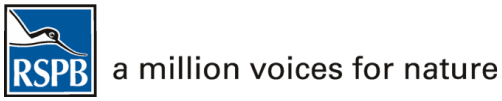
Concentrations of pefluorinated compounds (PFCs) in northern gannet, *Morus bassanus*, eggs: a Predatory Bird Monitoring Scheme (PBMS) Report

L.A Walker, M.G. Pereira, E.D. Potter, S. Lacorte & R.F. Shore[1]



[1]Corresponding author: RF Shore, Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK. E-mail: rfs@ceh.ac.uk. Website: http://www.ceh.ac.uk/

This report should be cited as:

Walker, L.A., Pereira, M.G., Potter, E.D., Lacorte S., & Shore R.F. (2014). Concentrations of peflurinated compounds (PFCs) in northern gannet, *Morus bassanus*, eggs: a Predatory Bird Monitoring Scheme (PBMS) Report, 11pp

Suggested keywords: Annual report; environmental contamination; monitoring; perfluorinated compounds; PFC; persistent organic pollutants; Great Britain; northern gannet; eggs

Centre for Ecology and Hydrology Project Number: NEC04288

E-copies of this report: This report can be requested through the Natural Environment Research Council's Open Research Archive http://nora.nerc.ac.uk/ or can be downloaded directly from the PBMS website http://pbms.ceh.ac.uk/

Contents

1. Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability contaminant monitoring and surveillance work on avian predators. By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

This report presents the results of a pilot study to quantify the concentrations of perfluorinated compounds (PFCs) in the eggs of the northern gannet, *Morus bassanus*, from the Ailsa Craig and Bass Rock colonies off the UK coast. The principle aim of this work determine the concentrations of PFCs that are accumulated in the eggs of gannets and whether there was any evidence of differences in accumulation between eggs from the two colonies studied.

The egg contents were analysed by Liquid Chromatograph – Mass Spectrometry (LC-MS) techniques. Compounds from both the perfluorinated carboxylate and perfluorinated sulfonate groups of PFCs were quantified.

PFCs were detected in all of the ten eggs analysed, with both carboxylate and sulfonate compounds present. Perfluorobutanoate (PFBA) and perfluorononanoate (PFNA) concentrations were both significantly higher in eggs from Ailsa Craig compared to those from Bass Rock but there was no difference between colonies in egg concentrations of sum PFC, sum carboxylate or sum sulphonate concengtrations.

Overall data from this limited one year of sampling suggests that gannet eggs from both Ailsa Craig and Bass Rock contain relatively low concentrations compared to eggs from some other species that have been examined. Concentrations of perfluorooctane sulfonate (PFOS) were with an order of magnitude of residues associated with adverse effects [and for one egg from Bass Rock these levels were exceeded] but the majority of eggs from both colonies contained PFOS residues that exceeded a suggested predicted no effect concentration (PNEC) for this compound.

2. The Predatory Bird Monitoring Scheme

2.1. Background

The Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.

By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. The PBMS provides the scientific evidence needed to determine how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory decisions about sustainable use of chemicals (for example, the EU Directive on the Sustainable Use of Pesticides). In addition, the outcomes from the monitoring work are used to assess whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently, the PBMS has two key objectives:

(i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value

(ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the PBMS website.

Perfluoroalkyl compounds (PFCs) are a class of compounds that are used world wide as surface treatments on textiles, leather and carpet, in paper products used for food preparation and storage, and in fire fighting foams, insecticides and floor polishes (Custer *et al.*, 2012). PFCs consist of a hydrocarbon chains where the hydrogen atoms have been substituted by a fluorine atom. Commonly a functional group, such as a carboxylate or sulfonate, is added to one of the carbons in the chain. The length (number of carbon atoms) in the chain and the type and position of the functional group determine the physiochemical properties of the individual compound and consequently its use and environmental fate and toxicity.

