Aquatic Toxicology 156 (2014) 65–73

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Toxicokinetics of perfluorooctanoate (PFOA) in rainbow trout (*Oncorhynchus mykiss*)☆

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ARTICLE INFO

Article history:
Received 12 May 2014
Received in revised form 30 July 2014
Accepted 31 July 2014
Available online 8 August 2014

Keywords:
Perfluoroalkyl acid
Perfluorooctanoate
Rainbow trout
Renal elimination
Tissue distribution
Membrane transporter

ABSTRACT

Rainbow trout (*Oncorhynchus mykiss*) confined to respirometer-metabolism chambers were dosed with perfluorooctanoate (PFOA) by intra-arterial (i.a.) injection and sampled to obtain concentration time-course data for plasma, urine, and expired water. The data were then analyzed by compartmental modeling to estimate rates of renal and branchial clearance. Averaged across all animals, the renal clearance rate (1.35 mL/h/kg) was more than ten times greater than the branchial clearance rate (0.12 mL/h/kg). The average whole-body elimination half-life was 12.6 d, which is somewhat longer than values obtained in previous studies with smaller trout. The tissue distribution of PFOA was assessed by collecting tissues at the end of chambered exposures and in a separate tissue time-course experiment. From the time-course study it appeared that an internal steady-state was established within 24 h of i.a. injection. Consistent with previous studies, the rank order of PFOA concentration in tissues at steady state was: plasma > liver > kidney > muscle. In a second set of chambered experiments, fish were exposed to PFOA in water to determine the rate of branchial uptake. Branchial uptake rates were too low to assess directly by measuring PFOA concentrations in inspired and expired water. Uptake rate constants (mean 0.19 L/d/kg; 0.1% uptake efficiency) were therefore estimated by compartmental modeling using plasma concentration time-course data and model parameters derived from the elimination experiments. It is clear from this effort that elimination of PFOA by trout occurs primarily via the renal route. This finding is consistent with numerous studies of mammals and suggests that trout possess membrane transporters that facilitate the movement of PFOA from plasma to urine.

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1. Introduction

Perfluoroalkyl acids (PFAAs) are used in stain-resistant coatings, and as surfactants, fire-fighting foams, and photographic developers (Lau et al., 2007; Renner, 2001). Concern that these chemicals may constitute an important class of environmental contaminants can be traced back to 2001, when Giesy and Kannan (2001) published their findings on the global distribution of perfluorooctane sulfonate (PFOS) in wildlife. Since then, PFAAs have been measured in humans, surface waters, and aquatic sediments, and have been found in environmental matrices far from any known sources (Prevedouros et al., 2006). Although initially thought to be biologically inert, several PFAAs have been shown to cause toxic effects in mammals, including developmental toxicity, immunotoxicity, and hepatotoxicity (Andersen et al., 2008; Lau et al., 2004, 2007). Toxicity studies with fish have largely, though not solely focused on PFOS as a model compound. These investigations have demonstrated reproductive effects (Han and Fang, 2010), developmental toxicity (Han and Fang, 2010; Huang et al., 2010; Shi et al., 2008), hepatotoxicity (Hoff et al., 2003; Wei et al., 2008) and behavioral effects (Huang et al., 2010).

Individual PFAAs have been shown to accumulate in aquatic species, although the extent of this accumulation varies widely. Measured log bioaccumulation factors (BAFs) for six PFAAs in Great

☆ The information in this document has been funded wholly by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

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http://dx.doi.org/10.1016/j.aquatox.2014.07.022
0166-445X/Published by Elsevier B.V.

Lakes lake trout (*Salvelinus namaycush*) ranged from 2.7 for perfluorohexane sulfonate (PFHxS) to 4.1 for PFOS (Furdui et al., 2007). Liu et al. (2011) reported BAFs for green mussels (*Perna viridis*) ranging from 15 for perfluoroctanoate (PFOA) to 859 for perfluorodecanoate (PFDA), along with evidence for dependence of these values on the exposure concentration. In general, the tendency of individual PFAAs to accumulate in fish is directly related to the length of a compound's fluorinated carbon chain as well as the identity of the terminal group (sulfonate or carboxylate), which confers to the molecule its amphipathic character. PFOA and other shorter chain carboxylates accumulate to a lesser extent than perfluorosulfonates of comparable chain length, to the point that they are not considered to be bioaccumulative based on standard regulatory criteria (e.g., BAF > 1000; Martin et al., 2003a,b; Conder et al., 2008).

Models commonly used to predict the accumulation of lipophilic chemicals in fish are generally thought to be inadequate for PFAAs. Such models describe uptake and accumulation as a consequence of passive diffusion across the gills and gut, and subsequent partitioning to whole-body lipid, taking into consideration the possibility of biotransformation (Arnot and Gobas, 2004). It is well known, however, that PFAAs do not partition to tissue lipid but instead bind to protein. Moreover, the character of this binding suggests high affinity for specific protein sub-classes including serum albumin and fatty-acid binding proteins in the liver (Han et al., 2003; Ng and Hungerbühler, 2013). Thus, PFAAs exhibit little tendency to accumulate in the white muscle of fish, which constitutes by far the largest store of total whole-body protein, and instead accumulate in blood plasma, liver, and kidney (Martin et al., 2003a). Complicating matters further, PFAAs are ionogenic and exist as a mixture of charged and neutral species. Uptake, distribution, and elimination are likely to depend, therefore, on an individual compound's pK_a and the pH of the local environment.

