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Short Communication

Perfluoroalkylsulfonic and carboxylic acids in earthworms (*Eisenia fetida*): Accumulation and effects results from spiked soils at PFAS concentrations bracketing environmental relevance

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HIGHLIGHTS

- First study on earthworms in PFAS-spiked soil at concentrations covering background levels.
- PFASs can be detected in earthworms even in the 0.1 μg kg^{-1} soil treatment after 21-d exposure.
- PFAS accumulation in earthworms follows the order: PFNA > PFHxS > PFHpA > PFBS.

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ABSTRACT

Effects of perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluoroheptanoic acid (PFHpA) on earthworms (*Eisenia fetida*) in soils contaminated with these compounds at 0.1, 1, 10, 1,000, and 100,000 μg kg^{-1} dry weight, covering concentration levels found in background, biosolid–amended, and facility–surrounding soils, were investigated. Earthworms were exposed to spiked soil for 21 days. Concentrations of these compounds in earthworms after 21-d exposure ranged from below detection to 127 mg kg^{-1} wet weight with the rank order of PFNA > PFHxS > PFHpA > PFBS; no mortality of earthworms was observed in all treatments including controls, except PFBS at 1,000 μg kg^{-1} and all PFASs at 100,000 μg kg^{-1}. The highest weight loss (29%) was observed for earthworms exposed to PFNA at 100,000 μg kg^{-1}, which was significantly different from all other treatments except PFHpA at 100,000 μg kg^{-1}. These results are expected to fill some data gaps in toxicity of PFASs in terrestrial environments and provide helpful information on the potential for trophic transport of PFASs from soil to higher organisms.

1Introduction

Per- and polyfluoroalkyl substances (PFASs) are widely used in the manufacturing of various industrial and consumer products including nonstick food packaging, stain repellents and aqueous film-forming foams. Among PFASs globally used, long–chain perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs) have drawn great interest after scientists found global contamination of perfluorooctane sulfonic acid ($C_8F_{17}SO_3H$, PFOS) in wildlife (Giesy and Kannan, 2001) and perfluorooctanoic acid ($C_7F_{15}COOH$, PFOA) in human blood (Hansen et al., 2001). Perfluorohexanesulfonic acid ($C_6F_{13}SO_3H$, PFHxS), perfluorononanoic acid ($C_8F_{17}COOH$, PFNA), and perfluoroheptanoic acid ($C_6F_{13}COOH$, PFHpA) are long–chain PFASs included in the Toxic Substances Control Act (USEPA, 2009), while perfluorobutanesulfonic acid ($C_4F_9SO_3H$, PFBS) is a short–chain PFAS used to replace PFOS. These compounds were frequently detected in food, human, and environmental samples along with PFOS and PFOA (Stahl et al., 2011; Pérez et al., 2014). Concerns about their potential adverse effects are increasing because of their persistence in the environment and

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accumulation in terrestrial organisms such as invertebrates (Conder et al., 2008; Rich et al., 2014).

Concentrations of PFASs in biosolid–amended natural soils and soils surrounding manufacturing facilities varied, ranging from not detectable (ND) to 2,500 μg kg^{-1} dry soil; the sum of all PFASs could be as high as 4–6 mg kg^{-1} dry soil (Washington et al., 2010; Sepulvado et al., 2011; Wang et al., 2013; Filipovic et al., 2015). PFASs in background surface soil samples collected from around the world were typically <100 μg kg^{-1} dry soil (Strynar et al., 2012; Wang et al., 2015; Rankin et al., 2016). The presence of PFASs in soils is of concern since they have the potential to adversely affect ecological receptors and terrestrial foodwebs. However, studies on their effect in terrestrial organisms are still limited especially for earthworm, which is one of the organisms serving as a possible carrier of PFASs in contaminated soils to higher (vertebrate) organisms. Among the few studies that have evaluated the effect of PFASs in soils on earthworms and bioaccumulation of these compounds in soil invertebrates (Zhao et al., 2013, 2014; Navarro et al., 2016; Zhao et al., 2016), most focus on PFOS and PFOA (Joung et al., 2010; Xu et al., 2013; Zareitalabad et al., 2013; D'Hollander et al., 2014; Das et al., 2015; Wen et al., 2015; He et al., 2016); little data exist for other PFASs including those used as substitutes for the regulated PFOS and PFOA. Moreover, these studies were conducted at soil concentrations ≥100 μg kg^{-1} (Zhao et al., 2013) or mg kg^{-1} levels (Zareitalabad et al., 2013; He et al., 2016), which are much higher than typical concentrations in natural soils and may be higher than concentrations at some sites with known aqueous film forming foam spills or municipal sludge application.

Our study was conducted to evaluate the concentration of PFBS, PFHxS, PFNA, and PFHpA in earthworms (*Eisenia fetida*) exposed to soil contaminated with these compounds at concentration levels typically found in background, biosolid–amended, and facility–surrounding soils. Effects of these compounds on earthworm survival and weight were also monitored. To the best of our knowledge, this is the first study to examine the effect of PFBS, PFHxS, PFNA, and PFHpA on earthworms conducted at concentration levels found in natural soils.

