Evaluation of Perfluorooctanesulfonic acid absorption and tissue concentrations in Organic Anion Transporting Polypeptide 2B1-deficient mice

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STEEP Sources, Transport, Exposure & Effects of PFASs
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ABSTRACT

Perfluorooctanesulfonic Acid (PFOS) is a perfluoroalkyl substance (PFAS) of ubiquitous exposure, detected in the serum of ~95% of the U.S. adult population and has a half-life of 4.1-8.67 years. PFOS exposure is associated with liver damage, dyslipidemia, and obesity. Intestinal PFOS absorption is highly efficient (99% absorbed), yet little is known about the mechanism(s) that drive gut PFOS absorption. Organic Anion Transporting Polypeptide 2B1 (OATP2B1) transports drugs/xenobiotic uptake and is located on the apical side of enterocytes and sinusoidal membrane of hepatocytes. PFOS is an OATP2B1 substrate in human and rat overexpressed cells, thus, it is hypothesized that OATP2B1 could contribute to PFAS uptake *in vivo*. In this study, adult male/female OATP2B1 null (OATP2B1$^{-/-}$, n = 7) and wild-type mice (OATP2B1$^{+/+}$, n = 10) were administered a single PFOS dose via oral gavage (1 mg/kg). Plasma was collected over the course of 5 days (female) and 14 days (male) at 30 minutes, 1, 3, 8, 12, 24, 48 hours, 5, 7, and 14 days, respectively. At the respective times of necropsy, tissues were snap-frozen in liquid nitrogen, and samples were later homogenized and processed utilizing QuEChERS extraction for LC/MS analysis. Liver PFOS concentration between OATP2B1$^{+/+}$ (7.75 ng/mg liver = female, 7.31 ng/mg liver = male) and OATP2B1$^{-/-}$ mice (6.81 ng/mg liver = female, 7.90 ng/mg liver = male) did not statistically vary, suggesting that OATP2B1 is not critical for PFOS uptake and deposition to liver. Plasma PFOS measurements are ongoing and will be presented. In summary, our work will present the contribution of OATP2B1 for PFOS uptake and distribution in mice after a single oral exposure to PFOS.

INTRODUCTION

PFAS are persistent man-made environmental toxicants, that are currently minimally regulated regarding the production and manufacturing of these compounds. The full array of adverse health impacts seen as a resultant of exposure is still yet to be completely understood. PFOS is a commonly known and studied chemical in this group of compounds. PFOS has been shown to induce hepatocyte peroxisome proliferation, liver hypertrophy, vacuolization, and hyperplasia in rats and mice.[1-3] Additionally, exposure causes elevated liver enzymes, liver enlargement, and hepatic steatosis in adult mice. OATP2B1 belongs to the Solute Carrier Family (SLC) and is located on the apical side of enterocytes and sinusoidal membrane of hepatocytes. It is responsible for transporting organic anionic endo- and xenobiotics, and shows pH dependent activity. Longer chain PFAS have been shown to be substrates for human and rat OATP2B1[4-5].

In this study, Oatp2b1-deficient mice were bred using a C57BL/6 background strain. Mice were generated using heterozygous embryos purchased from the University of California Davis Knockout Mouse (KOMP) Repository and bred to produce homozygous mice using Flp-Frt and Cre-loxP recombination.[6]

HYPOTHESIS

The OATP2B1 knockout model will give insight into the role of OATP2B1 in intestinal absorption of PFOS. The levels of PFOS measured will be lower in liver and plasma.

MATERIALS and METHODS

Treatment paradigm. A single PFOS dose was administered via oral gavage (1 mg/kg BW) at the start of the study. Treatment groups consisted of WT Male 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Male 1mg/kg PFOS (n=3), WT Female 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Female 1mg/kg PFOS (n=4). Blood was collected (alternating cheek pouch or orbital collection) and plasma was isolated at 30 minutes, 1, 3, 8, 12, 24, 48 hours, 5, 7, and 14 days. On day 5, all females were euthanized, and tissues collected. On day 14, all males were euthanized, and tissues collected **(Figure 1).**

*Tissues collected: liver, duodenum, jejunum, ileum, large intestine, kidney, adipose, brain, lung, muscle, bone

LC/MS Prep. PFOS was extracted according to the RoQ TM Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) manufacturer protocol (Phenomenex, Torrance, CA). An input of 5 µL of plasma and ~20 mg of liver tissue was used in respective sample preparations. All samples were spiked with matched isotope-labeled internal standard (M4PFOS) obtained from Wellington Laboratories (Ontario, Canada). Samples were processed on a Sciex 6500 QTRAP LC-MS/MS (Framingham, MA) and data was analyzed utilizing GraphPad Prism 9.5.0 (La Jolla, CA).

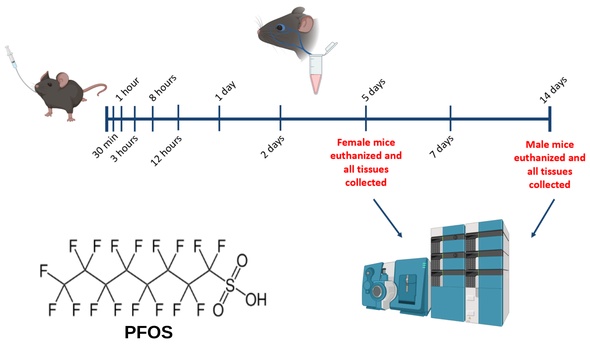

Figure 1. Schematic of blood sampling and necropsy timepoints.
- *WT Female:* 1mg/kg PFOS (n=5)
- *OATP2B1$^{-/-}$ Female:* 1mg/kg PFOS (n=4)
- *WT Male:* 1mg/kg PFOS (n=5)
- *OATP2B1$^{-/-}$ Male:* 1mg/kg PFOS (n=3)

