POLYFLUOROALKYLPHOSPHATE ESTERS (PAPS) IN HERRING GULL TISSUES, BLOOD, AND EGGS FROM THE NORTH AMERICAN GREAT LAKES

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Introduction

Fluorotelomer-based polyfluoroalkylphosphate mono-, di-, and triesters (mono-, di-, and triPAPs, respectively) are commercial chemicals that are used to water- and grease-proof food packaging materials made of paper and cardboard. PAPs have been identified as precursor compounds to perfluorocarboxylic acids (PFCAs), as their degradation to PFCAs has been shown in microbial and rat systems.[1,2]

DiPAPs have been identified as the main components in technical PAP mixtures (mono- and triPAPs are by-products)[3], and diPAPs with varying chain length have been detected in food packaging material and food items[4]and in human serum from Germany, Hong Kong, and the US.[5,6,7,8] Studies investigating the environmental fate of PAPs are limited. Loi et al. reported on the presence of 6:2/6:2 and 8:2/8:2 diPAP in water, sediment, and worms from Hong Kong[8], while Guo et al. detected these two diPAPs in lake trout (*Salvelinusnamaycush*) from the Great Lakes.[9]The detection of diPAPs in lake trout showed the presence of PAPs in the Great Lakes environment, and the trout is likely exposed to PAPs by consuming prey fish. Herring gulls (*Larusargentatus*) in the Great Lakes feed on the same prey fish as lake trout but are exposed to higher concentrations of perfluoroalkyl acids (PFAAs) compared to the trout.[9,10] To what extent herring gulls in the Great Lakes, or other avian top predators, are exposed to PAPs is presently unclear.

Recently, Gebbink et al. detected PAPs in food items by applying a sample clean-up method using weak anion exchange SPE.[4] This methodology has earlier also been used in a study thatidentified PFAAs and precursor compounds in herring gull tissues, blood, and eggs collected from the Great Lakes (Chantry Island) in 2010.[10]The aim of the present study was to screen herring gull tissues (liver, brain, muscle, and adipose), blood (plasma and red blood cells) and eggs (yolk and albumen) for PAPs with varying chain length by reanalyzing the sample extracts from Gebbink and Letcher.[10]

Materials and methods

Samples of organs and tissues, i.e., liver, blood, the whole brain, muscle and adipose, were collected from female herring gulls (n=8) from ChantryIsland, Lake Huron in April 2010. The blood samples were centrifuged on site to separate the plasma from the red blood cells (RBCs). Also, complete clutches of eggs (n=17) from the eight nests were collected (seven nests with two eggs, one nest with three eggs).The eggs were processed by separating and homogenizing the yolk and albumen. All individual samples were previously extracted, cleaned up, and fractionated by weak anion exchange SPE. The two fractions containing neutral (FOSAs and FTOHs) and ionic (PFSAs, PFCAs, and FTUCAs) compounds were analyzed and results published by Gebbink and Letcher.[10]Final extracts from that study were used for PAP analysis in the present work.Fractions containing ionic compounds were pooled for each tissue (n=8), while yolk and albumen samples were divided into 2 pools (n= 8 and 9), each pool containing 1 egg from each nest and one pool additionally containing a second egg from one nest. Final extracts of individual procedural blanks (n=8) were also pooled to determine the methodological background contamination. All pooled samples were concentrated and spiked with labeled internal standards (6:2 and 8:2 mono- and diPAPs) prior to analysis on anAcquity UPLC system connected to a Xevo TQ-S triple quadrupole MS (Waters).

Samples were analyzed for seven monoPAPs (4:2, 6:2, 8:2, 10:2, 12:2, 14:2, 16:2) and fourteen diPAPs (4:2/4:2,4:2/6:2, 6:2/6:2, 6:2/8:2, 8:2/8:2,6:2/10:2,8:2/10:2,6:2/12:2,10:2/10:2,8:2/12:2,6:2/14:2,10:2/12:2,8:2/14:2,and 6:2/16:2) of which 6:2, 8:2,

and 10:2 mono- and diPAPs were identified using authentic standards. Other PAPs were identified using a Zonyl-RP® technical mixture.[4]For both mono- and diPAPs the $H_2PO_4^-$ product ion (*m/z* 97) was the most abundant ion in the product ion spectra of $[M\text{-}H]^-$, however, additional product ions were included in the method. These were $[M\text{-}HF]^-$for monoPAPsand a monoPAP product ion for diPAPs. For diPAPs with mixed chain lengths (e.g, 4:2/6:2), the ion for the shorter chain length monoPAP (here 4:2) was identified as the more abundant product ion.[3]Further details on instrumental analysiscan be found in Gebbinket al.[4]

