

Supplemental Material

Exploring indirect sources of human exposure to perfluoroalkyl carboxylates (PFCAs): Evaluating uptake, elimination and biotransformation of polyfluoroalkyl phosphate esters (PAPs) in the rat

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Materials and Methods

Chemical purchase, synthesis and purity. The following perfluorinated carboxylic acids (PFCAs): perfluorobutanonoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA), were quantified using the PFC-MXA mix standard from Wellington labs (Guelph, ON). Perfluoroundecanoic acid (PFUnA) was also obtained from Wellington labs. 6:2, 8:2 and 10:2 fluorotelomer carboxylic acids (6:2 FTCA, 8:2 FTCA, 10:2 FTCA) were quantified using the FTA-MXA mix standard from Wellington labs. All mass-labeled internal standards were obtained from Wellington labs.

The 4:2 FTCA and all fluorotelomer unsaturated carboxylic acid (FTUCA) were synthesized as described in Achilefu et al. (1995). 8:2 fluorotelomer sulfate (8:2 FTOH-sulfate) and 8:2 fluorotelomer glucuronide (8:2 FTOH-glucuronide) were synthesized as described in Butt et al (2010).

Fluorotelomer alcohol (FTOH) concentrations in the polyfluoroalkyl phosphate diester (diPAP) doses were consistently below 0.1% of the diPAP of similar chain length, aside from the 10:2 FTOH which was present at 0.4% and 1% in the gavage and intravenous doses, respectively. FTOH concentrations were slightly higher in the polyfluoroalkyl phosphate monoester (monoPAP) doses, but were consistently below 1% (see Table 4). At a concentration of 1 mg/L diPAP, monoPAPs were not detected in the either diPAP dose. With an instrumental detection limit of 0.5 µg/L for the monoPAPs, this indicates there is < 0.05% monoPAPs in the diPAP doses.

Preparatory liquid chromatography. DiPAPs were isolated using a Gemini-NX prep LC column (100 x 21.2 mm, 5 μ m; Phenomenex, Torrance, CA) at a flow rate of 20 mL/min with 1 mL injections of about 50 mg/mL individual diPAP solutions. Details of solvent composition and elution time are provided in Table 2.

Extraction procedure. Blood and urine samples were extracted by adding 300 μ L of acetonitrile to whole blood samples, which ranged in size from 40 mg to 150 mg, or 300 μ L of urine. Following acetonitrile addition, the samples were sonicated for 30 minutes, centrifuged for 10 minutes (6000 rpm) and filtered using a 0.2 μ m nylon filter (Chromatographic Specialties Inc., Brockville, ON).

Feces samples were extracted using a solvent composition of 80:20 acetonitrile:water. The amount of solvent added to the feces sample ranged from 5 to 20 mL, and depended on the mass of feces in the sample, which varied from 0.1 g to 10 g. After the addition of solvent the feces samples were homogenized by vortexing for 5 minutes. The homogenates were then sonicated for 30 minutes, centrifuged for 10 minutes and a 1 mL aliquot removed and filtered using a 0.2 μ m nylon filter.

Instrumental analysis. Extracts from the blood, urine and feces samples were analyzed by liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS) using an API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Mississauga, ON) and an Agilent 1100 solvent delivery system (Mississauga, ON). Chromatography was performed using a Gemini-NX C18 analytical LC column (50mm \times 4.6 mm, 3 μ m) with a mobile phase composed of methanol and water, both with 10 mM ammonium acetate. Multiple reaction monitoring (MRM) transitions are provided in Table 8. Four separate LC methods were used in this analysis.

All LC-MS/MS analysis was performed at a flow rate of 500 $\mu\text{L}/\text{min}$ using 35 μL injections. LC method 1 was used for the analysis of the diPAPs. LC method 2 was used for the analysis of the monoPAPs. LC method 3 was used for the analysis of the C6-C11 PFCAs, as well as the 8:2 FTCA, 8:2 FTUCA, 7:3 FTCA, 10:2 FTCA, 10:2 FTUCA, 9:3 FTCA, 8:2 FTOH-sulfate, 8:2 FTOH-glucuronide, 10:2 FTOH-sulfate and 10:2 FTOH-glucuronide. LC method 4 was used for the analysis of PFBA and PFPeA, as well as the 4:2 FTCA, 4:2 FTUCA, 3:3 FTCA, 6:2 FTCA, 6:2 FTUCA, 5:3 FTCA, 4:2 FTOH-sulfate, 4:2 FTOH-glucuronide, 6:2 FTOH-sulfate and 6:2 FTOH-glucuronide.

LC method 1: Initial conditions of 65:35 methanol:water, increasing to 95:5 over 2 minutes ($t = 2 \text{ min}$), holding at 95:5 for 3 minutes ($t = 5 \text{ min}$), reverting to initial conditions of 65:35 over 30 seconds ($t = 5.5 \text{ min}$) and re-equilibrating for 2.5 minutes ($t = 8 \text{ min}$).

LC method 2: Initial conditions of 50:50 methanol:water, increasing to 80:20 over 1 minute ($t = 1 \text{ min}$), increasing to 90:10 over 2 minutes ($t = 3 \text{ min}$), increasing to 95:5 over 2 minutes ($t = 5 \text{ min}$), holding at 95:5 for 3 minutes ($t = 8 \text{ min}$), reverting to initial conditions of 50:50 over 30 seconds ($t = 8.5 \text{ min}$) and re-equilibrating for 2.5 minutes ($t = 11 \text{ min}$).

LC method 3: Initial conditions of 65:35 methanol:water, increasing to 95:5 over 3 minutes ($t = 3 \text{ min}$), holding at 95:5 for 2 minutes ($t = 5 \text{ min}$), reverting to initial conditions of 65:35 over 30 seconds ($t = 5.5 \text{ min}$) and re-equilibrating for 2.5 minutes ($t = 8 \text{ min}$).

LC method 4: Initial conditions of 30:70 methanol:water, increasing to 80:20 over 3 minutes ($t = 3 \text{ min}$), holding at 80:20 for 2 minutes ($t = 5 \text{ min}$), reverting to initial

conditions of 30:70 over 30 seconds ($t = 5.5$ min) and re-equilibrating for 2.5 minutes ($t = 8$ min).

For dose purity analysis, FTOHs were analyzed in the final dose material by dissolving 10 μL of dose in 990 μL ethyl acetate, followed by analysis by gas chromatography mass spectrometry using 1 μL injection into an Rtx-WAX column (30 m x 0.25 mm ID, 0.25 μm ; Restek, Bellefonte, PA) at a constant pressure of 8.2 psi. The temperature program started at 60 $^{\circ}\text{C}$, with a 1 minute hold ($t = 1$ min), followed by a 5 $^{\circ}\text{C}/\text{min}$ ramp to 75 $^{\circ}\text{C}$ ($t = 4$ min), a 10 $^{\circ}\text{C}/\text{min}$ ramp to 130 $^{\circ}\text{C}$ ($t = 9.5$ min), and a 50 $^{\circ}\text{C}/\text{min}$ ramp to 240 $^{\circ}\text{C}$ ($t = 11.7$ min), followed by a hold at 240 $^{\circ}\text{C}$ for 1 minute ($t = 12.7$ min). Both the inlet and the mass spectrometer transfer line were held at 220 $^{\circ}\text{C}$. The mass spectrometer was operated in single ion monitoring mode looking for the following m/z : 265 (4:2 FTOH), 365 (6:2 FTOH), 465 (8:2 FTOH), 565 (10:2 FTOH).

Analyte quantification. All PFCAs and FTCAs were quantified by addition of the following internal standards to the extract immediately prior to analysis: $^{13}\text{C}_4$ -PFBA (PFBA, PFPeA), $^{13}\text{C}_2$ -PFHxA (PFHxA, PFHpA), $^{13}\text{C}_4$ -PFOA (PFOA), $^{13}\text{C}_5$ -PFNA (PFNA), $^{13}\text{C}_2$ -PFDA (PFDA), $^{13}\text{C}_2$ -PFUnA (PFUnA), $^{13}\text{C}_2$ -6:2 FTCA (4:2 FTCA, 6:2 FTCA), $^{13}\text{C}_2$ -6:2 FTUCA (4:2 FTUCA, 3:3 FTCA, 4:2 FTOH-sulfate, 4:2 FTOH-glucuronide, 6:2 FTUCA, 5:3 FTCA, 6:2 FTOH-sulfate, 6:2 FTOH-glucuronide), $^{13}\text{C}_2$ -8:2 FTCA (8:2 FTCA), $^{13}\text{C}_2$ -8:2 FTUCA (8:2 FTUCA, 7:3 FTCA, 8:2 FTOH-sulfate, 8:2 FTOH-glucuronide), $^{13}\text{C}_2$ -10:2 FTCA (10:2 FTCA), $^{13}\text{C}_2$ -10:2 FTUCA (10:2 FTUCA, 9:3 FTCA, 10:2 FTOH-sulfate, 10:2 FTOH-glucuronide).

No standards were available for the analysis of the 4:2, 6:2 and 10:2 FTOH-sulfate, the 4:2, 6:2 and 10:2 FTOH-glucuronide or the 3:3, 5:3 and 9:3 FTCA. Therefore

the 8:2 FTOH-sulfate was used to quantify all of the FTOH-sulfate chain lengths, the 8:2 FTOH-glucuronide was used to quantify all the glucuronide chain lengths, and the 7:3 FTCA was used to quantify the 3:3, 5:3 and 9:3 FTCA. As the accuracy of the assumptions made is not known, the reported concentrations of these species should be considered approximate.

To account for any differences in PAPs purity between the gavage and intravenous doses, samples were quantified using standards made from the respective dose.

Spike and recovery experiments. Analyte recoveries from rat blood, urine and feces were determined by spiking approximately 50 mg rat blood, 300 μ L rat urine or approximately 0.50 g rat feces from a control animal ($n=4$) with 35 ng diPAPs, 350 ng monoPAPs, and 0.50 ng PFCAs, FTCAs, FTUCAs and the 8:2 FTOH-sulfate and FTOH-glucuronide. All spiked samples were stored for 24 hours at -20°C prior to extraction as described above. Results of these experiments are provided in Tables 5 and 6.

All of the monoPAP recoveries in blood and urine were above 100%, and so appear to be overestimated. This overestimation may be due to matrix enhancement, however as a quantification technique standard addition should account for any effects of the matrix on analyte quantification. Despite the apparent overestimation of monoPAP concentrations in blood and urine, the monoPAPs were not detected in either matrix.

Limits of detection and quantification. Analyte limits of detection (LOD) were defined empirically as the concentration producing a signal-to-noise (S/N) ratio of 3, and limits of quantification (LOQ) were defined as the concentration producing a S/N of 10.

Given the large number of analytes in the present study, the lowest calibration standard was used as the limit of quantitation. LOD and LOQ values are presented in Table 8.

Details of monoPAP analysis. LC-MS/MS analysis of the monoPAP congeners is nontrivial. The dianionic moiety of these phosphate monoesters is very surface active, often causing significant tailing during chromatographic analysis. Previous studies in our lab have used a low pH 1% formic acid buffer for monoPAP analysis with very good success (Lee et al 2010). Due the large number of analytes in the present study we used a relatively shallow LC gradient (LC method 2) with the mobile phase at pH 7 (10 mM ammonium acetate) to avoid changing buffering systems between analyses. The LC column used (Gemini-NX, Phenomenex) was chosen for its pH stability as this seems to decrease monoPAP peak tailing, potentially due to a decreased number of active sites. An example of the monoPAP chromatography achieved in this study is shown in Figure 2.

Calculation of pharmacokinetic parameters. Half-lives ($t_{1/2}$) were calculated using [1],

$$t_{1/2} = \frac{\ln(2)}{z} \quad [1]$$

where z is the elimination rate constant, which is the slope of the natural logarithm of the blood concentration over time (shown in Figures 4, 5 and 6). Half-lives for the diPAPs and PFCAs are provided in Tables 9 and 10.

