MECHANISMS FOR TISSUE-SPECIFIC ACCUMULATION AND PHASE I/II TRANSFORMATION OF 6:2 FLUOROTELOMER PHOSPHATE DIESTER IN EARTHWORM (*M. GUILLELMI*)

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Introduction

Per- and polyfluoroalkyl substances (PFASs) are extensively used and are ubiquitous in various environmental compartments throughout the world[1]. Telomer-based polyfluoroalkyl phosphate esters (PAPs) are used as oil- and water-repellent coating agents for food packaging materials as well as surfactants in personal care and cosmetic products. They are also widely identified in environmental compartments. DiPAPs are potential sources of the more persistent PFCAs in the environment due to biotransformation[2]. Research on terrestrial organisms is warranted considering that PAPs potentially partition to the soil to a great extent. The results based on geophagous *M. guillelmi* provide crucial information on the contributions of different uptake pathways, reflecting the actual risks induced by PFASs in soil.

This study aimed to investigate the tissue-specific accumulation and biotransformation of 6:2 diPAP in earthworms using geophagous *M. guillelmi* as the model organism.

Materials and methods

Exposure experiment

After acclimatization, three earthworms were transferred to a 5 L plastic beaker containing 500 g of dry soil. On days 0, 2, 4, 6, 10, 15, and 21 of uptake, three beakers (n=3) were sacrificed. At the end of the exposure, the remaining earthworms were transferred to plastic beakers containing clean soil. Following the same methodology, earthworms were sampled on days 23, 25, 27, and 31. Whole earthworm, gut, skin, organs (all organs except the gut) and body fluid were collected. On days 0, 2, 10, and 21, the soil was sampled from the beakers.

In vitro incubation experiments

In vitro metabolism tests were performed by incubating 6:2 diPAP with the homogenates extracted from the gut and organs of the earthworms respectively. An *in vitro* desorption experiment was conducted to investigate the desorption process of diPAPs from the soil in the earthworm digestive fluid. Briefly, the mixture of gut/organs fluid and spiked soil was incubated. After incubation, the obtained overlying fractions were combined.

Results

Tissue distribution of 6:2 diPAP in earthworm

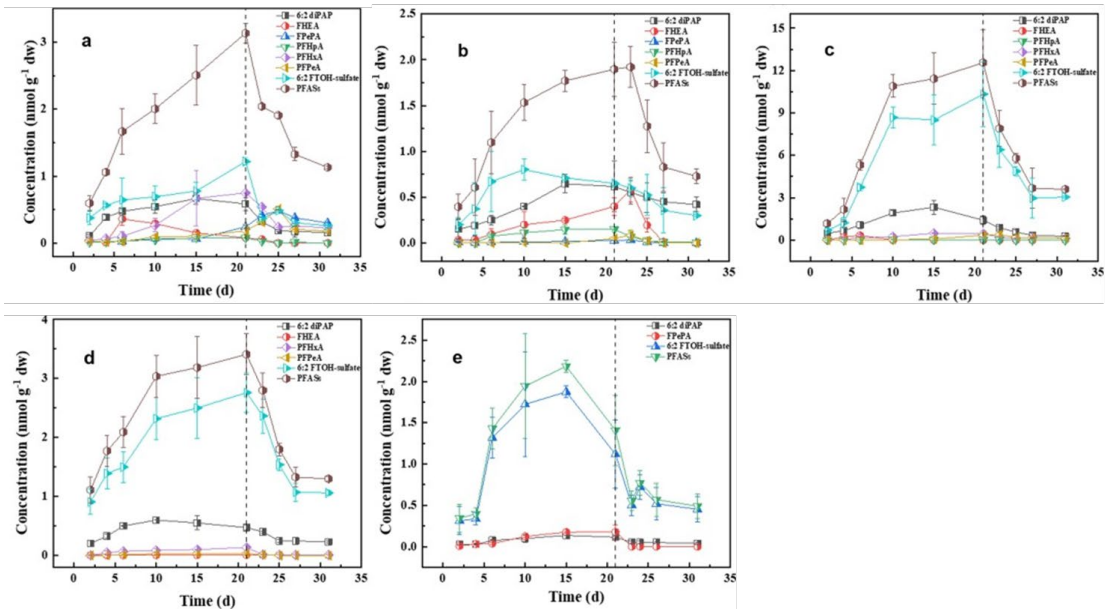

Figure 1. The concentrations of 6:2 diPAP, the transformation products, and total PFASs in the whole earthworm (a), skin (b), gut (c), organs (d), and body fluid (e) during the uptake and depuration phases (n=3).

