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Production of Perfluorinated Carboxylic Acids (PFCAs) from the Biotransformation of Polyfluoroalkyl Phosphate Surfactants (PAPS): Exploring Routes of Human Contamination

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Perfluorinated acids are detected in human blood worldwide, with increased levels observed in industrialized areas. The origin of this contamination is not well understood. A possible route of exposure, which has received little attention experimentally, is indirect exposure to perfluorinated acids through ingestion of chemicals applied to food contact paper packaging. The current investigation quantified the load of perfluorinated acids to Sprague−Dawley rats upon exposure to polyfluoroalkyl phosphate surfactants (PAPS), nonpolymeric fluorinated surfactants approved for application to food contact paper products. The animals were administered a single dose at 200 mg/kg by oral gavage of 8:2 fluorotelomer alcohol (8:2 FTOH) mono-phosphate (8:2 monoPAPS), or the corresponding di-phosphate (8:2 diPAPS), with blood taken over 15 days post-dosing to monitor uptake, biotransformation, and elimination. Upon completion of the time-course study the animals were redosed using an identical dosing procedure, with sacrifice and necropsy 24 h after the second dosing. Increased levels of perfluorooctanoic acid (PFOA), along with both 8:2 PAPS congeners, were observed in the blood of the dosed animals. In the 8:2 monoPAPS-dosed animals, 8:2 monoPAPS and PFOA blood concentrations peaked at 7900 ± 1200 ng/g and 34 ± 4 ng/g respectively. In the 8:2 diPAPS-dosed animals, 8:2 diPAPS peaked in concentration at 32 ± 6 ng/g, and 8:2 monoPAPS and PFOA peaked at 900 ± 200 ng/g and 3.8 ± 0.3 ng/g, respectively. Several established polyfluorinated metabolites previously identified in 8:2 FTOH metabolism studies were also observed in the dosed animals. Consistent with other fluorinated contaminants, the tissue distributions showed increased levels of both PFOA and the 8:2 PAPS congeners in the liver relative to the other tissues measured. Previous investigations have found that PAPS can migrate into food from paper packaging. Here we link ingestion of PAPS with *in vivo* production of perfluorinated acids.

Introduction

Since 1968 when Taves (*1*) discovered organic fluorine in human blood samples, fluorinated organic compounds have been observed in the blood of humans (*2*) and wildlife (*3*) worldwide. Commonly discussed fluorinated contaminants are the perfluorinated acids, which consist of perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs). Despite their low pK_a values (*4*), which render them relatively involatile, perfluorinated acids are widespread in the environment (*5*, *6*). This ubiquity is not intuitive, and as such has incited interest into possible modes of dissemination. Two fields of thought have emerged to explain current levels found in the environment. One theory relies on direct input of PFOA from production facilities (*7*, *8*), and the other relies on volatile fluorinated alcohol precursor emissions from manufactured materials as an indirect source of contamination (*9*−*11*). The hub of major debate has been Arctic contamination (*6*−*11*). The issue of human exposure is increasingly complicated as several sources are likely involved, with relative contributions varying with lifestyle and location (*2*, *12*). The reported concentrations of PFOA in human serum vary from nondetect to >30 ng/mL worldwide (*2*). Human contamination is of concern as perfluorinated acids have no known degradation pathway, are slowly excreted by humans (*13*), and an advisory board to the U.S. EPA has published a draft document proposing PFOA be deemed a rodent carcinogen with relevance to humans (*14*). To properly estimate human exposure, relative contributions of different exposure pathways need to be deciphered. Discussed here is an indirect source of human exposure to PFCAs via ingestion and metabolism of FTOH-based PAPS, which are approved for application to food contact materials.

Fluorinated chemicals have primarily been produced via two manufacturing processes: telomerization and electrochemical fluorination (ECF). ECF chemistry generates a characteristic distribution of 20−30% structural isomers (*4*), whereas telomerization produces only the straight chain isomer (*4*). 3M, a major manufacturer of ECF-based fluorochemicals, phased-out their perfluorooctyl-products in 2001 due to environmental concerns (*15*). The void left by 3M was largely replaced by telomer-based products, resulting in a dramatic shift in the market toward telomer, and hence linear, compounds (*16*, *17*). The PFCA isomer distribution in pooled human blood samples from the Midwestern United States between 2004 and 2005 was >98% linear (*18*). With a half-life for PFOA of 4.4 years in human serum (*13*), in the absence of significant isomer discrimination, the predominance of the linear isomer suggests exposure to current-use fluorinated materials and not the historical load present in the environment.

