**In Vitro Metabolic Formation of Perfluoroalkyl Sulfonamides from Copolymer Surfactants of Pre- and Post-2002 Scotchgard Fabric Protector Products**

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Supporting Information

**ABSTRACT:** Currently there is a scientific debate on whether fluorinated polymers (or copolymers) are a source, as a result of their degradation and subsequent formation, of perfluorinated carboxylic acids (PFCAs) and perfluorinated alkanesulfonates (PFASs). The present study investigated whether commercially available fluorinated surfactants, such as Scotchgard fabric protector (3M Company), can be metabolically degraded, using a model microsomal in vitro assay (Wistar-Han rats liver microsomes), and with concomitant formation of PFCAs, PFASs, and/or their precursors. The results showed that the main in vitro metabolite from the pre-2002 product was perfluorooctane sulfonamide (FOSA), and coincident with the detection of the major fabric protector components, which contains the N-ethyl-perfluorooctanesulfonyl chemical moiety (C₈F₁₇SO₂N(C₂H₅)−); the main in vitro metabolite of the post-2002 product was perfluorobutane sulfonamide (FBSA), which was coincident with the detection of the major fabric protector components, and contains the N-methyl-perfluorobutanesulfonyl chemical moiety (C₄F₉SO₂N(CH₃)−). FOSA or FBSA metabolite concentrations increased over the 0–60 min microsomal incubation period. However, concentrations of their small molecule precursors such as alkylated FOSAs or FBSAs were not detectable (<LODs) in these fabric protector original standard solutions. Thus, the FOSA or FBSA metabolites were derived from the copolymer product itself rather than nonreacted reagents in the Scotchgard products. This result is consistent with reports of high concentrations of PFAEs detected in the plasma of persons in households where Scotchgard products are heavily used.

**INTRODUCTION**

Poly- and perfluoroalkyl substances (PFASs) have been used in chemical products for decades. Their unique physical and chemical characteristics, such as water and oil repellency, surface tension lowering properties, ability to create stable foams and thermal stability, make them very useful for a wide range of industrial and consumer-use applications which includes metal plating; coating formulations; fire-fighting foams; oil and water repellents for leather, paper, and textiles; and industrial additives.

Since the 1970s there has been increasing concern over the fate of perfluoroalkyl acids (PFAAs) in the environment and possible adverse effects in biota including in wildlife and humans.5–7 The strength of the carbon–fluorine bond of PFAAs contributes to their extremely high thermal and chemical stability, and biological inertness,8 and these properties account for their resistance to degradation and high bioaccumulative potential. It was also reported that long-chain PFAAs (≥6 carbons for perfluorosulfonates and ≥8 carbons for perfluorocarboxylates) have long serum elimination half-life in both animals and humans.14,9 The most detectable PFAAs in the environment and humans include two main groups, perfluorinated carboxylic acids (PFCAs; e.g., perfluorooctanoic acid (PFOA)) and perfluorinated sulfonates (PFASs; e.g., perfluorooctanesulfonate (PFOS)).1 High levels of PFAAs such as PFOS have been reported in the tissues (mostly liver) of humans and other biota including mammals and birds from remote environments or environments in close proximity to human populations.10–13 However, the sources in the environment of PFAAs such as PFCAs and PFASs are not well understood.4,14–16 Sources of PFCAs and PFASs to the environment include direct manufacturing discharge to air and water, and the degradation and/or precursor release from final commercial and consumer products. Transport pathways in the environment include atmospheric and ocean water long-range transport of PFAAs and precursors.14

Recent focus has been on whether perfluorinated polymers (or copolymers) can degrade and produce intermediates that can be transformed to PFAAs found in the environment.17

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Considering the large amount of carpet and fabric stain repellants and paper coatings manufactured from these polymers, if degradation is substantial, release from these products can potentially be an important source of PFAAs to the environment. There are a few published examples showing the degradation of fluorotelomer polymer in soil microcosms, and under aerobic and anaerobic conditions. Furthermore, Xu et al. reported using rat liver microsomes, cytosol, and slices, and with expressed rat and human cytochromes P450, PFSA precursor degradation by biotransformation pathways are important for, e.g., N-ethyl-perfluorooctane sulfonamide (N-EtFOSA) and/or N-methyl-perfluorooctane sulfonamide (N-MeFOSA).

