

# Bioavailability and Biotransformation of Technical Mixtures Containing Side-chain Fluorinated Copolymers in a Soil-earthworm System

Felicia. Fredriksson<sup>1\*</sup>, Ulrika. Eriksson<sup>1</sup>, Anna. Kärman<sup>1</sup>, Leo WY. Yeung<sup>1</sup>

<sup>1</sup> Man-Technology-Environment (MTM) Research Centre, School of Science and Technology, Örebro University, Sweden, SE-701 82, [felicia.fredriksson@oru.se](mailto:felicia.fredriksson@oru.se)

## 1 Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large group of anthropogenic compounds used in a wide range of commercial and industrial applications due to their unique physicochemical properties such as water-, oil- and dust repellence and thermal and chemical stability<sup>1</sup>. Several PFAS have been found to pose a risk to the environment and humans due to their persistence, mobility, toxicity, and bioaccumulative potential<sup>1-3</sup>. However, only a minor part of these several thousand compounds is commonly monitored and even less is regulated.

A large proportion of the several thousands of PFAS registered on the global market are fluorinated polymers; one group included is side-chain fluorinated polymers. Side-chain fluorinated polymers are fluorinated polymers with a structure of a non-fluorinated polymer backbone with fluorinated side-chains<sup>1</sup>. They are commonly used to give oil-, dust- and water repellency to materials. There are a large knowledge and data gap regarding side-chain fluorinated polymers in their occurrence and environmental levels, especially the bioavailability, bioaccumulation, and biotransformation.

Two suspected side-chain fluorinated copolymers have been identified in impregnation sprays from the trademark Scotchgard of 3M Company<sup>4</sup>. Their chemical identities are a trade secret of 3M Company, and therefore their structures are not yet fully understood. At the moment, we know that these two compounds differ in fluorinated side-chain lengths (C8-based vs C4-based); one is produced before (C8-based) and the other after the phase-out of POSF (C4-based). Chu and Letcher<sup>4</sup> identified the perfluorinated side-chain in both formulations; in the technical mixture of the Scotchgard™ before the year 2002 (pre-2002), N-ethyl-perfluorooctane sulfonamide (Et-FOSA) was observed. Whereas, in the Scotchgard™ formulation in production after 2002 (post-2002), N-methyl-perfluorobutane sulfonamide (Me-FBSA) was observed. Besides the already reported C4- and C8-fluoroalkylsulfonamido (FASA) side-chains, our recent study identified the molecular structure for the perfluorooctane (C8) sulfonamide-urethane copolymer in the pre-2002 formulation (CAS no: 21055-88-9, molecular formula of C<sub>33</sub>H<sub>26</sub>F<sub>34</sub>N<sub>4</sub>O<sub>8</sub>S<sub>2</sub>)<sup>5</sup>. Further identification of the C4-FASA-based copolymer in the technical mixture of post-2002 could not be provided and are still a mystery.

Both the C4- and C8-FASA-based copolymers have been found in the environment in matrices as biosolids-augmented soil, sediments, biosolids, and sludge<sup>6-8</sup>. Recent results from a sorption test indicate that these FASA-based copolymers are not mobile in soil<sup>5</sup>, which is supported by previous findings. However, even if they are not mobile, they may be available for uptake in terrestrial organisms (i.e. earthworms). Further studies are needed to understand how different physicochemical parameters affect the sorption of these FASA-based copolymers, their environmental pathway, and their bioavailability.

This project investigated the fate of the two FASA-based copolymers in a soil-earthworm system by studying the bioavailability and biotransformation of the two FASA-based copolymers in earthworms (*Eisenia fetida*).

## 2 Materials and Methods

Technical mixtures of Scotchgard™ pre-2002 formulation (1000 µg/mL in methanol) and Scotchgard™ post-2002 formulation (1000 µg/mL in methanol) used for the exposure study were purchased from AccuStandard Inc. (New Haven, USA). Native and mass labeled PFAS standards used for the target analysis of potential transformation products, included perfluoroalkyl sulfonic acids (PFSA), perfluoroalkyl carboxylic acids (PFCA), FASAs, and perfluorooctane sulfonamidoacetates (FOSAAs) were obtained from Wellington Laboratories (Guelph, Canada).

