Quality Assurance Review of "A Model Template Approach for Rapid Evaluation of Physiologically Based Pharmacokinetic Models for use in Human Health Risk Assessments"

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Introduction

A quality assurance (QA) review of the PBPK model template described in the manuscript "A model template approach for rapid evaluation of physiologically based pharmacokinetic models for use in human health risk assessment" (Bernstein et al., 2021) has been conducted. This document describes how the QA criteria outlined in Section B of *An Umbrella Quality Assurance Project Plan (QAPP) for PBPK models* (U.S. EPA, 2018) have been met. The document is divided into sections that correspond to subsections of Section B of the QAPP. Excel (.xlsx) documents referenced in this document are available upon request by contacting Dustin Kapraun (kapraun.dustin@epa.gov) or Paul Schlosser (schlosser.paul@epa.gov).

B1: Data Review, Verification, Validation, and Usability

B1.1 ADME Data Evaluation and Selection

The following ADME data sets for PFHxS, PFNA, PFDA, PFOA, and PFOS were used by Bernstein et al. (2021) for purposes of comparison with model simulation output. These data sets were selected because PBPK models for PFHxS (Kim et al., 2018), PFNA (Kim et al., 2019), PFDA (Kim et al., 2019), PFOA (Loccisano et al., 2012), and PFOS (Loccisano et al., 2012) were used as case studies for the PBPK model template described by Bernstein et al. (2021).

- PFHxS concentration in plasma, liver, and kidney vs. time and cumulative amount of PFHxS excreted in urine vs. time for female rats given a single oral dose of 4 mg/kg (Kim et al., 2018).
- PFHxS concentration in plasma, liver, and kidney vs. time and cumulative amount of PFHxS excreted in urine vs. time for male rats given a single oral dose of 10 mg/kg (Kim et al., 2018).
- PFNA concentration in plasma, liver, and kidney vs. time and cumulative amount of PFNA excreted in urine vs. time for female rats given a single oral dose of 3 mg/kg (Kim et al., 2019).
- PFNA concentration in plasma, liver, and kidney vs. time and cumulative amount of PFNA excreted in urine vs. time for male rats given a single oral dose of 3 mg/kg (Kim et al., 2019).
- PFDA concentration in plasma, liver, and kidney vs. time and cumulative amount of PFDA excreted in urine vs. time for female rats given a single oral dose of 1 mg/kg (Kim et al., 2019).
- PFOA concentration in plasma and liver vs. time for male rats given a single intravenous dose of 0.041 mg/kg (Loccisano et al., 2012; Kudo et al., 2007).
- PFOA concentration in plasma and liver vs. time for male rats given a single intravenous dose of 16.56 mg/kg (Loccisano et al., 2012; Kudo et al., 2007).
- PFOA concentration in plasma vs. time and cumulative percentage of dose excreted in urine and feces vs. time for male rats given a single oral dose of 25 mg/kg (Loccisano et al., 2012; Kemper, 2003).
- PFOS concentration in plasma and liver vs. time for male rats given a single oral dose of 15 mg/kg (Loccisano et al., 2012).

The quality assurance procedures that were conducted to ensure fidelity in the extraction of these data sets are described in Section B1.2 of this document.

B1.2 Extraction of Quantitative ADME Data and PK Model Parameters

ADME Data

Quality assurance for the data sets shown in Figure 3 of Bernstein et al. (2021) was conducted as follows.

- PFHxS concentration in plasma vs. time for female rats given a single oral dose of 4 mg/kg. This data was extracted from Figure 8a of Kim et al. (2018) and was reproduced in Figure 3 (upper left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8a.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".
- PFHxS concentration in liver vs. time for female rats given a single oral dose of 4 mg/kg. This data was extracted from Figure 8b of Kim et al. (2018) and was reproduced in Figure 3 (upper right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8b.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".
- PFHxS concentration in kidney vs. time for female rats given a single oral dose of 4 mg/kg. This data was extracted from Figure 8c of Kim et al. (2018) and was reproduced in Figure 3 (lower left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8c.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".
- Cumulative amount of PFHxS excreted in urine vs. time for female rats a single oral dose of 4 mg/kg. This data was extracted from Figure 8f of Kim et al. (2018) and was reproduced in Figure 3 (lower right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8f.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".

Quality assurance for the data sets shown in Figure 4 of Bernstein et al. (2021) was conducted as follows.