Use of an in vitro mammalian microsomal model system has high utility from an enzyme-mediated metabolism standpoint, to examine fluorinated copolymer surfactant degradation. The objective of the present study is to determine whether the most common fluorinated surfactants known as Scotchgard, which are produced by the 3M Company, can be biotransformed to PFAAs or their small molecular precursors in a model in vitro assay based on mammalian hepatic microsomes.

**EXPERIMENTAL SECTION**

**Chemicals and Materials.** The commercial fluorinated copolymer surfactants currently under study, Scotchgard pre-2002 formulation (Tech mix) (100 μg/mL in methanol) and Scotchgard post-2002 formulation (Tech mix) (100 μg/mL in methanol), were purchased from AccuStandard Inc. (New Haven, CT). As detailed by the supplier (AccuStandard Inc.), these two standard solutions were prepared from commercial product of Scotchgard fabric protector produced by the 3M Company. These standard solutions were used directly for in vitro metabolism assay without any further purification.

Standard solutions of potassium perfluoro-1-butanesulfonate (PFBS), sodium perfluoro-1-octane-sulfonate (PFOS), perfluoro-n-butanoic acid (PFBA), perfluoro-n-octanoic acid (PFOA), perfluoro-1-octane-sulfonamide (FOSA), N-methyl-perfluoro-1-octane-sulfonamide (N-MeFOSA), N-ethyl-perfluoro-1-octane-sulfonamide (N-EtFOSA), perfluoro-1-octane-sulfonamidoacetic acid (FOSAA); N-methyl-perfluorooctane-sulfonamidoacetic acid (N-MeFOSAA) and N-ethyl-perfluorooctane-sulfonamidoacetic acid (N-EtFOSAA) were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Perfluoro-1-[13C₆]-octanoic-sulfonamide (M8FOSA) was used as internal standard and purchased from Wellington Laboratories Inc.

Perfluoro-1-butane-sulfonamide (FBSA) was synthesized and purified in our lab according the method described by Benfodda et al. with some modifications. FBSA was synthesized by reaction of perfluorobutanesulfonyl fluoride (Sigma-Aldrich, Oakville, ON, Canada) with an excess of dry ammonia. The raw product was dissolved in diethyl ether and washed with NH₄OH aqueous solution and water to reduce impurities (byproduct of PFBS), and then purified by crystallization in ethanol. The purity of FBSA was tested by LC/MS method and found to be >97%. HPLC grade methanol and diethyl ether were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada) and VWR International (Mississauga, ON, Canada), respectively. Utrapure water was obtained from a Milli-Q system. Ammonium acetate was obtained from Sigma-Aldrich (Oakville, ON, Canada). A suspension of rat liver microsomes (protein content 20 mg/mL) from pooled adult male Wistar-Han rats (BD Gentest Brand), NADPH regeneration system solutions (A) and (B), and 0.5 M potassium phosphate (pH 7.4) solution were obtained from BD Biosciences (Woburn, MA). The NADPH regeneration system was composed of NADP+ and glucose-6-phosphate (Glc-6-P) (solution A) and glucose-6-phosphate dehydrogenase (G6PDH) (solution B).

**In vitro Metabolism Assay.** All assays were performed according to the optimal assay parameters for CYP and NADPH-dependent enzymes outlined by BD Biosciences with some modifications. Briefly, in a 15 mL test tube, 1426 μL of water, 400 μL of 0.5 M potassium phosphate buffer (pH 7.4), A 50 μL volume of rat liver microsomes suspension, 100 μL of NADPH regeneration system solution A, and 20 μL of NADPH regenerating system solution B were combined. Reactions were initiated by addition of 4 μL of the copolymer substrate solution (100 μg/mL in methanol) and incubated at 37 °C in a water bath with shaking. At the 0, 10, 20, 30, 40, 50, and 60 min time points during the incubations, 200 μL of the suspension were transferred into a conical centrifuge tube containing 5 mL of 0.01 ng/mL M8FOSA diethyl ether solution (internal standard). After vortexing and separation by centrifugation, the solution was frozen at −20 °C and then diethyl ether phase was separated from the frozen aqueous phase. The diethyl ether phase was concentrated to dryness under a stream of nitrogen, and then the residue was dissolved in 50 μL methanol containing 2 mM ammonium acetate for quantitative analysis by LC-MS/MS. Three replicates were performed for each set of in vitro assays.