Increasing production over previous decades has lead to increasing exposure to birds and fish species to these compounds(Gebbink and Letcher, 2012). Other factors, such as the proximity to sources, have been shown to influence the concentrations of these compounds in wildlife (Gebbink *et al.*, 2009). Long-chain perfluorinated chemicals (C9-C13) are bioaccumulative in wildlife and humans, and are persistent in the environment. To date, significant adverse effects have not been found in the general human population but adverse effects have been identified in laboratory animals and wildlife (USEPA, 2009). These effects include reduced hatchability and chick survival in birds, reduced cumulative fecundity and fertility in fish and delays in growth and metamorphosis in amphibians.

The use of one prevalent PFC, perfluorooctane sulfonate (PFOS) is restricted due to its inclusion in Annex B[2] of the Stockholm Convention and is subject to action plans, aimed at reducing the environmental impact of PFOS, and it's precursors, both in the USA and the United Kingdom (Defra and EA, 2004, USEPA, 2009)..

The PBMS has utilized its tissue archive to carry out a pilot study to determine whether PFCs can be detected in the eggs of the northern gannet, *Morus bassanus*, from Ailsa Craig and Bass Rock. Both colonies consist of between 30,000 and 35,000 breeding pairs of gannet which combined equates to approximately 16% of the world population of this species. As a top predator, gannets are likely to be exposed to chemical contaminants that biomagnify through the food chain due to their persistence and bioaccumulative properties.

The aim of this pilot study is to determine whether PFCs are present at detectable levels in gannet eggs and to identify the compounds that are most prevalent. In addition the magnitude of residues found in eggs from Ailsa Craig and Bass Rock will be compared to those previously reported in eggs and to residue levels thought to cause adverse effects.

[2] http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx

3. Methods

3.1. Collection of eggs

The eggs used in this study were collected under licence as part of the long-term monitoring programme of the Predatory Bird Monitoring. Ten fresh eggs were taken during laying or the early incubation period from separate nests from the Ailsa Craig and Bass Rock colonies (Fig. 3.1). The length, breadth and weight of each egg were measured and contents were collected by cracking the eggs open. The contents were homogenised, kept at -20ºC and then analysed.

Figure 3.1 Location of Ailsa Craig (West Scotland) and Bass Rock (East Scotland).

Eggs collected in 2007 from two major colonies of northern gannet were selected for this pilot study. This is the most recent year from which we have samples from both colonies, thus allowing comparisons between the two colonies inter-year variation potentially being a confounding factor in contributing to inter-colony variation.

3.2. Analytical methods

PFASs were solid-liquid extracted from homogenized wet samples using acetonitrile. One g of eggs was weighted in polypropylene tubes and internal standards (m-PFOS and m-PFOA) were added at a concentration of 100 ng/g, and incubated for 18 hours at 4ºC. Nine mL of acetonitrile were added and the samples were thoroughly mixed using a vortex mixer. Samples were extracted in an ultrasonic bath for 10 min at room temperature. This procedure (vortexing and ultrasonic extraction) was repeated 3 times without changing the solvent. Afterwards, the samples were centrifuged at 2.500 rpm for 5 min. The supernatant was transferred to a new vial and evaporated to dryness. Then, 1 mL of acetonitrile was added to the dried sample and incubated for 10 min in the ultrasonic bath. The samples were purified by adding 25 mg of activated carbon and 50 µL of glacial acetic acid and were vigorously mixed for 1 minute. Afterwards, the samples were centrifuged for 10 min at 10.000 rpm. The supernatant was transferred to a clean micro vial, and 250 µL of this were diluted with 250 µL of water with 10 mM ammonium acetate buffer of mobile phase. PFASs were measured using an Acquity Ultra Performance Liquid Chromatography

system connected to a Triple Quadruple Mass Spectrometry Detector (Waters, USA) with an Acquity UPLC BEH C18 column (1.7 µm particle size, 100 mm x 2.1 mm, Waters, USA) using MRM. Five µL of extract were injected. Internal standard quantification was performed using m-PFOS to quantify PFBS, PFHxS, PFOS and PFDS and m-PFOA to quantify PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTriDA, PFTeDA, PFHxDA and PFODA.

