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Application of physiologically-based pharmacokinetic modeling to explore the role of kidney transporters in renal reabsorption of perfluorooctanoic acid in the rat☆

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ABSTRACT

Renal elimination and the resulting clearance of perfluorooctanoic acid (PFOA) from the serum exhibit pronounced sex differences in the adult rat. The literature suggests that this is largely due to hormonally regulated expression of organic anion transporters (OATs) on the apical and basolateral membranes of the proximal tubule cells that facilitate excretion and reabsorption of PFOA from the filtrate into the blood. Previously developed PBPK models of PFOA exposure in the rat have not been parameterized to specifically account for transporter-mediated renal elimination. We developed a PBPK model for PFOA in male and female rats to explore the role of Oat1, Oat3, and Oatp1a1 in sex-specific renal reabsorption and excretion of PFOA. Descriptions of the kinetic behavior of these transporters were extrapolated from *in vitro* studies and the model was used to simulate time-course serum, liver, and urine data for intravenous (IV) and oral exposures in both sexes. Model predicted concentrations of PFOA in the liver, serum, and urine showed good agreement with experimental data for both male and female rats indicating that *in vitro* derived physiological descriptions of transporter-mediated renal reabsorption can successfully predict sex-dependent excretion of PFOA in the rat. This study supports the hypothesis that sex-specific serum half-lives for PFOA are largely driven by expression of transporters in the kidney and contribute to the development of PBPK modeling as a tool for evaluating the role of transporters in renal clearance.

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1. Introduction

Perfluorinated compounds (PFCs) have been used since the 1950's in a variety of industrial applications and consumer products. Perfluorooctanoic acid (PFOA) is among one of the most well studied members of this class of compounds (ATSDR, 2009) and is frequently found in oil, stain, grease, and water repellent coatings on carpets, textiles, leather, and paper (ATSDR, 2009). PFOA is particularly environmentally and biologically persistent due to its eight-carbon backbone, strong carbon-fluorine bonds, and metabolic stability (Calafat et al., 2007; Bartell et al., 2010). Despite recent reductions in manufacturing, PFOA has been identified as a 'contaminant of emerging concern' by the U.S. Environmental Protection Agency (EPA) due to its frequent detection in water systems across the United States (USEPA, 2008; USEPA, 2009; Bartell et al., 2010) as well as in the blood of the general U.S. population (Calafat et al., 2006; Calafat et al., 2007; Olsen et al., 2007; Olsen et al., 2012; USEPA, 2014a).

Epidemiological studies of occupational and community exposure to PFCs indicate associations between blood serum levels of PFOA and high cholesterol, other liver effects including increased liver enzymes and decreased bilirubin levels, chronic kidney disease, and early menopause (Olsen et al., 2000; Olsen et al., 2007; Sakr et al., 2007a; Sakr et al., 2007b; Vaughn et al., 2013; USEPA, 2014b). In rodent studies, PFOA has resulted in body-weight changes, developmental effects, liver effects, and decreased serum total cholesterol (Ikeda et al., 1985; Kawashima et al., 1995; Butenhoff et al., 2004; Guruge et al., 2006; Lau et al., 2006; Cui et al., 2009).

The pharmacokinetics of PFOA are well studied in rats. PFOA is known to be well absorbed in the gastrointestinal tract, highly bound in the serum albumin, not metabolized, and excreted unchanged primarily via the kidneys (Johnson et al., 1979; Vanden Heuvel et al., 1991). PFOA reaches steady-state in the serum very rapidly with daily dosing, but is eliminated slowly (Bartell et al., 2010). The serum half-life for PFOA is estimated to be between four and six days in the male rat and between 2 and 4 h in the female rat (Kemper, 2003). In contrast, the serum half-life for PFOA in humans has been estimated to be between 2.3 and 3.8 years (Olsen et al., 2007; Bartell et al., 2010). This

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Agency for Toxic Substances and Disease Registry.

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sex and species specificity in serum half-life is hypothesized to be due to hormonally-regulated, saturable renal reabsorption of PFOA via organic anion transporters (OATs) expressed on the apical and basolateral membranes of the proximal tubule cells (Kudo et al., 2002; Andersen et al., 2006; Nakagawa et al., 2007). The rat model described here uses *in vitro* to *in vivo* extrapolation (IVIVE) to incorporate physiological descriptions of these transporters to predict sex specific renal clearance of PFOA in the adult rat.

Oat1 (*Slc22a6*) and Oat3 (*Slc22a8*) are expressed most highly in the proximal tubule cells of the rat kidneys and are localized to the basolateral membrane (Buist et al., 2002; Weaver et al., 2010). An extensive list of diverse substrates has been identified for Oat1, including PFOA. Oat3 has also been shown to be capable of PFOA transport (Nakagawa et al., 2007). Together, these basolateral membrane transporters translocate PFOA from the blood into the proximal tubule cells and facilitate renal secretion. Oatp1a1 (*Slco1a1*) is expressed on the apical membrane of the proximal tubule cells in the rat and has been shown to transport PFOA from the urine back into the proximal tubule cells, thereby facilitating renal reabsorption (Weaver et al., 2010). The expression of these transporters is known to be sex-hormone regulated and have sex specific expression patterns in the adult rat (Buist et al., 2002). OATs are responsible for the movement of many pharmaceuticals and chemicals in the kidney. Sex-specific clearance and biological half-life has been demonstrated for several substrates of OATs including *p*-aminohippurate (PAH) (Reyes et al., 1998), zenarestat (Tanaka et al., 1991; Morris et al., 2003), S-pentachlorophenyl-N-acetyl-L-cysteine (Smith and Francis, 1983), carnitine (Carter and Stratman, 1982), nilvadipine metabolite (M3) (Terashita et al., 1995) and 1-aminocyclohexanecarboxylic acid (Anton et al., 1986).