As defined by [1] and [4] in the main text both the % bioavailability and % biotransformation parameters require the area under the concentration-time curve (AUC). A graphical representation of the AUC_t , which is the AUC for the time period captured in the study, for both the diPAP and PFCA congeners are shown in Figure 8 (blood concentrations expressed in mass (ng/g)) and Figure 9 (blood concentrations expressed in

moles (nmol/g)). AUC_t values were determined using the *Area below curve* macro in SigmaPlot® for Windows Version 11.0. Calculation of the pharmacokinetic parameters requires the AUC to infinity (AUC_∞), which was calculated using the AUC_t as described in [2],

$$AUC_\infty = AUC_t + \frac{C_t}{z} \quad [2]$$

where z is the elimination rate constant, and C_t is the concentrations at the last time point. Bioavailability calculations used the AUC_∞ calculated from the blood concentrations expressed in mass (ng/g, Figure 8 and Table 11), whereas biotransformation calculations used the AUC_∞ calculated from the blood concentrations expressed in moles (nmol/g, Figure 9 and Table 12).

Modelling PFOA serum concentrations. PFOA serum elimination was modelled using [3],

$$[PFOA] = [PFOA]_0 e^{-zt} \quad [3]$$

The calculation performed to predict the production of PFOA from the biotransformation of 8:2 diPAP is demonstrated graphically in Figure 10. From the 8:2 diPAP concentrations observed in our previous study (D'eon et al 2009), we assumed a constant concentration of 0.15 $\mu\text{g/L}$ (0.15 nmol/L) 8:2 diPAP in human sera. We then divided the x-axis (time) into one month intervals, and calculated the AUC_t for 8:2 diPAP one month exposure ($AUC_t(\text{diPAP}) = 0.15 \text{ nmol L}^{-1} \text{ month}^{-1}$, shown as blue squares in Figure 10). Using the 8:2 diPAP AUC_t we can calculate the PFOA AUC_t for this one month time period for a given % biotransformation using [4].

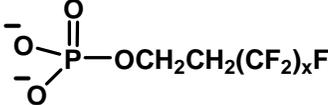
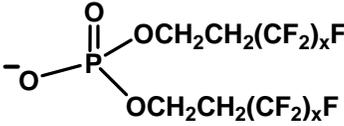
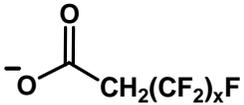
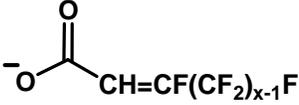
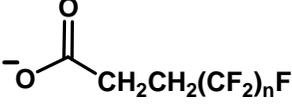
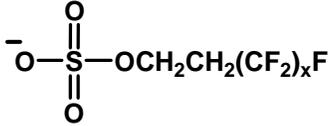
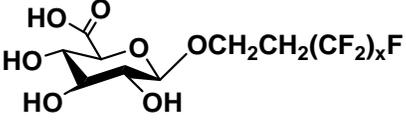
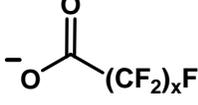
$$(AUC_t)_{\text{PFOA}} = \frac{2(AUC_t)_{8:2\text{diPAP}}(\% \text{biotransformation})}{100} \quad [4]$$

Using a 10% biotransformation yield this gives a PFOA AUC_t of $0.030 \text{ nmol month}^{-1} \text{ L}^{-1}$ (shown as yellow squares in Figure 10), which results in an increase in PFOA blood concentrations of $0.030 \text{ nmol L}^{-1}$ or $0.0125 \text{ } \mu\text{g L}^{-1}$ per month. During this one month PFOA is produced from the biotransformation of 8:2 diPAPs, PFOA is also depurated. PFOA depuration was modelled in one month intervals using [3]. PFOA blood concentrations were then iteratively calculated each month, which, as demonstrated by the dotted red line in Figure 2 in the main text, overtime this results in an equilibrium between increased PFOA exposure from 8:2 diPAP biotransformation and PFOA depuration.

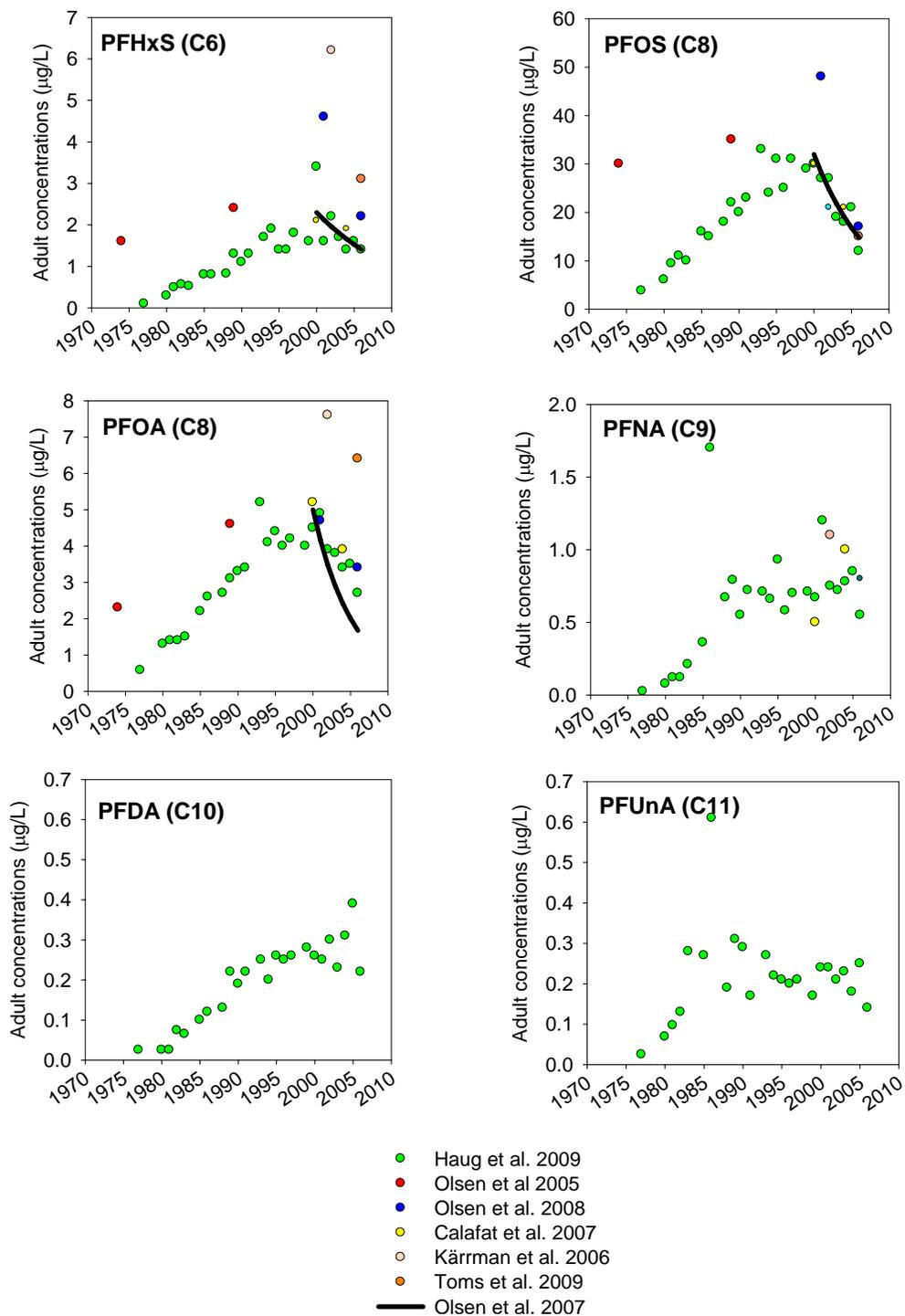
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- Lee H, D'eon JC, Mabury SA. 2010. Biodegradation of polyfluoroalkyl phosphates (PAPs) as a source of perfluorinated acids to the environment. *Environ Sci Technol* 44:3305-3310.

Supplemental Material, Table 1. Structures, acronyms, and congeners monitored.

Structure	Congeners Monitored	Acronym
Administered chemicals: Polyfluoroalkyl phosphoric acids (PAPs)		
	4 congeners x = 4, 6, 8, 10	x:2 monoPAP
	4 congeners x = 4, 6, 8, 10	x:2 diPAP
Intermediate metabolites: Fluorotelomer carboxylic acids (FTCAs)		
	4 congeners x = 4, 6, 8, 10	x:2 FTCA
	4 congeners x = 4, 6, 8, 10	x:2 FTUCA
	4 congeners n = 3 ^a , 5 ^a , 7, 9 ^a	n:3 FTCA
Phase II metabolites: Fluorotelomer sulfates and glucuronides		
	4 congeners x = 4 ^a , 6 ^a , 8, 10 ^a	x:2 FTOH-sulfate
	4 congeners x = 4 ^a , 6 ^a , 8, 10 ^a	x:2 FTOH-glucuronide
Final degradation products: Perfluorinated carboxylic acids (PFCAs)		
	8 congeners x = 3-10	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA

^a No analytical standards available



Supplemental Material, Figure 1. Temporal trends in human sera for PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA, plotted with the arithmetic human serum half-lives for PFHxS, PFOS and PFOA. (References in the main text)

Supplemental Material, Table 2. Preparatory LC conditions used to purify the diPAPs.

All solvents contained 1 mM ammonium acetate.

Analyte	Solvent composition (Acetonitrile:Water)	Peak collections (min)
4:2 diPAP	35:65	4-6
6:2 diPAP	55:45	2-4
8:2 diPAP	65:35	4-6
10:2 diPAP	80:20	5-7

Supplemental Material, Table 3. PAP concentrations in the gavage and intravenous doses.

Analyte	Conc of Dose (mg/mL)	Volume delivered (mL)	Conc to the animal (mg/kg)	Mass of chemical to the animal (mg)	Mass of the animal (kg)
Gavage Experiment					
4:2 diPAP	16.6	1	66	17	
6:2 diPAP	11.5	1	46	12	0.253 ±
8:2 diPAP	10.4	1	42	10	0.006
10:2 diPAP	9.6	1	39	10	
4:2 monoPAP	11.3	1	45	11	
6:2 monoPAP	10.4	1	42	10	0.250 ±
8:2 monoPAP	6.0	1	24	6.0	0.009
10:2 monoPAP	8.5	1	34	8.5	
Intravenous Experiment					
4:2 diPAP	8.5	0.5	17	4.3	
6:2 diPAP	4.9	0.5	9.8	2.4	0.257 ±
8:2 diPAP	4.3	0.5	8.7	2.2	0.010
10:2 diPAP	9.4	0.5	19	4.7	
4:2 monoPAP	4.3	0.5	8.6	2.2	
6:2 monoPAP	4.2	0.5	8.4	2.1	0.278 ±
8:2 monoPAP	9.4	0.5	19	4.7	0.005
10:2 monoPAP	17.3	0.5	35	8.6	

Supplemental Material, Table 4. Concentrations of FTOHs in the diPAP and monoPAP doses.

Dose	Percentage (%) ^a			
	4:2 FTOH	6:2 FTOH	8:2 FTOH	10:2 FTOH
Gavage Experiment				
diPAP dose	0.03	0.06	0.1	0.4
monoPAP dose	0.3	0.8	1	0.4
Intravenous Experiment				
diPAP dose	0.04	0.1	0.1	1
monoPAP dose	1	1	0.3	0.09

^a FTOH percentages are defined as the mass of FTOH with respect to the mass of diPAP or monoPAP of similar chain length.

Supplemental Material, Table 5. Recoveries of the diPAP and monoPAP congeners of interest.

Analyte	Recovery (%)		
	Whole blood	Urine	Feces
monoPAPs			
4:2 monoPAP	180 ± 20	140 ± 30	N/A ^a
6:2 monoPAP	160 ± 20	160 ± 30	76 ± 44
8:2 monoPAP	140 ± 20	140 ± 30	150 ± 90
10:2 monoPAP	240 ± 40	240 ± 70	81 ± 29
diPAPs			
4:2 diPAP	110 ± 10	89 ± 19	89 ± 6
6:2 diPAP	110 ± 40	180 ± 20	110 ± 10
8:2 diPAP	130 ± 20	75 ± 45	88 ± 23
10:2 diPAP	160 ± 30	100 ± 40	96 ± 31

^a For unexplained reasons the 4:2 monoPAP was not properly recovered from the feces.

Supplemental Material, Table 6. Recoveries for the final PFCA degradation products, metabolic intermediates and phase II metabolites.