2. Materials and methods

2.1. Chemicals and reagents

PFBS (purity > 98%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). PFHxS, PFNA, and PFHpA (purity > 97%) were obtained from Sigma–Aldrich (Saint Louis, MO). Acetonitrile and methanol were Optima–LC/MS grade and obtained from Fisher Scientific (Fair Lawn, NJ). QuEChERS extraction salts (4,000 mg $MgSO_4$/1,000 mg NaCl) were purchased from UCT, Inc. (Bristol, PA). Ultrapure water (>18 MΩ) was prepared by a Barnstead NANOpure infinity ultrapure water system (Dubuque, IA). Standard solutions of test compounds were prepared in methanol.

2.2. Soil preparation and experimental setup

Sandy loam soil (Terry County, Texas) was air dried and sieved (2 mm) prior to use. The soil was slightly basic (pH = 8.3) and consisted of 74% sand, 10% silt, and 16% clay with 1.3% organic carbon. The texture and properties of the test soil were determined by A&L Midwest Laboratories (Omaha, NE). In preliminary assays using similar extraction and analysis procedures as those described below, we concluded that the soil was free from PFAS contamination; we did not detect any PFBS, PFHxS, PFNA and PFHpA contamination in the unspiked soil.

The experiment was conducted in 125–mL jars containing 100 g of dry soil spiked with PFBS, PFHxS, PFNA, or PFHpA in methanol to obtain final soil concentrations of 0.1, 1, 10, 1,000, and 100,000 μg kg^{-1} dry weight with four replicates for each treatment. Each compound was tested individually. Stock solutions used to spike the soils were tested by LC–MS/MS to ensure that their concentrations were 90–110% of nominal. Once spiked, jars were held at room temperature for 24 h to allow the methanol to evaporate. A control set with unspiked soil was also included. Milli–Q water was added to each jar to hydrate the soil to 15% of soil weight (approximately 80% of soil water holding capacity) and the soil was mixed thoroughly.

Results of spike-recovery tests for PFASs (100 μg kg^{-1}) using acetonitrile as the extraction solvent were as follows: 91 ± 5.5% for PFBS, 82 ± 2.7% for PFHxS, 71 ± 4.2% for PFNA, and 89 ± 5.6% for PFHpA. Because the spike-recovery tests were reasonable and consistent with recovery limits in PFAS methods that we are aware of, we chose to present the results based on nominal PFAS concentrations.

2.3. Earthworms and observation of PFAS effects on earthworms

Adult earthworms (*Eisenia fetida*) were purchased from Yelm Earthworm and Castings (Yelm, WA) and acclimated at room temperature (approximately 20 °C) before use; worms were allowed to depurate their gut contents on moist filter paper overnight prior to the test. Five earthworms (approximately 1 g wet weight, ww) of similar size with clitellum were selected, weighed, and placed into each jar containing 100-g soil with four replicates in each treatment. Jars were closed loosely with lids to allow aeration and kept in the dark at room temperature for 21 days. Every 2–3 days, Milli–Q water was added to the soil to maintain soil moisture. After 21-d exposure, live earthworms were counted, rinsed with Milli–Q water, depurated on filter paper for 24 h, and weighed. Worms were put in 50–mL polypropylene centrifuge tubes and frozen at −20 °C until extraction.

Our test method was modified from OECD guideline 317 (OECD, 2010). We had concerns about the ability to detect PFASs in earthworms after 21-d exposure, especially in the low concentration treatments. To have more earthworm mass for analysis, we used a lower soil:worm ratio (20 g dry weight of soil per worm) than is prescribed by the OECD guideline (50 g dry weight of soil per worm).

2.4. Extraction and analysis of PFASs

Earthworms in each tube were thawed, ground, and extracted with 10 mL of acetonitrile for an hour in ultrasonic bath. Samples were added to 5 g of QuEChERS extraction salt, vortexed, and centrifuged at 3,000 rpm for 5 min. Supernatants were collected, evaporated to dryness under nitrogen, and reconstituted in methanol. Samples were filtered through 0.2 μm cellulose acetate syringe filters into 1–mL polypropylene LC vials and analyzed using LC–MS/MS. Blank samples (1 g_{ww} of non–contaminated worm for 1–mL extract preparation) were extracted in the same manner as other samples and used to prepare a matrix–matched calibration curve (spiked blank samples) or for sample dilution (if necessary). This extraction method was developed in our lab (Lanza et al., 2017) to utilize and introduce the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique for sample cleanup, instead of solid phase extraction (SPE) which has been frequently used to extract PFASs from earthworms by others (Zhao et al., 2013, 2014; Wen et al., 2015; Navarro et al., 2016; Zhao et al., 2016). Recovery of PFASs in earthworms (100 μg $kg^{-1}{}_{ww}$) from our preliminary experiments was 87 ± 3.0% for PFBS, 76 ± 4.9% for PFHxS, 75 ± 6.1% for PFNA, and 61 ± 4.4% for PFHpA. Method detection limits based on USEPA guidelines (USEPA, 2000) from the analysis of spiked