PFCAs may be present in consumer articles treated with fluorinated polymers. Extraction tests and product information suggest that direct exposure to PFOA from the appropriate use of products treated with fluorochemicals is not a significant source of contamination to the general population (*19*). FTOHs and perfluorinated sulfonamides have been observed in both indoor (*20*) and outdoor (*20*−*22*) air. FTOHs are metabolized to PFCAs (*23*), while perfluorinated sulfonamides are metabolized to PFSAs (*24*). As such, inhalation of these neutral precursors may contribute to the load of perfluorinated acids observed in human blood. Indirect exposure to perfluorinated acids may also occur by ingestion of PAPS, which are used to impart oil and water repellency to certain food contact paper products (*25*). Begley et al. (*26*) have shown that PAPS will migrate into food simulants under appropriate test conditions. Cleavage of the phosphate ester linkage of PAPS within a biological system would likely result in production of perfluorinated acids via metabolism of the released fluorinated alcohol. Although

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dephosphorylation processes are common in biological systems (*27*), the significance of the fluorinated chains present in PAPS-based materials on this process is not known.

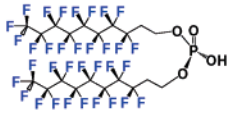
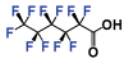
N-methyl perfluorooctane sulfonamido ethanol (NMeFOSE) and *N*-ethyl perfluorooctane sulfonamido ethanol (NEtFOSE) were the functional units used by 3M in their fluorinated coatings (*28*). NMeFOSE was primarily incorporated into polymeric surface treatment products for fabrics and carpets, a technology that was commercialized in the 1950s (*28*, *29*), whereas the primary application of NEtFOSE was in PAPS-based nonpolymeric surfactant materials used in food contact paper products, for which human food contact applications were introduced in 1974 (*28*, *29*). In a study of the historical load of fluorochemicals in human blood, Olsen et al. (*29*) observed a 4-fold increase in the acetate adduct of perfluorooctane sulfonamide (PFOSA) between 1974 and 1989. The authors attribute this increase to the incorporation of NEtFOSE-PAPS materials into human food contact paper products (*29*). A study published in 2006 by Calafat et al. (*12*) found the acetate adducts of both NMeFOSE and NEtFOSE in pooled blood samples from the United States from 2001 and 2002. The presence of these fluorinated metabolites, which are relatively quickly eliminated or metabolized to perfluorooctane sulfonate (PFOS) (*24*, *29*), suggests recent exposure to indirect fluorochemical sources. The presence of the NEtFOSE acetate adduct suggests this indirect source may be a PAPS-based material.

The relevance of PAPS to human fluorochemical exposure extends beyond their use in food packaging applications, as PAPS are approved by the U.S. EPA as an inert defoaming additive to pesticide formulations (*30*). Although the load of PAPS used in this capacity is not publicly known, nonpolymeric fluorinated surfactants akin to PAPS account for 20% of the 12 million kg of fluorinated materials produced annually (*16*).

To interrogate whether PAPS can contribute to the load of PFCAs observed in the human population, we dosed male Sprague−Dawley rats with in-house synthesized and purified 8:2 FTOH mono- or di-substituted PAPS (8:2 monoPAPS, 8:2 diPAPS). We were particularly interested in the biological availability of FTOH-based PAPS as they are currently commercially available for food contact paper applications (*31*−*33*), and specific restrictions, which take effect in 2008, have been placed on their use as inert additives within pesticide formulations (*34*).

The animals were administered a single dose of either 8:2 PAPS congener by oral gavage with subsequent blood sampling for 15 days post-dosing. As 8:2 FTOH is the fluorinated moiety in both 8:2 PAPS congeners, PFOA is the major PFCA biotransformation product expected (*23*, *35*). In addition to PFOA, several established 8:2 FTOH intermediate metabolites were monitored to link PFOA production with 8:2 FTOH exposure via 8:2 PAPS biotransformation (*23*, *35*). Perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), and perfluorohexanoic acid (PFHxA) were monitored to quantify contributions from α-oxidation, and any potential degradation of the perfluorinated chain (*23*, *35*). Direct absorption of the 8:2 PAPS congeners was also monitored. All analytes are shown in Table 1. To investigate the distribution of 8:2 PAPS among select tissues, the animals were redosed upon completion of the time-course, with sacrifice and necropsy 24 h after the second dosing. Hydrolysis experiments were performed in concert with the biological experiments to characterize the abiotic degradation potential of the 8:2 PAPS congeners.