Analyte	Recovery (%)		
	Whole blood	Urine	Feces
Metabolic Intermediates			
4:2 FTCA	80 ± 3	64 ± 7	91 ± 17
6:2 FTCA	140 ± 20	76 ± 17	52 ± 11
8:2 FTCA	160 ± 10	93 ± 14	64 ± 26
10:2 FTCA	97 ± 9	71 ± 11	74 ± 32
4:2 FTUCA	85 ± 7	64 ± 4	79 ± 4
6:2 FTUCA	93 ± 7	55 ± 9	76 ± 7
8:2 FTUCA	73 ± 9	58 ± 6	57 ± 15
10:2 FTUCA	97 ± 9	54 ± 8	46 ± 14
7:2 FTCA	80 ± 3	120 ± 20	170 ± 50
Phase II metabolites			
8:2 FTOH-sulfate	150 ± 20	100 ± 20	130 ± 40
8:2 FTOH-glucuronide	120 ± 10	120 ± 10	190 ± 20
PFCAs			
PFBA	110 ± 40	72 ± 26	200 ± 10
PFPeA	110 ± 10	97 ± 16	210 ± 10
PFHxA	92 ± 32	50 ± 13	190 ± 30
PFHpA	91 ± 7	51 ± 14	150 ± 20
PFOA	60 ± 3	81 ± 9	85 ± 8
PFNA	88 ± 6	65 ± 11	63 ± 16
PFDA	82 ± 7	67 ± 10	57 ± 17
PFUnA	79 ± 3	58 ± 10	56 ± 18

Supplemental Material, Table 7. MRM transitions and MS parameters for the analytes of interest.

Analyte	MRM transition	Declustering potential (V)	Collision Energy (V)
PAPs			
4:2 monoPAP	343 > 79	-35	-65
6:2 monoPAP	443 > 79	-40	-75
8:2 monoPAP	543 > 79	-45	-90
10:2 monoPAP	643 > 79	-50	-105
4:2 diPAP	589 > 343	-50	-25
	589 > 97		-50
6:2 diPAP	789 > 443	-65	-27
	789 > 97		-65
8:2 diPAP	989 > 543	-80	-33
	989 > 97		-75
10:2 diPAP	1189 > 643	-80	-40
	1189 > 97		-85
Metabolic Intermediates			
4:2 FTCA	277 > 193	-35	-18
4:2 FTUCA	257 > 193	-25	-18
6:2 FTCA	377 > 293	-35	-18
6:2 FTUCA	357 > 293	-35	-18
8:2 FTCA	477 > 393	-55	-19
8:2 FTUCA	457 > 393	-50	-19
10:2 FTCA	577 > 493	-55	-19
10:2 FTUCA	557 > 493	-50	-19
3:3 FTCA ^a	241 > 137	-60	-17
5:3 FTCA ^a	341 > 237	-60	-17
7:3 FTCA	441 > 337	-70	-17
9:3 FTCA ^a	541 > 437	-70	-17

Analyte	MRM transition	Declustering potential (V)	Collision Energy (V)
Phase II metabolites			
4:2 FTOH-sulfate ^a	343 > 97	-70	-70
6:2 FTOH-sulfate ^a	443 > 97	-70	-70
8:2 FTOH-sulfate	543 > 97	-70	-70
10:2 FTOH-sulfate ^a	643 > 97	-70	-70
4:2 FTOH-glucuronide ^a	439 > 193	-60	-30
6:2 FTOH-glucuronide ^a	539 > 193	-60	-30
8:2 FTOH-glucuronide	639 > 193	-60	-30
10:2 FTOH-glucuronide ^a	739 > 193	-60	-30
PFCAs			
PFBA	213 > 119	-25	-13
PFPeA	263 > 219	-25	-13
PFHxA	313 > 269	-30	-14
PFHpA	363 > 319	-30	-14
PFOA	413 > 369	-35	-15
PFNA	463 > 419	-35	-15
PFDA	513 > 469	-45	-15
PFUnA	563 > 519	-45	-17

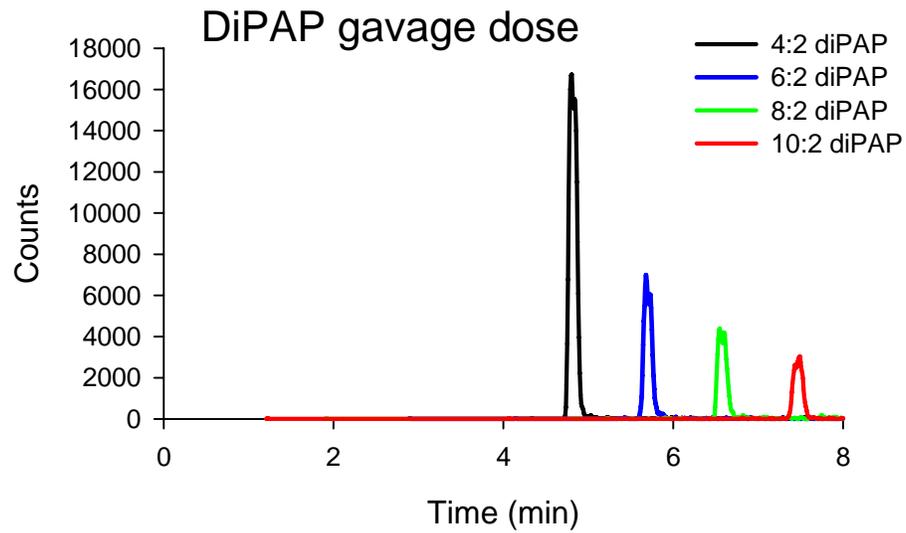
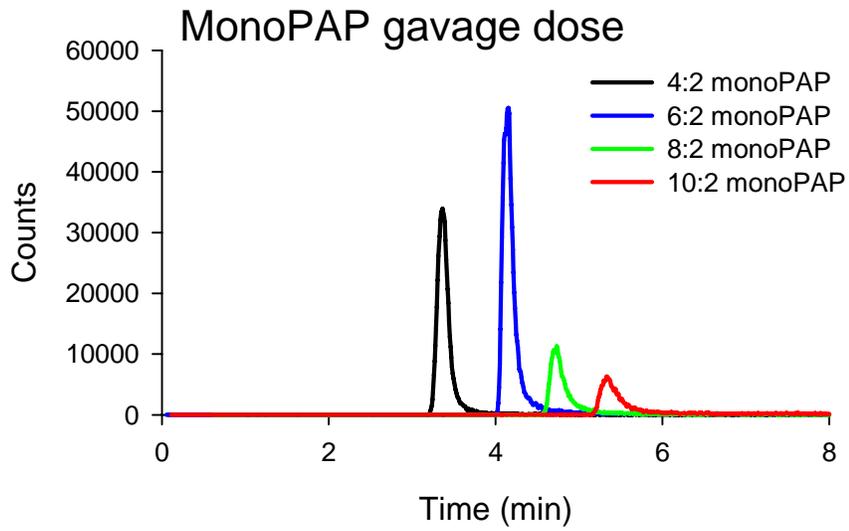
^a No standards were available or synthesized for these compounds. MRM transitions and MS parameters were inferred from compounds with the same functionality but a different chain length

Supplemental Material, Table 8. Matrix-specific limits of detection and limits of quantitation for the analytes of interest.

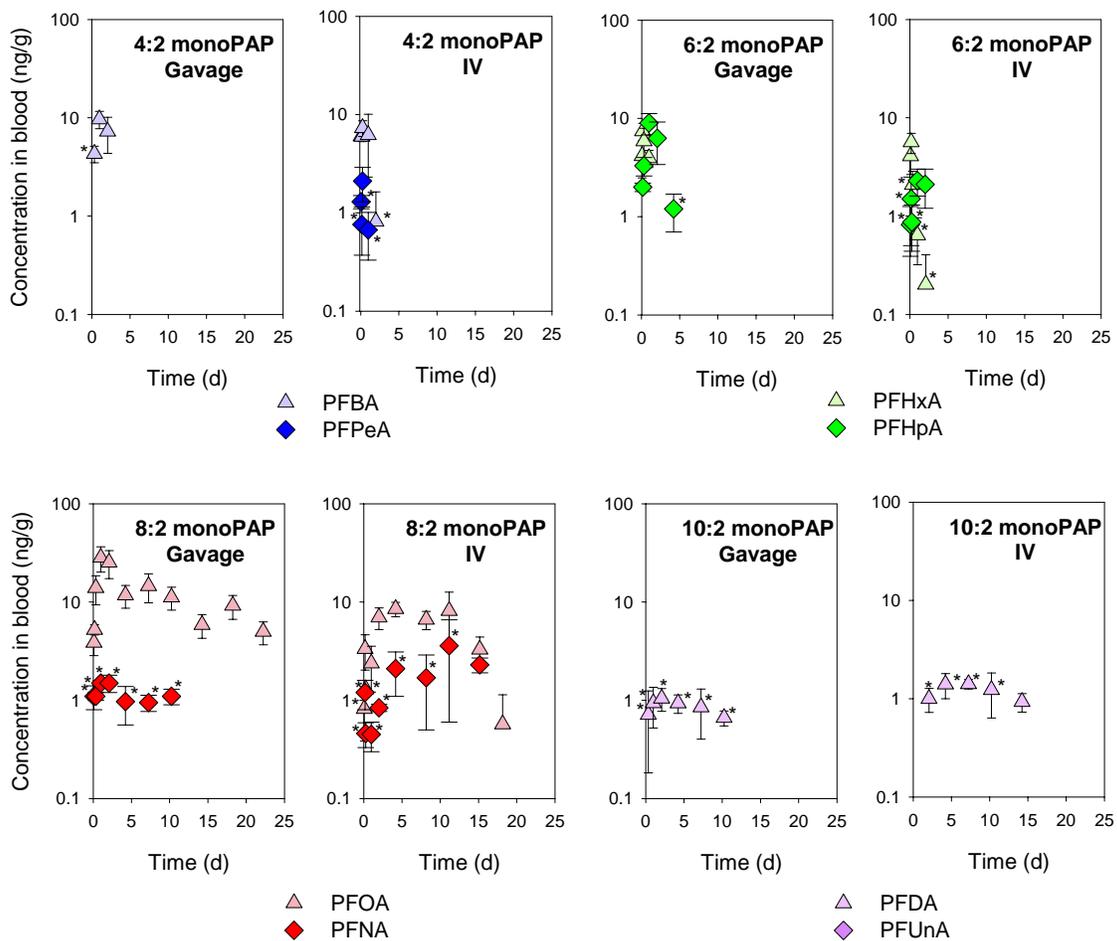
Analyte	Instrumental (ng/mL)	Blood (ng/g)	Urine (ng/g)	Feces (ng/g)
monoPAPs				
4:2 monoPAP	LOD: 0.5 LOQ: 2	N/A ^a	N/A ^a	N/A ^a
6:2 monoPAP	LOD: 0.5 LOQ: 2	10 40	5 20	5 20
8:2 monoPAP	LOD: 0.5 LOQ: 2	10 40	5 20	5 20
10:2 monoPAP	LOD: 0.5 LOQ: 2	10 40	5 20	5 20
diPAPs				
4:2 diPAP	LOD: 0.10 LOQ: 0.25	2 5	1 3	1 3
6:2 diPAP	LOD: 0.10 LOQ: 0.25	2 5	1 3	1 3
8:2 diPAP	LOD: 0.10 LOQ: 0.25	2 5	1 3	1 3
10:2 diPAP	LOD: 0.10 LOQ: 0.25	2 5	1 3	1 3
Metabolic Intermediates				
4:2 FTCA	LOD: 0.3 LOQ: 1	6 20	3 10	3 10
4:2 FTUCA	LOD: 0.03 LOQ: 0.06	0.5 1.3	0.3 0.7	0.3 0.7
6:2 FTCA	LOD: 0.3 LOQ: 1	6 20	3 10	3 10
6:2 FTUCA	LOD: 0.03 LOQ: 0.06	0.5 1.3	0.3 0.7	0.3 0.7
8:2 FTCA	LOD: 0.3 LOQ: 1	6 20	3 10	3 10

Analyte	Instrumental (ng/mL)	Blood (ng/g)	Urine (ng/g)	Feces (ng/g)
8:2 FTUCA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
10:2 FTCA	LOD: 0.3	6	3	3
	LOQ: 1	20	10	10
10:2 FTUCA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
7:3 FTCA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
Phase II metabolites				
8:2 FTOH-sulfate	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
8:2 FTOH-glucuronide	LOD: 0.3	6	3	3
	LOQ: 1	20	10	10
PFCAs				
PFBA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFPeA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFHxA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFHpA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFOA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFNA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFDA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7
PFUnA	LOD: 0.03	0.5	0.3	0.3
	LOQ: 0.06	1.3	0.7	0.7

