Dietary exposure of rainbow trout to 8:2 and 10:2 fluorotelomer alcohols and perfluorooctanesulfonamide: Uptake, transformation and elimination

Sicco H. Brandsma [a], Marla Smithwick [b,c], Keith Solomon [b], Jeff Small [c], Jacob de Boer [a], Derek C.G. Muir [c,*]

[a] Institute for Environmental Studies, VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands
[b] Dept. of Environmental Biology, University of Guelph, Guelph, Canada ON N1G 2W1
[c] Water Science and Technology Directorate, Environmental Canada, Burlington, Canada ON L7R 4A6

ABSTRACT

The bioaccumulation of perfluorooctanesulfonamide (PFOSA) and two fluorotelomer alcohols (8:2 FTOH, 10:2 FTOH) by rainbow trout (*Oncorhynchus mykiss*) through dietary exposure, including depuration rates and metabolism was investigated. Concentrations in the spiked feed ranged from 10.9 $\mu g\,g^{-1}$ wet weight (wet wt) for PFOSA and 6.7 $\mu g\,g^{-1}$ wet wt for 8:2 FTOH to 5.0 $\mu g\,g^{-1}$ wet wt for 10:2 FTOH. Trout was fed at 1.5% body weight per day for 30 d and depuration was followed for up to 30 d following previously published dietary exposure protocols. Perfluorooctanesulfonate (PFOS) was the major perfluoroalkylsulfonate (PFSA) detected in fish following dietary exposure to PFOSA. Half-lives of PFOS and PFOSA were 16.9 ± 2.5 and 6.0 ± 0.4 d, respectively. A biomagnification factor (BMF) of 0.023 was calculated for PFOSA which indicates that dietary exposure to PFOSA does not result in biomagnification in the rainbow trout. PFOS had a BMF of 0.08. The fluorotelomer saturated acids (8:2 FTCA, 10:2 FTCA) and fluorotelomer unsaturated acids (8:2 FTUCA, 10:2 FTUCA) were the major products detected in rainbow trout following dietary exposure to 8:2 FTOH and 10:2 FTOH, respectively. Half-lives were 3.7 ± 0.4, 2.1 ± 0.5, 3.3, and 1.3 d for 10:2 FTCA, 10:2 FTUCA, 8:2 FTCA, and 8:2 FTUCA, respectively. Small amounts of perfluorooctanoate (PFOA) and perfluorodecanoate (PFDA) were also detected in the FTOH exposed fish.

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1. Introduction

The unique properties of perfluoroalkylated compounds (PFCs) have led to their use in a wide range of applications (Kissa, 1994, 2001; Hekster et al., 2003) and to the global distribution of their perfluoroalkylsulfonate (PFSA) and perfluorocarboxylate (PFCA) degradation products (Giesy and Kannan, 2001; Giesy et al., 2006; Houde et al., 2006b). PFCs typically have a hydrophilic head and a hydrophobic tail. The hydrophobic tail of PFCs contains fluorinated carbons, which give them oleophobic properties. The C–F bond is a strong bond and as a result PFCs are very stable to acids, alkali, oxidation and reduction, and even to relatively high temperatures. The largest use of PFCs based on perfluoroalkyl sulfonamide and fluorotelomer chemistry has been in the production of fluorinated polymers (Kissa, 1994, 2001; 3M Company, 1999a). Because of their unique physical and chemical properties, fluorinated polymers are used for many applications to provide water and stain repellency to the treated product like carpets, textile, leather and paper products (Kissa, 2001; Schultz et al., 2003; Prevedouros

et al., 2006). Most applications of these products have involved the use of perfluoroalkylsulfamide alcohols or fluorotelomer alcohols (FTOHs), with 8–10 carbon atoms, which are bonded to a polymer backbone (3M Company, 1999a,b; Russell et al., 2008). During the production or use of the PFC-treated polymers the residual alcohols, or PFCAs (e.g. PFOA) used as polymer processing aids, can be released into the environment. PFSAs have also been used directly in aqueous film forming foams (AFFFs) used for fire-fighting, in photo-imaging, in semi-conductor manufacturing, and metal plating (Moody and Field, 2000; Schultz et al., 2003; UNEP, 2007).