The bioavailability was assessed according to OECD guidelines for bioaccumulation of chemicals in soil oligochaetes (OECD317) and ISO11268-1. Briefly, triplicate experiments of earthworms cultivated in our laboratory were exposed to a non-contaminated soil (artificial soil) spiked either with the technical mixtures pre-2002 or post-2002 formula (1000 µg). Both technical mixtures were used in the exposure study without any purification. Triplicate of a non-spiked soil with earthworms was set up as blank controls, it was performed under identical conditions as the spiked soil. Each glass container contained approximately 500 g dry mass of artificial soil and ten mature earthworms with a weight between 300-600 mg. The earthworms were exposed to the soil for 30 days before they were removed from the soil. Then, they were washed with water and allowed to purge on clean filter paper for 48 hours. For each glass container the earthworm was pooled together, frozen overnight, and freeze-dried. The freeze-dried earthworms were homogenized by cutting them into small pieces and ground.

Analysis of the FASA side-chain fluorinated copolymers and potential degradation products of the side-chain fluorinated copolymers were conducted in both soil and earthworms. The C8- and C4-FASA side-chain fluorinated copolymers were extracted according to a previous study<sup>6</sup>. Extraction and clean-up for anionic and neutral PFAS (transformation products) followed the procedure used by Golovko et al.<sup>9</sup>. The FASA-based copolymers and the anionic and neutral PFAS were analyzed by a Water Acquity Ultra Performance Liquid Chromatography (UPLC) with a C18 BEH column (2.1 x 100 mm, 1.7  $\mu$ m) coupled to a Waters XEVO TQ-S tandem mass spectrometer. Further analysis of unknown transformation products will be done with a Waters Acquity UPLC coupled to a Waters Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometer.

The transformation products (e.g. PFCAs, PFSAs, FASAs, and FOSAAs) were quantified by internal calibration. No suitable internal standards were available for the FASA-based copolymers and therefore quantified by matrix match quantification. Single standard addition was spiked prior extraction, to take into account both the matrix effect and extraction efficiency. The percentage of the copolymers in the technical mixture was not known and an overestimation may occur if the levels of the copolymers were quantified by using the concentration of available technical mixtures. Since both copolymers' perfluoroalkyl chains have been identified and the total fluorine content of the technical mixture is reported<sup>5</sup>, fluorinated sulfonamide side-chain equivalent was calculated for both copolymers. In order to compare the levels of the copolymers with other PFAS, the concentration of FASA-based copolymers was converted to fluorinated side-chain equivalents (FSC eq.).

Bioaccumulation factor (BAF) was determined for the earthworms after the exposure phase in each soil. This was done by dividing the level of the FASA-based copolymer in the earthworms by the level of the FASA-based copolymer in the soil, both concentrations were reported on a dry weight basis. Biota soil accumulation factor (BSAF) was determined by dividing the level of FASA-based copolymer in the earthworms, reported in wet weight, by the level of the FASA-based copolymer in the soil, reported in dry weight, and normalized for the organic carbon content of the soil. The BSAF value was not normalized to lipids, due to the fact that the accumulation of PFAS is mainly driven by proteins and not lipids.

### 3 Results

During exposure phase, all earthworms had healthy growth and no mortality occurred; no sign of toxicity of the spiked FASA-based copolymers to the earthworms was observed. No FASA-based copolymers were detected in the earthworms of the blank control tests. Both, the C8- and C4-FASA-based copolymers were detected in the earthworms after exposure (i.e., 30 days). Average concentrations of  $11.4 \pm 4.4$  ng FSC eq/g d.w. and  $0.79 \pm 0.23$  ng FSC eq/g d.w. were detected in the earthworms after exposure to the C8-FASA-based and C4-FASA-based copolymers, respectively.

The bioaccumulation factor values were estimated for the FASA-based copolymers (Figure 1). A BAF value above one indicates a higher level of the FASA-based copolymer in the earthworm than in the exposed soil. No BAF value above one was observed. The BAF values for the C8-FASA-based copolymer ranged from 0.24 to 0.46, whereas the BAF values for the C4-FASA-based were twenty times lower than the C8-FASA-based copolymer and were in a range of 0.009 to 0.016.