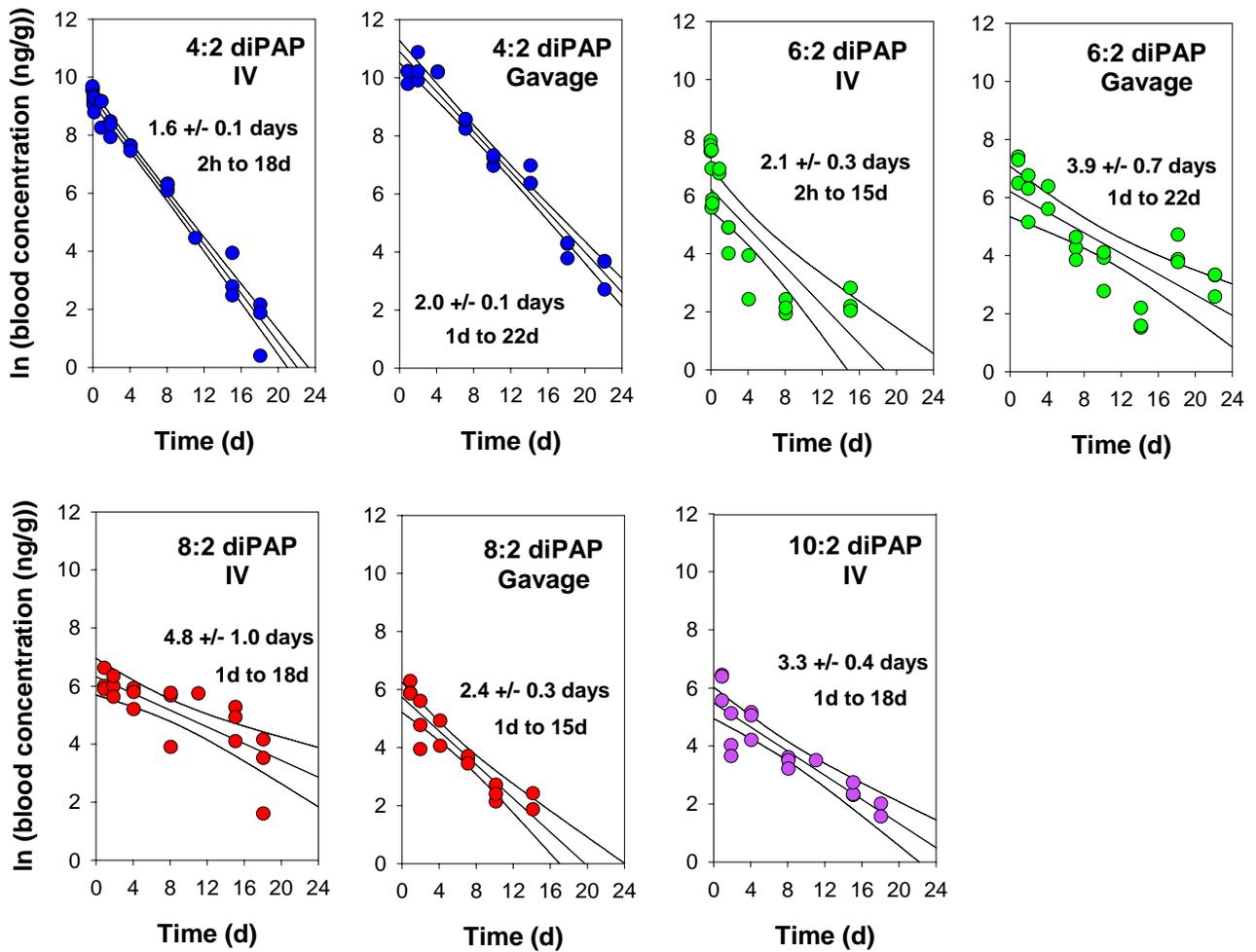
^a 4:2 monoPAP was not recoverable from the feces samples, and so no LOD or LOQ could be determined.



Supplemental Material, Figure 2. Sample chromatogram of the gavage dose administered to the monoPAP-dosed animals and that administered to the diPAP-dosed animals.



Supplemental Material, Figure 3. Arithmetic mean and standard error of PFCA concentrations observed in rats ($n = 3$) after monoPAP dose administered either via oral gavage or intravenously (IV). Values below the limit of detection are not shown on this plot, while those with any value below the limit of quantitation are indicated by an asterisk. (Note: The monoPAPs were dosed as a mixture, for clarity the PFCA biotransformation products are plotted together based on the expected parent monoPAP)



Supplemental Material, Figure 4. Plots to determine diPAP serum elimination half-lives.

Supplemental Material, Table 9. DiPAP half-lives after gavage and intravenous dosing.

Analyte	Half-life after intravenous dosing (days)	Half-life after gavage dosing (days)
4:2 diPAP	1.6 ± 0.1 ^a	2.0 ± 0.1 ^b
6:2 diPAP	2.1 ± 0.3 ^c	3.9 ± 0.7 ^d
8:2 diPAP	4.8 ± 1.0 ^e	2.4 ± 0.3 ^f
10:2 diPAP	3.3 ± 0.4 ^e	N/A ^g

^a Parameter was calculated using blood concentrations from 2h-18d post-dosing

^b Parameter was calculated using blood concentrations from 1d-22d post-dosing

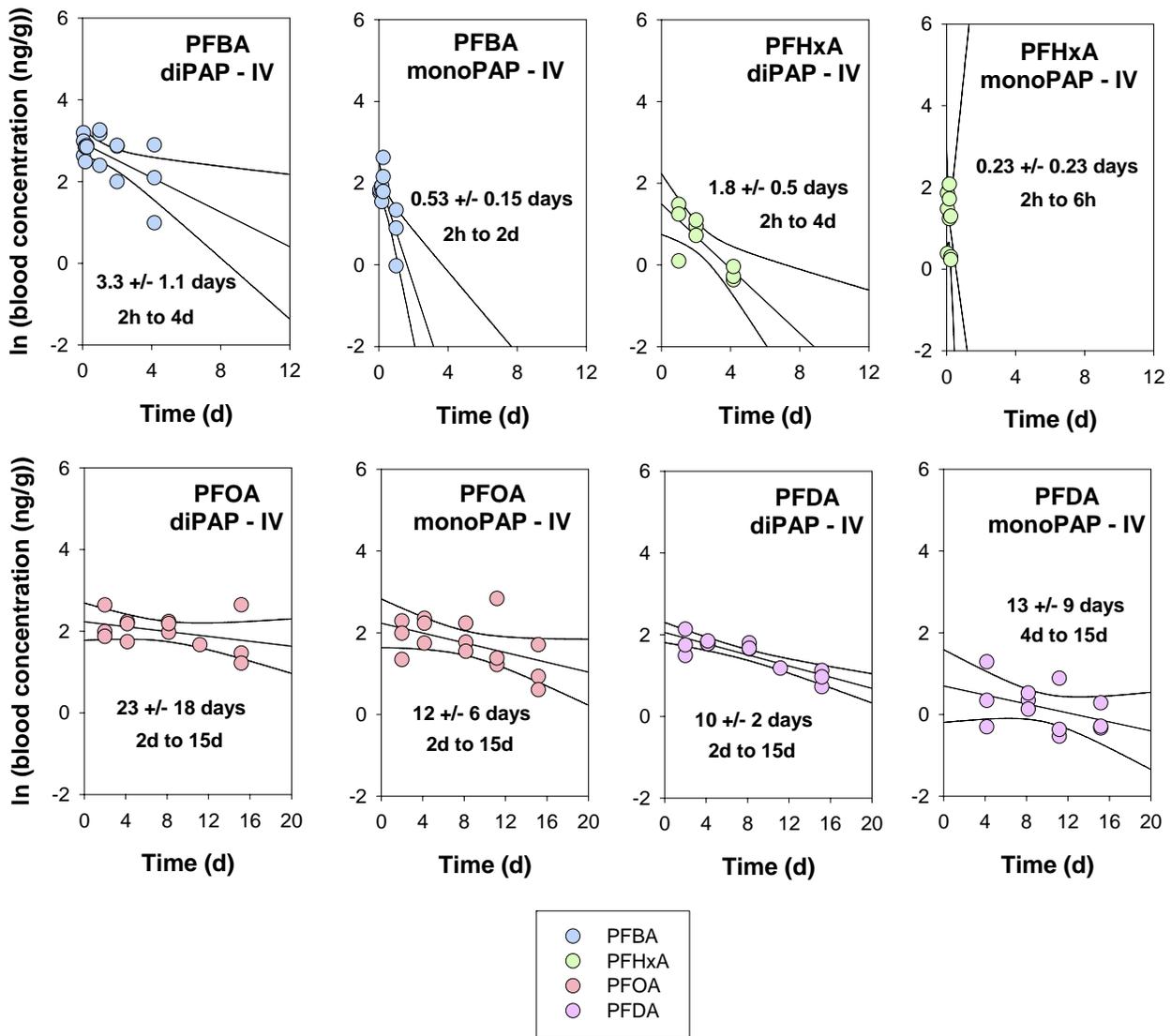
^c Parameter was calculated using blood concentrations from 2h-15d post-dosing

^d Parameter was calculated using blood concentrations from 1-22d post-dosing

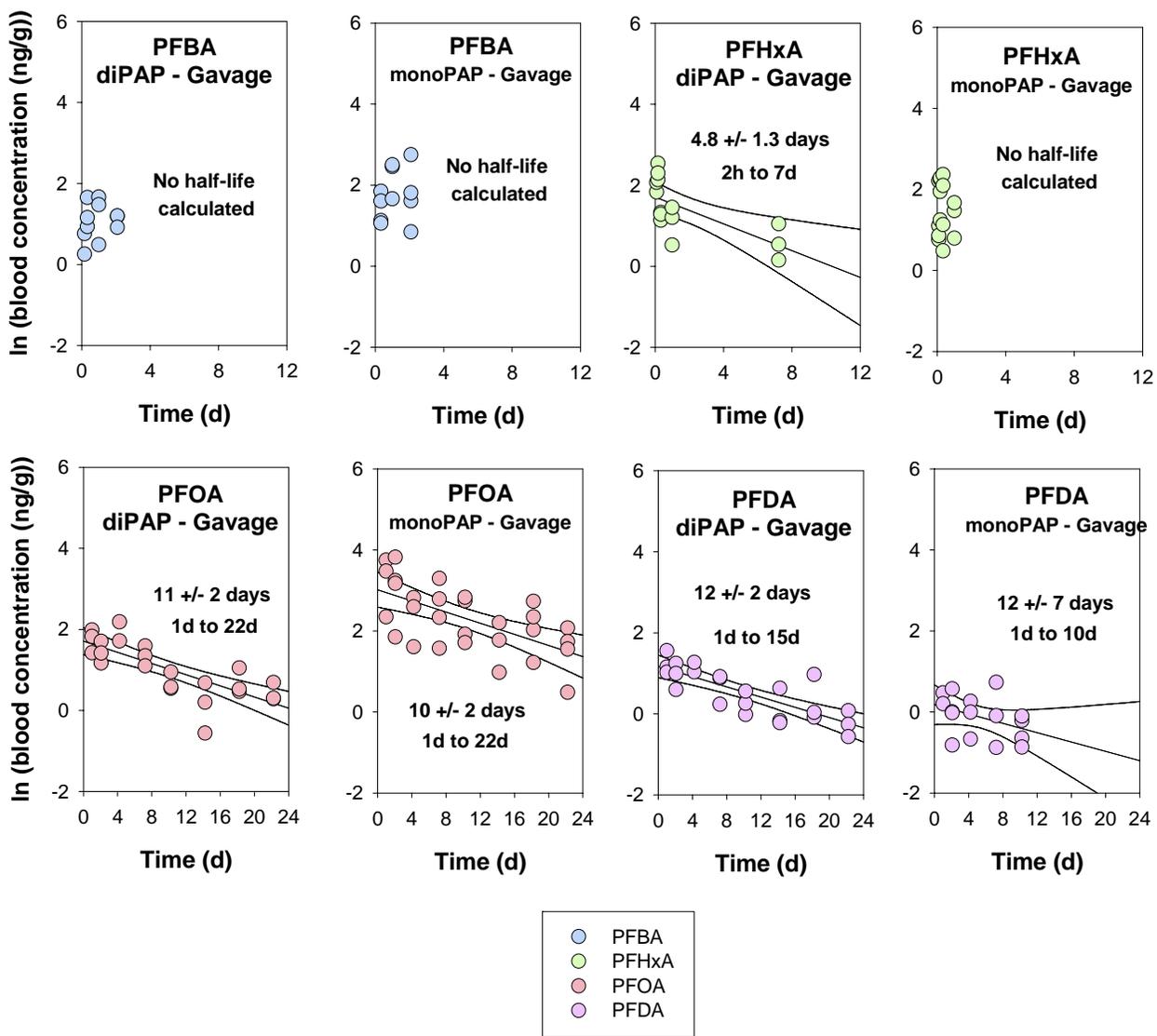
^e Parameter was calculated using blood concentrations from 1d-18d post-dosing

^f Parameter was calculated using blood concentrations from 1d-15d post-dosing

^g Parameter cannot be calculated because 10:2 diPAP was not observed above the limit of detection in the oral gavage experiment



Supplemental Material, Figure 5. Plots to determine the half-lives of the even chain length PFCAs after intravenous dose administration.