In mammals, large differences in the biological half-life of specific PFAAs have been noted for different test organisms (Butenhoff et al., 2004a; Andersen et al., 2008; Han et al., 2012). These differences have been attributed to different rates of renal elimination (Andersen et al., 2006; Butenhoff et al., 2004b; Han et al., 2012) which are due, in turn, to differences in the activities of specific transporters including the organic anion transporters Oat1 and Oat3, the organic anion transporting polypeptide Oatp1a1, and uric acid transporter 1 (Urat1), all of which facilitate reuptake of PFAAs from renal filtrate in the proximal tubule (Kudo et al., 2002; Nakagawa et al., 2008; Weaver et al., 2010). The presence/absence and activities of these transporters are also thought to explain large differences in elimination that often exist between the sexes, as the expression of many renal transporters is known to be under the control of sex hormones (Cheng and Klaassen, 2009; Ljubojević et al., 2007; Morris et al., 2003).

Recent efforts to model PFAA accumulation in fish have attempted to account for some or all of these characteristic behaviors. For example, a model given by Armitage et al. (2013) predicts the uptake and accumulation of PFAAs based on partitioning of neutral and ionized chemical species to both neutral lipids and phospholipids. This model does not, however, account for the possible role of renal clearance. A second model, given by Ng and Hungerbühler (2013) explicitly describes protein binding interactions in several tissue compartments and includes a renal clearance description which operates against the unbound chemical mass in kidney tissue. Model terms that describe renal elimination were based on data from studies with mammals, as data for fish were lacking.

Given the importance of PFAAs as a class of environmental contaminants as well as the need to advance current modeling efforts, it is critical to obtain mechanistic information regarding the kinetics of these compounds in fish, and in particular data regarding renal clearance. Although direct evidence for renal elimination of PFAAs in fish is lacking, indirect evidence suggests that it may play a role in the elimination of some compounds. For example, Lee and Schultz (2010) suggested that differences in renal elimination may have accounted for observed sex-linked differences in elimination of PFOA by fathead minnows. The goal of the present study was to characterize the routes and rates of PFOA elimination in large rainbow trout which had been chronically catherized to permit continuous collection of urine. Additional experiments were performed to evaluate the uptake of PFOA across trout gills and the kinetics of its distribution to tissues.

2. Materials and methods

2.1. Chemicals

PFOA (>98% pure) was purchased from Sigma Aldrich (St. Louis, MO). PFOA and mass-labeled [^{13}C] PFOA standards were purchased from Wellington Laboratories (Guelph, ON, Canada). HPLC grade acetonitrile, methanol, and glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Animals

Rainbow trout (*Oncorhynchus mykiss*) weighing approximately 100 g were obtained from the USGS Upper Midwest Environmental Sciences Center in La Crosse, WI, and grown up to the desired size for each experiment. The animals were held in sand-filtered Lake Superior water at 11 ± 1 °C under a natural photoperiod and fed a commercial trout chow (Silver Cup, Nelson and Sons Inc., Murray, UT). Water chemistry characteristics were: total hardness 45–46 mg/L as $CaCO_3$; alkalinity 41–44 mg/L as $CaCO_3$; pH 7.6–7.8; total ammonia < 1 mg/L; dissolved O_2 85–100% of saturation.

2.3. Surgical preparation of animals

Elimination and branchial uptake studies were performed using trout confined to respirometer-metabolism chambers (McKim and Goeden, 1982). All of these fish had gone through at least one spawning cycle prior to use, and varied with respect to their state of sexual maturity at the time of data collection. Fish weighing 700–1500 g were sedated with tricaine methanesulfonate (MS-222; Finquel, Argent Laboratories, Redmond, WA), weighed, and placed on a surgical table designed to provide continuous anesthesia with temperature-controlled, oxygenated water. Each fish was immobilized by spinal transection and surgically fitted with a dorsal aortic cannula (Intramedic PE 50; Becton, Dickinson and Co., Parsippany, NJ) to permit periodic blood sampling. Fish used for elimination experiments also were fitted with a urinary catheter (Intramedic PE 90) for continuous collection of urine. A latex membrane sewn to the fish's mouth allowed separate collection of inspired and expired water. A second latex membrane just posterior to the pectoral fins prevented further dilution of expired water. Fish were allowed 24 h to recover from surgery before starting an experiment. Water temperature (T; °C), ventilation volume (V_{VOL}; mL/min), and oxygen consumption (VO_2; mg/h/kg) were monitored continuously using an automated data collection system and customized software (Carlson et al., 1989). This information was then evaluated to assess the viability and representativeness of each preparation, and generate average values for the experimental time period.

2.4. Elimination experiments

Eight trout of mixed sex were given a bolus intra-arterial (i.a.) injection of PFOA dissolved in trout serum (1 mL/kg), with each