Results and discussion

Analysis of the pooled blank extract showed the presence of traces of 6:2 monoPAP and 4:2/6:2, 6:2/6:2, 6:2/8:2, 8:2/8:2,6:2/10:2,8:2/10:2,6:2/12:2,10:2/10:2,8:2/12:2,and 6:2/14:2diPAPs. Detection of these PAPs in the sample extracts were considered a positive detect when peak areas were at least three times higher than the corresponding peak area in the blank extract. Table 1 shows the detected mono- and diPAPs in the eggs, tissues, and blood.Of these positive detects, PAP peak areas in the sample extracts were subtracted with one time the blank area (blank correction) for estimation of concentrations (see below).

By monitoring the $H_2PO_4^-$ product ion, the 6:2, 8:2, and/or 10:2 monoPAPs were detected in herring gull egg (yolk and albumen), tissues (liver, brain, adipose) and plasma, while the $[M\text{-}HF]^-$product ion was not detected (Table 1, Figure 1). The liver and yolk showed the highest detection frequenciesof diPAPs, while the 8:2/8:2 and 6:2/10:2 diPAPswere detected in most samples. All diPAPs were identified by both the $H_2PO_4^-$ and monoPAP product ions.Ratios of the two product ions in the samples were comparable to the ratios in standard solutions or Zonyl-RP® (Table 1). Previous studies have shown wildlife exposure to 6:2/6:2 and 8:2/8:2 diPAPs.[8,9]The present results show that herring gulls from the Great Lakes were not only exposed to these diPAPs, but also to monoPAPs and other chain length diPAPs. These are PAPs that have been identified in technical mixtures as well as in food packaging materials.[4]

Table 1 Peak area ratio between two main product ions of detected mono- and diPAPs in herring gull eggs, tissues, and blood[1]

	Standard/	Egg		Tissue				Blood	
	Zonyl-RP[2]	Yolk[3]	Albumen[3]	Liver	Brain	Muscle	Adipose	Plasma	RBC
monoPAP									
6:2	6.39			√[4]					
8:2	6.42	√/√	√/√	√	√		√	√	
10:2	10.9	√/√						√	
diPAP									
4:2/6:2	*2.14*	1.55/1.91							
6:2/6:2	1.14	1.23/1.28		1.34				1.30	
6:2/8:2	*2.26*	2.57/2.48	2.40/nd	2.42	2.47			2.45	
8:2/8:2	1.45	1.57/1.51		1.59	1.74	1.58		1.69	1.60
6:2/10:2	*2.07*	1.95/2.10		2.17	2.25	2.35		2.00	2.73
8:2/10:2	*3.64*	nd/4.22		2.54		5.19			
6:2/12:2	*1.67*	nd/2.07		2.32					
10:2/10:2	n.a.[5]			#[6]					
8:2/12:2	n.a.[5]			#[6]					
6:2/14:2	*1.62*			4.00					

[1]monoPAP product ion ratio: $H_2PO_4^-$ / $[M\text{-}HF]^-$; diPAP product ions ratio: $H_2PO_4^-$ / monoPAP ion

[2] Product ion ratios in *italics* were determined using Zonyl-RP®, other PAPs with authentic standards

[3]Two yolk and albumen pools were generated from individual extracts

[4] √ indicates detection of $H_2PO_4^-$ product ion but not of $[M\text{-}HF]^-$ product ion

[5]10:2/10:2 and 8:2/12:2 diPAPscoeluted, therefore a ratio could not be calculated

[6] Both product ions were detected for 10:2/10:2 and 8:2/12:2 diPAPs but a ratio could not be calculated

In all analyzed herring gull tissues, eggs, and blood compartments, one or more mono-and/or diPAPs were detected. Previous studies on biota have measured PAPs in whole organisms (worms)[8] or trout fillet samples.[9] Although PAPs have been shown to undergo degradation, the present data indicates that PAPs are persistent enough to be distributed through the environment/food web into the gull's body, resulting in accumulation in all the analyzed tissues, including the liver. Maternal transfer of mono- and diPAPs occurred as they were detected in the egg yolk and/or albumen. Egg proteins are produced in the liver and likely PAPs bind to these proteins in the liver and are transferred to the ovaries. The detection of PAPs in the eggs is again an indication of their persistence as the PAPs pass the liver, although metabolism in the liver could have occurred. For monitoring purposes of PAPs in wildlife species, analysis of egg yolk or liver samples would be preferred as the highest detection frequency and signalintensities of mono- and diPAPs were found in these tissues, although tissue-specific differences in the detection of single diPAPs were observed (Figure 1).