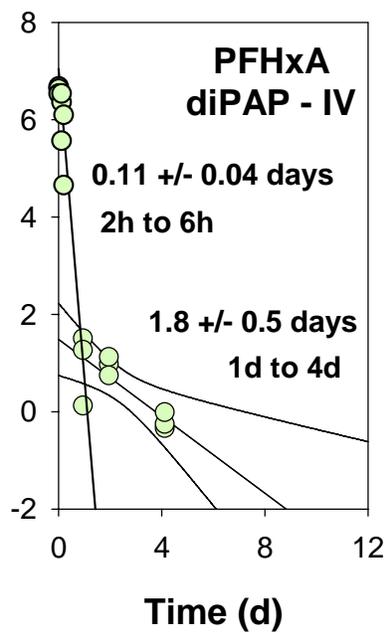


Supplemental Material, Figure 6. Plots to determine the half-lives of the even chain length PFCAs after gavage dose administration.

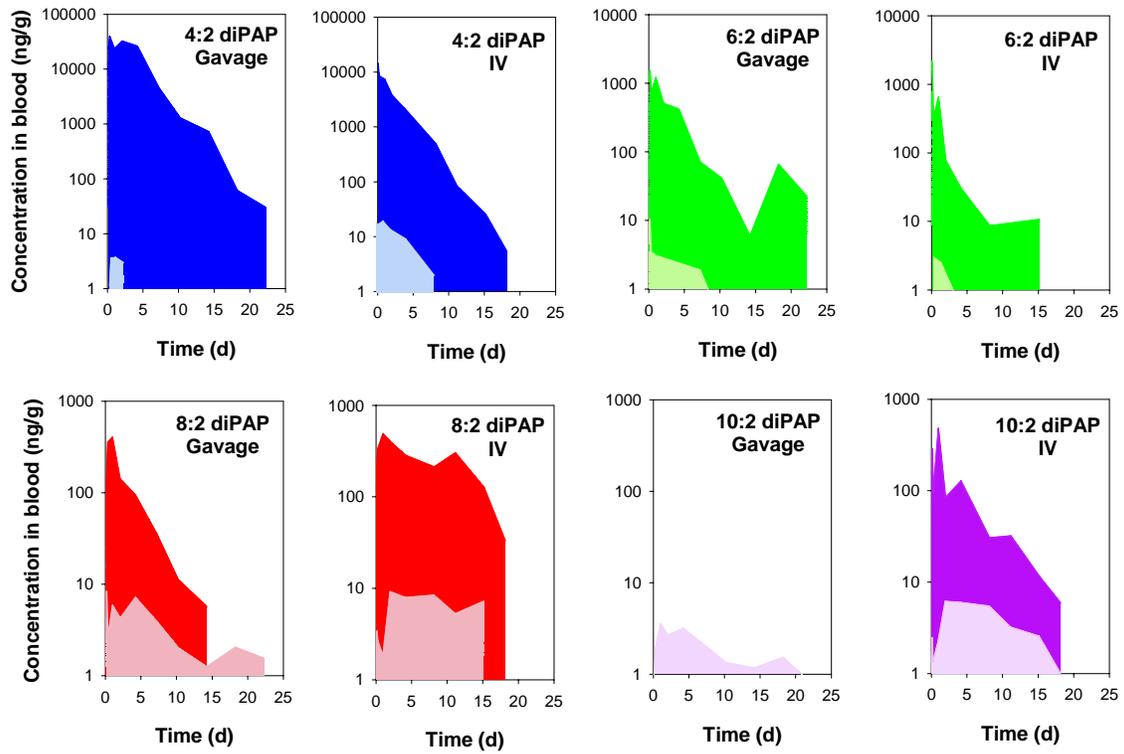
Supplemental Material, Table 10. Half-lives of the PFCA degradation products after gavage and intravenous dosing.

Analyte	Half-life (days)	
	diPAPs-dose	monoPAP-dose
Gavage Experiment		
PFBA	N/A ^a	N/A ¹
PFHxA	4.2 ± 1.3	N/A ¹
PFOA	11 ± 2	10 ± 2
PFDA	11 ± 2	12 ± 8
Intravenous Experiment		
PFBA	3.3 ± 1.2	0.53 ± 0.15
PFHxA	1.8 ± 0.51	0.23 ± 0.23
PFOA	23 ± 17	12 ± 6
PFDA	10 ± 2	13 ± 9

^a No clear decreasing trend observed for PFBA in the gavage experiments, and so an appropriate half-life could not be calculated.



Supplemental Material, Figure 7. Concentration-time plot of PFHxA in rats ($n = 3$) after administration of the intravenous dose of diPAP congener that was contaminated with PFHxA. The initial PFHxA, from dose contamination, and the subsequent half-life of PFHxA, from diPAP biotransformation are shown.



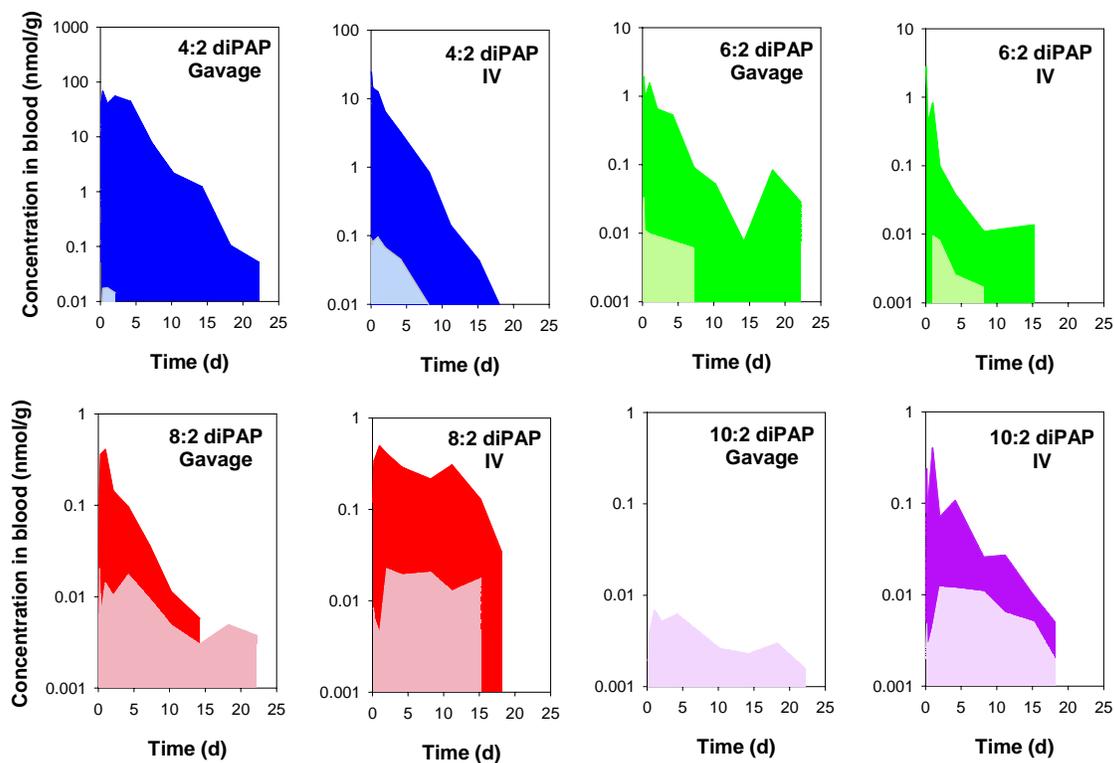
Supplemental Material, Figure 8. Pictorial representation of the area under the curve for the time period captured in the concentrations-time plots in blood (AUC_t) for both the diPAPs and the major even chain length PFCA biotransformation products after intravenous or gavage dosing. Concentrations are expressed in mass (ng/g).

Supplemental Material, Table 11. Area under the curve (AUC) using the blood concentrations expressed in mass (ng/g) for the diPAPs, and the resulting % bioavailability.

Analyte	AUC _t (ng days/g)	AUC _∞ ^a (ng days/g)	Bioavailability (%)
PAPs			
Uptake Experiment (gavage dose)			
4:2 diPAP	182,000	182,000	190
6:2 diPAP	4190	4250	74
8:2 diPAP	1150	1170	5
10:2 diPAP	N/A ^b	N/A ^{2b}	N/A ^b
Elimination Experiment (intravenous dose)			
4:2 diPAP	25,600	25,600	-
6:2 diPAP	1210	1220	-
8:2 diPAP	4410	4600	-
10:2 diPAP	1290	1320	-

^a Half-lives reported in Table S8 were used to calculate AUC_∞

^b Parameter cannot be calculated because 10:2 diPAP was not observed above the limit of detection in the oral gavage experiment



Supplemental Material, Figure 9. Pictoral representation of the area under the curve for the time period captured in the concentrations-time plots in blood (AUC_t) for both the diPAPs and the major even chain length PFCA biotransformation products after intravenous or gavage dosing. Concentrations are expressed in moles (nmol/g).

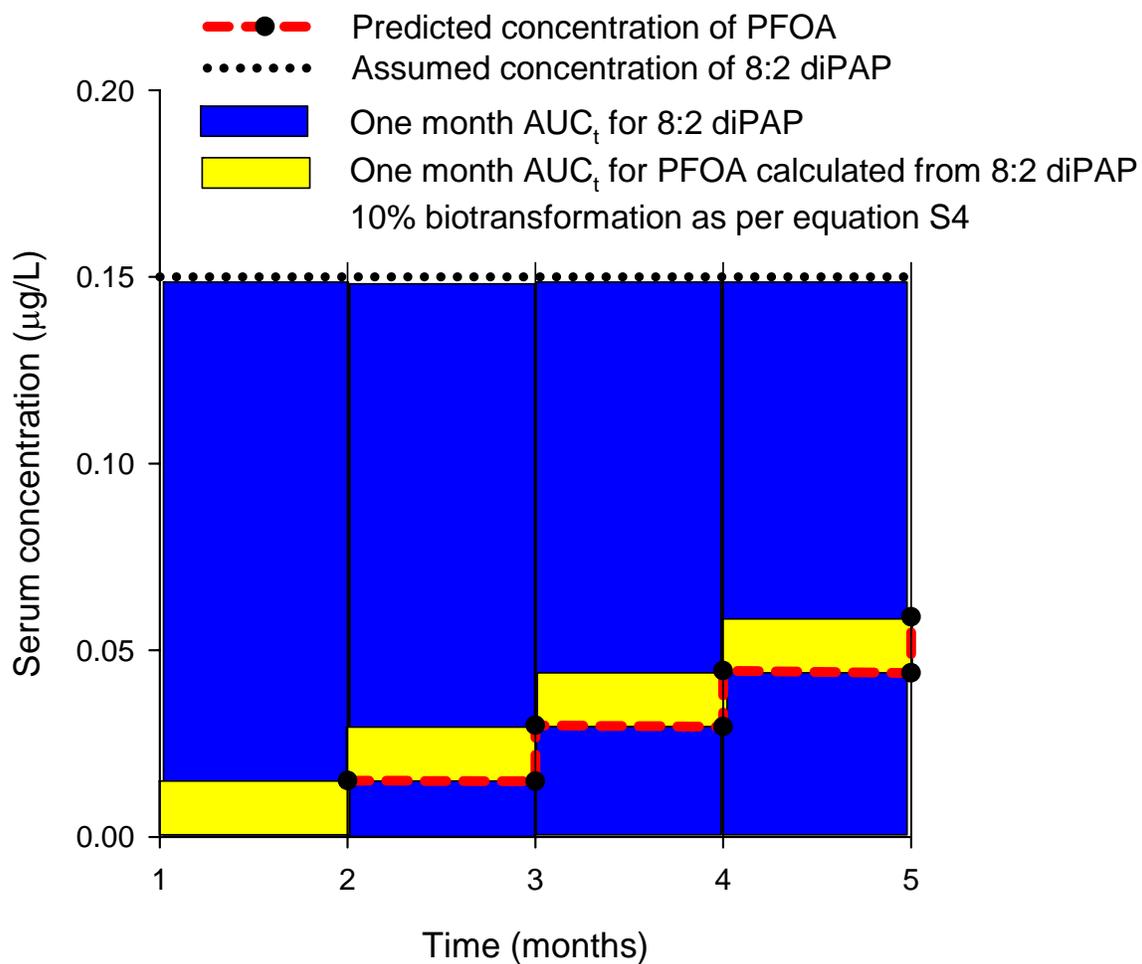
Supplemental Material, Table 12. Area under the curve (AUC) using blood concentrations expressed in moles (nmol/g) for the diPAPs and PFCAs, and the resulting % biotransformation.

