples were extracted and prepared as described previously[7]. From the 2004 sample set 11 plasma samples, 8 egg samples, 6 liver samples, all from shag, and 10 eider egg samples were selected for PFC analyses. Briefly, tissue and egg samples were extracted with acetonitrile in an ultrasonic bath. After centrifugation, the supernatant solution was added to 25 mg ENVI-Carb and 50µL glacial acetic acid. After additional centrifugation an aliquot of the solution was transferred in an autoinjector vial and a recovery standard (3,5-bis(trifluoromethyl)phenyl acetic Acid, 20µL of a 0.25 ng/µL solution in methanol) and aqueous ammonium acetate was added. For quantitation perfluoro-3,7-di-methyl-octanoic acid was used as internal standard.

Chromatographic separation and quantification

POPs: A 8560 Mega gas chromatograph (CE Instruments, Milan, Italy) was equipped with a 30 m DB5-MS column (0.25 mm id and 0.25 µm film thickness; J&W, Folsom, USA), a guard column (0.53 mm id, 2.5 m length deactivated, J&W) and a restriction capillary (0.18 mm id, 1.5 m length deactivated, J&W). Helium (6.0 quality, Hydrogas, Porsgrunn, Norway) was used as carrier gas at a flow rate of 1 mL/min. Two µL of the sample extract were injected on-column with an AS800 automatic injection system, (CE Instruments). The following temperature program was used: 70 °C (2 min), then 15 °C/min to 180°C and 5 °C/min to 280 °C (10 min isothermal). Quantification was carried out by low resolution mass spectrometry (LRMS) using a MD 800 mass spectrometer (Finnigan, San Jose, CA, USA) with an ionisation energy of 70 eV. The transfer line temperature was held at 280 °C and the source temperature was set to 220 °C. A Varian 1200 mass spectrometer was used in ECNI mode, under similar conditions as described above, for identification and quantification of the

pesticides (except DDTs), monitoring single ions, source temperature at 160 °C. The limit of detection (three times signal/noise ratio) for the analysed BFR PCB, DDTs and organochlorines ranged between 2 - 20 pg/g ww.
PFCs: Aliquots (50 μL) were injected automatically on a HPLC (Agilent 1100; Agilent Technologies, Palo Alto, CA) coupled to ESI time-of-flight-high-resolution MS in the negative ion mode (LCT, Micromass, Manchester, England). Compounds were separated on an ACE C18 column (150 x 2.1 mm, 3 μm particle size) (ACT, Aberdeen, U.K.) using a gradient of 200 μL/min methanol and water (both with 2 mM NH_4OAc). The initial mobile phase condition was 50:50 methanol/water, followed by a 5 min ramp increase to 85:15, a 5 min hold at 85:15, a 0.5 min ramp to 99:1, and hold until reverting to initial condition after min 15. Full scan (*m/z* 165-720) high resolution mass spectra were monitored throughout the chromatograms.

Results and Discussion

Because PFCs bind to proteins, lipid normalization is not useful in order to compare concentrations. Our data are given on wet weight basis. Liver concentrations of sumPFC in European shag were slightly higher compared to plasma and egg samples (Figure 2). PFOS was the dominating compound in all analysed samples. With median concentration of 29 ng/g ww PFOS in egg, 27 ng/g ww in liver and 28 ng/g in plasma concentrations were approximately four times lower than reported for glaucous gull from the Norwegian Arctic[8] presumably because the European shag feeds on a lower trophic level. Perfluorooctane sulfonamide (PFOSA) was detected in all shag liver samples, and varied between 5 and 20 ng/g ww, but was much lower in egg and plasma samples. On the other hand, the pattern of the perfluorinated carboxylates (PFCA) varied considerably between the analysed sample types. The perfluorooctanoate (PFOA) was detected in all plasma samples in concentrations between 2-6 ng/g ww, whilst it was not detectable in the shag eggs and only occasionally found in the liver. We found high proportions of the perfluorodecanoic acid (PFDcA) in the liver and plasma but not in the eggs indicating different transport mechanisms within the body compartments. However, long and odd numbered PFCA were found only in the shag eggs (up to perfluorotridecanoic acid), indicating a selective transportation mechanism for these compounds under egg production. The common eider eggs showed the lowest sumPFC concentration with the fewest number of detectable compounds of all analysed sample types.

Eggs from European shag were the most contaminated samples analysed (sumPCB: 4750 ng/g lw; sumDDT: 1540 ng/g lw; sumPBDE: 90 ng/g lw; sumPest: 1610 ng/g lw). Liver and plasma showed between 20 – 40 times lower concentrations for the analysed compounds when related to lipid content. European shag egg samples from our study (Figure 3) show ca. 3 times lower POPs levels compared to a recent study on yolc sac of European shag hatchlings from the same site in 2002 [9]. The difference of the levels is difficult to explain. We did not see any considerable differences between our two years of sampling. Compared to a recent study done on liver of black guillemot from Svalbard[11], the here presented liver data are ca. 10 times lower for sumPCB and sumDDT. PBDEs could be detected in plasma and liver from European shag only occasionally and in very small concentrations. The eggs from the common eider showed slightly lower concentrations for all investigated POPs, with sumPBDE as an exception, when compared on wet weight basis. By relating to lipid content, the difference increases in favor of the shag , as the shag eggs has a much lower lipid content (4 % in shags, 16 % in eiders). However, a clear PBDE pattern difference was found between the two bird species. Similar to [9], BDE 100 dominates the PBDE pattern in the European shag egg, followed by BDE 47, 154, 28, 153 and 99, whilst the common eider shows the usual PBDE pattern for marine biota with BDE 47 as the dominating PBDE.