For metabolite identification, and using the same assay parameters that have already been described, the maximum microsomal assay incubation time of 60 min was used. All resulting incubation solutions were extracted using diethyl ether. Afterward, the organic phase was separated, concentrated and reconstituted in 100 μL methanol containing 2 mM ammonium acetate for LC-Q-TOF-MS/MS identification.

Negative control in vitro metabolism assays were performed with heat-treated rat liver microsomes (heated at 100 °C for 5 min) and containing all of the necessary reaction components. This negative control in vitro metabolism assays also evaluated for the occurrence of any possible nonmetabolic transformation of test substrate, and the possibility of metabolite contamination. For the positive control assay, N-ethylperfluoro-1-octanesulfonamide (N-EtFOSA) was used as substrate (4 μL × 5 mg/mL) in place of the copolymer substrate solution.

**Identification of Scotchgard Fabric Protector Components and Metabolites.** An Agilent 1200 LC system coupled to an Agilent 6520A liquid chromatograph-quadrupole time-of-flight mass spectrometer (LC-Q-TOF-MS) system (Agilent Technologies, Mississauga, ON, Canada) was used to identify components in the pre-2002 and post-2002 Scotchgard fabric protector and metabolites formed as a result of in vitro assay incubation. A volume of 10 μL sample was injected into the LC system. LC separation was carried out on a Luna C8(2) column (50 mm × 2.0 mm, 3 μm particle size) (Phenomenex Com., Torrance, CA). LC mobile phases were water (A) and methanol (B), and with both containing 2 mM of ammonium acetate. The mobile phase flow rate was 0.3 mL/min. The gradient for LC system started at 5% B, increasing to 95% B in 10 min, and was held for 10 min. Thereafter the mobile phase composition was returned to initial conditions and the column was allowed to equilibrate for 15 min between runs. The electrospray ionization (ESI) interface was operated in negative mode and the capillary voltage was 4000 V. The
fragmentor and skimmer voltages were 200 and 100 V, respectively. Nitrogen was used as drying and nebulizing gas, and helium was used as the collision gas when the system was operated in MS/MS mode. The gas temperature was 300 °C, dry gas flow rate was 10 L/min and nebulizer pressure was 25 psi. Full-scan data acquisition was performed by scanning from m/z 50 to 3000. When operated in the MS/MS mode, the collision energy was 40 eV. The resolution of MS (TOF) was >20 000 at m/z 622.028413.

Quantitative Analysis Of Metabolites In In Vitro Incubation Solutions. To Increase sensitivity, the metabolite concentrations in individual liver microsomal sample extracts (incubation times 0–60 min) were quantitatively analyzed by liquid chromatography-triple quadrupole mass spectrometer (LC-MS/MS). The LC separation was carried out using a Waters 2695 liquid chromatographic system (Waters Limited, Milford, MA) and the separation parameters were as the same as that described for LC-Q-TOF-MS analysis, with an exception that LC flow rate was 0.2 mL/min. The mass spectrometer was a Waters Quattro Ultima triple quadrupole mass spectrometer (Waters Limited, Milford, MA). Nitrogen was used as nebulizing gas and dissolvent gas. Argon was used as collision gas. Analyses were performed in negative ion ESI mode using multiple reaction monitoring (MRM) mode. The capillary voltage was 4.5 kV. The source temperature and probe temperature were 120 and 320 °C, respectively. Cone and desolvation gas flow rate were 150 and 700 L/h, respectively. The compound dependent operation parameters and retention times are listed in Table 1. Concentrations of metabolites in the extracts were measured with internal standard method (M8FOSA was used as internal standard). The limits of detection (LODs) and limits of quantification (LOQs) were defined as the concentration of target compounds in spiked sample producing a peak in chromatogram with a S/N ratio of 3 and 10 (peak to peak), respectively, and are listed in Table 1.