Analyte	AUC _t (nmol days/g)	AUC _∞ ^a (nmol days/g)	Biotransformation (%)
PAPs			
Uptake Experiment (gavage dose)			
4:2 diPAP	309	309	-
6:2 diPAP	5.31	5.47	-
8:2 diPAP	1.17	1.19	-
10:2 diPAP	N/A ^b	N/A ^b	-
Elimination Experiment (intravenous dose)			
4:2 diPAP	43.5	43.5	-
6:2 diPAP	1.52	1.56	-
8:2 diPAP	4.46	4.69	-
10:2 diPAP	1.08	1.11	-
PFCAs			
Uptake Experiment (gavage dose)			
PFBA	0.0309	0.0326 ^c	0.005
PFHxA	0.0275	0.0645	0.6
PFOA	0.165	0.225	9
PFDA			
Elimination Experiment (intravenous dose)			
PFBA	0.394	0.440	0.5
PFHxA	0.0289	0.0333	1
PFOA	0.255	0.835	9
PFDA	0.141	0.170	8

^a Half-lives reported in Table 8 and 9 were used to calculate AUC_∞.

^b Parameter cannot be calculated because 10:2 diPAP was not observed above the limit of detection in the oral gavage experiment

^c No half-life was able to be calculated and so the literature value of 2 hours was used (Chang et al 2009)



Supplemental Material, Figure 10. PFOA sera concentrations predicted from a 10% biotransformation yield of 0.15 µg/L 8:2 diPAP in human sera over 5 months.

Supplemental Material, Table 13. Arithmetic mean and standard error for the diPAP and PFCA concentrations observed in the blood after intravenous diPAP dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after diPAP IV dose (ng/g)			
	4:2 diPAP	6:2 diPAP	8:2 diPAP	10:2 diPAP
2h	14,000 ± 1000	2200 ± 200	120 ± 40	150 ± 80
4h	9600 ± 800	1000 ± 500	270 ± 80	280 ± 40
6h	8400 ± 2100	320 ± 20	340 ± 50	96 ± 5
1d	7400 ± 1800	650 ± 260	490 ± 120	480 ± 110
2d	3800 ± 600	78 ± 26	400 ± 80	84 ± 38
4d	1900 ± 100	30 ± 19	290 ± 60	130 ± 30
8d	490 ± 30	(9 ± 1)	210 ± 80	41 ± 3
11d	83 ^a	nd ¹	300 ¹	32 ¹
15d	26 ± 12	11 ± 2	130 ± 40	12 ± 2
18d	(5.4 ± 2.1)	nd	33 ± 16	(5.9 ± 1.1)

Time	Analyte concentrations in blood after diPAP IV dose (ng/g)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
2h	19 ± 3	7.1 ± 0.6	730 ± 30 ^b	5.0 ± 0.3	3.4 ± 2.2	nd	(1.6 ± 0.1)	nd
4h	16 ± 2	1.3 ± 0.1	500 ± 120 ¹	7.3 ± 0.6	3.4 ± 0.5	(1.2 ± 0.2)	(2.8 ± 0.2)	nd
6h	17 ± 1	6.3 ± 4.9	270 ± 170 ¹	7.1 ± 0.1	2.7 ± 1.4	(1.2 ± 0.3)	(1.4 ± 0.1)	nd
1d	20 ± 5	(1.2 ± 0.2)	(3.0 ± 1.0)	5.3 ± 1.2	(1.8 ± 0.6)	(0.63 ± 0.10)	(2.6 ± 0.4)	nd
2d	14 ± 3	nd	2.5 ± 0.3	5.8 ± 1.0	9.3 ± 2.4	(1.3 ± 0.4)	6.2 ± 1.2	nd
4d	9.6 ± 4.5	nd	(0.80 ± 0.08)	3.4 ± 1.2	8.0 ± 1.1	(1.2 ± 0.2)	6.0 ± 0.1	nd
8d	(2 ± 2)	nd	(0.53 ± 0.03)	(0.84 ± 0.32)	8.5 ± 0.7	(1.9 ± 1.1)	5.5 ± 0.3	nd
11d	nd	nd	1.4	nd	5.3 ¹	nd	3.2 ¹	nd
15d	nd	nd	nd	nd	7.2 ± 3.4	(4.1 ± 3.3)	2.6 ± 0.3	nd
18d	nd	nd	nd	nd	(0.7 ± 0.7)	nd	(1.0 ± 0.6)	nd

^a Only one blood sample was available for analysis at the 11 day timepoint.

^b The high PFHxA concentrations observed in the first three timepoints after intravenous diPAP gavage dosing resulted from PFHxA contamination in the dose and not biotransformation. See text for details.

Supplemental Material, Table 14. Arithmetic mean and standard error for the metabolic intermediate concentrations observed in the blood after intravenous diPAP dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after diPAP IV dose (ng/g)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
2h	nd	nd	nd	nd	nd	nd	nd	nd
4h	nd	nd	nd	nd	nd	nd	nd	nd
6h	nd	nd	nd	nd	nd	nd	nd	nd
1d	nd	nd	nd	nd	nd	nd	nd	nd
2d	nd	nd	nd	nd	nd	nd	(8.3 ± 1.6)	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
8d	nd	nd	nd	nd	nd	nd	nd	nd
11d	nd	nd	nd	nd	nd	nd	nd	nd
15d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte concentrations in blood after diPAP IV dose (ng/g)			
	3:2 FTCA	5:3 FTCA ^a	7:3 FTCA	9:3 FTCA
2h	nd	57 ± 7	nd	nd
4h	nd	48 ± 5	nd	nd
6h	nd	62 ± 11	nd	nd
1d	nd	11 ± 1	nd	nd
2d	nd	(1.4 ± 0.7)	nd	nd
4d	nd	nd	nd	nd
8d	nd	(1.2 ± 0.7)	nd	nd
11d	nd	nd	nd	nd
15d	nd	nd	nd	nd
18d	nd	nd	nd	nd

^a 7:3 FTCA was used to quantify the 5:3 FTCA, the appropriateness of this assumption is not known, so the reported 5:3 FTCA concentrations should be considered approximate.

Supplemental Material, Table 15. Arithmetic mean and standard error for the phase II metabolite concentrations observed in the blood after intravenous diPAP dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after diPAP IV dose (ng/g)							
	4:2 FTOH-sulfate	6:2 FTOH-sulfate	8:2 FTOH-sulfate	10:2 FTOH-sulfate	4:2 FTOH-glucuronide	6:2 FTOH-glucuronide	8:2 FTOH-glucuronide	10:2 FTOH-glucuronide
2h	nd	nd	nd	nd	nd	nd	nd	nd
4h	nd	nd	nd	nd	nd	nd	nd	nd
6h	nd	nd	nd	nd	nd	nd	nd	nd
1d	nd	nd	nd	nd	nd	nd	nd	nd
2d	nd	nd	nd	nd	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
8d	nd	nd	nd	nd	nd	nd	nd	nd
11d	nd	nd	nd	nd	nd	nd	nd	nd
15d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd

Supplemental Material, Table 16. Arithmetic mean and standard error for the diPAP and PFCA concentrations observed in the blood after diPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after diPAP gavage dose (ng/g)			
	4:2 diPAP	6:2 diPAP	8:2 diPAP	10:2 diPAP
2h	26,000 ± 4000	1300 ± 100	31 ± 8	nd
4h	24,000 ± 13,000	1500 ± 500	140 ± 40	nd
6h	39,000 ± 4000	720 ± 190	360 ± 100	nd
1d	23,000 ± 3000	1200 ± 300	400 ± 60	nd
2d	32,000 ± 10,000	510 ± 190	140 ± 60	nd
4d	26,000 ± 1000	420 ± 160	95 ± 38	nd
7d	4600 ± 500	71 ± 15	36 ± 3	nd
10d	1300 ± 100	41 ± 13	11 ± 2	nd
14d	720 ± 160	5.9 ± 1.4	5.7 ± 3.1	nd
18d	61 ± 10	65 ± 21	nd	nd
22d	30 ± 7	22 ± 5	nd	nd

Time	Analyte concentrations in blood after diPAP gavage dose (ng/g)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
2h	nd	nd	7.1 ± 0.7	(0.97 ± 0.55)	2.3 ± 0.2	(1.1 ± 0.5)	(0.99 ± 0.05)	nd
4h	(1.1 ± 0.6)	nd	10 ± 1.2	(1.3 ± 0.67)	8.4 ± 3.7	(0.56 ± 0.56)	(1.1 ± 0.3)	nd
6h	3.6 ± 0.8	nd	3.5 ± 0.2	(1.6 ± 0.4)	(2.6 ± 0.8)	(0.71 ± 0.04)	2.0 ± 0.3	nd
1d	3.8 ± 1.0	nd	3.1 ± 0.8	2.6 ± 0.5	5.8 ± 0.9	(0.78 ± 0.04)	3.6 ± 0.6	nd
2d	3.0 ± 0.3	nd	nd	1.4 ± 0.1	4.3 ± 0.7	(0.80 ± 0.06)	2.7 ± 0.5	nd
4d	nd	nd	nd	nd	7.2 ± 1.7	3.0 ± 1.7	3.2 ± 0.4	nd
8d	nd	nd	1.9 ± 0.5	nd	3.9 ± 0.6	6.4 ± 5.2	2.1 ± 0.4	nd
11d	nd	nd	nd	nd	2.0 ± 0.3	nd	(1.3 ± 0.2)	nd
15d	nd	nd	nd	nd	(1.3 ± 0.4)	nd	(1.2 ± 0.4)	nd
18d	nd	nd	nd	nd	2.0 ± 0.4	(0.63 ± 0.40)	(1.5 ± 0.6)	nd
22d	nd	nd	nd	nd	1.5 ± 0.2	nd	0.81 ± 0.15	nd

Supplemental Material, Table 17. Arithmetic mean and standard error for the metabolic intermediate concentrations observed in the blood after diPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after diPAP gavage dose (ng/g)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
2h	nd	nd	nd	nd	nd	nd	nd	nd
4h	nd	nd	nd	nd	nd	nd	nd	nd
6h	73 ^a	120 ¹	36 ¹	65 ¹	nd	11 ¹	22 ± 7	nd
1d	nd	nd	nd	nd	nd	nd	nd	nd
2d	nd	nd	nd	nd	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
7d	nd	nd	nd	nd	nd	nd	nd	nd
10d	nd	nd	nd	nd	nd	nd	nd	nd
14d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd
22d	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte concentrations in blood after diPAP gavage dose (ng/g)			
	3:2 FTCA	5:3 FTCA	7:3 FTCA	9:3 FTCA
2h	nd	nd	nd	nd
4h	nd	nd	nd	nd
6h	nd	nd	nd	nd
1d	nd	nd	nd	nd
2d	nd	nd	nd	nd
4d	nd	nd	nd	nd
7d	nd	nd	nd	nd
10d	nd	nd	nd	nd
14d	nd	nd	nd	nd
18d	nd	nd	nd	nd
22d	nd	nd	nd	nd

^a The metabolic intermediates were only observed in the blood of one animal at this timepoint