TABLE 1. Common Names, Acronyms, and Structures for the Analytes of Interest

Common Name	Acronym	Structure
8:2 fluorotelomer alcohol di-substituted phosphate surfactant	8:2 diPAPS	
8:2 fluorotelomer alcohol mono-substituted phosphate surfactant	8:2 monoPAPS	
8:2 fluorotelomer alcohol	8:2 FTOH[a]	
8:2 saturated fluorotelomer carboxylic acid	8:2 FTCA	
8:2 unsaturated fluorotelomer carboxylic acid	8:2 FTUCA	
7:3 saturated fluorotelomer carboxylic acid	7:3 Acid	
7:3 unsaturated fluorotelomer carboxylic acid	7:3 uAcid[b]	
Perfluorohexanoic acid	PFHxA	
Perfluoroheptanoic acid	PFHpA	
Perfluorooctanoic acid	PFOA	
Perfluorononanoic acid	PFNA	

[a] 8:2 FTOH was not monitored in this investigation. [b] No analytical standards were available for the 7:3 uAcid.

Experimental Section

Synthesis of the Polyfluoroalkyl Phosphate Surfactants (PAPS). 8:2 monoPAPS, 8:2 diPAPS, and 9:1 diPAPS (using 9:1 FTOH, $F(CF_2)_9CH_2OH$) were synthesized using the following equilibrium reaction, which is a bench-scale variation of a 1963 patented process (*36*). To establish anhydrous conditions, triethylamine (TEA) was dried by distillation under nitrogen, and tetrahydrofuran (THF) was dried by reflux over sodium with benzophenone as an indicator.

Under nitrogen atmosphere at −78 °C, 3 mol equiv of dry TEA in 10 mL of dry THF was added over 10 min to 1 mol equiv of phosphorus oxychloride in 10 mL of dry THF. For the di-substituted surfactant, 2 mol equiv of either 8:2 FTOH or 9:1 FTOH was added to the reaction mixture in 30 mL of dry THF over 1 h. For the monosubstituted surfactant 0.3 mol equiv (to minimize production of di- and tri-substituted 8:2 PAPS congeners) of 8:2 FTOH was added to the reaction mixture in 30 mL of dry THF over 1 h. After addition of the alcohol the reaction mixture was allowed to warm to room temperature (1−2 h), then 50 mL of distilled deionized water was added over 1 h. Product isolation and purity analysis are described in the Supporting Information. Both 8:2 PAPS congeners were >97% pure with respect to fluorinated materials of interest. 8:2 monoPAPS contained <0.01% PFOA and 0.6% 8:2 FTOH. 8:2 diPAPS contained <0.02% PFOA, 1.5% 8:2 FTOH, and <1% 8:2 monoPAPS. Unless otherwise stated all percentages are expressed on a per mole basis.

Abiotic Hydrolysis Procedure. The hydrolysis procedure involved detection of 8:2 FTOH, produced by cleavage of the phosphate ester linkage from either 8:2 PAPS congener at 50 °C and pH 9, via a purge-and-trap system described elsewhere (*9*), with analysis using a Hewlett-Packard 6890 gas chromatograph coupled to a 5973 inert mass spectrometer operating in single ion monitoring mode (GC−MS). Experimental and chromatographic details are provided in the Supporting Information.

***In Vivo* Model.** This research was conducted under an animal use protocol approved by the University Animal Care Committee, and was supervised by a licensed veterinarian. Twelve 7-week-old male Sprague−Dawley rats were obtained from Charles River Laboratories Inc. (Wilmington, MA). The animals were doubly housed and randomly assigned into three groups corresponding to 8:2 monoPAPS exposure, 8:2 diPAPS exposure, and control. Food and water were available *ad libitum* throughout the experiment. After one week acclimatization, the animals in the exposure groups were administered a single bolus dose of either 8:2 monoPAPS or 8:2 diPAPS at 200 mg/kg in 0.5% aqueous methylcellulose at 5 mL/kg by oral gavage without prior fasting. Shaking by hand and sonication were used to dissolve the 8:2 PAPS congeners in 0.5% aqueous methylcellulose. Although the administered doses were cloudy, they were uniform and void of visible lumps. Control animals received 5 mL/kg of undosed 0.5% aqueous methylcellulose. In accordance with the approved animal use protocol, the dose concentration corresponded to a no observable adverse effect level (NOAEL) for 8:2 FTOH from the literature (*37*). Whole blood was harvested from the animals via heparinized syringes using the lateral tail vein 24 h prior to dosing and at 0.2 (4 h), 1, 2, 3, 5, 9, and 15 days post-dosing. The total volume of blood collected per animal over the course of the experiment was limited to 10% of their total blood volume (estimated using 50 mL of blood per kg of animal). Individual blood samples ranged from 50 to 500 mg, with a mean mass of 189 mg. Whole blood samples were stored at −20 °C until extraction and analysis. Urine was not collected, however feces samples were obtained, if available, from animals during blood sampling. Five days after the last time-course sample was taken, the animals were redosed using the same procedure as above, with sacrifice by carbon dioxide asphyxiation 24-h post-dosing. Blood, liver, kidney, muscle, fat, spleen, and brain were harvested from the animals and stored at −20 °C until extraction and analysis. The animals did not show any clinical signs of toxicity throughout the course of the experiment, and the liver somatic index was not statistically different among the three groups of animals (8:2 monoPAPS-dosed, 8:2 diPAPS-dosed, control), as determined using a Kruskal−Wallis test ($p = 0.397$), suggesting the administered dose had little toxicological significance.