Table 1. Compound Dependent Operation Parameters, Retention Times, Limits of Detections (LODs) and Limits of Quantifications (LOQs) for Individual Compound on LC-ESI(-)-MS/MS

<table>
<thead>
<tr>
<th>no.</th>
<th>compound</th>
<th>RT (min)</th>
<th>precursor ion (m/z)</th>
<th>product ion (m/z)</th>
<th>cone voltage (V)</th>
<th>collision energy (eV)</th>
<th>LOD (ng/L)*</th>
<th>LOQ (ng/L)*</th>
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<tr>
<td>1</td>
<td>FBSA</td>
<td>11.48</td>
<td>298</td>
<td>78</td>
<td>60</td>
<td>70</td>
<td>9.9</td>
<td>32.9</td>
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<tr>
<td>2</td>
<td>FOBSA</td>
<td>13.72</td>
<td>497.9</td>
<td>78</td>
<td>60</td>
<td>70</td>
<td>1.9</td>
<td>6.4</td>
</tr>
<tr>
<td>3</td>
<td>PFBS</td>
<td>10.27</td>
<td>298.9</td>
<td>80.1</td>
<td>35</td>
<td>40</td>
<td>2.9</td>
<td>9.6</td>
</tr>
<tr>
<td>4</td>
<td>PFOS</td>
<td>12.74</td>
<td>498.9</td>
<td>80.1</td>
<td>35</td>
<td>40</td>
<td>5.2</td>
<td>17.4</td>
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<tr>
<td>5</td>
<td>N-MeFOSA</td>
<td>14.34</td>
<td>512</td>
<td>169</td>
<td>35</td>
<td>25</td>
<td>3.8</td>
<td>12.8</td>
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<tr>
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<td>14.57</td>
<td>526</td>
<td>169</td>
<td>50</td>
<td>25</td>
<td>3.8</td>
<td>12.7</td>
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<tr>
<td>IS</td>
<td>M8FOSA</td>
<td>13.72</td>
<td>506</td>
<td>78</td>
<td>60</td>
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</table>

*Concentration in incubation solution.
RESULTS

Identification of Components in Scotchgard Fabric Protectors. For commercial usage, the concentration of active components in Scotchgard products has been reported to be <3%.24 The standard solutions of Scotchgard fabric protector, which were used in this study, were characterized using LC separation and full-scan TOF with ESI source (− and +). With the ESI source in the negative mode, in the mass chromatogram of pre-2002 product the main detectable component peak was at retention time of 15.8 min, and in the mass chromatogram of the post-2002 product the main detectable component peak was at retention time of 14.5 min (Figure 1). The main detectable chromatographic peak in pre-2002 product showed a mass spectral peak at m/z 1315.0591, while that of the post-2002 product showed mass spectral peak with m/z 1634.3120 (Figure 1A and B). Direct MS injection (with no prior LC separation) was also performed and no other notable component ions were found. Although it was not possible to determine their detailed molecular structure with only mass spectral information, MS/MS data provided further insight. The product mass spectrum of m/z 1315.0591 (pre-2002 product) showed two main ions with m/z 418.9718 and 525.9786 (Figure 2A), which corresponded to the \([\text{C}_8\text{F}_{17}]^−\) and \([\text{C}_8\text{F}_{17}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)]^−\) fragment ions, respectively. The product mass spectrum of m/z 1634.3120 (post-2002 product) showed two main ions with m/z 218.9860 and 311.9751 (Figure 2B), which corresponded to the \([\text{C}_4\text{F}_{9}]^−\) and \([\text{C}_4\text{F}_{9}\text{SO}_2\text{N}(\text{CH}_3)]^−\) fragment ions, respectively. These data were coincident with the announcement that the 3M Company had voluntarily phased out PFOS related products by the end of 2000.25 Impurities in the Scotchgard products were identified by LC-Q-TOF and quantitatively analyzed by LC-MS/MS. Any possible unreacted fluorinate compounds, such as \(\text{N}-\text{EtFOSA}, \text{N}-\text{MeFOSA}\) and FOSA, in the initial incubation solution (0 min) were below their respective LODs.