Supplemental Material, Table 18. Arithmetic mean and standard error for the phase II metabolite concentrations observed in the blood after diPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after diPAP gavage dose (ng/g)							
	4:2 FTOH-sulfate	6:2 FTOH-sulfate	8:2 FTOH-sulfate	10:2 FTOH-sulfate ^a	4:2 FTOH-glucuronide	6:2 FTOH-glucuronide	8:2 FTOH-glucuronide	10:2 FTOH-glucuronide
2h	nd	nd	nd	nd	nd	nd	nd	nd
4h	nd	nd	nd	nd	nd	nd	nd	nd
6h	nd	nd	nd	nd	nd	nd	nd	nd
1d	nd	nd	nd	6.6 ± 1.8	nd	nd	nd	nd
2d	nd	nd	nd	3.8 ± 0.5	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
7d	nd	nd	nd	nd	nd	nd	nd	nd
10d	nd	nd	nd	nd	nd	nd	nd	nd
14d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd
22d	nd	nd	nd	nd	nd	nd	nd	nd

^a 8:2 FTOH-sulfate was used to quantify the 10:2 sulfate, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

Supplemental Material, Table 19. Arithmetic mean and standard error for the PFCA concentrations observed in the blood after intravenous monoPAP dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after monoPAP IV dose (ng/g)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
2h	6.0 ± 0.1	(1.3 ± 0.2)	4.1 ± 1.4	(0.82 ± 0.43)	(0.82 ± 0.43)	nd	nd	nd
4h	6.0 ± 0.7	(0.76 ± 0.39)	5.6 ± 1.3	(1.5 ± 1.0)	3.3 ± 1.3	(1.2 ± 0.4)	nd	nd
6h	7.3 ± 1.3	2.1 ± 0.8	(2.1 ± 0.1)	(0.87 ± 0.43)	(1.3 ± 0.1)	(0.46 ± 0.13)	nd	nd
1d	(2.4 ± 0.8)	(0.67 ± 0.34)	(1.8 ± 1.3)	2.3 ± 0.7	2.4 ± 1.2	(0.45 ± 0.15)	nd	nd
2d	nd	nd	(1.1 ± 0.8)	2.1 ± 0.9	7.0 ± 1.7	(0.84 ± 0.07)	(1.0 ± 0.2)	nd
4d	nd	nd	nd	nd	8.5 ± 1.4	(2.1 ± 1.0)	(1.4 ± 0.4)	nd
8d	nd	nd	nd	nd	6.6 ± 1.4	(1.7 ± 1.2)	(1.4 ± 0.2)	nd
11d	nd	nd	nd	nd	8.1 ± 4.5	(3.6 ± 3.0)	(1.2 ± 0.6)	nd
15d	nd	nd	nd	nd	3.3 ± 1.1	2.3 ± 0.4	0.93 ± 0.20	nd
18d	nd	nd	nd	nd	(0.6 ± 0.6)	nd	nd	nd

Supplemental Material, Table 20. Arithmetic mean and standard error for the metabolic intermediate concentrations observed in the blood after intravenous monoPAP dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after monoPAP IV dose (ng/g)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
2h	23 ± 3	2.0 ± 0.6	42 ± 6	6.5 ± 1.5	nd	nd	nd	nd
4h	nd	nd	nd	(1.1 ± 0.1)	nd	nd	nd	nd
6h	nd	nd	nd	(2.1 ± 0.8)	nd	nd	nd	nd
1d	nd	nd	nd	nd	nd	nd	nd	nd
2d	nd	nd	nd	nd	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
8d	nd	nd	nd	nd	nd	nd	nd	nd
11d	nd	nd	nd	nd	nd	nd	nd	nd
15d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte concentrations in blood after monoPAP IV dose (ng/g)			
	3:2 FTCA	5:3 FTCA ^a	7:3 FTCA	9:3 FTCA
2h	nd	2.9 ± 1.4	nd	nd
4h	nd	(1.1 ± 0.1)	nd	nd
6h	nd	(2.6 ± 0.7)	nd	nd
1d	nd	nd	nd	nd
2d	nd	nd	nd	nd
4d	nd	nd	nd	nd
8d	nd	nd	nd	nd
11d	nd	nd	nd	nd
15d	nd	nd	nd	nd
18d	nd	nd	nd	nd

^a 7:3 FTCA was used to quantify the 5:3 FTCA, the appropriateness of this assumption is not known, so the reported 5:3 FTCA concentrations should be considered approximate.

Supplemental Material, Table 21. Arithmetic mean and standard error for the phase II metabolite concentrations observed in the blood after intravenous monoPAP dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after monoPAP IV dose (ng/g)							
	4:2 FTOH-sulfate ^a	6:2 FTOH-sulfate	8:2 FTOH-sulfate	10:2 FTOH-sulfate	4:2 FTOH-glucuronide ^b	6:2 FTOH-glucuronide	8:2 FTOH-glucuronide	10:2 FTOH-glucuronide
2h	4.4 ± 0.3	nd	nd	nd	1500 ± 300	nd	nd	nd
4h	nd	nd	nd	nd	nd	nd	nd	nd
6h	nd	nd	nd	nd	nd	nd	nd	nd
1d	nd	nd	nd	nd	nd	nd	nd	nd
2d	nd	nd	nd	nd	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
8d	nd	nd	nd	nd	nd	nd	nd	nd
11d	nd	nd	nd	nd	nd	nd	nd	nd
15d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd

^a 8:2 sulfate was used to quantify the 4:2 sulfate, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^b 8:2 glucuronide was used to quantify the 4:2 glucuronide, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

Supplemental Material, Table 22. Arithmetic mean and standard error for the PFCA concentrations observed in the blood after monoPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after monoPAP gavage dose (ng/g)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
2h	nd	nd	4.2 ± 1,7	nd	3.9 ± 1.0	(1.1 ± 0.3)	nd	nd
4h	nd	nd	7.4 ± 1.5	2.0 ± 0.2	5.2 ± 0.6	nd	nd	nd
6h	(4.3 ± 0.8)	nd	5.9 ± 2.1	3.3 ± 0.7	14 ± 5	(1.1 ± 0.1)	(0.72 ± 0.53)	nd
1d	9.7 ± 1.9	nd	4.0 ± 0.7	9.0 ± 2.2	28 ± 8	(1.5 ± 0.09)	(0.94 ± 0.42)	nd
2d	7.3 ± 2.9	nd	2.5 ± 0.3	6.3 ± 2.9	25 ± 8	(1.5 ± 0.3)	(1.1 ± 0.27)	nd
4d	9.6 ± 4.5	nd	nd	(1.2 ± 0.5)	12 ± 3	(0.97 ± 0.41)	(0.94 ± 0.20)	nd
8d	nd	nd	nd	nd	15 ± 5	(0.95 ± 0.18)	(0.85 ± 0.45)	nd
11d	nd	nd	nd	nd	11 ± 3	(1.1 ± 0.2)	(0.66 ± 0.11)	nd
15d	nd	nd	nd	nd	5.9 ± 1,5	nd	nd	nd
18d	nd	nd	nd	nd	9.2 ± 2.5	nd	nd	nd
22d	nd	nd	nd	nd	5.0 ± 1.3	nd	nd	nd

Supplemental Material, Table 23. Arithmetic mean and standard error for the metabolic intermediate concentrations observed in the blood after monoPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after monoPAP gavage dose (ng/g)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
2h	nd	nd	(32 ± 9)	2.9 ± 1.0	(14 ± 7)	(1.0 ± 0.5)	nd	nd
4h	nd	nd	(53 ± 31)	(1.7 ± 1.2)	nd	nd	nd	nd
6h	nd	nd	(40 ± 13)	2.8 ± 1.0	nd	(1.3 ± 0.5)	nd	nd
1d	nd	nd	nd	nd	nd	nd	nd	nd
2d	nd	nd	nd	nd	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
7d	nd	nd	nd	nd	nd	nd	nd	nd
10d	nd	nd	nd	nd	nd	nd	nd	nd
14d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd
22d	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte concentrations in blood after monoPAP gavage dose (ng/g)			
	3:2 FTCA	5:3 FTCA ^a	7:3 FTCA	9:3 FTCA
2h	nd	6.6 ± 2.4	3.2 ± 1.5	nd
4h	nd	9.4 ± 2.6	4.8 ± 2.1	nd
6h	nd	8.8 ± 3.7	5.8 ± 2.3	nd
1d	nd	8.6 ± 3.8	5.1 ± 3.3	nd
2d	nd	4.5 ± 2.2	2.8 ± 1.5	nd
4d	nd	2.5 ^b	(1.7 ± 0.8)	nd
7d	nd	nd	nd	nd
10d	nd	nd	nd	nd
14d	nd	nd	nd	nd
18d	nd	nd	nd	nd
22d	nd	nd	nd	nd

^a 7:3 FTCA was used to quantify the 5:3 FTCA, the appropriateness of this assumption is not known, so the reported 5:3 FTCA concentrations should be considered approximate

^b The metabolic intermediates were only observed in the blood of one of the four animals at this timepoint

Supplemental Material, Table 24. Arithmetic mean and standard error for the phase II metabolite concentrations observed in the blood after monoPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte concentrations in blood after monoPAP gavage dose (ng/g)							
	4:2 FTOH-sulfate	6:2 FTOH-sulfate	8:2 FTOH-sulfate	10:2 FTOH-sulfate ^a	4:2 FTOH-glucuronide ^b	6:2 FTOH-glucuronide ²	8:2 FTOH-glucuronide	10:2 FTOH-glucuronide
2h	nd	nd	nd	nd	45 ± 25	34 ± 12	nd	nd
4h	nd	nd	nd	nd	nd	39 ^c	nd	nd
6h	nd	nd	(0.88 ± 0.15)	nd	65 ± 21	25 ± 10	nd	nd
1d	nd	nd	4.5 ± 1.2	9.0 ± 1.6	nd	nd	nd	nd
2d	nd	nd	2.1 ± 1.0	(3.5 ± 1.7)	nd	nd	nd	nd
4d	nd	nd	nd	nd	nd	nd	nd	nd
7d	nd	nd	nd	nd	nd	nd	nd	nd
10d	nd	nd	nd	nd	nd	nd	nd	nd
14d	nd	nd	nd	nd	nd	nd	nd	nd
18d	nd	nd	nd	nd	nd	nd	nd	nd
22d	nd	nd	nd	nd	nd	nd	nd	nd

^a 8:2 sulfate was used to quantify the 10:2 sulfate, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^b 8:2 glucuronide was used to quantify the 4:2 and 6:2 glucuronide, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^c 6:2 glucuronide was only observed in the blood of one of the four animals at this timepoint.

Supplemental Material, Table 25. Arithmetic mean and standard error for the mass of diPAPs and PFCAs observed in the urine after diPAP intravenous dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte mass in urine after diPAP IV dose (ng)			
	4:2 diPAP	6:2 diPAP	8:2 diPAP	10:2 diPAP
0h-2h	nd and 97 ^a	nd	nd	nd
2h-8h	nd and 48 ¹	nd	nd	nd
8h-24h	117 and 241 ^b	nd	nd	nd
24h-33h	144 and 126 ²	nd	nd	nd
33h-48h	nd	nd	nd	nd

Time	Analyte mass in urine after diPAP IV dose (ng)							
	PFBA	PFPeA	PFHxA ^c	PFHpA	PFOA	PFNA	PFDA	PFUnA
0h-2h	nd and 59 ¹	340 and 430 ¹	5900 and 11,000 ¹	nd and 2.5 ¹	nd	nd	nd	nd
2h-8h	850 and 28 ¹	25 and 170 ¹	2000 and 6100 ¹	0.7 and 4.8 ¹	nd	nd	nd	nd
8h-24h	520 ± 170	130 ± 40	16,000 ± 3000	110 ± 40	nd	nd	nd	nd
24h-33h	140 ± 20	16 ± 2	210 ± 74	10 ± 1	nd	nd	nd	nd
33h-48h	300 ± 10	46 ± 6	1800 ± 500	120 ± 10	nd	nd	nd	nd

^a Urine was only available from two out of three animal at this timepoint.

^b 4:2 diPAP was only observed in urine from two out of three animals at this timepoint.

^c PFHxA contamination of the diPAP intravenous dose likely resulted in the high concentrations of PFHxA in the first three urine timepoints.