- PFHxS concentration in plasma vs. time for male rats given a single oral dose of 10 mg/kg. This data was extracted from Figure 7a of Kim et al. (2018) and was reproduced in Figure 4 (upper left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7a.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".
- PFHxS concentration in liver vs. time for male rats given a single oral dose of 10 mg/kg. This data was extracted from Figure 7b of (Kim et al., 2018) and was reproduced in Figure 4 (upper right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7b.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".
- PFHxS concentration in kidney vs. time for male rats given a single oral dose of 10 mg/kg. This data was extracted from Figure 7c of (Kim et al., 2018) and was reproduced in Figure 4 (lower left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7c.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".
- Cumulative amount of PFHxS excreted in urine vs. time for male rats given a single oral dose of 10 mg/kg. This data was extracted from Figure 7f of (Kim et al., 2018) and was reproduced in Figure 4 (lower right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7f.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFHxS".

Quality assurance for the data sets shown in Figure 5 of Bernstein et al. (2021) was conducted as follows.

- PFNA concentration in plasma vs. time for female rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7a of Kim et al. (2019) and was reproduced in Figure 5 (upper left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7a.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".
- PFNA concentration in liver vs. time for female rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7c of Kim et al. (2019) and was reproduced in Figure 5 (upper right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7c.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".
- PFNA concentration in kidney vs. time for female rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7d of Kim et al. (2019) and was reproduced in Figure 5 (lower left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7d.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".
- Cumulative amount of PFNA excreted in urine vs. for female rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7b of Kim et al. (2019) and was reproduced in Figure 5 (lower right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7b.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".

Quality assurance for the data sets shown in Figure 6 of Bernstein et al. (2021) was conducted as follows.

- PFNA concentration in plasma vs. time for male rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7a of Kim et al. (2019) and was reproduced in Figure 6 (upper left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7a.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".
- PFNA concentration in liver vs. time for male rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7c of Kim et al. (2019) and was reproduced in Figure 6 (upper right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7c.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".
- PFNA concentration in kidney vs. time for male rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7d of Kim et al. (2019) and was reproduced in Figure 6 (lower left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7d.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".
- Cumulative amount of PFNA excreted in urine vs. for male rats given a single oral dose of 3 mg/kg. This data was extracted from Figure 7b of Kim et al. (2019) and was reproduced in Figure 6 (lower right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig7b.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFNA".

Quality assurance for the data sets shown in Figure 7 of Bernstein et al. (2021) was conducted as follows.

- PFDA concentration in plasma vs. time for female rats given a single oral dose of 1 mg/kg. This data was extracted from Figure 8a of Kim et al. (2019) and was reproduced in Figure 7 (upper left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8a.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFDA".
- PFDA concentration in liver vs. time for female rats given a single oral dose of 1 mg/kg. This data was extracted from Figure 8c of Kim et al. (2019) and was reproduced in Figure 7 (upper right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8c.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFDA".
- PFDA concentration in kidney vs. time for female rats given a single oral dose of 1 mg/kg. This data was extracted from Figure 8d of Kim et al. (2019) and was reproduced in Figure 7 (lower left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8d.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFDA".
- Cumulative amount of PFDA excreted in urine vs. for female rats given a single oral dose of 1 mg/kg. This data was extracted from Figure 8b of Kim et al. (2019) and was reproduced in Figure 7 (lower right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8b.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFDA".

Quality assurance for the data sets shown in Figure 8 of Bernstein et al. (2021) was conducted as follows.

- PFOA concentration in plasma vs. time for male rats given a single intravenous dose of 0.041 mg/kg. This data was extracted from Figure 8 (upper left panel) of Loccisano et al. (2012) and was reproduced in Figure 8 (left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8_Kudo_lowPlasma.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOA".
- PFOA concentration in liver vs. time for male rats given a single oral dose of 0.041 mg/kg. This data was extracted from Figure 8 (upper right panel) of Loccisano et al. (2012) and was reproduced in Figure 8 (right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8_Kudo_lowLiver.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOA".

Quality assurance for the data sets shown in Figure 9 of Bernstein et al. (2021) was conducted as follows.

- PFOA concentration in plasma vs. time for male rats given a single intravenous dose of 16.56 mg/kg. This data was extracted from Figure 8 (lower left panel) of Loccisano et al. (2012) and was reproduced in Figure 9 (left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8_Kudo_highPlasma.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOA".
- PFOA concentration in liver vs. time for male rats given a single oral dose of 16.56 mg/kg. This data was extracted from Figure 8 (lower right panel) of Loccisano et al. (2012) and was reproduced in Figure 9 (right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig8_Kudo_highLiver.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOA".

Quality assurance for the data sets shown in Figure 10 of Bernstein et al. (2021) was conducted as follows.

- PFOA concentration in plasma vs. time for male rats given a single oral dose of 25 mg/kg. This data was extracted from Figure 9 (lower left panel) of Loccisano et al. (2012) and was reproduced in Figure 10 (left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig9_Kemper_OraldosePlasma.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOA".
- Cumulative percentage of dose excreted in urine and feces vs. time for male rats given a single oral dose of 25 mg/kg. This data was extracted from Figure 9 (lower right panel) of Loccisano et al. (2012) and was reproduced in Figure 10 (right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig9_Kemper_OraldoseExcretion.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOA".

