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PFCAS, PFSAS AND FOSA IN HARBOUR PORPOISES (PHOCOENA PHOCOENA) STRANDED OR BYCAUGHT IN THE UK DURING 2012-2014

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Introduction

Marine mammals make excellent sentinels of pollution due to their high trophic position in marine food chains. Chemicals that are bioaccumulative can build up to high concentrations in their tissues, and this may result in harmful effects. The UK Cetacean Strandings Investigation Program (CSIP) has been running for 25 years. In this program selected animals which have been found stranded, or were bycaught around the UK, are taken for post-mortem study in order to establish cause of death. Contaminant analyses are conducted on a subset of these to establish current contaminant levels and to investigate spatial and temporal trends in concentration (1). Studies have been carried out with the aim of investigating possible links between contaminant burden and death due to infectious disease and, recently, reproductive impairment and failure (2-3). Although a variety of species are regularly collected, only harbour porpoises (Phocoena phocoena) are present throughout the UK and are consistently sampled in large numbers, so contaminant studies have focused mostly on this species. Perfluoroalkyl substances (PFAS), such as PFOS, are a group of man-made chemicals whose emission into the environment has caused concern due to the persistence, bioaccumulative and toxic properties held by some of them. PFOS concentrations in UK harbour porpoises have previously been determined for animals collected between 1992-2003 (4), and were among the highest that had been measured at the time. In this follow-up study, we analysed animals obtained in 2012-14 for a suite of 15 PFAS, to see if concentrations of PFOS had changed since the last study, and to see what other PFAS could be detected using an updated method.

Materials and methods

51 porpoises from 2012-12 that had been collected between 2012-2014 were selected for PFAS analysis. Only freshly dead to moderately decomposed animals were considered, and animals were chosen to provide a good geographical coverage, whilst also being an even mix of stranded or bycaught animals, and containing a similar proportion of adult males, adult females and juveniles in the different regions. The distribution of sampling locations around the UK is shown in Figure 1.

Figure 1. Map of UK with the standing locations where samples were collected for our study.

Liver samples were stored frozen at -20°C prior to analysis and were then thawed and homogenized before extraction. Samples (1 g) were spiked with 20 μL of a mixture of isotopically mass-labelled recovery/internal standards (ISTDs) in methanol containing 0.2 ng/μL of each ISTD ($^{13}C_2$-PFHxA, $^{13}C_4$-PFOA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{13}C_2$-PFTeDA, $^{13}C_8$-PFOSA, $^{18}O_2$-PFHxS and $^{13}C_4$-PFOS, all from Wellington, Guelph, Canada) in polypropylene tubes. The samples were extracted twice with 5 mL of acetonitrile in an ultrasonic bath (15 min, room temperature). Concentrated extracts underwent dispersive clean-up on 25 mg graphitized carbon (Supelclean ENVI-Carb 120/400, Supelco, Sigma-Aldrich, Stockholm, Sweden) and 50 μL glacial acetic acid in Eppendorf tubes. Aliquots of 0.5 mL of the cleaned-up extracts were diluted with 0.5 mL of 4 mM aqueous ammonium acetate and kept at 4°C until the day of analysis. The extracts were allowed to warm to room temperature, vortex mixed and centrifuged before the clear solution was transferred to an autoinjector vial, together with 10 μl of a mixture of isotopically mass-labelled injection standards containing 500 ng/μl of $^{13}C_8$-PFOA and $^{13}C_8$-PFOS. The analysis of PFAS was performed using an ultra-performance liquid chromatograph Acquity (Waters Ltd, Elstree, Hertfordshire, UK) using a BEH C18 analytical column (50 mm x 2.1 mm and 1.7 μm particle size) from Waters. The UPLC system was coupled to a TQ MS Xevo triple quadrupole mass spectrometer (Waters Ltd, Elstree, Hertfordshire, UK),

using an electro spray ionization (ESI) probe in negative mode. Where isomers were present in samples, only the linear isomer was quantified against the linear PFAS present in standards and results are reported for the linear isomer only, as recommended by Berger et al. (2011). For QA/QC purposes, a blank and reference material sample (flounder tissue from 6th Interlaboratory Study on PFASs in Environmental Samples 2013) were analysed with every 10 samples.

