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Aerobic biotransformation of polyfluoroalkyl phosphate esters (PAPs) in soil☆

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ABSTRACT

Microbial transformation of polyfluoroalkyl phosphate esters (PAPs) into perfluorocarboxylic acids (PFCAs) has recently been confirmed to occur in activated sludge and soil. However, there lacks quantitative information about the half-lives of the PAPs and their significance as the precursors to PFCAs. In the present study, the biotransformation of 6:2 and 8:2 diPAP in aerobic soil was investigated in semi-dynamics reactors using improved sample preparation methods. To develop an efficient extraction method for PAPs, six different extraction solvents were compared, and the phenomenon of solvent-enhanced hydrolysis was investigated. It was found that adding acetic acid could enhance the recoveries of the diPAPs and inhibit undesirable hydrolysis during solvent extraction of soil. However 6:2 and 8:2 monoPAPs, which are the first breakdown products from diPAPs, were found to be unstable in the six solvents tested and quickly hydrolyzed to form fluorotelomer alcohols. Therefore reliable measurement of the monoPAPs from a live soil was not achievable. The apparent DT_{50} values of 6:2 diPAP and 8:2 diPAP biotransformation were estimated to be 12 and > 1000 days, respectively, using a double first-order in parallel model. At the end of incubation of day 112, the major degradation products of 6:2 diPAP were 5:3 fluorotelomer carboxylic acid (5:3 acid, 9.3% by mole), perfluoropentanoic acid (PFPeA, 6.4%) and perfluorohexanoic acid (PFHxA, 6.0%). The primary product of 8:2 diPAP was perfluorooctanoic acid (PFOA, 2.1%). The approximately linear relationship between the half-lives of eleven polyfluoroalkyl and perfluoroalkyl substances (PFASs, including 6:2 and 8:2 diPAPs) that biotransform in aerobic soils and their molecular weights suggested that the molecular weight is a good indicator of the general stability of low-molecular-weight PFAS-based compounds in aerobic soils.

1. Introduction

Polyfluoroalkyl phosphate esters (PAPs), as an important class of anionic fluorinated surfactants, have received considerable attention as contaminants of emerging concerns. They have been widely detected in the environment and shown the potential to degrade into perfluoroalkyl carboxylic acids (PFCAs) (De Silva et al., 2012; Eriksson and Kärrman, 2015; Lee et al., 2013). Long-chain PFCAs containing more than seven perfluorinated carbons are subject to strict regulatory scrutiny and use restrictions due to persistence, toxicity, and bioaccumulation potential (U.S. EPA, 2009). PAPs are formulated into oil- and water-resistant papers and synthetic fibers (Funaki and Seki, 2000; Yoshida et al., 1998), and used in semiconductor materials (Schultz, 2007) and personal care products including shampoos and cosmetics (Fujii et al., 2013). A PAP molecule comprises a phosphate hydrophile and fluorine-containing hydrophobic chains (Kissa, 2001). For the PAPs reported in the recent literature, the fluorine-containing chains are either fluorotelomer-based perfluoroethyl groups [$F(CF_2CF_2)_nCH_2CH_2-$, n = 2–9] (Eriksson and Kärrman, 2015) or *N*-ethyl perfluorooctane sulfonamido groups [$F(CF_2)_8SO_2NCH_2CH_2-$] (Benskin et al., 2013). Fluorotelomer-based PAPs have exhibited many variations in the number of fluorine-containing chains (i.e., mono-, di-, and tri-ester) and the size of the chains (i.e., n = 2–9) (Gebbink et al., 2012). The fluorine-containing chains within the same molecules are not necessarily the same. For instance, two different sizes of perfluoroethyl groups have been detected many diPAP species, such as 6:2/8:2 diPAP and 8:2/10:2 diPAP (De Silva et al., 2012; Eriksson and Kärrman, 2015).

Detection of a range of PAPs in human serum samples was attributed to migration of PAPs from treated food package paper

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(D'Eon and Mabury, 2007, 2011). The recent discovery of the ubiquitous presence of PAPs in indoor dust in private households suggested dust inhalation and ingestion another possible significant exposure route to humans (De Silva et al., 2012; Eriksson and Kärrman, 2015). With median levels of ∑monoPAPs and ∑diPAPs ranging from 3.7 to 1023 ng/g and 3.6–692 ng/g, respectively, PAPs are so far the most dominant class of polyfluoroalkyl and perfluoroalkyl substances (PFASs) found in indoor dust (Eriksson and Kärrman, 2015). The findings suggest the possible significant presence of PAPs in household products and subsequently high levels in waste streams, sewage, and biosolids. DiPAPs (6:2, 6:2/8:2, and 8:2) have been detected at ppb levels in sewage sludge, sub-ppb levels in wastewater influent and effluent, as well as sub-ppt levels in ocean surface waters (D'eon et al., 2009; Loi et al., 2013) while monoPAPs (8:2 and 10:2) were identified at ppb level in sewage sludge (Liu et al., 2013). Human exposure to PAPs has raised concerns about their impact on human health. Rosenmai et al. (2013) observed that the presence of 8:2 diPAP and 8:2 monoPAP led to decreased levels of androgens *in vivo*, and, therefore, suggested that these diPAPs could inhibit male sex hormone synthesis. Toxicity rising from PAPs is also associated with their degradation products or metabolites. Metabolism of 6:2 diPAP in rats lead to covalent protein binding with intermediate metabolites in plasma, liver, and kidney, which could induce toxicity (Rand and Mabury, 2014).