Extraction Procedure. Whole blood samples were extracted using a modified version of the ion-pairing method developed by Hansen et al. (*38*). Tissue and feces samples, except fat, were extracted using the abovementioned ion-pairing method with the addition of a fluorosolvent protein precipitation step described in detail in Furdui et al. (*39*). Due to the high lipid content, fat samples were extracted using a methanol extraction technique. Extractions are described in detail in the Supporting Information.

Instrumental Analysis. All samples were analyzed by liquid chromatography coupled to negative electrospray ionization tandem mass spectrometry (LC−MS/MS). Blood samples from the time-course were analyzed for PFHxA, PFHpA, PFOA, PFNA, 8:2 FTCA, 8:2 FTUCA, 7:3 Acid, 7:3 uAcid, 8:2 monoPAPS, and 8:2 diPAPS, using an API 4000 Q Trap (Applied Biosystems/MDS Sciex) coupled to an Agilent 1100 autosampler. All tissue samples, including blood obtained at necropsy, were analyzed for PFOA, 8:2 monoPAPS, and 8:2 diPAPS, using a Micromass Ultima (Micromass, Manchester, United Kingdom) coupled to a Waters 717 plus autosampler (Waters, Milford, United Kingdom). Chromatographic details and mass transitions are provided in the Supporting Information.

Quality Control Procedure. Concentration and spike and recovery values are reported using the mean concentration and standard error. Units are reported as mass of analyte (ng) per mass of blood or tissue extracted (g). Analytes were quantified using internal calibration with the following internal standards: $^{13}C_2$-PFHxA (PFHxA), $^{13}C_2$-PFOA (PFHpA, PFOA), $^{13}C_5$-PFNA (PFNA), $^{13}C_2$-8:2 FTUCA (8:2 FTCA, 8:2 FTUCA, 7:3 Acid, 7:3 uAcid), $^{13}C_2$-perfluorodecanoic acid (8:2 monoPAPS), and 9:1 diPAPS (8:2 diPAPS). Internal calibration of 8:2 monoPAPS with $^{13}C_2$-perfluorodecanoic acid was validated using standard addition, where three samples analyzed by both methods were statistically similar using a paired *t*-test ($p = 0.411$). A spike and recovery experiment ($n = 4$) was performed using blood harvested upon necropsy from a control animal. Whole rat blood (300 μL) was stored for 24 h at −20 °C with 2.5 ng of all analytes of interest (Table 1). Extraction and analysis was performed as described above. The spike and recovery results were as follows: 107 ± 4% (PFHxA), 64 ± 5% (PFHpA), 111 ± 6% (PFOA), 108 ± 7% (PFNA), 101 ± 8% (8:2 FTCA), 63 ± 5% (8:2 FTUCA), 174 ± 19% (7:3 Acid), 70 ± 16% (8:2 monoPAPS), and 66 ± 8% (8:2 diPAPS). Reported values were not corrected for recovery. Procedural contamination was quantified by including two extraction blanks with each set of samples. Samples were blank corrected where appropriate using the mean blank value obtained from all analyses. For analytes present in the procedural blanks (PFHxA, PFHpA, PFOA (time-course), PFNA) the limit of detection (LOD) was defined as three standard deviations (3σ) from the mean blank level, and the limit of quantitation (LOQ) was defined as 10σ from the mean blank level (*40*). For analytes absent in the procedural blanks (PFOA (tissue distribution), 8:2 FTCA, 8:2 FTUCA, 7:3 Acid, 8:2 monoPAPS, 8:2 diPAPS) the LOD was empirically determined as the concentration producing a signal-to-noise ratio of 3, and the LOQ was defined as the concentration producing a signal-to-noise ratio of 10 (*40*). LOD and LOQ values were transformed from extract concentrations to blood or tissue concentrations using the mean mass of blood harvested over the time-course (189 mg) and the mean mass of tissue extracted (0.715 g). LOD and LOQ values for all analyses are provided in the Supporting Information. Values less than LOD are reported as nondetect (nd) and given a value of zero, values less than LOQ are reported unaltered but are indicated using brackets in tables and an asterisk (*) in figures. Background concentrations in the control animals were either nondetect or below LOQ for all analytes, and are reported in the Supporting Information.