The FTOHs and perfluorooctanesulfonamide (PFOSA) can be degraded through atmospheric oxidation down to the more stable PFCAs and PFSAs which are potentially toxic and bioaccumulative (Dinglasan et al., 2004; Ellis et al., 2004). Perfluorooctanesulfonate (PFOS) and the PFCAs with 9–11 carbons are the most important degradation products and are found in the liver, tissue and blood of humans and wildlife all over the world, including remote locations such as the Arctic (Houde et al., 2006b; Butt et al., 2010a). The distribution pathways for PFSAs and PFCAs are still unclear, although atmospheric degradation of the volatile FTOHs to PFCAs may be an important route (Ellis et al., 2004; Young et al., 2007).

* Corresponding author. Tel.: +905 319 6921; fax: +905 336 6430.
E-mail address: Derek.Muir@ec.gc.sa (Derek C.G. Muir).

The presence of PFSAs can be explained by degradation of the neutral compounds *N*-methyl perfluorooctane sulfonamidoethanol and *N*-ethyl perfluorooctane sulfonamide which have been found in urban and rural air samples along with the FTOHs (Stock et al., 2004; Shoeib et al., 2006; Jahnke et al., 2007).

Because of concern about the worldwide detection of the persistent PFCs in humans and wildlife, the main manufacturing company 3 M voluntarily began phasing out the perfluorooctanesulfonyl fluoride (POSF $C_8F_{17}SO_2F$)-based materials and its electrochemical cell fluorination (EFC) production of perfluorooctanoic acid (PFOA) in 2000 (3M Company, 2000). The FTOHs remain in production, however, the manufacturers have agreed to reductions of PFOA associated with production of these products (US EPA, 2006).

High levels of long chain PFCAs and PFOS are found in higher trophic-level organisms (Kannan et al., 2002; Van De Vijver et al., 2005; Houde et al., 2006b). Evidence for bioconcentration of PFOS has been found in laboratory studies with fish (Martin et al., 2003a). PFCAs and PFSAs with perfluoroalkyl chain of 6 carbons or more showed BCFs that increase with the length of the perfluoroalkyl chain, ranging from 4 to 23 000 based on wet weight concentrations (Martin et al., 2003a). BMFs calculated from dietary exposure of fish ranged from 0.038 to 1 (Martin et al., 2003b). BMFs of 2.5 and 0.7 have been reported for PFOSA in zooplankton to fish (Tomy et al., 2004a; Houde et al., 2006a). However, limited information is available on biomagnification of PFOSA (Houde et al., 2006b).

FTOHs were detected in rain, surface water and waste water treatment plant (WWTP) effluent by Mahmoud et al. (2009). The total concentration of FTOHs observed in WWTP effluents and surface water was 23.2 ng L^{-1} and 10.8 ng L^{-1}, respectively. The results of Mahmoud et al. (2009) indicate that surface water can be contaminated by FTOHs released from WWTP effluents or by wet deposition of FTOHs. Therefore, not only air breathing organisms can be exposed to FTOHs but also aquatic organisms.

Studies have shown that FTOHs can be metabolized in rats and mice to PFCAs (Hagen et al., 1981; Martin et al., 2005; Kudo et al., 2005; Fasano et al., 2006). However, information on the accumulation and metabolism of FTOHs by fish has been very limited until recently. Nabb et al. (2007) identified the following metabolites after in vitro incubation with trout hepatocytes; PFOA, PFHxA, perfluoropentanoic acid (PFPeA), PFHpA, PFNA, 8:2 FTCA, 8:2 FTUCA, 8:2 FTOH-glucuronide, 8:2 uFTOH-glutathione and the 8:2 FTUA-glutathione. These were identical to those observed in earlier rat and mice in vitro and in vivo studies. Other metabolites not previously reported were: 7:2 ketone, 7:3 β-keto acid, 7:3 aldehyde, 7:3α-β unsaturated aldehyde and the 7:3 acid taurine conjugate. Butt et al. (in press) exposed rainbow trout to 8:2 FTOH acrylate (8:2 FTAc) and showed that it was rapidly biotransformed in the gut or liver. Metabolites observed in the fish liver were PFOA, 8:2 FTCA, 8:2 FTUCA, 7:3 fluorotelomer acid (7:3 FTCA), and the conjugated metabolite 8:2 FTOH-glucuronide in the faeces (Butt et al., in press). Butt et al. (2010b) demonstrated conclusively that 8:2 FTCA and FTUCA formed PFOA, and that the 7:3 FTCA formed PFHpA in rainbow trout during dietary exposure. A study with rainbow trout microsomes showed that *N*-methyl and *N*-ethyl perfluorooctane-sulfonamido-ethanol were transformed into PFOSA and finally to the stable end product PFOS (Tomy et al., 2004b). However, to our knowledge no previous fish dietary exposure studies have been conducted with PFOSA, nor directly with fluorotelomer alcohols.