The metabolism or microbial transformation of PAPs leading to persistent PFCAs has been reported in rats (D'Eon and Mabury, 2007), activated sludge (Lee et al., 2010), and aerobic soil (Lee et al., 2013). These studies showed that the biotransformation process begins with hydrolysis of the phosphate ester bonds to produce fluorotelomer alcohols (FTOHs). Then biotransformation of FTOHs leads to the formation of not only PFCAs of several carbon chain lengths, but also a number of characteristic fluorotelomer carboxylic acids and volatile intermediates (Liu et al., 2010b; Wang et al., 2009). Biotransformation pathways and kinetics of 6:2 and 8:2 FTOHs have been well elucidated (Liu and Mejia Avendaño, 2013). As FTOHs generally exhibit very short half-lives (e.g., <2 days for 6:2 FTOH and <7 days for 8:2 FTOH in aerobic soils), the stability of the phosphate ester bonds and the ester hydrolysis rates become the critical factors in determining how fast PFCAs would be generated from PAPs. Lee et al. (2013) have qualitatively demonstrated soil biodegradation of diPAPs and subsequent uptake of their degradation products by plants; however, reliable estimation of PAP hydrolysis rates or half-lives and the formation yields to PFCAs is still lacking. The information is particularly lacking for soil, which is one of the major reservoirs of pollutants. Such data is critical in the estimation of the contributions of indirect sources of PFCAs, such as from precursor substances like PAPs, and in predicting with high confidence the future trends of PFCAs in the environment.

One of the challenges of investigating the environmental fate in soil of fluorotelomer-based substances with hydrolyzable function groups (e.g., carboxylic acid and phosphate esters) is solvent-enhanced hydrolysis during solvent extraction of the environmental samples. Dasu et al. (2010) first reported the rare but significant phenomenon. They discovered that 8:2 fluorotelomer stearate ester (8:2 FTSE) can undergo rapid hydrolysis in certain organic solvents (e.g., acetonitrile and methanol) during solvent extraction of live or even γ-irradiated soils. Such experimental artifact would lead to substantial overestimation of the rate of hydrolysis and underestimation of the environmental stability of fluorotelomer-based ester compounds. Though the fundamental mechanisms behind the phenomenon are still elusive, it appears that residual microbial enzymes are not deactivated in those organic solvents, rather they exhibit greatly enhanced hydrolytic activity towards the ester bonds. We have noticed that several distinct solvents (e.g., methanol, methanol containing ammonium hydroxide, and tetrahydrofuran containing acetic acid) have been used for extracting PAPs from solid environmental matrices. However, there was no investigation whether any solvent-enhanced hydrolysis would occur to PAPs (De Silva et al., 2012; Lee et al., 2013; Liu and Mejia Avendaño, 2013). Furthermore, given that hydrolysis rates of phosphorous esters are likely to be sensitive to pH (Larson and Weber, 1994), it is unlikely that a broad range of pH used in various recent studies would be equally suitable for PAP extraction unless PAPs are highly resistant to hydrolysis irrelevant of pH.

Therefore, the overall goal of this study was to determine the half-lives of PAPs and the formation yields to PFCAs through biotransformation processes in soil. It is of primary importance to first evaluate the suitability and efficiency of different solvents for PAP extraction without leading to the undesirable solvent-enhanced hydrolysis. 6:2 and 8:2 diPAPs, which are the most dominant PAPs in the environment, were chosen as test compounds. In the end, we compare the stability of diPAPs in soil to that of other fluorotelomer derivatives with hydrolyzable functional groups to evaluate the factors that impact their environmental recalcitrance. Such information would be useful for predicting the stability of many fluorotelomers that have not been subject to experimental studies.

2. Materials and methods

2.1. Chemicals and reagents

6:2 and 8:2 diPAPs (>97%) were purchased from Toronto Research Chemicals (Toronto, Canada), 6:2 and 8:2 monoPAPs (>97%) acquired from University of Toronto. The rest of PFAS standards and isotopically labeled internal standards were obtained from a variety of sources as listed in Table S1 in the Supporting Information (SI). HPLC-grade solvents including acetonitrile (ACN), methanol (MeOH), methyl tert-butyl ether (MTBE), and ethyl acetate (EA), LC/MS-grade water and acetic acid (HAc), certified sodium hydroxide (NaOH, 5 N) and hydrochloric acid (HCl, 6 N), and ACS-grade calcium chloride ($CaCl_2$) were purchased from Fisher Scientific (Ottawa, ON).

2.2. Soil microcosm setup

St-Bernard soil was collected from McGill University McDonald campus in Sainte-Anne-de-Bellevue, QC, at the same location where the soil used for a previous aerobic biotransformation study of perfluoroalkyl sulfonamide derivatives was collected (Mejia Avendaño and Liu, 2015). The soil was sieved via a 2-mm sieve immediately upon collection and stored at −4 °C and used within three months. Part of the soil was rendered sterile via autoclaving. Three cycles of autoclaving were performed at 121 °C for 60 min per cycle, and the soil was incubated at room temperature for 24 h in between cycles. To further inhibit microbial or enzymatic activities, three antibiotics (kanamycin sulfate, chloramphenicol, and cycloheximide) were added to the autoclaved soil to reach a concentration of 100 mg kg^{-1} soil (Liu et al., 2010b). The soil moisture content was adjusted with a sterile $CaCl_2$ solution to gravimetric moisture content of 22%, which approximated 80% of the water holding capacity of the soil.

The same semi-dynamics setup using 500-mL glass bottles as the one used by Mejia Avendaño and Liu (2015) was used to incubate ~110 g soil (oven dry weight, 103 °C). The airtight caps were fitted with two openings: one connected to an SPE C18 cartridge (Maxi-Clean™, Alltech) to trap volatile fluorinated