Results and Discussion

Abiotic Study. PFCA production from 8:2 PAPS will likely proceed via cleavage of the phosphate ester linkage, releasing free 8:2 FTOH, with subsequent biotransformation to PFOA. Although phosphate triesters are relatively labile toward hydrolysis (*41*), phosphate monoesters and diesters are stable, with lifetimes on the order of several years with respect to hydrolysis at environmental conditions (*42*). To confirm these findings, the hydrolytic stability of both 8:2 monoPAPS and 8:2 diPAPS was investigated under aggressive conditions of pH 9 and 50 °C. We observed <0.1% degradation over a 2-week period for both 8:2 PAPS congeners, corresponding to a minimum lifetime of 26 years with respect to hydrolysis. As this lifetime is consistent with previous investigations (*42*), we expect both 8:2 monoPAPS and 8:2 diPAPS to be stable toward abiotic hydrolysis throughout the entire gastrointestinal tract. In biological systems phosphates are subject to dephosphorylation by phosphatase enzymes, which are prevalent in the body as the phosphate anion is involved in energy transfer reactions and as a major component of hydroxyapatite (*27*). Phosphatase enzymes are also present in the intestinal tract and are involved in the dephosphorylation of certain nutrients, such as the phosphate ester of thiamin (*27*). As both 8:2 PAPS congeners are insensitive to abiotic hydrolysis, any dephosphorylation presumably in-