The uptake and elimination data fit the respective first-order uptake and decay kinetic models very well (R^2=0.544 and 0.906, $p<0.05$). At the end of the exposure period, the concentration of 6:2 diPAP followed the order of gut>skin>organs>body fluid (Figure 1). The biota-to-soil accumulation factor (BSAF) in the gut (1.15 $g_{oc} \cdot g_{dw}^{-1}$) was 3-6 times higher than that in the skin and organs, even though the gut exhibited an elimination rate comparable to that of the other three tissues. 6:2 diPAP had the highest BSAF in the gut. The *in vitro* desorption experiment indicated that 96.2 ± 1.7% of 6:2 diPAP in the soil was released in the gut digestive fluid, which was significantly higher than that released in the digestive organ fluid (60.5% ± 6.8%), and both were remarkably higher than in the control group with PBS buffer as the solution (3.25% ± 0.14%).

Biodegradation of 6:2 diPAP in soil

6:2 fluorotelomer phosphate monoester (6:2 monoPAP), 3-perfluoropentyl propanoic acid (FPePA), and perfluorohexanoic acid (PFHxA) were detected in the soil on day 2. The yields of each transformation product in the soil were monoPAP (0.27 mol% yield), FPePA (0.27 mol% yield), and PFHxA (7.76 mol% yield) (Figure 2). This suggested that 6:2 diPAP experienced biodegradation in the soil and PFHxA might be the terminal product.

In the test groups with earthworms, the estimated degradation rate (k0) of 6:2 diPAP in the soil was 0.031 d-1 (95% confidence interval: 0.023-0.039), which was significantly higher than that in the control group (0.018 d-1, 95% confidence interval: 0.017-0.019) ($p < 0.05$). The mass of the total transformation products in the soil with earthworms at the end of incubation was 1.81 times that in the control.

Biotransformation of 6:2 diPAP in earthworm

The transformation product concentrations increased over time during the exposure phase (Figure 1a). PFHxA was detected as the predominant phase I product (0.750 ± 0.011 nmol g^{-1} dw) in the whole earthworm at the end of the exposure. During the depuration phase, 6:2 diPAP, FHEA, PFHxA, and PFHpA displayed a distinct decreasing trend, whereas the concentrations of FPePA and PFPeA in the earthworm still increased (Figure 1a). In the earthworms, PFCAs with even-numbered carbon chain lengths (PFHxA, 0.750 ± 0.011 nmol g^{-1}) had much higher concentrations than those with odd-numbered PFCAs, such as PFPeA (0.185 ± 0.098 nmol g^{-1}) and PFHpA (0.075 ± 0.003 nmol g^{-1}) (Figure 1a). This strongly supported that 6:2 diPAP mainly experienced *β*-oxidation in the earthworms.

The phase I transformation products exhibited the highest concentrations in the gut, followed by the organs, skin, and body fluid. PFHxA was the dominant phase I transformation product detected in both the gut and organs. The biotransformation ratio in the gut (33.2%) was significantly higher than that in the organs (19.1%). The yields of PFHxA from 6:2 diPAP, PFOA from 8:2 diPAP, and PFDA from 10:2 diPAP in rats were 1%, 9%, and 8%, respectively.[3]

Identification and quantification of 6:2 FTOH-sulfate in earthworms

As we analyzed the earthworm samples, a peak area of an unknown compound increased continuously with time during the exposure period. This unknown compound was supposed to be 6:2 FTOH-sulfate ($C_8H_4F_{13}O_4S^-$). To confirm this hypothesis, we synthesized and identified 6:2 FTOH-sulfate using HRMS. The highest concentration took place in the gut (10.3 ± 2.3 nmol g^{-1}), followed by the organs (2.76 ± 0.01 nmol g^{-1}), whole earthworm (1.22 ± 0.05 nmol g^{-1}), body fluid (1.12 ± 0.42 nmol g^{-1}) and skin (0.653 ± 0.027 nmol g^{-1}), and accounted for 82.2%, 80.9%, 21.8%, 79.4% and 34.4% of the total PFASs, respectively (Figure 2).