A mixture of native perfluoroalkylcarboxylic acids (PFCAs)[3] (and native perfluoroalkylsulfonates (PFSAs)[4] was supplied by Wellington Laboratories (Ontario, Canada). Stock standard solutions were prepared in acetonitrile at a concentration of 5 ng/µl for all native compounds and were stored at -18ºC. Perfluoro-n-(1,2,3,4-13C4) octanoic acid (m-PFOA) and sodium perfluoro-1-(1,2,3,4-13C4) octanesulfonate (m-PFOS), also from Wellington Laboratories, were used as surrogate standards. HPLC grade water and acetonitrile were supplied by Merck (Darmstadt, Germany) and glacial acetic acid from Panreac (Barcelona, Spain). Average recoveries for internal PF recovery standards ranged between 72% and 144%.

3.3. Data expression, format and analysis

Throughout this report, egg concentrations of perfluorinated compounds are reported as ng/g wet weight (wet wt). A correction factor was applied for desiccation by multiplying concentrations by the total egg weight/volume ratio. Egg volume was estimated using the equation $V = 0.51 \times LB^2$, where L is egg length and B is egg breadth(Hoyt, 1979). When summed PFC concentrations were calculated, individual compound concentrations below the limit of detection (non-detected) were assigned a zero value. All statistical tests were performed using GraphPAD Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

[3] perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTriDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA) and perfluorooctadecanoic acid (PFODA)

[4] perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS) and perfluorodecane sulfonic acid (PFDS)

4. Results and Discussion

4.1. PFC profile in the eggs of northern gannets from Ailsa Craig and Bass Rock.

Perfluorinated compounds were detected in all the eggs analysed with Perfluorooctane sulphonate (PFOS), Perfluorotridecanoate (PFTriDA) and Perfluoroundecanoate (PFUnA) the most prevalent compounds on a mass concentration basis. Compounds can be split into two broad classes, the carboxylates and sulphonates, and individual sum concentrations for the two classes ranged from 7.25 to 34.5 ng.g^{-1} wet wt. and 25.0 to 204 ng.g^{-1} wet wt., respectively. PFOS accounted for 98-99% of sum sulphonate concentrations, although it should be noted that only four sulphonate compounds were quantified in this study.

Sum perfluorinated carboxylate (Mann Whitney U = 4, P=0.095) and sum perfluorinated sulpohonate (Mann Whitney U = 3, P=0.055) concentrations did not differ between eggs from the two colonies studied, although concentrations of some individual compounds did. Perfluorobutanoate (PFBA) and perfluorononanoate (PFNA) concentrations were both significantly higher in eggs from Ailsa Craig compared to those from Bass Rock (Fig. 4.1). However as the residues of these compounds are relatively low compared to other PFC compounds detected in this study, less than 5% of sum PFC concentration, these differences were not statistically significant in sum PFC, sum carboxylate PFC, and sum sulphonate residues.