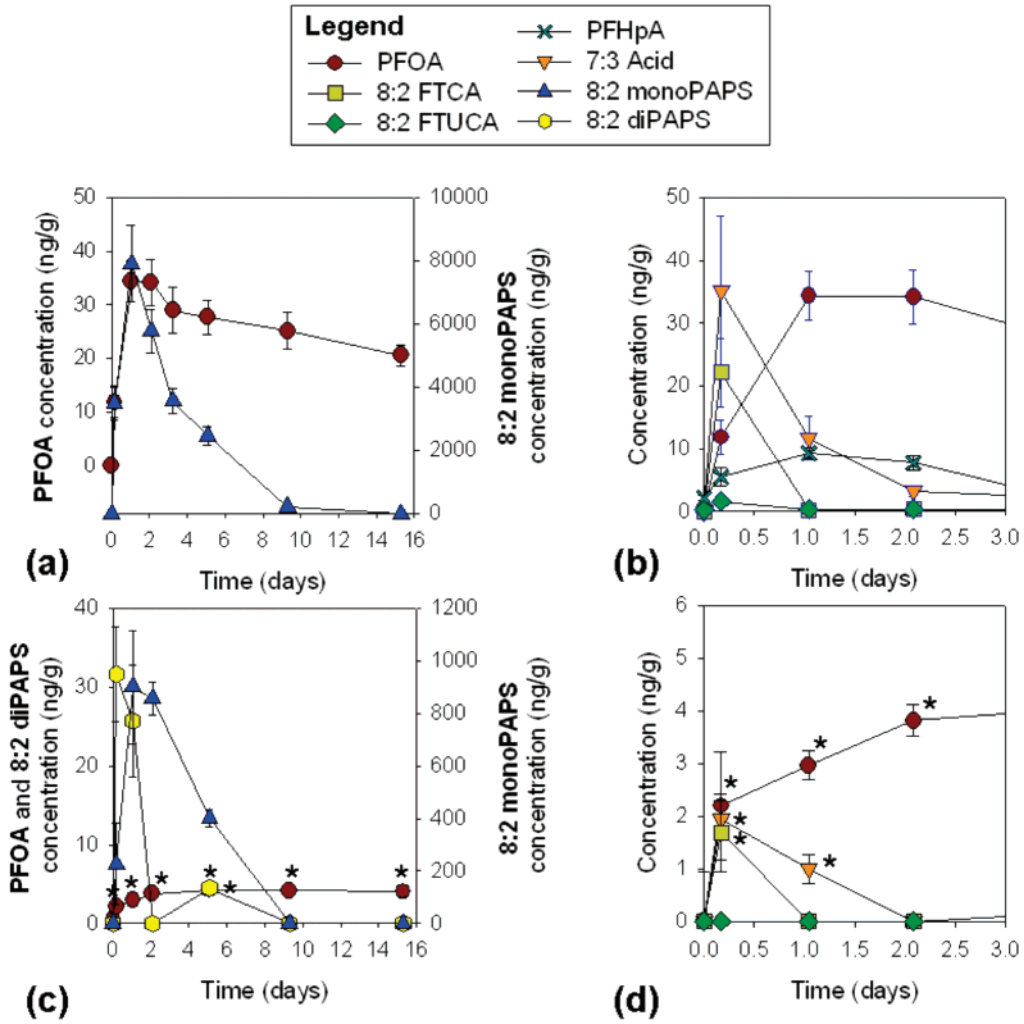


FIGURE 1. Mean concentrations in whole blood. Error bars indicate standard error. (a) 15-Day time-course for 8:2 monoPAPS and PFOA in the 8:2 monoPAPS-dosed animals. (b) 3-Day time-course for PFOA, PFHpA, 8:2 FTCA, 8:2 FTUCA, and 7:3 Acid in the 8:2 monoPAPS-dosed animals. (c) 15-Day time-course for 8:2 diPAPS, 8:2 monoPAPS, and PFOA in the 8:2 diPAPS-dosed animals. (d) 3-Day time-course for PFOA, 8:2 FTCA, 8:2 FTUCA, and 7:3 Acid in the 8:2 diPAPS-dosed animals. Values less than LOD are reported as zero, and values less than LOQ are indicated with an asterisk (*).

volves phosphatase enzymes either in the gut or within the body.

***In Vivo* Study.** Elevated levels of PFOA were observed in the blood from both dose groups as compared to background levels in the control animals; where 80% of the control samples were below the LOD of 1.8 ng/g, with a maximum observed concentration of 2.0 ng/g. In the 8:2 monoPAPS-dosed animals, 8:2 monoPAPS was the most prevalent fluorinated contaminant observed (Figure 1a), with a peak concentration of 7900 ± 1200 ng/g 24 h post-dosing. Overlaid with 8:2 monoPAPS in Figure 1a is the time-course for PFOA. PFOA peaked in concentration between 24 and 48 h post-dosing, with a mean value of 34 ± 4 ng/g at both time-points. This plateau suggests the true concentration peak was likely not captured by the sampling routine. Using 24 h as the peak uptake value, we calculated an elimination half-life of PFOA from blood of 20 days. This half-life is longer than the value of 4.1−9.0 days reported by Fasano et al. (*35*) for the elimination half-life of PFOA from the blood of adult male rats administered a single bolus dose of 8:2 FTOH at 125 mg/kg by oral gavage. The additional dephosphorylation step involved in PAPS metabolism may be responsible for the longer half-life observed here.

In addition to 8:2 monoPAPS and PFOA, several polyfluorinated metabolites, consistent with 8:2 FTOH exposure, were observed in the blood of the 8:2 monoPAPS-dosed animals. The time-course from 0 to 3 days post-dosing for the 7:3 Acid, 8:2 FTCA, and 8:2 FTUCA is shown in Figure 1b. The 7:3 uAcid was not observed, however this analyte was monitored using a mass transition obtained from Martin et al. (*23*) without further optimization as no analytical standards were available. The transient nature of the observed metabolites in blood is evident from their concentration peak at 4 h post-dosing, with subsequent rapid decrease or absence in the remainder of the time-points. The biological mechanism involved in the transformation from 8:2 FTOH to PFOA has been discussed in detail in previous studies (*23*, *35*), and is not the focus of this investigation, however these transient species provide considerable evidence that the observed increase in PFOA resulted from 8:2 FTOH exposure. As well as the transient metabolites, Figure 1b also includes the initial 3-day time-course for PFOA and PFHpA. Aside from PFOA, PFHpA was the only PFCA observed above background levels in the control animals. PFHpA attained a maximum concentration of 6.1 ± 1.1 ng/g 24 h post-dosing. PFHpA has previously been observed in rats dosed with 8:2 FTOH (*35*).

Concentrations observed in the blood of the 8:2 diPAPS-dosed animals are displayed in Figure 1c and d. 8:2 diPAPS peaked at 4 h post-dosing with a concentration of 32 ± 6 ng/g. 8:2 monoPAPS was observed at a maximum concentration of 900 ± 200 ng/g 24 h post-dosing. The 15-day time-course for PFOA shows a clear uptake profile, which plateaus

TABLE 2. Mean Concentrations in Tissues Harvested at Necropsy (Errors are Reported Using the Standard Error; Values Less than LOD are Reported as Nondetect (nd) and Values Less than LOQ are Reported in Brackets)

	concentration (ng/g)[a,b]					
	8:2 monoPAPS-dosed			8:2 diPAPS-dosed		
tissue[c]	PFOA	8:2 monoPAPS	8:2 diPAPS	PFOA	8:2 monoPAPS	8:2 diPAPS
blood	130 ± 15	{130 ± 21}	-	480 ± 100	820 ± 90	{85 ± 11}
liver	320 ± 50	1700 ± 300	-	1500 ± 200	9200 ± 600	600 ± 200
kidneys	100 ± 20	{440 ± 70}	-	230 ± 30	3700 ± 200	nd