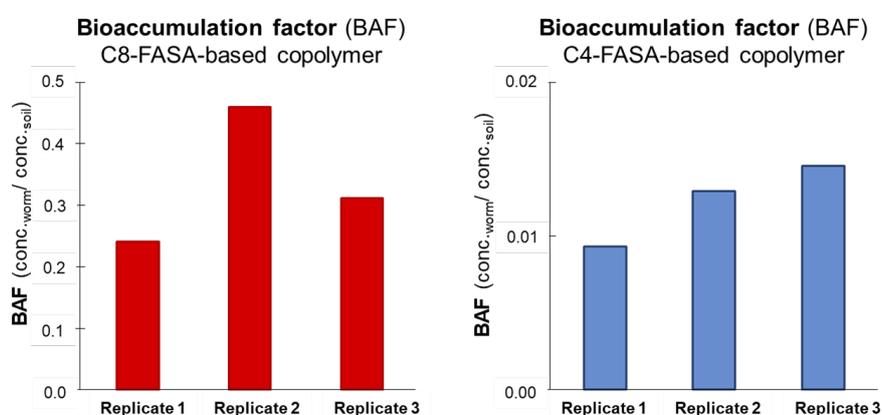


Figure 1: Bioaccumulation factor (BAF) values for the C8-FASA-based exposure test (red), and C4-FASA-based exposure test (blue).

The estimated BSAF values ranged from 0.0072 to 0.0002, and the C8-FASA-based copolymer had the highest values (Figure 2). The lower BAF and BSAF values for the C4-FASA-based copolymer compared to the C8-FASA-based copolymer suggest the C8-FASA-based copolymer to be more bioavailable to earthworms compared to the C4-FASA-based copolymer.

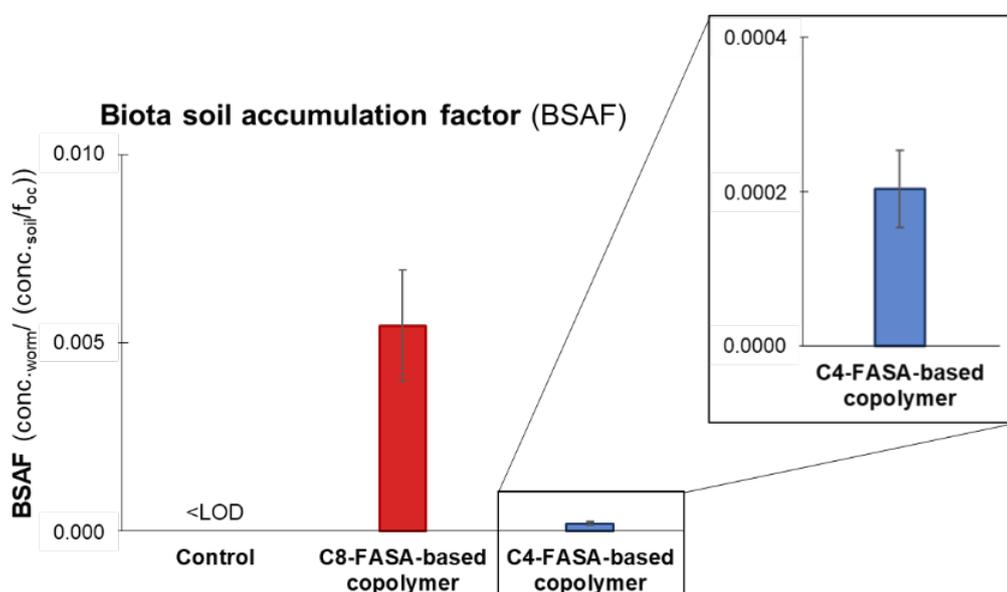


Figure 2: Biota solid accumulation factor (BSAF) values for the blank control, C8-FASA-based spiked, and C4-FASA-based spiked experiment. The error bars represent the standard deviation of the experiment.

The earthworms and soil from the exposure experiments were extracted and analyzed for anionic and neutral PFAS in order to estimate any transformation products that may be formed. Except for the spiked compounds, no detectable PFAS were noted in the soil spiked with the technical mixture of pre-2002 or post-2002. Figure 3 represents the uptake and biotransformation products in earthworms after exposure to the FASA-based copolymer for 30 days. For the earthworms exposed to the technical mixture of pre-2002, perfluorooctane sulfonamide (FOSA) was detected; whereas perfluorobutane sulfonamide (FBSA), and perfluorobutane sulfonate (PFBS) were detected after exposed to the technical mixture of post-2002.