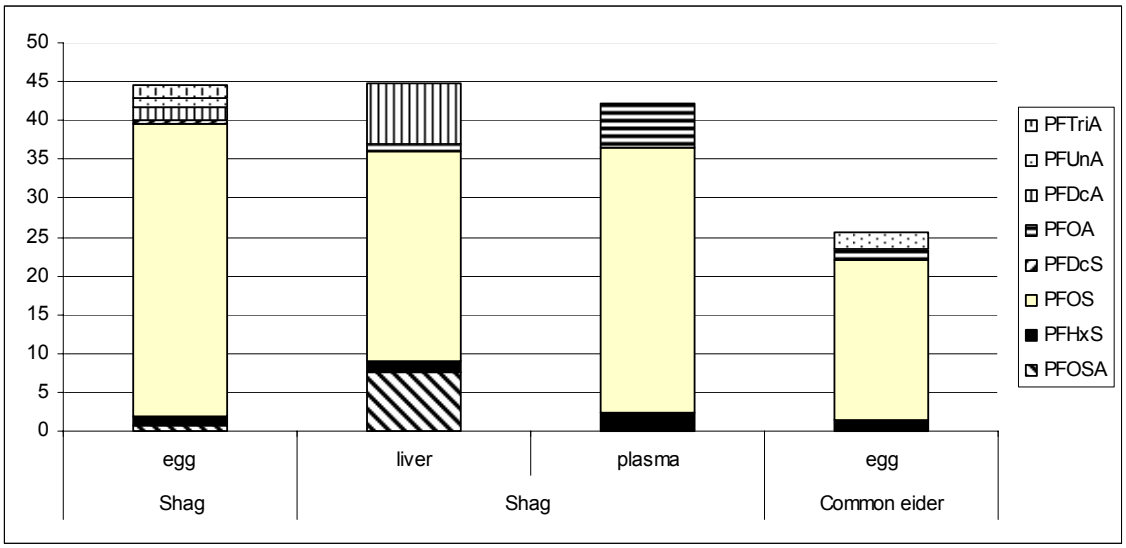

Figure 2: Median values of PFCs in shag and common eider from Sklinna, Norway

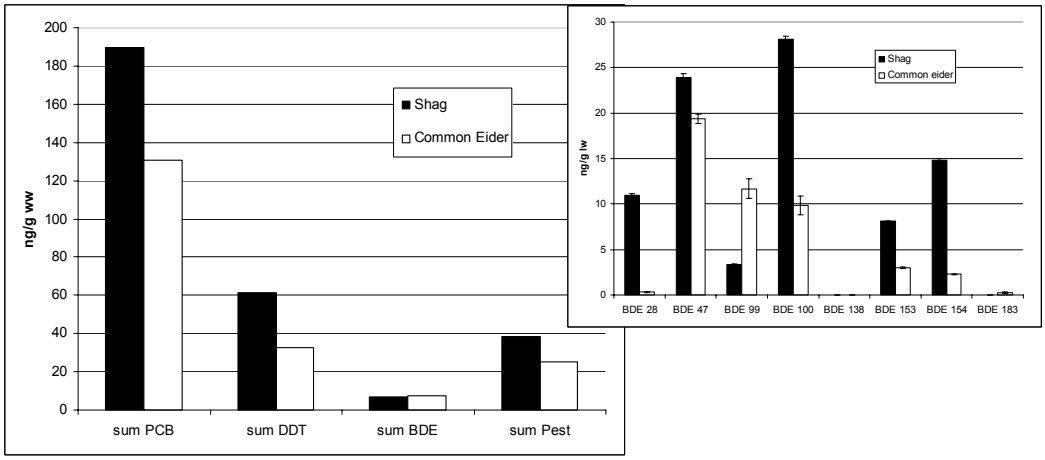

Figure 3: POP levels in eggs of shag and common eider from Sklinna, Norway (Lipid content: European shag: 4%; Common eider: 16%).

References

1) Kissa,E, Fluorinated Surfactants and Repellents, Marcel Dekker, New York, 2nd edn., 2001.
2) Giesy,JP and K. Kannan, Environ. Sci. Technol., 2001, 35, 1339.
3) Smithwick,M, RJ Norstrom, SA Mabury, K Solomon, TJ Evans, I Stirling, MK Taylor, DCG Muir, 2006, Environ. Sci. Technol, v. 40, p. 1139-1143.
4) Kannan,K, J. Koistinen, K. Beckmen, T. Evans, J. F. Gorzelany, K. J. Hansen, P. D. Jones, E. Helle, M. Nyman and J. P. Giesy, Environ. Sci. Technol., 2001, 35, 1593.
5) X. Han, T. A. Snow, R. A. Kemper and G. W. Jepson, Chem. Res. Toxicol., 2003, 16, 775.
6) Herzke D., R Kallenborn, T Nygård, 2002, STOTEN, v. 291, p. 59-71.
7) Powley, C.R., R.C. Buck, 2005, poster presentation at the 15th annual SETAC Europe meeting, Lille, France
8) Verreault,J, M Houde, GW Gabrielsen, U Berger, M Haukås, R J Letcher, D C G Muir, 2005, Env. Sci. Techn, v. 39, p. 7439-7445.
9) Murvoll,KM, J U Skaare, E Anderssen, B M Jenssen, 2006, Env. Tox. Chem., v. 25, p. 190-198.
10) Toschik,PC, BA Rattner, PC McGowan, MC Christman, DB Carter, RC Hale, CW Matson, MA Ottinger, 2005, Env. Tox. Chem., v. 24, p. 617-628.
11) Haukås, M, 2005, Master Thesis; Dep. of Aquatic Biosciences; University of Tromso; Norway.