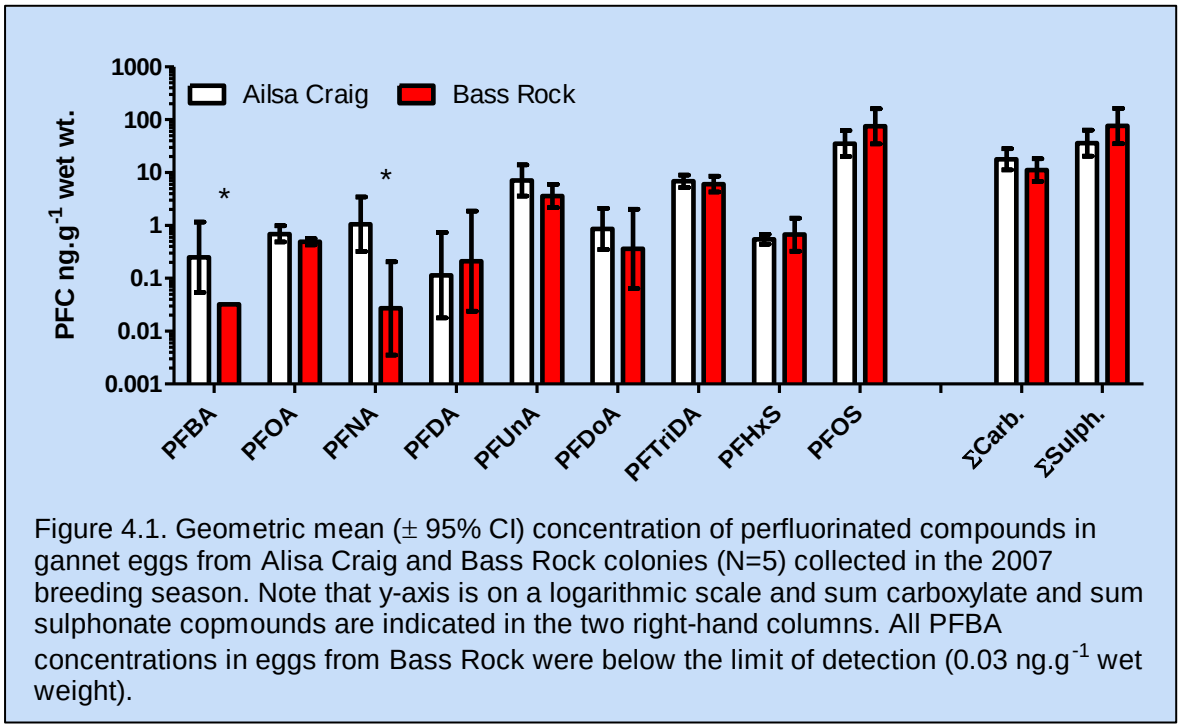


Figure 4.1. Geometric mean (± 95% CI) concentration of perfluorinated compounds in gannet eggs from Alisa Craig and Bass Rock colonies (N=5) collected in the 2007 breeding season. Note that y-axis is on a logarithmic scale and sum carboxylate and sum sulphonate copmounds are indicated in the two right-hand columns. All PFBA concentrations in eggs from Bass Rock were below the limit of detection (0.03 ng.g^{-1} wet weight).

4.2. How do our findings compare to other studies?

As the suite of PFC compound quantified varies among studies, it is only valid to compare concentrations of individual compounds rather than sum PFC residues. Concentrations of the primary PFC compound, PFOS, in eggs from Ailsa Craig and Bass Rock are relatively low compared to those reported in bird eggs from previous studies. The high residues of PFOS in bird eggs, 1249 $ng.g^{-1}$ wet wt., were measured in double-crested cormorant, *Phalacrocorax auritus*, from the heavily urbanised estuary of San Francisco Bay between 2006 and 2009 (Sedlak and Greig, 2012). The average concentrations of PFOS measured in our study are in the lowest 10% of those previously reported (Custer *et al.*, 2013, Sedlak and Greig, 2012). The gannet eggs from our study also had relatively low concentrations of PFNA, PFDA, PFDoA compared to other studies while concentrations of PFOA, PFUnA and PFHxS were within the inter-quartile range of the mean concentrations reported in other studies. As in studies on other species, PFHpA was not detected in gannet eggs. Overall data from this one year of sampling suggests that gannet eggs from both Ailsa Craig and Bass Rock contain relatively low concentrations compared to eggs from some other species. Further analysis of samples from other years would be necessary to ascertain whether this is consistently the case