Results and discussion

Of the 15 PFAS analysed, 6 were ubiquitous (PFOS, FOSA, PFNA, PFDcA, PFUnA and PFTrDA), a further 5 were present in the majority of samples (PFHxS, PFDcS, PFHpA, PFDoDA and PFTeDA), 2 were detected fairly frequently (PFHxA and PFOA) and the other 2 were only occasionally detected (PFPeA and PFBuS) (see Table 1). Unsurprisingly, PFOS was present at the highest concentrations, with the maximum value of 1144 ng/g ww found in an animal bycaught in Merseyside (Liverpool). FOSA was the next highest concentration, on average, as was observed in Norwegian Atlantic harbour porpoises (5), with the maximum value of 89.7 ng/g ww found in the animal from Merseyside. From the perfluoroalkyl carboxylates (PFCAs), PFUnDA was usually found at the highest concentration, although occasionally PFDcA and PFTrDA were the highest. Highest PFUnDA, PFDcA and PFTrDA concentrations of 88.8, 80.8 and 50.1 ng/g ww were found in Anglesey (North Wales), Merseyside and The Gower (South Wales), respectively. Highest concentrations of individual PFAS were consistently found in the Irish Sea region. Concentrations of individual PFAS ranged over 2-3 orders of magnitude between lowest and highest values, suggesting local sources were important. In comparison with data obtained for PFOS in UK harbour porpoises from 1992-2003 (4), average concentrations in 2012-14 are about 1/3rd of those found earlier, suggesting environmental levels have decreased in response to the phase-out of PFOS.

Table 1. Concentrations and frequency of detection of PFAS in stranded and bycaught harbour porpoises.

Typically, there was a predominance of odd chain PFCAs over even chain PFCAs, which has been suggested might be indicative for degradation of fluorotelomer-based precursors (6), with PFNA>PFOA, PFUnA>PFDcA, and PFTrDA>PFDoDA. The south east corner of England, from Hampshire to Kent, differed from this pattern, with PFDcA≥PFUnA in most samples.

If samples are grouped into those from Scotland, the East and West of the rest of the UK, there are some interesting patterns. For PFOS and FOSA, East and West sample concentrations are similar but higher than those from Scotland. However, for PFCAs, West and Scotland sample concentrations are similar but higher than those from East. These differences result in concentrations of ΣPFSAs being much higher than ΣPFCAs in east samples, but similar to each other in Scotland.

Sex and age differences were found in the dataset. Concentrations in adult females were lower than those in adult males or juveniles of either sex, which in turn were similar to each other. Concentrations in females decreased with increasing body length but no trend was found in males. This data is consistent with females offloading some of their contaminant burden to their offspring. Previous studies have shown a difference in blubber PCB concentrations between stranded (=unhealthy) and bycaught (=healthy) animals, with stranded animals significantly higher than bycaught ones, i.e. PCB levels were having a detrimental effect on immune response (7). This was not the case for any PFAS in this study, with liver concentrations tending to be higher in the bycaught animals than the stranded ones.

Levels of PFAS obtained in this study will be compared with other published values for marine mammals and an assessment of potential exposure health effects will be made. A comparison with predicted health effects of other pollutants for which data is available in UK harbour porpoises will be presented.

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References

1) Law, R.J., Barry, J., Barber, J.L., Bersuder, P., Deaville, R., Reid, R.J., Brownlow, A., Penrose, R., Barnett, J., Loveridge, J., Smith, B., Jepson, P.D., 2012. Contaminants in cetaceans from UK waters:

status as assessed within the Cetacean Strandings Investigation Programme from 1990 to 2008. Mar. Pollut. Bull. 64, 1485-1494.
2) Murphy, S., Barber, J.L., Learmonth, J.A., Read, F.L., Deaville, R., Perkins, M.W., Brownlow, A., Davison, N., Penrose, R., Pierce, G.J., Law, R.J., Jepson, P.D., 2015. Reproductive failure in UK Harbour porpoises Phocoena phocoena: Legacy of pollutant exposure? PloS one 10.7, e0131085.
3) Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, Al., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., ten Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Giménez, J., Loveridge, J., Llavona, A., Martin, Vi., Mazwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez, A., Law, R.J., 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Scientific reports 6, Article number: 18573
4) Law, R.J., Bersuder, P., Mead, L.K., Jepson, P.D., 2008. PFOS and PFOA in the livers of harbour porpoises (Phocoena phocoena) stranded or bycaught around the UK. Mar. Pollut. Bull. 56, 792-97.
5) Huber, S., Ahrens, L., Bardsen, B.J., Siebert, U., Bustnes, J.O., Vikingsson, G.A., Ebinghaus, R., Herzke, D., 2012. Temporal trends and spatial differences of perfluoroalkylated substances in livers of harbour porpoise (Phocoena phocoena) populations from Northern Europe, 1991-2008. Sci. Total Environ. 419, 216-224.
6) Butt, C., Muir, D.C., Stirling, I., Kwan, M., Mabury, S.A., 2007. Rapid response of arctic ringed seals to
changes in perfluoroalkyl production. Environ. Sci. Technol. 41, 42–9.
7) Jepson, P.D., Bennett, P.M., Deaville, R., Allchin, C.R., Baker, J.R., Law, R.J., 2005. Relationships between PCBs and health status in harbour porpoises (Phocoena phocoena) stranded in the United Kingdom. Environ. Toxicol. Chem. 24, 238-248.

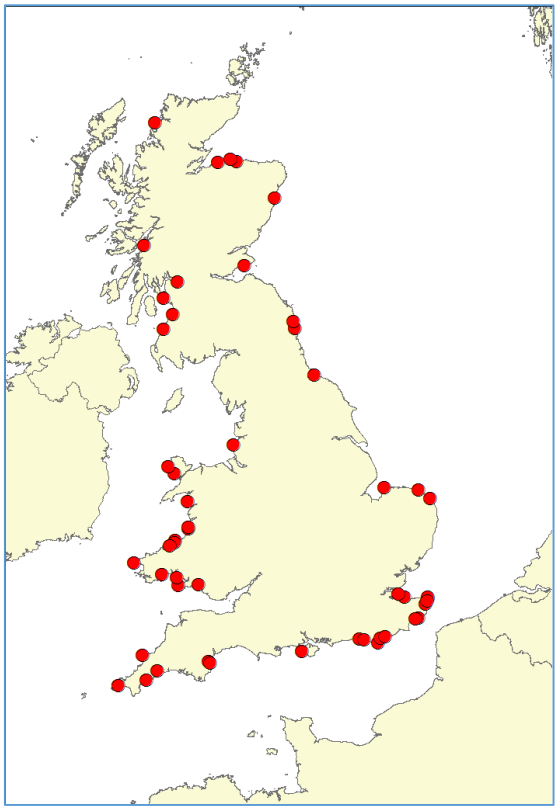

Figure 1. Map of UK with the standing locations where samples were collected for our study.

Table 1. Concentrations and frequency of detection of PFAS in stranded and bycaught harbour porpoises.

PFAS	No of detects (/51)	Concentration in ng/g ww		
		Mean of detected values	Minimum	Maximum
PFPeA	6	0.193	<0.05	0.508
PFHxA	10	0.095	<0.05	0.164
PFHpA	31	0.171	<0.05	0.43
PFOA	24	0.255	<0.05	0.818
PFNA	51	2.71	0.087	18.5
PFDcA	51	9.06	0.535	80.8
PFUnA	51	16.0	1.53	88.0
PFDoDA	50	2.82	<0.1	14.9
PFTrDA	51	7.39	1.02	50.1
PFTeDA	39	1.60	<0.2	7.06
Sum PFCAs	**51**	**39.4**	**6.25**	**181**
PFBuS	3	0.104	<0.05	0.125
PFHxS	49	1.04	<0.05	4.62
PFOS	51	178	6.56	1144
PFDcS	46	0.735	<0.1	5.84
Sum PFSAs	**51**	**180**	**6.56**	**1152**
FOSA	51	17.7	0.206	89.7