RESULTS

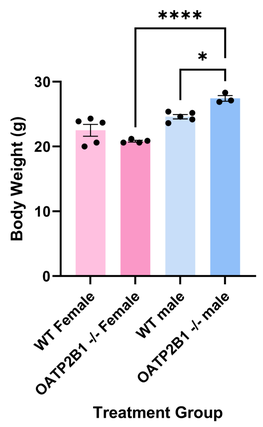

Figure 2. Average body weight of mice (g) after single oral dose of 1 mg/kg BW PFOS. Treatments include: WT Male 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Male 1mg/kg PFOS (n=3), WT Female 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Female 1mg/kg PFOS (n=4). There was a significant difference observed between OATP2B1$^{-/-}$ male and OATP2B1$^{-/-}$ females, p value <0.0001. There was a significant difference observed between male WT and male OATP2B1$^{-/-}$, p value = 0.0484.

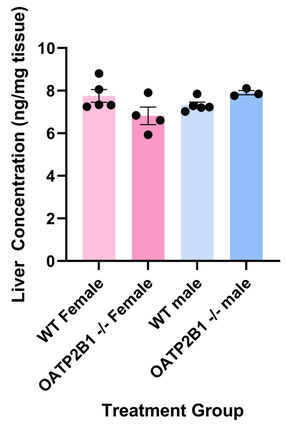

Figure 3. Average liver PFOS concentration (ng/mg tissue) after single oral dose of 1 mg/kg BW PFOS, measured at 5 days (female) and 14 days (male). . Treatments include: WT Male 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Male 1mg/kg PFOS (n=3), WT Female 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Female 1mg/kg PFOS (n=4). There was no significant difference observed between treatment groups

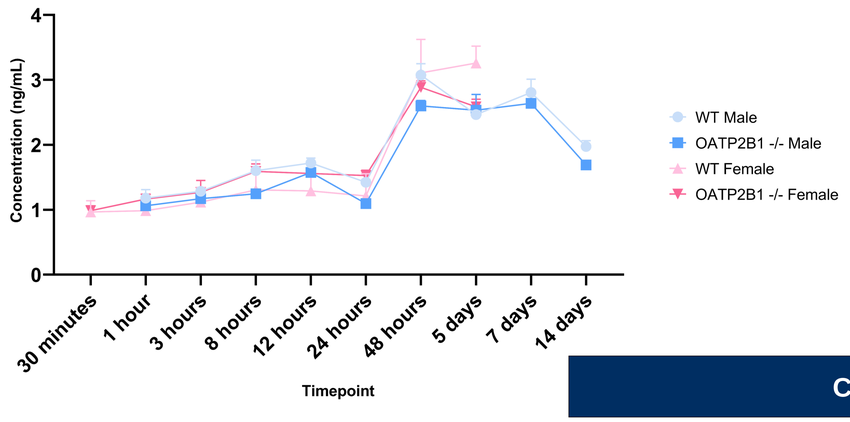

Figure 4. Average plasma PFOS concentration after single oral dose of 1 mg/kg BW PFOS, measured over 5 days (female) and 14 days (male). Treatments include: WT Male 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Male 1mg/kg PFOS (n=3), WT Female 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Female 1mg/kg PFOS (n=4).

Table 1. Average PFOS plasma and liver concentrations after single oral dose of 1 mg/kg BW PFOS. Average plasma concentrations at 30 minutes, 1, 3, 8, 12, 24, 48 hours, 5, 7, and 14 days. Average liver concentrations at Day 5 (Female) and Day 14 (Male). Treatments are: WT Male 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Male 1mg/kg PFOS (n=3), WT Female 1mg/kg PFOS (n=5), OATP2B1$^{-/-}$ Female 1mg/kg PFOS (n=4).

Plasma Timepoint	WT Female (ng/mL)	OATP2B1$^{-/-}$ Female (ng/mL)	WT Male (ng/mL)	OATP2B1$^{-/-}$ Male (ng/mL)
30 minutes	0.965	0.985	-	-
1 hour	0.985	1.164	1.178	1.061
3 hours	1.118	1.268	1.284	1.171
8 hours	1.305	1.590	1.602	1.245
12 hours	1.289	1.559	1.719	1.575
24 hours	1.212	1.527	1.423	1.095
48 hours	3.108	2.886	3.075	2.598
5 days	3.260	2.586	2.465	2.532
7 days	-	-	2.806	2.638
14 days	-	-	1.975	1.690

Liver Concentration (ng/mg tissue)	7.751	6.816	7.318	7.903

CONCLUSIONS

The results indicate that OATP2B1 may not play as important of a role in the intestinal absorption of PFOS as hypothesized. **Figure 2** displays bodyweight data showing a significant difference observed between OATP2B1$^{-/-}$ male and OATP2B1$^{-/-}$ females, p value <0.0001. There was also a significant difference observed between male WT and male OATP2B1$^{-/-}$, p value = 0.0484. **Figure 3** revealed no significant differences observed between average liver concentrations between treatments. **Figure 4** displayed the time course of average plasma PFOS concentrations for each treatment group over the course of 5 days (female) and 14 days (male), showing no marked differences between treatment groups. **Table 1** highlights the average plasma PFOS concentrations for each treatment from 30 minutes – 5 days (female) and 1 hour – 14 days (male). In addition to the average liver concentrations at day 5 (female) and day 14 (male) for each treatment. Overall, no marked differences between plasma or liver concentrations were observed, suggesting OATP2B1 is not critical for PFOS uptake and deposition.

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