Metabolite Identification from Scotchgard Fabric Protector Components. The extracts of the in vitro incubation solutions of the pre-2002 and post-2002 Scotchgard products (after 60 min of incubation time) were analyzed by LC-Q-TOF-MS(/MS) to identify any metabolites that may be formed. For the pre-2002 incubation extract, only FOSA was detectable, and with no detectable concentrations of PFOS, N-MeFOSA, N-EtFOSA, N-MeFOSAA or N-EtFOSAA. Subsequently, the same sample extract was reanalyzed by LC-triple quadrupole mass spectrometer (LC-MS/MS) in MRM mode, and showed that only FOSA was detectable. Similarly, in the extract of incubation solution of the post-2002 Scotchgard product (after 60 min of incubation time) only FBSA was detectable.

In Vitro Time-Dependent Trends for Scotchgard Component and Metabolite Concentrations. Because FOSA and FBSA were detected in the extracts of in vitro incubation solutions of the pre-2002 and post 2002 products, respectively, the concentration changes over the 0–60 min incubation period were determined by LC-MS/MS data provided further insight. The product mass spectrum of m/z 1315.0591 (pre-2002 product) showed two main ions with m/z 418.9718 and 525.9786 (Figure 2A), which corresponded to the \([\text{C}_8\text{F}_{17}]^−\) and \([\text{C}_8\text{F}_{17}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)]^−\) fragment ions, respectively. The product mass spectrum of m/z 1634.3120 (post-2002 product) showed two main ions with m/z 218.9860 and 311.9751 (Figure 2B), which corresponded to the \([\text{C}_4\text{F}_{9}]^−\) and \([\text{C}_4\text{F}_{9}\text{SO}_2\text{N}(\text{CH}_3)]^−\) fragment ions, respectively. These data were coincident with the announcement that the 3M Company had voluntarily phased out PFOS related products by the end of 2000.25 Impurities in the Scotchgard products were identified by LC-Q-TOF and quantitatively analyzed by LC-MS/MS. Any possible unreacted fluorinate compounds, such as \(\text{N}-\text{EtFOSA}, \text{N}-\text{MeFOSA}\) and FOSA, in the initial incubation solution (0 min) were below their respective LODs.

Attempts to detect concentration changes of the active components in the Scotchgard products (with m/z 1634.3120 for post-2002 product and m/z 1315.0591 for pre-2002 product, respectively) were not successful in our study (Figure 4, A and B). The large concentration variations resulted from...
the fact that there was no suitable internal standard to be used, and thus the MS responses could not be corrected due to substantial ESI(−) matrix effects.

In positive control samples (using N-EtFOSA as substrate) in the same situation N-EtFOSA was metabolized much faster than Scotchgard products. After 10 min most of N-EtFOSA (>90%) was metabolized to FOSA (see the results in the Supporting Information).

■ DISCUSSION

Previous studies have shown that in most cases, there is minimal direct human exposure to residual amounts of PFCAs and PFASs in consumer products.26,27 Several studies have suggested that there may be other additional indirect sources of human exposure to PFCAs and PFASs.22,26,28 There has been a continual debate on whether fluorinated polymers (or copolymers) are potential environmental sources of PFAAs.17 Russell et al.19 published an important paper on a 2-year degradation experiment for a fluoropolymer stain repellent solution under aerobic conditions in four different soils. Fluoropolymer degradation was reported to be dependent on unbound impurities of PFOA and PFCAs. It was also concluded that the fluoropolymer degraded very slowly with a half-life of 1200−1700 years. This implied that such an extremely long half-life of the polymer would mean high stability, and minimal degradation of the fluoropolymer stain repellent, and thus it was unlikely that the fluoropolymer itself was a source of PFNA release into the environment. However, Renner17 pointed out that the application of fluorinated polymers to soil was not comparable due to the differing degradation processes that occur for e.g. fluoropolymer-containing commercial products such as textiles (e.g., carpets), and influents to wastewater treatment plants.20