4.3. How do our findings compare to residues associated with adverse effects?

Toxicity data for PFCs in birds is limited. Effects on hatching and pipping success in domestic chickens eggs are associated with residues ranging from 0.1 µg $PFOS.g^{-1}$ egg (lowest observed adverse effect level) to 100 $\mu g.g^{-1}$ (Molina *et al.*, 2006, O'Brien *et al.*, 2009) injected into the air space of the egg. PFOS concentrations in one of the gannet eggs from Bass Rock exceeded the lower of these concentrations with the majority of eggs from both colonies within an order of magnitude of the 0.1 µg $PFOS.g^{-1}$ reference residue level. Newstead et al (2005) suggests a predicted no effect level (PNEC) for eggs, based on reproductive effects in dietary exposed northern bobwhite quail, of 1µg PFOS/ml egg yolk. This PNEC would be equivalent to 29 ng PFOS/g egg; assuming a yolk specific gravity of 1.0 and after adjusting concentration in yolk to concentrations in the whole eggs using a 29% yolk to 71% albumen mass ratio measured in herring gull eggs. The majority of eggs from both colonies contained PFOS residues that exceeded this PNEC value. Further studies are needed to determine if such exceedence is typical and how PFOS concentrations may be changing over time.

5. Acknowledgements

We thank Scottish Natural Heritage staff for collecting the eggs from both Ailsa Craig and Bass Rock. The Predatory Bird Monitoring Scheme is co-funded by the Centre for Ecology & Hydrology, the Campaign for Responsible Rodenticide Use, the Department for Environment and Rural Affairs, Natural England, Scottish Natural Heritage, the Scottish Environment Protection Agency and the Royal Society for the Protection of Birds.

6. References

Custer, C. M., Custer, T. W., Schoenfuss, H. L., Poganski, B. H. & Solem, L. 2012. Exposure and effects of perfluoroalkyl compounds on tree swallows nesting at Lake Johanna in east central Minnesota, USA. *Reprod Toxicol,* 33**,** 556-62.

Custer, T. W., Dummer, P. M., Custer, C. M., Wu, Q., Kannan, K. & Trowbridge, A. 2013. Perfluorinated compound concentrations in great blue heron eggs near St. Paul, Minnesota, USA, in 1993 and 2010-2011. *Environ Toxicol Chem,* 32**,** 1077-83.

Defra & Ea 2004. Perfluorooctane Sulphonate: Risk Reduction Strategy and Analysis of Advantages and Drawbacks. Department for environmnet, Food and Rural Affairs & the Environment Agency for England and Wales.

Gebbink, W. A., Herbert, C. E. & Letcher, R. J. 2009. Perfluorinated carboxylates and sulfonates and precursor compounds in herring gull eggs from colonies spanning the Laurentian Great Lakes of North America. *Environmental Science & Technology,* 43**,** 7443-7449.

Gebbink, W. A. & Letcher, R. J. 2012. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environ Pollut,* 162**,** 40-7.

Hoyt, D. F. 1979. Practical methods of estimating volume and fresh weight of birds eggs. *Auk,* 96**,** 73-77.

Molina, E. D., Balander, R., Fitzgerald, S. D., Giesy, J. P., Kannan, K., Mitchell, R. & Bursian, S. J. 2006. Effects of air cell injection of perfluorooctane sulfonate before incubation on development of the white leghorn chicken (Gallus domesticus) embryo. *Environmental Toxicology and Chemistry,* 25**,** 227-232.

Newstead, J. L., Jones, P. D., Coady, K. & Giesy, J. P. 2005. Avian Toxicity Reference Values for Perfluorooctane Sulfonate. *Environ Sci Technol,* 2005**,** 9357-9362.

O'brien, J. M., Carew, A. C., Chu, S., Letcher, R. J. & Kennedy, S. W. 2009. Perfluorooctane sulfonate (PFOS) toxicity in domestic chicken (Gallus gallus domesticus) embryos in the absence of effects on peroxisome proliferator activated receptor alpha (PPARalpha)-regulated genes. *Comp Biochem Physiol C Toxicol Pharmacol,* 149**,** 524-30.

Sedlak, M. D. & Greig, D. J. 2012. Perfluoroalkyl compounds (PFCs) in wildlife from an urban estuary. *Journal of Environmental Monitoring,* 14**,** 146-154.

USEPA 2009. Long-Chain Perfluorinated Chemicals (PFCs) Action Plan. *In:* UNITED STATES ENVIRONMENT PROTECTION AGENCY (ed.). Link: http://www.epa.gov/opptintr/existingchemicals/pubs/pfcs_action_plan1230_09.pdf