Supplemental Material, Table 26. Arithmetic mean and standard error for the mass of metabolic intermediates and phase II metabolites observed in the urine after diPAP intravenous dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte mass in urine after diPAP IV dose (ng)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
0h-2h	nd	nd	nd	nd	nd	nd	nd	nd
2h-8h	nd	nd	nd	nd	nd	nd	nd	nd
8h-24h	nd	nd	nd	nd	nd	nd	nd	nd
24h-33h	nd	nd	nd	nd	nd	nd	nd	nd
33h-48h	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte mass in urine after diPAP IV dose (ng)			
	3:2 FTCA	5:3 FTCA	7:3 FTCA	9:3 FTCA
0h-2h	nd	nd	nd	nd
2h-8h	nd	nd	nd	nd
8h-24h	nd	nd	nd	nd
24h-33h	nd	nd	nd	nd
33h-48h	nd	nd	nd	nd

Time	Analyte mass in urine after diPAP IV dose (ng)							
	4:2 FTOH-sulfate ^a	6:2 FTOH-sulfate	8:2 FTOH-sulfate	10:2 FTOH-sulfate	4:2 FTOH-glucuronide ^b	6:2 FTOH-glucuronide	8:2 FTOH-glucuronide	10:2 FTOH-glucuronide
0h-2h	nd	nd	nd	nd	18,000 and 30,000 ¹	nd	nd	nd
2h-8h	nd	nd	nd	nd	32,000 and 40,000 ¹	nd	nd	nd
8h-24h	80 ± 41	nd	nd	nd	76,000 ± 38,000	nd	nd	nd
24h-33h	98 ± 43	nd	nd	nd	170,000 ± 37,000	nd	nd	nd
33h-48h	200 ± 60	nd	nd	nd	100,000 ± 10,000	nd	nd	nd

^a 8:2 sulfate was used to quantify the 4:2 sulfate, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^b 8:2 glucuronide was used to quantify the 4:2 glucuronide, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

Supplemental Material, Table 27. Arithmetic mean and standard error for the mass of diPAPs and PFCAs observed in the urine after diPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte mass in urine after diPAP gavage dose (ng)				
	4:2 diPAP	(4:2 dose %)	6:2 diPAP	8:2 diPAP	10:2 diPAP
0h-8h	34 and 690 ^a	(0.002%)	nd	nd	nd
8h-24h	740 ± 260	(0.004%)	nd	nd	nd
24h-33h	1700 ± 100	(0.01%)	nd	nd	nd
33h-48h	4600 ± 1100	(0.03%)	nd	nd	nd

Time	Analyte mass in urine after diPAP gavage dose (ng)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
0h-8h	7.4 and 30 ¹	33 and 110 ¹	270 and 1400 ¹	nd and 11 ¹	nd	nd	nd	nd
8h-24h	130 ± 20	62 ± 9	560 ± 40	38 ± 7	9.5 ± 1.0	nd	nd	nd
24h-33h	61 ± 11	17 ± 3	130 ± 10	19 ± 3	3.5 ± 0.7	nd	nd	nd
33h-48h	130 ± 20	37 ± 3	200 ± 40	47 ± 12	6.3 ± 4.0	nd	nd	nd

^a Urine was only collected from two out of three animal at this timepoint

Supplemental Material, Table 28. Arithmetic mean and standard error for the mass of metabolite intermediates and phase II metabolites observed in the urine after diPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte mass in urine after diPAP gavage dose (ng)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
0h-8h	nd	nd	nd	nd	nd	nd	nd	nd
8h-24h	nd	nd	nd	nd	nd	nd	nd	nd
24h-33h	nd	nd	nd	nd	nd	nd	nd	nd
33h-48h	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte mass in urine after diPAP gavage dose (ng)			
	3:2 FTCA	5:3 FTCA	7:3 FTCA	9:3 FTCA
0h-8h	nd	nd	nd	nd
8h-24h	nd	nd	nd	nd
24h-33h	nd	nd	nd	nd
33h-48h	nd	nd	nd	nd

Time	Analyte mass in urine after diPAP gavage dose (ng)							
	4:2 FTOH-sulfate ^a	6:2 FTOH-sulfate	8:2 FTOH-sulfate	10:2 FTOH-sulfate	4:2 FTOH-glucuronide ^b	6:2 FTOH-glucuronide	8:2 FTOH-glucuronide	10:2 FTOH-glucuronide
0h-8h	nd	nd	nd	nd	10,000 and 65,000 ^c	nd	nd	nd
8h-24h	nd	nd	nd	nd	nd	nd	nd	nd
24h-33h	80 ± 41	nd	nd	nd	20,000 ^d	nd	nd	nd
33h-48h	98 ± 43	nd	nd	nd	190,000 ± 20,000	nd	nd	nd

^a 8:2 sulfate was used to quantify the 4:2 sulfate, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^b 8:2 glucuronide was used to quantify the 4:2 glucuronide, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^c Urine was only collected from two out of three animal at this timepoint

^d Analyte was only observed in one of the urine samples.

Supplemental Material, Table 29. Arithmetic mean and standard error for the mass of PFCAs, metabolic intermediates and phase II metabolites observed in the urine after monoPAP intravenous dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte mass in urine after monoPAP IV dose (ng)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
0h-2h	59 and 200 ^a	8.2 and 20 ¹	140 and 150 ¹	0.9 and 3.4 ¹	nd	nd	nd	nd
2h-8h	88 ^b	9.2 ²	120 ²	2.4 ²	nd	nd	nd	nd
8h-24h	640 ± 60	120 ± 10	1000 ± 100	240 ± 30	nd	nd	nd	nd
24h-33h	190 ± 30	35 ± 14	100 ± 50	30 ± 4	nd	nd	nd	nd
33h-48h	220 ± 60	44 ± 27	290 ± 160	160 ± 30	nd	nd	nd	nd

Time	Analyte mass in urine after monoPAP IV dose (ng)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
0h-2h	14 and 21 ²	2.6 and 3.6 ²	nd	nd	nd	nd	nd	nd
2h-8h	32 ²	4.9 ²	nd	nd	nd	nd	nd	nd
8h-24h	nd	nd	nd	nd	nd	nd	nd	nd
24h-33h	nd	nd	nd	nd	nd	nd	nd	nd
33h-48h	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte mass in urine after monoPAP IV dose (ng)			
	3:3 FTCA	5:3 FTCA	7:3 FTCA	9:3 FTCA
all	nd	nd	nd	nd

Time	Analyte mass in urine after monoPAP IV dose (ng)							
	4:2 sulfate ^c	6:2 sulfate	8:2 sulfate	10:2 sulfate	4:2 gluc ^d	6:2 gluc ⁴	8:2 gluc	10:2 gluc
0h-2h	nd	nd	nd	nd	490,000 ± 90,000 ¹	1700 ± 300 ¹	nd	nd
2h-8h	nd	nd	nd	nd	670,000 ²	2700 ²	nd	nd
8h-24h	410 ± 60	nd	nd	nd	40,000 ± 18,000	nd	nd	nd
24h-33h	420 ± 60	nd	nd	nd	65,000 ± 15,000	nd	nd	nd
33h-48h	450 ± 60	nd	nd	nd	28,000 ± 10,000	nd	nd	nd

^a Urine was only collected from two out of three animals at this timepoint.

^b Urine was only collected from one out of three animals at this timepoint.

^c 8:2 sulfate was used to quantify the 4:2 sulfate.

^d 8:2 glucuronide was used to quantify the 4:2 and 6:2 glucuronide

Supplemental Material, Table 30. Arithmetic mean and standard error for the mass of PFCAs, metabolic intermediates and phase II metabolites observed in the urine after monoPAP gavage dosing. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Analyte mass in urine after monoPAP gavage dose (ng)							
	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA
0h-8h	37 ± 9	160 ± 10	470 ± 100	13 ± 3	nd	nd	nd	nd
8h-24h	400 ± 90	250 ± 110	2500 ± 800	140 ± 30	29 ± 9	nd	nd	nd
24h-33h	130 ± 30	59 ± 12	380 ± 100	80 ± 30	13 ± 3	nd	nd	nd
33h-48h	190 ± 30	56 ± 19	360 ± 70	150 ± 30	30 ± 11	nd	nd	nd

Time	Analyte mass in urine after monoPAP gavage dose (ng)							
	4:2 FTCA	4:2 FTUCA	6:2 FTCA	6:2 FTUCA	8:2 FTCA	8:2 FTUCA	10:2 FTCA	10:2 FTUCA
all	nd	nd	nd	nd	nd	nd	nd	nd

Time	Analyte mass in urine after monoPAP gavage dose (ng)			
	3:3 FTCA	5:3 FTCA	7:3 FTCA	9:3 FTCA
all	nd	nd	nd	nd

Time	Analyte mass in urine after monoPAP gavage dose (ng)							
	4:2 sulfate ^a	6:2 sulfate	8:2 sulfate	10:2 sulfate	4:2 gluc ^b	6:2 gluc ²	8:2 gluc	10:2 gluc
0h-2h	nd	nd	nd	nd	250,000 ± 80,000	2200 and 4800 ^c	nd	nd
2h-8h	nd	nd	nd	nd	nd	nd	nd	nd
8h-24h	410 ± 60	nd	nd	nd	100,000 ± 70,000	nd	nd	nd
24h-33h	420 ± 60	nd	nd	nd	39,000 ± 9000	nd	nd	nd
33h-48h	450 ± 60	nd	nd	nd	28,000 ± 10,000	nd	nd	nd

^a 8:2 sulfate was used to quantify the 4:2 sulfate, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^b 8:2 glucuronide was used to quantify the 4:2 and 6:2 glucuronide, the appropriateness of this assumption is not known, so the reported concentrations should be considered approximate.

^c Analyte was only observed in two of the urine samples.

Supplemental Material, Table 31. Arithmetic mean and standard error of the cumulative analyte mass observed in the feces. The percentage of the dose that can be accounted for by the mass excreted is also provided. For the purposes of calculating means, values below the limit of detection are assigned a value of zero and those below the limit of quantitation but above the limit of detection are used unaltered, but are indicated by parentheses.

Time	Cumulative analyte mass in feces after diPAP IV dose (μg)							
	4:2 diPAP	(dose %)	6:2 diPAP	(dose %)	8:2 diPAP	(dose %)	10:2 diPAP	(dose %)
8h-24h	76 \pm 3	2%	120 \pm 20	5%	10 \pm 3	0.5%	2.6 ^d	-
24h-33h	86 \pm 4	2%	140 \pm 10	6%	12 \pm 3	0.6%	1.4 \pm 0.9	0.03%
33h-48h	140 \pm 30	3% _s	220 \pm 10	9%	23 \pm 4	1%	1.8 \pm 0.8	0.04%

Time	Cumulative analyte mass in feces after diPAP gavage dose (μg)							
	4:2 diPAP	(dose %)	6:2 diPAP	(dose %)	8:2 diPAP	(dose %)	10:2 diPAP	(dose %)
0h-3h	0.35 \pm 0.14	0.002%	nd	-	nd	-	nd	-
3h-8h	10 \pm 10	0.06%	47 \pm 47	0.4%	17 \pm 17	0.2%	nd	-
8h-24h	3400 \pm 1000	20%	7100 \pm 1200	62%	3200 \pm 600	31%	nd	-
24h-48h	3500 \pm 1000	21%	7500 \pm 1100	65%	3600 \pm 700	34%	nd	-

Time	Cumulative analyte mass in feces after monoPAP IV dose (μg)							
	4:2 monoPAP	(dose %)	6:2 monoPAP	(dose %)	8:2 monoPAP	(dose %)	10:2 monoPAP	(dose %)
all	nd	-	nd	-	nd	-	nd	-

Time	Cumulative analyte mass in feces after monoPAP gavage dose (μg)							
	4:2 monoPAP	(dose %)	6:2 monoPAP	(dose %)	8:2 monoPAP	(dose %)	10:2 monoPAP	(dose %)
0h-3h	nd	-	nd	-	nd	-	nd	-
3h-24h ^e	nd	-	4.7 \pm 1.1	0.05%	52 \pm 16	0.9%	74 \pm 21	0.9%
24h-48h	nd	-	nd	-	nd	-	nd	-

^d 10:2 diPAP was only detected in one of the feces samples at this timepoint.

^e Of the four animals in the monoPAP gavage experiment, monoPAPs were observed in the 3h-8h feces sample for one animal and in the 8h-24h feces sample in the remaining three animals. As we expect this represents the time at which the gavage dose was observed in the feces these two timepoints were amalgamated into one 3h-24h mass excreted.