Quality assurance for the data sets shown in Figure 11 of Bernstein et al. (2021) was conducted as follows.

- PFOS concentration in plasma vs. time for male rats given a single oral dose of 15 mg/kg. This data was extracted from Figure 4 (upper left panel) of Loccisano et al. (2012) and was reproduced in Figure 11 (left panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig4_3M_OralPlasma.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOS".
- PFOS concentration in liver vs. time for male rats given a single oral dose of 15 mg/kg. This data was extracted from Figure 4 (lower left panel) of Loccisano et al. (2012) and was reproduced in Figure 11 (right panel) of Bernstein et al. (2021). Verification of fidelity in data extraction is provided in the file "Fig4_3M_OralLiver.xlsx" in the source code subdirectory called "Data/Digitized_Data_PFOS".

Model Parameters

In the tables that follow, parameter values used by Bernstein et al. (2021) are listed in the "Value" column, parameter values from the relevant published paper describing a model are listed in the "Value from Paper" column, and parameter values used in the source code provided by the paper's authors are listed in the "Value from Source Code" column. When the value in the paper differs from the value in the source code, Bernstein et al. (2021) used the value from the source code (except where otherwise noted). When the value in the paper and the value in the authors' source code differs by a small amount (usually because of rounding to a different number of significant figures), we highlighted the number in the "Value from Paper" column in pink. When the value in the paper and the value in the authors' source code differs by a larger, more significant amount, we highlighted the number in the "Value from Paper" or "Value from Source Code" column (whichever is assumed to be incorrect) in red. In many cases, a large discrepancy was found when different units (e.g., mg vs. g) were used or reported in the paper and the source code; in these cases, the discrepancy may have just been a reporting error or a typographical error.

PFHxS PBPK model parameters for female rats

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFHxS PBPK model of Kim et al. (2018) for female rats in the spreadsheet document "PFAS_template_parameters_PFHxS.xlsx" on the worksheet "FKimRecreateBW". These values were taken from the paper (Kim et al., 2018) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the source code were used. The general parameter values for simulations of female rats are provided in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code
sim.time	End Time of Simulation	days	14	14	340 / 24
Q_cardiacc	Cardiac Output	mL/h/BW^.75	7297.341982	2580 / $0.25^{0.75}$	N/A
hcrit	Hematocrit		N/A	N/A	N/A
BW	Body Weight	kg	0.25	0.25	N/A
dose	Dose	ng/kg BW	4000000	4 * 10^6	N/A
F_free	Free Fraction (initial value)		0.000694	0.00069	0.000694
delta	free fraction adjustment constant		N/A	N/A	N/A
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	N/A	N/A	N/A
F_unabs	Fraction Unabsorbed		0.61	N/A	0.61
T_mc	Transport Maximum	ng/h/BW^0.75	2.57×10^3	910 * 10^3 / $0.25^{0.75}$	910 / $0.25^{0.75}$
K_t	Transport Affinity Constant	ng/mL	1.59×10^4	15851 * 10^3	15851
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	N/A	N/A	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	0.417900108	0.591 / $0.25^{-0.25}$	0.591 / $0.25^{-0.25}$
K_f	Rate Constant to Feces from GI	1/h	2.240280702	36.8 * 0.00069 / 0.0114 (Free * Kf / Kgi)	36.8 * 0.000694 / 0.0114 (Free * Kf / Kgi)
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	N/A	N/A	N/A
K_abs	Oral Absorption Rate	1/h	3.73	3.73	3.73
K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	N/A	N/A	N/A
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	81.81225458	115.7 / $0.25^{-0.25}$	115.7 / $0.25^{-0.25}$
K_b	affinity constant for liver binding (saturable)	ng/mL	N/A	N/A	N/A
Bmax	maximum binding capacity (liver, saturable)	ng/mL	N/A	N/A	N/A
k_off	dissociation rate constant (liver)	1/h	N/A	N/A	N/A

The compartment-specific values for simulations of female rats are provided in the table below.