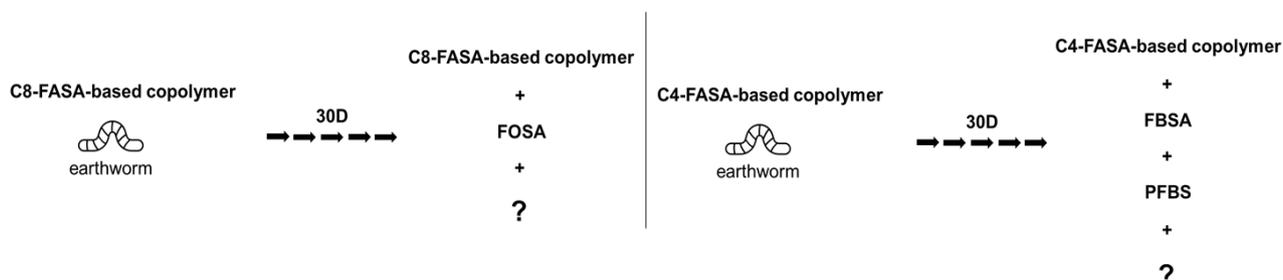


Figure 3: Scheme of uptake and biotransformation products in earthworms after exposure to the FASA-based copolymer for 30 days. C8-FASA-based copolymer (left), C4-FASA-based copolymer (right).

#### 4 Discussion

Findings in the study indicate the bioavailability of the FASA-based copolymers to terrestrial organisms. How rapidly the earthworms took up the copolymer and if equilibrium was reached could not be estimated in the present study. Levels of both FASA-based copolymers have been found in soil, sludge, and biosolids<sup>6-8</sup>. Based on previous findings, these FASA-based copolymers are not mobile in soil<sup>5</sup>. A plausible environmental fate of these FASA-based copolymers in the terrestrial system would be terrestrial organisms taking up these compounds when sludge/biosolids were applied as fertilizer in agricultural fields.

The biota soil accumulation factor (BSAF) has been studied in several PFAS. Zhao et al.<sup>10</sup> exposed earthworms to artificially soils spiked with ten PFAS, at the highest exposure concentration the BSAF value for C6-C12 PFCAs ranged from 0.008 to 0.261, C4, C6, and C8 PFSAs ranged from 0.008 to 0.097. The biota soil accumulation factor was observed to increase with increasing fluorinated carbon chain length. The same trend for PFCAs was seen by Rich et al.<sup>11</sup>, however, the opposite trend was seen for the PFSAs, where the BSAF decreases with increasing fluorinated carbon chain length. In the present study, the C4- and C8-FASA-based copolymers, the BSAF values were found to be 0.005 and 0.0002, respectively. Higher BSAF values for the C8-FASA-based copolymer demonstrate a higher uptake than the C4-FASA-based copolymer. The reason for the higher BSAF value for the C8-FASA-based copolymer compared to the C4-FASA-based may be the longer fluorinated side-chain length in the C8-FASA-based copolymer, which is aligned with the previous study<sup>10</sup>. The C8-FASA-based copolymer BSAF value is in the lower range compared to other PFAS, whereas the C4-FASA-based copolymer is one magnitude lower compared to other PFAS. The reason might be that these large

molecular weight copolymers have lower bioavailability compared to other low molecular weight perfluoroalkyl acids (PFAAs) and/or a higher degradation rate to other transformation products.

One common and important discussion is about side-chain fluorinated polymers' stability and their potential to be indirect sources of other PFAS in the environment<sup>12,13</sup>. Our result observed that these copolymers biodegrade to C8 and C4 sulfonamides, e.g., FOSA and FBSA, respectively in earthworms. This result is supported by an earlier *in vitro* metabolic study on these FASA-based copolymers, where the same formation was observed<sup>4</sup>. The transformation product of FOSA is PFOS<sup>14</sup>, similar biotransformation pathway can be expected for FBSA. Elevated levels of PFBS in earthworm after exposure to the C4-FASA-based copolymer was observed and support this pathway. Both copolymers biodegrade to other low molecular PFAS which demonstrates that both copolymers have the potential to be an indirect source of other PFAS in the environment.

## 5 Conclusions

The result of present exposure study demonstrates that the C8- and C4-FASA-based copolymers were taken up by the earthworms, and thereafter were metabolized further to FOSA for the C8-FASA-based copolymer and FBSA and PFBS for the C4-FASA-based copolymer. Further studies are needed to understand the bioaccumulation kinetics for these compounds and their environmental pathways. The present study demonstrates the potential side-chain fluorinated polymers have to contribute to other anionic and neutral low molecular PFAS in the environment. Since fluorinated polymers are produced in high quantity, more research on bioavailability and degradation are essential for other types of fluorinated polymers.

## 6 Acknowledgments

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## 7 References

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