[a] Analysis was performed using a Micromass Ultima mass spectrometer. [b] Tissues harvested from the control animals were nondetect for the analytes of interest. [c] Muscle, fat, and brain samples from the dosed animals were nondetect for the analytes of interest. Spleen and testes from the dosed animals were nondetect for all analytes except PFOA observed in the testes of the 8:2 diPAPS-dosed animals at 57 ± 7 ng/g and PFOA observed in the spleen of the 8:2 diPAPS-dosed animals at 5.5 ± 0.7 ng/g.

around 48 h at 3.8 ± 0.3 ng/g, however the excretion profile is not obvious, with a PFOA concentration of 4.0 ± 0.7 ng/g present in the blood of the animals 15 days post-dosing. It must be noted that the LOQ for PFOA in the whole blood samples analyzed here is 6.1 ng/g (see Supporting Information), driven by minor contamination present in the procedural blanks. Values are provided as illustration of the observed trends, without further interpretation. The 3-day time-course for PFOA and the intermediate metabolites for the 8:2 diPAPS-dosed animals is displayed in Figure 1d. Despite the low levels of PFOA observed, both the 7:3 Acid and 8:2 FTCA were detected 4 h post-dosing. Aside from PFOA, no PFCAs were observed above background levels present in the control animals for the 8:2 diPAPS-dosed animals.

Upon completion of the time-course the animals were dosed a second time, with sacrifice and necropsy 24 h post-dosing. In contrast to the results for the time-course, the levels observed in the 8:2 diPAPS-dosed animals were greater than those in the 8:2 monoPAPS-dosed animals. Reasons for this discrepancy are not apparent, and with only one time point subsequent to the second dosing the two situations are difficult to compare. Concentrations observed in the analyzed tissues are displayed in Table 2. Within the dose groups, concentrations for each analyte were greatest in the liver, which is consistent with previous rat studies involving PFOA (*43*).

The quality of the reported results depends on the purity of the administered dose. There is considerable evidence that the compounds observed in the dosed animals are a result of PAPS metabolism and not due to contamination within the dosed materials. Byproducts of the 8:2 PAPS synthetic routine include congeners of different substitutions and residual starting materials. After purification, both 8:2 monoPAPS and 8:2 diPAPS were >97% pure with respect to relevant fluorinated materials. Of particular concern within this purity range is residual 8:2 FTOH. The percent by mass of 8:2 FTOH in the synthesized 8:2 monoPAPS and 8:2 diPAPS was 0.6% and 0.7%, respectively. The 8:2 monoPAPS-dosed animals had almost 1 order of magnitude more PFOA in their blood 24 h post-dosing compared to the 8:2 diPAPS-dosed animals (Figure 1). As 8:2 FTOH contamination is consistent by mass between the doses, the difference in PFOA exposure between the dose groups must be due to increased biological processing of 8:2 monoPAPS as compared to 8:2 diPAPS, and not from residual 8:2 FTOH present in the dosed materials.

The concentration profiles for 8:2 diPAPS, 8:2 monoPAPS, and PFOA in the 8:2 diPAPS-dosed animals, shown in Figure 1c, have staggered maxima. This absorption pattern is consistent with the sequential dephosphorylation of 8:2 diPAPS shown schematically in Figure 2. The administered 8:2 diPAPS dose contained <1% by mass 8:2 monoPAPS, however feces collected with blood at 4 and 24 h post-dosing contained 3% and 9% by mass 8:2 monoPAPS relative to 8:2 diPAPS. Changes of this magnitude in the chemical composition of the feces cannot be explained by uptake of 8:2 diPAPS or excretion of 8:2 monoPAPS via enterohepatic circulation, as the sum of these compounds observed in the blood of the animals was <0.05% of the administered dose. As a result, the increasing amount of 8:2 monoPAPS relative to 8:2 diPAPS in the feces of the 8:2 diPAPS-dosed animals suggests that 8:2 diPAPS was dephosphorylated in the gut of the animals. Intestinal dephosphorylation of 8:2 diPAPS to 8:2 monoPAPS releases a unit of 8:2 FTOH into the gut of the animal, which may continue through the metabolic pathway to PFOA (Figure 2). Although relative contributions from reaction byproducts and from biotransformation of 8:2 diPAPS cannot be fully delineated, the concentration profiles in the blood and feces suggest there is a measurable contribution from biotransformation of 8:2 diPAPS to the load of 8:2 monoPAPS and PFOA observed in the 8:2 diPAPS-dosed animals.