Code	Model parameter	Units	Value	Value from Paper	Value from Source Code
V_blc	plasma volume fraction	L/kg BW	0.0816	20.4 * 10^{-3} / 0.25	20.4 * 10^{-3} / 0.25
V_gic	GI volume fraction	L/kg BW	0.04	10.0 * 10^{-3} / 0.25	10.0 * 10-3 / 0.25
Q_gic	GI blood flow fraction	fraction of Q_cardiac	0.174418605	450 / 2580	450 / 2580

K_gi	GI:blood partition coefficient	none	0.0114	shown in Fig. 5	0.0114
V_lic	liver volume fraction	L/kg BW	0.032	8.0 * 10^{-3} / 0.25	8.0 * 10^{-3} / 0.25
Q_lic	liver blood flow fraction	fraction of Q_cardiac	0.0320930233	828 / 2580	828 / 2580
K_li	liver:blood partition coefficient	none	0.0704	shown in Fig. 5	0.0704
V_kic	kidney volume fraction	L/kg BW	0.0072	1.8 * 10^{-3} / 0.25	1.8 * 10^{-3} / 0.25
Q_kic	kidney blood flow fraction	fraction of Q_cardiac	0.201550388	520 / 2580	520 / 2580
K_ki	kidney:blood partition coefficient	none	0.047	shown in Fig. 5	0.047
V_filc	filtrate volume fraction	L/kg BW	0.00072	0.18 * 10^{-3} / 0.25	0.18 * 10^{-3} / 0.25
Q_filc	filtrate blood flow fraction	fraction of Q_cardiac	0.1007752	260 / 2580	260 / 2580
V_st1c	[lung] volume fraction	L/kg BW	0.004	1.0 * 10^{-3} / 0.25	1.0 * 10^{-3} / 0.25
Q_st1c	[lung] blood flow fraction	fraction of Q_cardiac	1.0	2580 / 2580	2580 / 2580
K_st1	[lung]:blood partition coefficient	none	0.0358	shown in Fig. 5	0.0358
V_st2c	[heart] volume fraction	L/kg BW	0.0032	0.8 * 10^{-3} / 0.25	0.8 * 10^{-3} / 0.25
Q_st2c	[heart] blood flow fraction	fraction of Q_cardiac	0.090697674	234 / 2580	234 / 2580
K_st2	[heart]:blood partition coefficient	none	0.0176	shown in Fig. 5	0.0176
V_st3c	[brain] volume fraction	L/kg BW	0.0028	0.7 * 10^{-3} / 0.25	0.7 * 10^{-3} / 0.25
Q_st3c	[brain] blood flow fraction	fraction of Q_cardiac	0.006976744	18 / 2580	18 / 2580
K_st3	[brain]:blood partition coefficient	none	0.0028	shown in Fig. 5	0.005
V_rbc	rest-of-body volume fraction	L/kg BW	0.5332	(10 + 122 + 1.3) * 10^{-3} / 0.25	133.3 * 10^{-3} / 0.25
Q_rbc	rest-of-body blood flow fraction	fraction of Q_cardiac	0.197674419	(450 + 24 + 36) / 2580	510 / 2580
K_rb	rest-of-body:blood partition coefficient	none	0.0161	0.027	0.0161

PFHxS PBPK model parameters for <u>male</u> rats

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFHxS PBPK model of Kim et al. (2018) for <u>male</u> rats in the spreadsheet document "PFAS_template_parameters_PFHxS.xlsx" on the worksheet "MKimRecreateBW". These values were taken from the paper (Kim et al., 2018) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the source code were used. The general parameter values for simulations of <u>male</u> rats are provided in the table below.

Code	**Model Parameter**	**Units**	**Value**	**Value from Paper**	**Value from Source Code**
sim.time	End Time of Simulation	days	14	14	340 / 24
Q_cardiacc	Cardiac Output	mL/h/BW^.75	7297.341982	2580 / $0.25^{0.75}$	N/A
hcrit	Hematocrit		N/A	N/A	N/A
BW	Body Weight	kg	0.25	0.25	N/A
dose	Dose	ng/kg BW	10000000	10 * 10^6	N/A
F_free	Free Fraction (initial value)		0.000758	0.00076	0.000758

delta	free fraction adjustment constant		N/A	N/A	N/A
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	N/A	N/A	N/A
F_unabs	Fraction Unabsorbed		0.47	N/A	0.47
T_mc	Transport Maximum	ng/h/BW^0.75	2.41×10^5	$85300 * 10^3 / 0.25^{0.75}$	$85300 / 0.25^{0.75}$
K_t	Transport Affinity Constant	ng/mL	1.59×10^4	$15851 * 10^3$	15851
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	N/A	N/A	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	0.176776695	$0.25 / 0.25^{-0.25}$	$0.25 / 0.25^{-0.25}$
K_f	Rate Constant to Fecal Storage from GI	1/h	0.000522759	0.02 * 0.00076 / 0.029 (Free * Kf / Kgi)	0.02 * 0.000758 / 0.029 (Free * Kf / Kgi)
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	N/A	N/A	N/A
K_abs	Oral Absorption Rate	1/h	3.73	3.73	3.73
K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	N/A	N/A	N/A
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	7.424621202	$10.5 / 0.25^{-0.25}$	$10.5 / 0.25^{-0.25}$
K_b	affinity constant for liver binding (saturable)	ng/mL	N/A	N/A	N/A
Bmax	maximum binding capacity (liver, saturable)	ng/mL	N/A	N/A	N/A
k_off	dissociation rate constant (liver)	1/h	N/A	N/A	N/A

The compartment-specific values for simulations of <u>male</u> rats are provided in the table below.