Our in vitro metabolism assay results clearly demonstrated that the detected FOSA and FBSA in incubation solutions did not come from any small molecular product impurities in the pre-2002 and post-2002 Scotchgard, respectively. For the pre-2002 product, since the concentration of low molecular weight FOSA precursors such as N-MeFOSA and N-EtFOSA were not detectable in the incubation solution, the detected FOSA (20−35 ng/L) in the in vitro metabolism assay incubation solution could not be the metabolite of these impurities. It is clear that even when manufacturers attempt to limit and/or remove the impurities from their polymeric products, the use of these products can still have an environmental impact via enzyme-mediated degradation to form perfluoralkyl sulfonamides.

It is well-known that most of high molecular weight, commercial anthropogenic polymers are relatively stable and have been found to be highly resistant to biodegradation if their
molecular weight is greater than 1000 amu.\textsuperscript{29} In a study by Russell et al.,\textsuperscript{19} an aqueous dispersion of a fluorooctyl polymers with an average molecular weight of 40 000 amu was found to be highly resistant to biodegradation. However, the detectable active components in the Scotchgard products we studied have much lower molecular weights. Therefore, it is more than likely that the detected FOSA and FBSA in the present \textit{in vitro} incubation solutions were metabolites of the copolymer products.

Using the present rat liver microsomal suspensions, for the pre-2002 product, FOSA formation occurred, which increased progressively over the incubation period, but with no concomitant formation of PFOS. This demonstrated that the microsomes possessed NADPH catalyzed \textit{N}-deethylation activity to give a final FOSA degradation product via a phase I metabolic pathway. FOSA is the penultimate precursor of PFOS.\textsuperscript{30} Biotransformation of FOSA to PFOS was observed \textit{in vitro} as a result of FOSA incubation with enzymatically viable liver slices although the rate of this transformation was slow.\textsuperscript{22} Similar to the pre-2002 product and FOSA metabolite results, incubation with the post-2002 product resulted in FBSA formation, which increased progressively over the incubation period, but with no concomitant formation of PFBS.

In a very recent study, exceptionally high concentrations of PFOS and PFHxS have been reported in the serum samples in persons in a household environment where Scotchgard carpet products were heavily used.\textsuperscript{7} Such exposures in that study were pre-2002, and thus related to human exposures to pre-2002 Scotchgard carpet protector products (which are not currently available on the market). However, post-2002 Scotchgard carpet and rug protector product (3M Canada, London, ON, Canada) is commercially available. To see if our Scotchgard post-2002 formulation (Tech mix) (from AccuStand Inc.) is comparable to the content in commercially available rug and carpet protectors, we compared \textit{in vitro} incubation results. We compared LC-Q-TOF analysis results and it revealed the presence of the same main copolymer (with a peak of \textit{m/z} 1634.3104), which was found in AccuStandard standard solution (post-2002), as well as some other copolymer PFBS-substances and other nonfluorinated surfactants in the post-2002 Scotchgard carpet and rug protector product. Considering that the 3M Company also produced PFHS for specific postmarket carpet treatment applications, and PFHxS is known to have a much longer half-life in serum than PFOS,\textsuperscript{4,5} this might explain why high concentrations of PFHxS have been found in recent human serum samples.\textsuperscript{7}