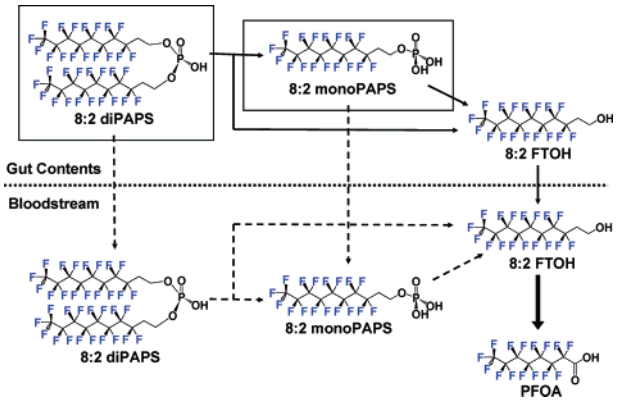


FIGURE 2. Schematic outlining two potential pathways for PFOA production from the biotransformation of the 8:2 PAPS congeners. Dosed compounds are indicated by boxes. The solid arrows represent intestinal dephosphorylation followed by absorption of the free 8:2 FTOH. The dotted arrows indicate absorption of 8:2 PAPS followed by *in vivo* dephosphorylation.

Implications to Human Exposure. The overall conclusions from this study are twofold. First, the phosphate ester bonds in both 8:2 PAPS congeners are biologically labile. Animals from both exposure groups had increased levels of PFOA and intermediate 8:2 FTOH metabolites from 8:2 PAPS dephosphorylation and subsequent biotransformation of 8:2 FTOH. Second, both 8:2 PAPS congeners are bioavailable, with both 8:2 monoPAPS and 8:2 diPAPS observed in the blood of their respective dose groups.

Possible exposure pathways leading to the production of PFOA from either 8:2 monoPAPS or 8:2 diPAPS are shown schematically in Figure 2. The administered compounds are indicated with boxes. Two distinct mechanisms are shown in Figure 2. Following the solid arrows, the 8:2 PAPS congeners are intestinally dephosphorylated with absorption of free

8:2 FTOH, whereas the dotted arrows depict direct absorption of 8:2 PAPS followed by dephosphorylation within the animal. There is evidence to support the participation of both mechanisms. The presence of both 8:2 PAPS congeners in the blood of the animals establishes that dephosphorylation could occur after absorption, and the concentration profile of 8:2 monoPAPS in the feces of the 8:2 diPAPS-dosed animals supports the hypothesis that intestinal dephosphorylation is contributing to the observed transformation. As the kinetics of the individual transformations are not known, contributions from each mechanism cannot be inferred from the pharmacokinetics observed here.

Determining sources of human exposure to PFCAs may have implications beyond controlling the final concentration observed in human blood. Although direct PFCA exposure and exposure to precursor compounds with subsequent metabolism to PFCAs both manifest as elevated PFCA serum levels, their toxicological significance may not be equivalent. The metabolic pathway from FTOHs to PFCAs proceeds via several reactive intermediates including the polyfluorinated carboxylic acids monitored here. The toxicology of the 10:2 FTCA has been investigated using *Daphnia magna*, and was found to be 4 orders of magnitude more toxic than the corresponding PFCA (*44*). In addition to the studied toxicological endpoints for FTCAs, several polyfluorinated aldehydes have been observed in the transformation from 8:2 FTOH to PFOA (*23*, *35*). Saturated and unsaturated aldehydes have well-documented toxicity and carcinogenic activity (*45*). Due to their inherent reactivity, exposure to these transient metabolites is likely of greater toxicological concern than exposure to PFCAs alone. Unlike bioaccumulation potential, which decreases with chain length (*6*), these toxicological concerns regarding FTOH exposure may not be mitigated by decreasing the length of the fluorinated chain.

PAPS may be a significant source of PFCA contamination to the human population. We have shown here that oral exposure of rats to either 8:2 monoPAPS or 8:2 diPAPS will result in increased PFOA blood levels, and that both 8:2 PAPS congeners themselves absorb from the gut into the bloodstream. The significance of PAPS exposure to the overall load of PFCAs observed in the general population is difficult to ascertain. PAPS products are manufactured with a variety of different molecular structures (*26*), and the relative contribution of different formulations to the commercial market is not known. The overall prevalence of PAPS incorporation into applications such as paper food packaging (*25*) and as pesticide defoaming agents (*30*) is expected to be significant, though specific values are not available. For the specific application of food-contact paper packaging, the extent of migration into food is not well documented, with only one study looking at only one PAPS formulation (*26*). Once an appreciation for these variables is available, human exposure to PFCAs via PAPS exposure can be properly assessed.

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Supporting Information Available

A comprehensive list of chemicals; isolation and purity analysis for the 8:2 PAPS congeners; abiotic hydrolysis procedure; complete extraction procedures for blood, tissues, and feces; details regarding LC−MS/MS analysis including MRM transitions and LC separation method; LOD and LOQ values for all analyses; concentrations observed in control animals; animal weights at dosing and necropsy; liver weights and the liver somatic index. This material is available free of charge via the Internet at http://pubs.acs.org.

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