Code	**Model parameter**	**Units**	**Value**	**Value from Paper**	**Value from Source Code**
V_blc	plasma volume fraction	L/kg BW	0.0816	$20.4 * 10^{-3} / 0.25$	$20.4 * 10^{-3} / 0.25$
V_gic	GI volume fraction	L/kg BW	0.04	$10.0 * 10^{-3} / 0.25$	10.0 * 10-3 / 0.25
Q_gic	GI blood flow fraction	fraction of Q_cardiac	0.174418605	450 / 2580	450 / 2580
K_gi	GI:blood partition coefficient	none	0.029	shown in Fig. 5	0.029
V_lic	liver volume fraction	L/kg BW	0.032	$8.0 * 10^{-3} / 0.25$	$8.0 * 10^{-3} / 0.25$
Q_lic	liver blood flow fraction	fraction of Q_cardiac	0.0320930233	828 / 2580	828 / 2580
K_li	liver:blood partition coefficient	none	0.127	shown in Fig. 5	0.127
V_kic	kidney volume fraction	L/kg BW	0.0072	$1.8 * 10^{-3} / 0.25$	$1.8 * 10^{-3} / 0.25$
Q_kic	kidney blood flow fraction	fraction of Q_cardiac	0.201550388	520 / 2580	520 / 2580
K_ki	kidney:blood partition coefficient	none	0.065	shown in Fig. 5	0.065
V_filc	filtrate volume fraction	L/kg BW	0.00072	$0.18 * 10^{-3} / 0.25$	$0.18 * 10^{-3} / 0.25$
Q_filc	filtrate blood flow fraction	fraction of Q_cardiac	0.1007752	260 / 2580	260 / 2580
V_st1c	[lung] volume fraction	L/kg BW	0.004	$1.0 * 10^{-3} / 0.25$	$1.0 * 10^{-3} / 0.25$
Q_st1c	[lung] blood flow fraction	fraction of Q_cardiac	1.0	2580 / 2580	2580 / 2580

K_st1	[lung]:blood partition coefficient	none	0.068	shown in Fig. 5	0.068
V_st2c	[heart] volume fraction	L/kg BW	0.0032	0.8 * 10^{-3} / 0.25	0.8 * 10^{-3} / 0.25
Q_st2c	[heart] blood flow fraction	fraction of Q_cardiac	0.090697674	234 / 2580	234 / 2580
K_st2	[heart]:blood partition coefficient	none	0.033	shown in Fig. 5	0.033
V_st3c	[brain] volume fraction	L/kg BW	0.0028	0.7 * 10^{-3} / 0.25	0.7 * 10^{-3} / 0.25
Q_st3c	[brain] blood flow fraction	fraction of Q_cardiac	0.006976744	18 / 2580	18 / 2580
K_st3	[brain]:blood partition coefficient	none	0.005	shown in Fig. 5	0.005
V_rbc	rest-of-body volume fraction	L/kg BW	0.5332	(10 + 122 + 1.3) * 10^{-3} / 0.25	133.3 * 10^{-3} / 0.25
Q_rbc	rest-of-body blood flow fraction	fraction of Q_cardiac	0.197674419	(450 + 24 + 36) / 2580	510 / 2580
K_rb	rest-of-body:blood partition coefficient	none	0.027	0.045	0.027

PFNA PBPK model parameters for female rats

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFNA PBPK model of Kim et al. (2019) for female rats in the spreadsheet document "PFAS_template_parameters_PFNA.xlsx" on the worksheet "FKimRecreateBW". These values were taken from the paper (Kim et al., 2019) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the source code were used. The general parameter values for simulations of female rats are provided in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code
sim.time	End Time of Simulation	days	60	60	1440 / 24
Q_cardiacc	Cardiac Output	mL/h/BW^.75	7297.341982	2580 / $0.25^{0.75}$	N/A
hcrit	Hematocrit		N/A	N/A	N/A
BW	Body Weight	kg	0.25	0.25	N/A
dose	Dose	ng/kg BW	3000000	3 * 10^6	N/A
F_free	Free Fraction (initial value)		0.00332	0.00332	0.00332
delta	free fraction adjustment constant		N/A	N/A	N/A
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	N/A	N/A	N/A
F_unabs	Fraction Unabsorbed		0.47	N/A	0.47
T_mc	Transport Maximum	ng/h/BW^0.75	9.77 × 10^4	34530 * 10^3 / $0.25^{0.75}$	34530 / $0.25^{0.75}$
K_t	Transport Affinity Constant	ng/mL	2.49 × 10^4	24850 * 10^3	24850
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	N/A	N/A	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	6.243752878	8.83 / $0.25^{-0.25}$	8.83 / $0.25^{-0.25}$
K_f	Rate Constant to Fecal Storage from GI	1/h	0.51704918	0.00332 * 0.95 / 0.0061 (Free * Kf / Kgi)	0.00332 * 0.95 / 0.0061 (Free * Kf / Kgi)
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	N/A	N/A	N/A
K_abs	Oral Absorption Rate	1/h	1.2	1.20	1.2