The objective of the present study was to investigate the biomagnification, depuration and metabolism PFOSA, 8:2 FTOH and 10:2 FTOH in rainbow trout after dietary exposure.

2. Materials and methods

2.1. Standards and reagents

Standards of PFHxS (99.99%), PFOS (98%), PFHxA (95%), PFHpA (99%), PFOA (96%), PFNA (97%), perfluorodecanoic acid (PFDA, 98%), perfluoroundecanoic acid (PFUA, 95%), perfluorododecanoic acid (PFDoA, 95%) and perfluorotetradecanoic acid (PFTA, 97%) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada).The fluorotelomer acids (8:2 FTCA and 10:2 FTCA), the unsaturated acids (8:2 FTUCA, 10:2 FTUCA) and the fluorotelomer alcohols (10:2 and 8:2 FTOH) were purchased from Oakwood Research Chemicals (West Columbia, SC, USA). PFOSA (99.9%) was provided by 3M (St. Paul, MN, USA). The 1, 2-^{13}C$_2$ PFOA (98%) from Perkin–Elmer Life and Analytical Sciences, (Woodbridge, ON, Canada) was used as an internal standard. Methyl-tert-butyl ether (MTBE) (99.8%), methanol (99%), tetrabutylammonium hydrogen sulphate (TBAS, 97%), ammonium acetate (>99%), and graphitized nonporous carbon (Envi-Carb cartridges) were purchased from Sigma–Aldrich. Acetonitrile (99.9%) and glacial acetic acid (100%) were purchased from Fischer Scientific (Pittsburg, PA, USA) and anhydrous sodium carbonate from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Dietary exposure

The fish feed was prepared according to Martin et al. (2003b). The feed was spiked with a solution of all the test compounds in methanol. Concentrations PFOSA, 10:2 FTOH and 8:2 FTOH in the methanol solution were verified by GC–MS using the method of Martin et al. (2002). The methanol was removed with a rotary evaporator under vacuum and the feed was further dried in an oven at 60 °C for 3 h. The feed was analysed for PFOSA, PFOS and major PFCAs immediately after preparation using the method described in Martin et al. (2003b). No PFOS or PFCAs were detected in the food above the detection limit, and only PFOSA was detectable at a concentration of 10.9 µg g^{-1}. Unfortunately actual concentrations of the 8:2 and 10:2 FTOHs in the feed could not be verified because an analytical method was not available at the time. Nominal concentrations based on the spike solution were 5.0 µg g^{-1} 10:2 FTOH and 6.7 µg g^{-1} 8:2 FTOH.

The control feed was prepared in the same manner, but without the test compounds. The juvenile rainbow trout were held at 12 °C for two weeks prior to the experiment to climatize to the laboratory conditions. Carbon filtered and dechlorinated water was used in a flow-through system at 12 °C, and a 12 h photoperiod was used. All fish were fed throughout the experiment at a rate of 1.5% body weight/day. The test was carried out in three tanks which received a continuous replacement of water at 2 L per minute. Two tanks in which the fish was fed with the spiked feed and one tank with the control fish. The feed was typically consumed within 5 s after offering. Water samples were not collected to check for presence of PFOS and PFCAs. However, previous work using identical flow rates and tanks found no detectable PFCAs in water (<5 ng L^{-1}) 10 min after feeding of PFOS and PFCAs spiked feed (Martin et al., 2003b). The water was not contaminated by the spiked feed and the bioaccumulation was considered to be exclusively related to the feed.

Three fish were randomly taken from each exposure group, including the control group, after 1, 2, 10, 20 and 30 d of feeding and sacrificed. The depuration phase began at 30 d. Three fish were randomly taken from each tank, including the control group at 32, 40, 50, and 60 d. Whole fish were lightly dabbed with a paper towel and weighed. The sampled fish were filleted and separated into liver, gastrointestinals and carcass, placed in plastic bags and stored at −20 °C until extraction and analysis.