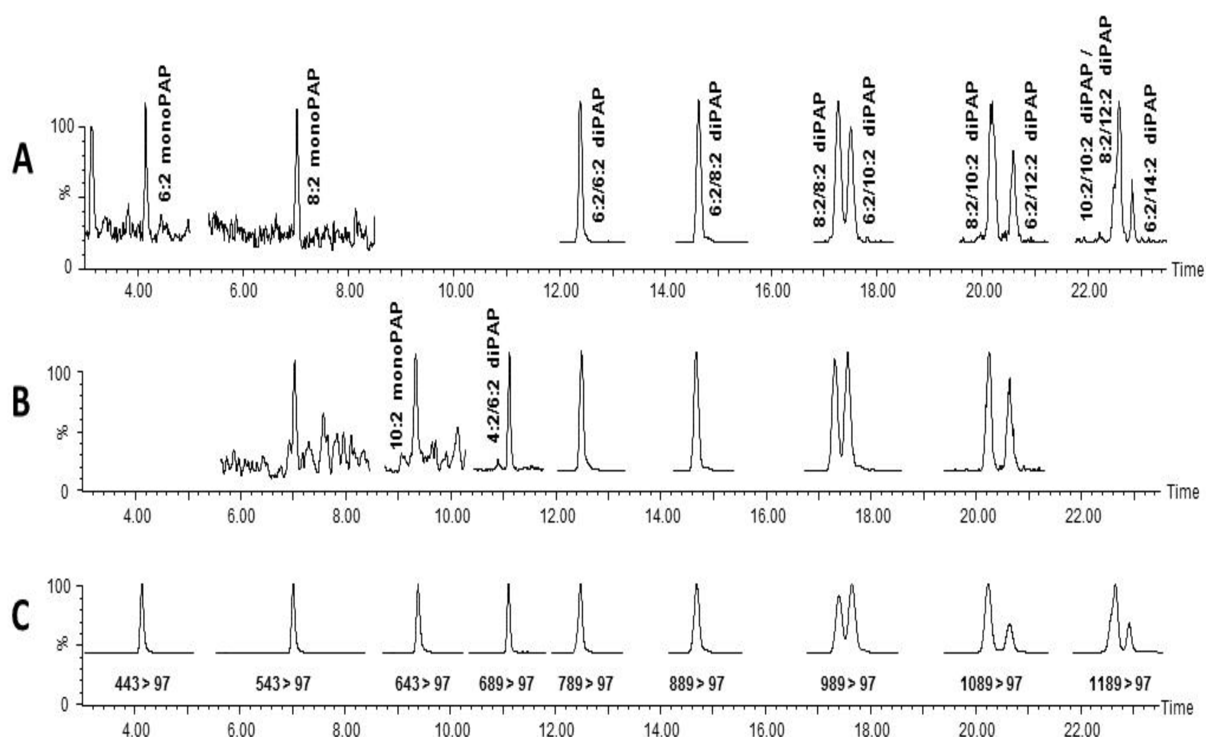

Figure 1 Chromatograms of detectable mono- and diPAPs in pooled liver extract (A) and pooled yolk extract (B), and the corresponding mono- and diPAPs in Zonyl-RP® (C)

Reanalysis of existing sample extracts showed the presence of mono- and diPAPs, however, accurate quantificationwas not possible. As mentioned, background contamination occurred during extraction and clean-up. Also, prior to extraction the samples were not spiked with isotopically mass-labeled mono- and diPAP internal standards, so no recovery correction could be made. However, a semi-quantitative estimation was made of the concentrations found in the tissues. Concentrations were calculated for 6:2 and 8:2 monoPAPs, and 6:2/6:2 and 8:2/8:2 diPAPs as both native and labeled standards were available.Labeled standards have been shown to be crucial for quantitative PAP determination in order to correct for matrix effects.[4]Sample extracts were spiked with labeled internal standards prior to LC-MSMS analysis, which corrected for some of the matrix effectsoccurring during instrumental analysis, e.g., due to sorption to the injection vial or during ionization.