One of the main applications of polyfluorinated organic compounds is in polymers, for example in N-EtFOSE or N-methyl perfluorooctane sulfonamidoethanol (N-MeFOSE)-based polymers.\textsuperscript{31} Because of the high production amounts of these polymers and extensive commercial usage, they could be or may become a significant source of PFCAs and PFASs in the environment and in humans.\textsuperscript{1} Moreover, some new formulations are being brought to market where little is known about their environmental disposition and the toxicity of the compounds that they contain.\textsuperscript{7} There is a dearth of published studies on the degradation of fluorinated polymers due to complexity of the subject and difficulties and challenges in the experimental setup.\textsuperscript{19,21,32} Most fluorinated polymers are strongly adsorbed on particles or container surfaces, and this behavior poses a challenge in obtaining reproducible analytical results.\textsuperscript{33} In general, biodegradation is one of the most important mechanisms by which organic contaminants are removed from the environment. While nonfluorinated functional groups in the polymers can be easily degraded and removed, the fluorine substituents in fluorinated polymers remain stable and cannot achieve mineralization.\textsuperscript{8} In the present study, FOSA and FBSA metabolites were detected as products in the \textit{in vitro} metabolism assay. Although FOSA has been shown to biodegrade to PFOS,\textsuperscript{17,22,34} it is relatively stable and is commonly detectable in wildlife and human samples.\textsuperscript{35} In some wildlife tissue samples, and particularly in liver, the concentration of FOSA has been shown to be as much as 10% of the concentration of PFOS.\textsuperscript{13,33} There are also reports of liver concentrations of PFOS that are lower than FOSA concentrations including arctic beluga whales from Alaska,\textsuperscript{36} and melon-headed whales from Japan.\textsuperscript{37} FOSA is also often found in human blood samples as well.\textsuperscript{38,39} As recently reviewed by Galatius et al.,\textsuperscript{40} PFOS to FOSA concentration ratios on wildlife is highly variable, and it was concluded that a general pattern could not be observed.

Although FBS-based products have been in the market for more than 10 years since the 3M Company phased out PFOS related products, there is a dearth of information available about its environmental behavior, degradation, and metabolism. For most environmental investigations, PFBS and FBSA are not included on PFAA monitoring lists.\textsuperscript{3,33} Manufacturers claim that PFBS has a shorter half-life in people than PFOS\textsuperscript{41} although it was expected to be persistent in the environment. For example, relatively high PFBS concentrations were recently reported in water samples from China.\textsuperscript{42} In the present study, FOSA and FBSA concentrations increased with the microsomal incubation time with the pre-2002 and post-2002 products, respectively (Figure 3 A and B). There was a plateau in the amount of FOSA formed after 20 min incubation time, but in contrast there was a lack of a FBSA formation plateau up to 60 min of maximum incubation time. Considering the high volume production of PFBS-based polymers and their extensive commercial usage, the accumulation of FBSA, a major intermediate precursor for FBS, is of concern in biota including in the tissues of wildlife and humans.

In comparison to other persistent organic pollutants (POPs), sources of human exposure to PFAAs are more complex. Although copolymer surfactants have low water solubility, they can form stable polymeric micelles in water,\textsuperscript{16} and subsequently enter water systems during the washing of textiles such as carpets. Finally, because these micelles are relatively stable, they can become latent sources of PFAA exposure to wildlife and humans. When polymer (or copolymer based) surfactants are used via spray application, a very common consumer usage in households, aerosols will form and human exposure can be via direct inhalation. Once inhaled, these surfactants may be metabolized and form terminal and stable PFAAs that can be accumulated in body. Bioaccumulation models exist for PFSA and PFCA in wildlife.\textsuperscript{4,14,15,30,44,45} Generally, these models assume that there were two pathways for wildlife and humans to accumulate these pollutants. The first is direct exposure to PFSA and PFCA contaminated food and water. The second pathway is via human and wildlife accumulation of PFSA and PFCA precursors, which are subsequently biotransformed to PFASs in the body. Until now these bioaccumulation models include only lower molecular weight PFAA precursors, but do not include commercially available and molecularly larger, fluorinated polymer surfactants.\textsuperscript{17} As this study has shown, the metabolic stability of fluorinated...
copolymer surfactants is put into question. One also has to consider that cytochrome P450 enzyme isofoms and activity vary among animals, and thus the ability to degrade fluorinated copolymer surfactants.

ASSOCIATED CONTENT

Supporting Information
Additional information is available concerning N-EtFOSA substrate concentration changes over the in vitro incubation period of 0 to 40 min as positive control in the rat hepatic microsomal incubation solutions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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