K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	N/A	N/A	N/A
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	1.42128463	2.01 / $0.25^{-0.25}$	2.01 / $0.25^{-0.25}$
K_b	affinity constant for liver binding (saturable)	ng/mL	N/A	N/A	N/A
Bmax	maximum binding capacity (liver, saturable)	ng/mL	N/A	N/A	N/A
k_off	dissociation rate constant (liver)	1/h	N/A	N/A	N/A

The compartment-specific values for simulations of <u>female</u> rats are provided in the table below.

Code	**Model parameter**	**Units**	**Value**	**Value from Paper**	**Value from Source Code**
V_blc	plasma volume fraction	L/kg BW	0.0816	20.4 * 10^{-3} / 0.25	20.4 * 10^{-3} / 0.25
V_gic	GI volume fraction	L/kg BW	0.04	10.0 * 10^{-3} / 0.25	10.0 * 10-3 / 0.25
Q_gic	GI blood flow fraction	fraction of Q_cardiac	0.174418605	450 / 2580	450 / 2580
K_gi	GI:blood partition coefficient	none	0.0061	shown in Fig. 5a	0.0061
V_lic	liver volume fraction	L/kg BW	0.032	8.0 * 10^{-3} / 0.25	8.0 * 10^{-3} / 0.25
Q_lic	liver blood flow fraction	fraction of Q_cardiac	0.0320930233	828 / 2580	828 / 2580
K_li	liver:blood partition coefficient	none	0.4665	shown in Fig. 5a	0.4665
V_kic	kidney volume fraction	L/kg BW	0.0072	1.8 * 10^{-3} / 0.25	1.8 * 10^{-3} / 0.25
Q_kic	kidney blood flow fraction	fraction of Q_cardiac	0.201550388	520 / 2580	520 / 2580
K_ki	kidney:blood partition coefficient	none	0.2471	shown in Fig. 5a	0.2471
V_filc	filtrate volume fraction	L/kg BW	0.00072	0.18 * 10^{-3} / 0.25	0.18 * 10^{-3} / 0.25
Q_filc	filtrate blood flow fraction	fraction of Q_cardiac	0.1007752	260 / 2580	260 / 2580
V_st1c	[lung] volume fraction	L/kg BW	0.004	1.0 * 10^{-3} / 0.25	1.0 * 10^{-3} / 0.25
Q_st1c	[lung] blood flow fraction	fraction of Q_cardiac	1.0	2580 / 2580	2580 / 2580
K_st1	[lung]:blood partition coefficient	none	0.0562	shown in Fig. 5a	0.0562
V_st2c	[heart] volume fraction	L/kg BW	0.0032	0.8 * 10^{-3} / 0.25	0.8 * 10^{-3} / 0.25
Q_st2c	[heart] blood flow fraction	fraction of Q_cardiac	0.090697674	234 / 2580	234 / 2580
K_st2	[heart]:blood partition coefficient	none	0.0339	shown in Fig. 5a	0.0339
V_st3c	[brain] volume fraction	L/kg BW	N/A	N/A	N/A
Q_st3c	[brain] blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_st3	[brain]:blood partition coefficient	none	N/A	N/A	N/A
V_rbc	rest-of-body volume fraction	L/kg BW	0.536	(10 + 122 + 1.3 + 0.7) * 10^{-3} / 0.25	134 * 10^{-3} / 0.25
Q_rbc	rest-of-body blood flow fraction	fraction of Q_cardiac	0.204651163	(450 + 24 + 36 + 18) / 2580	528 / 2580