One other PBPK model for PFOA administration in the adult rat exists in the published literature (Loccisano et al., 2012). This model describes saturable reabsorption of PFOA from the filtrate compartment back into the kidney compartment via a single transporter with transporter maximum (T_m) and affinity constant (K_t) based on *in vitro* data describing Oatp1a1 uptake of PFOA. While this model was able to successfully describe PFOA kinetics in the adult rat, the information necessary to scale *in vitro* measurements of transporter activity to *in vivo* values for the transporters involved in both the excretion and renal reabsorption was not available at the time (Loccisano et al., 2012). The model described here applies recent *in vitro* data to expand upon the existing model by including physiological descriptions of both basolateral and apical membrane transporters in order to describe the sex specific kinetics of excretion and reabsorption in the kidneys. This evidence-based model confirms the findings of prior hypothesis-driven modeling efforts by showing that saturable reabsorption is necessary to achieve a consistent description of the experimental data. Further, it supports the hypothesis that sex-specific serum half-lives for PFOA are largely driven by expression and activity of transporters in the kidney and contributes to the development of PBPK modeling as a tool for evaluating the role of transporters in renal clearance.

2. Materials and methods

2.1. Key pharmacokinetic studies in male and female rats

Pharmacokinetic data for PFOA in the adult rat were available for both oral gavage and intravenous (IV) dosing routes (Kemper, 2003; Kudo et al., 2007) for male and female Sprague–Dawley and Wistar rats. Datasets reporting PFOA concentrations in serum, urine, feces, and liver tissue following single IV and oral administration were used for development and evaluation of the model (Table 1).

For IV dosing, two datasets were available. In the first, four adult male and four adult female Sprague–Dawley rats were administered 1.0 mg/kg body weight [carbonyl-^{14}C] ammonium perfluorooctanoate (^{14}C-PFOA) via a surgically implanted jugular vein cannula (Kemper, 2003). In male rats, whole blood samples were collected from the cannula or from the tail vein pre-dose and at 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 24 h post-dose, at 24-hour intervals through 192 h, and then at 48-hour intervals from 192 through 528 h. In female rats, whole blood samples were collected from the cannula or the tail vein pre-dose and at 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, 36, 48, and 72 h post-dose. Serum samples were analyzed for ^{14}C-PFOA by liquid scintillation counting (LSC).

In the second IV dosing study, nine-week old male Wistar rats (four animals per dose group) were administered an IV dose of either 0.041 or 16.56 mg/kg body weight [1-^{14}C] PFOA (Kudo et al., 2007). Whole blood samples were collected from the vena cava two hours after injection, after which animals were euthanized and tissue samples, including liver, kidney, intestine, testis, spleen, fat, heart, lung, brain, and stomach were collected. Serum and tissue samples were analyzed for ^{14}C-PFOA by LSC.

One extensive dataset was available for oral dosing of PFOA and included measurement of PFOA in urine, feces and serum (Kemper, 2003). Four male and four female Sprague–Dawley rats per dose group were administered ^{14}C-PFOA in a water vehicle via oral gavage at doses of 0.1, 1.0, 5.0 or 25.0 mg/kg body weight. For male rats, whole blood samples were collected from a surgically implanted jugular vein cannula or from the tail vein pre-dose and at 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 24 h post-dose, at 24-hour intervals through 192 h, and then at 48-hour intervals from 192 through 528 h. In female rats, whole blood samples were collected from the cannula or tail vein pre-dose at 0.25, 0.5, 1, 2, 4, 8, 12, 16, 24, 36, 48, 72, and 96 h post-dose.

In a separate set of experiments (Kemper, 2003), four adult male and four adult female Sprague–Dawley rats per dose group were administered ^{14}C-PFOA in a water vehicle via oral gavage at doses of 1.0, 5.0, or 25.0 mg/kg body weight. Following dosing, rats were housed individually in glass metabolism cages that allowed for collection of urine, feces, expired air, and volatile organics. For male rats, urine and feces were collected at 4, 8, 12, and 24 h post-dose, and at 24-hour intervals through 336 h. For female rats, urine and feces were collected at 4, 8, 12, and 24 h post-dose, and at 24-hour intervals through 168 h. Serum, urine, and feces samples were analyzed for ^{14}C-PFOA by LSC.

Table 1
Key pharmacokinetic studies in male and female rats.

	Dose (mg/kg BW)	Route	Sex	Endpoint	Use in model development
Kemper, Experiment 1	1.0	Oral	M and F	Cumulative dose in urine and feces	Calibration
Kemper, Experiment 2	5.0	Oral	M and F	Cumulative dose in urine and feces	Evaluation
Kemper, Experiment 3	25.0	Oral	M and F	Cumulative dose in urine and feces	Evaluation
Kemper, Experiment 5	0.1	Oral	M and F	Serum concentration	Evaluation
Kemper, Experiment 6a	1.0	Oral	M and F	Serum concentration	Calibration
Kemper, Experiment 6b	1.0	IV	M and F	Serum concentration	Calibration (M), evaluation (F)
Kemper, Experiment 7	5.0	Oral	M and F	Serum concentration	Evaluation
Kemper, Experiment 8	25.0	Oral	M and F	Serum concentration	Evaluation
Kudo, Experiment 1	0.041	IV	M	Serum and liver concentration	Evaluation
Kudo, Experiment 2	16.56	IV	M	Serum and liver concentration	Evaluation

References: Kemper (2003) and Kudo et al. (2002).