Estimated concentrations of the 8:2 monoPAP ranged between 1 and 7 pg/g wet weight (ww) in the yolk, albumen, plasma, and the brain, while the adipose and liver contained 34 and 79pg/gww, respectively (Table 2). The 6:2 monoPAP was only quantified in the liver at 56 pg/gww. In the RBC and muscle neither monoPAPs were detected.The 6:2/6:2 and/or 8:2/8:2 diPAP concentrations in the plasma, RBC, muscle, and brain ranged between 1 and 4 pg/gww. In the liver, 6:2/6:2 and 8:2/8:2 diPAP concentrations were 20 and 46 pg/gww, respectively. The two yolk pools were both made up of 1 egg from each nest (one pool additionally containing a second egg from one of the nests), however, 6:2/6:2 diPAP concentrations in one pool was ten times higher compared to the other pool (343 and 3371 pg/gww).This was also seen for 8:2/8:2 diPAP (36 and 366 pg/gww). It is not clear why there was a tenfold difference in the concentrations of these two diPAPs in the two pools.

MonoPAPs have previously not been identified in wildlife samples.The presence of monoPAPs in the herring gull samples could result from dietary exposure tomonoPAPs, for example by consuming prey fish. Alternatively, the presence of monoPAPs could result from degradation of diPAPswithin the herring gulls. DiPAPs have been shown to be metabolized to monoPAPs and other compounds by rats.[1]Dietary intake is likely a dominant exposure route for diPAPs, asdiPAPs have also been detected in lake trout from Lake Huron[9], the same lake as where the herring gull colony is located, and both the trout and gulls feed on the same prey fish species [alewife (*Alosapseudoharengus*) and smelt (*Osmerusmordax*)]. Mono- and diPAPs could potentially be or had already been metabolized to PFCAs by the herring gulls.Thus, the PAPs could be a secondary source for PFCAs besides direct accumulation.

Table 2 Semi-quantitative concentrations (pg/g wet weight) of 6:2 and 8:2 monoPAPs and 6:2/6:2 and 8:2/8:2 diPAPs in herring gull eggs, tissues, and blood[1]

	Egg		Tissue				Blood	
	Yolk[2]	Albumen[2]	Liver	Brain	Muscle	Adipose	Plasma	RBC
monoPAP								
6:2			56					
8:2	7/3	4/4	79	1		34	2	
diPAP								
6:2/6:2	343/3371		20				3	
8:2/8:2	36/366		46	4	1		1	3

[1] Internal standard quantification was performed with the internal standards spiked to the final extracts
[2] Two yolk and albumen pools were generated from individual extracts

In summary, this is the first reporting of the presence of mono- and diPAPs in an avian top predator. Three mono- and ten diPAP were detected in the analyzed herring gull tissues, blood, and/or eggs.The highest detection frequencies of PAPs were in liver and yolk samples. By their detection in a top predator, mono- and diPAPshave been shown to be persistent enough to accumulate through the aquatic food chain, although the gulls might be able to metabolize PAPs, which could result in increased PFCA exposure. Further analyses are necessary to accurately determine PAP concentrations in tissues such as liver or eggs, in order to assess the exact PAP exposure to top predators, such as herring gulls.

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References

1. D'eon J and Mabury SA (2011)*Environ Health Perspect.* 119: 344-350
2. Lee H, D'eon J, Mabury SA (2010) *Environ SciTechnol.* 44: 3305-3310
3. Trier X, Nielsen NJ, Christensen JH (2011) *Environ SciPollut Res.* 18: 1422-1432
4. Gebbink WA, Ullah S, Sandblom O, Berger U (2013) *Environ SciPollut Res.* DOI 10.1007/s11356-013-1596-y
5. D'eon JC, Crozier PW, Furdui VI, Reiner EJ, Libelo EL, MaburySA (2009) *Environ SciTechnol.* 43: 4589-4594
6. Lee H,Mabury SA (2011) *Environ Sci Technol.* 45: 8067-8074
7. Yeung LWY, Robinson SJ, Koschorreck J, Mabury SA (2013) *Environ Sci Technol.*doi.org/10.1021/es303716k
8. LoiEIH, Yeung LWY, Mabury SA, Lam PKS (2013) *Environ Sci Technol.*doi.org/10.1021/es303805k
9. Guo R, Reiner EJ, Bhavsar SP, Helm PA, Mabury SA, Braekevelt E, Tittlemeir SA (2012) *Anal Bioanal Chem.* 404: 2699-2709
10. GebbinkWA, Letcher RJ (2012) *Environ Pollut.* 162:40-47