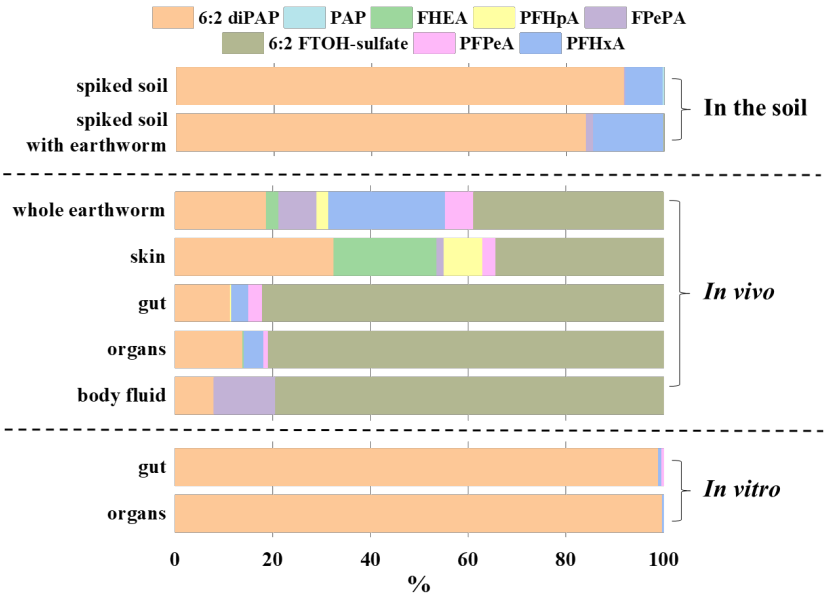

Figure 2. Assuming the total moles of all detected PFASs accounted for 100% in the soil and different tissues, the molar percentage of 6:2 diPAP and its transformation products in the soil, *in vivo* and *in vitro* tests at the end of the exposure period (day 21)

***In vitro* biotransformation of 6:2 diPAP in earthworm**

In agreement with the *in vivo* experiment, 6:2 diPAP decreased during incubation. Meanwhile, PFHxA, PFHpA, and PFPeA were detected in the gut homogenate, and their concentrations increased before leveling off at 12 h. FHEA was only present at 12 h, which further supported that it was intermediate. The dominant transformation product in the gut homogenate was PFHxA (0.615 mol% yield), showing consistency with the results of the *in vivo* experiment (Figure 2). In the organ homogenate incubation, only PFHxA was detected. These phenomena confirmed that 6:2 diPAP was actively transformed in the gut and organs of earthworms, and the gut contributed to this process via more robust enzymatic activities.

Discussion

The present study provides the first line of evidence that 6:2 diPAP was actively taken up by earthworms from the soil and experienced active phase I and phase II transformation. As a kind of geophagous earthworm, both skin absorption and gut processes served as major pathways for M. guillelmi to accumulate 6:2 diPAP from the soil. The fluid in the gut and other organs (the former in particular) promoted the desorption of 6:2 diPAP from the soil particles. 6:2 diPAP could be biodegraded in soil, and the presence of earthworms stimulated this process, which might be attributed to the enhanced microbial activities.[4] Many persistent PFCAs, including PFHxA, PFHpA, and PFPeA, were produced by earthworms, supplying strong evidence that diPAPs may be potential sources of these PFCAs in the environment.

The direct sulfation of 6:2 FTOH might form 6:2 FTOH-sulfate via a sulfotransferase,[5] which catalyzed the transfer of a sulfonyl group ($-SO_3^-$) from the cosubstrate 3′-phosphoadenosine 5′-phosphosulfate to a substrate containing a hydroxyl group. 3′-Phosphoadenosine 5′-phosphosulfate is supposed to be actively synthesized in the gut of *M. guillelmi*, since sulfate is mainly absorbed via dietary sources[6]. This may explain the highest concentration of 6:2 FTOH-sulfate observed in the gut. The retention time of 6:2 FTOH-sulfate (3.08 min) on the BEH C18 UPLC column was much shorter than that of 6:2 diPAP (4.20 min), suggesting that 6:2 FTOH-sulfate, with a smaller molecular size, was less hydrophobic than 6:2 diPAP, which may promote the elimination of pollutants from organisms. It is speculated that the earthworms tried to protect themselves from the impacts of 6:2 diPAP by forming a phase II product of 6:2 FTOH-sulfate.

The bioaccumulation and biotransformation of 6:2 diPAP in earthworms highlights the importance of probing the relative contribution of multiple uptake pathways and the potential transformation products of 6:2 diPAP across various species. Future studies are needed to clarify the governing factors controlling the different distributions and biodegradation of 6:2 diPAP and other related compounds in earthworms.

References

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