K_rb	rest-of-body:blood partition coefficient	none	0.0127	N/A	0.0127

PFNA PBPK model parameters for male rats

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFNA PBPK model of Kim et al. (2019) for male rats in the spreadsheet document "PFAS_template_parameters_PFNA.xlsx" on the worksheet "MKimRecreateBW". These values were taken from the paper (Kim et al., 2019) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the source code were used. The general parameter values for simulations of male rats are provided in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code
sim.time	End Time of Simulation	days	60	60	1440 / 24
Q_cardiacc	Cardiac Output	mL/h/BW^.75	7297.341982	2580 / $0.25^{0.75}$	N/A
hcrit	Hematocrit		N/A	N/A	N/A
BW	Body Weight	kg	0.25	0.25	N/A
dose	Dose	ng/kg BW	3000000	3 * 10^6	N/A
F_free	Free Fraction (initial value)		0.00272	0.00272	0.00272
delta	free fraction adjustment constant		N/A	N/A	N/A
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	N/A	N/A	N/A
F_unabs	Fraction Unabsorbed		0.6	N/A	0.6
T_mc	Transport Maximum	ng/h/BW^0.75	2.29×10^5	81030 * 10^3 / $0.25^{0.75}$	81030 / $0.25^{0.75}$
K_t	Transport Affinity Constant	ng/mL	2.49×10^4	24850 * 10^3	24850
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	N/A	N/A	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	2.48901587	3.52 / $0.25^{-0.25}$	3.52 / $0.25^{-0.25}$
K_f	Rate Constant to Fecal Storage from GI	1/h	0.502787879	0.00272 * 1.22 / 0.0066 (Free * Kf / Kgi)	0.00272 * 1.22 / 0.0066 (Free * Kf / Kgi)
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	N/A	N/A	N/A
K_abs	Oral Absorption Rate	1/h	6.24	6.24	6.24
K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	N/A	N/A	N/A
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	0.262336616	0.371 / $0.25^{-0.25}$	0.371 / $0.25^{-0.25}$
K_b	affinity constant for liver binding (saturable)	ng/mL	N/A	N/A	N/A
Bmax	maximum binding capacity (liver, saturable)	ng/mL	N/A	N/A	N/A
k_off	dissociation rate constant (liver)	1/h	N/A	N/A	N/A

The compartment-specific values for simulations of male rats are provided in the table below.

Code	Model parameter	Units	Value	Value from Paper	Value from Source Code
V_blc	plasma volume fraction	L/kg BW	0.0816	20.4 * 10^{-3} / 0.25	20.4 * 10^{-3} / 0.25
V_gic	GI volume fraction	L/kg BW	0.04	10.0 * 10^{-3} / 0.25	10.0 * 10-3 / 0.25
Q_gic	GI blood flow fraction	fraction of Q_cardiac	0.174418605	450 / 2580	450 / 2580
K_gi	GI:blood partition coefficient	none	0.0066	shown in Fig. 5a	0.0066
V_lic	liver volume fraction	L/kg BW	0.032	8.0 * 10^{-3} / 0.25	8.0 * 10^{-3} / 0.25
Q_lic	liver blood flow fraction	fraction of Q_cardiac	0.0320930233	828 / 2580	828 / 2580
K_li	liver:blood partition coefficient	none	1.1861	shown in Fig. 5a	1.1861
V_kic	kidney volume fraction	L/kg BW	0.0072	1.8 * 10^{-3} / 0.25	1.8 * 10^{-3} / 0.25
Q_kic	kidney blood flow fraction	fraction of Q_cardiac	0.201550388	520 / 2580	520 / 2580
K_ki	kidney:blood partition coefficient	none	0.1277	shown in Fig. 5a	0.1277
V_filc	filtrate volume fraction	L/kg BW	0.00072	0.18 * 10^{-3} / 0.25	0.18 * 10^{-3} / 0.25
Q_filc	filtrate blood flow fraction	fraction of Q_cardiac	0.1007752	260 / 2580	260 / 2580
V_st1c	[lung] volume fraction	L/kg BW	0.004	1.0 * 10^{-3} / 0.25	1.0 * 10^{-3} / 0.25
Q_st1c	[lung] blood flow fraction	fraction of Q_cardiac	1.0	2580 / 2580	2580 / 2580
K_st1	[lung]:blood partition coefficient	none	0.0292	shown in Fig. 5a	0.0292
V_st2c	[heart] volume fraction	L/kg BW	0.0032	0.8 * 10^{-3} / 0.25	0.8 * 10^{-3} / 0.25
Q_st2c	[heart] blood flow fraction	fraction of Q_cardiac	0.090697674	234 / 2580	234 / 2580
K_st2	[heart]:blood partition coefficient	none	0.0186	shown in Fig. 5a	0.0186
V_st3c	[brain] volume fraction	L/kg BW	N/A	N/A	N/A
Q_st3c	[brain] blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_st3	[brain]:blood partition coefficient	none	N/A	N/A	N/A
V_rbc	rest-of-body volume fraction	L/kg BW	0.536	(10 + 122 + 1.3 + 0.7) * 10^{-3} / 0.25	134 * 10^{-3} / 0.25
Q_rbc	rest-of-body blood flow fraction	fraction of Q_cardiac	0.204651163	(450 + 24 + 36 + 18) / 2580	528 / 2580
K_rb	rest-of-body:blood partition coefficient	none	0.0073	N/A	0.0073

PFDA PBPK model parameters for female rats

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFDA PBPK model of Kim et al. (2019) for female rats in the spreadsheet document "PFAS_template_parameters_PFDA.xlsx" on the worksheet "FKimRecreateBW". These values were taken from the paper (Kim et al., 2019) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the source code were used. The general parameter values for simulations of female rats are provided in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code

sim.time	End Time of Simulation	days	150	150	3600 / 24
Q_cardiacc	Cardiac Output	mL/h/BW^.75	7297.341982	2580 / $0.25^{0.75}$	N/A
hcrit	Hematocrit		N/A	N/A	N/A
BW	Body Weight	kg	0.25	0.25	N/A
dose	Dose	ng/kg BW	1000000	1 * 10^6	N/A
F_free	Free Fraction (initial value)		0.000122	0.000122	0.000122
delta	free fraction adjustment constant		N/A	N/A	N/A
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	N/A	N/A	N/A
F_unabs	Fraction Unabsorbed		0.65	N/A	0.65
T_mc	Transport Maximum	ng/h/BW^0.75	1.14×10^5	40250 * 10^3 / $0.25^{0.75}$	40250 / $0.25^{0.75}$
K_t	Transport Affinity Constant	ng/mL	3.01×10^4	30051 * 10^3	30051
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	N/A	N/A	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	0.481539718	0.681 / $0.25^{-0.25}$	0.681 / $0.25^{-0.25}$
K_f	Rate Constant to Fecal Storage from GI	1/h	N/A	N/A	N/A
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	0.276478751	0.391 / $0.25^{-0.25}$	0.391 / $0.25^{-0.25}$
K_abs	Oral Absorption Rate	1/h	1.44	1.44	1.44
K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	N/A	N/A	N/A
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	0.53598694	0.758 / $0.25^{-0.25}$	0.758 / $0.25^{-0.25}$
K_b	affinity constant for liver binding (saturable)	ng/mL	N/A	N/A	N/A
Bmax	maximum binding capacity (liver, saturable)	ng/mL	N/A	N/A	N/A
k_off	dissociation rate constant (liver)	1/h	N/A	N/A	N/A

The compartment-specific values for simulations of <u>female</u> rats are provided in the table below.

Code	**Model parameter**	**Units**	**Value**	**Value from Paper**	**Value from Source Code**
V_blc	plasma volume fraction	L/kg BW	0.0816	20.4 * 10^{-3} / 0.25	20.4 * 10^{-3} / 0.25
V_gic	GI volume fraction	L/kg BW	0.04	10.0 * 10^{-3} / 0.25	10.0 * 10-3 / 0.25
Q_gic	GI blood flow fraction	fraction of Q_cardiac	0.174418605	450 / 2580	450 / 2580
K_gi	GI:blood partition coefficient	none	0.0102	shown in Fig. 5b	0.0102
V_lic	liver volume fraction	L/kg BW	0.032	8.0 * 10^{-3} / 0.25	8.0 * 10^{-3} / 0.25
Q_lic	liver blood flow fraction	fraction of Q_cardiac	0.0320930233	828 / 2580	828 / 2580
K_li	liver:blood partition coefficient	none	0.607	shown in Fig. 5b	0.607
V_kic	kidney volume fraction	L/kg BW	0.0072	1.8 * 10^{-3} / 0.25	1.8 * 10^{-3} / 0.25
Q_kic	kidney blood flow fraction	fraction of Q_cardiac	0.201550388	520 / 2580	520 / 2580
K_ki	kidney:blood partition coefficient	none	0.2328	shown in Fig. 5b	0.2328

V_filc	filtrate volume fraction	L/kg BW	0.00072	0.18 * 10^{-3} / 0.25	0.18 * 10^{-3} / 0.25
Q_filc	filtrate blood flow fraction	fraction of Q_cardiac	0.1007752	260 / 2580	260 / 2580
V_st1c	[lung] volume fraction	L/kg BW	0.004	1.0 * 10^{-3} / 0.25	1.0 * 10^{-3} / 0.25
Q_st1c	[lung] blood flow fraction	fraction of Q_cardiac	1.0	2580 / 2580	2580 / 2580
K_st1	[lung]:blood partition coefficient	none	0.1002	shown in Fig. 5b	0.1002
V_st2c	[heart] volume fraction	L/kg BW	0.0032	0.8 * 10^{-3} / 0.25	0.8 * 10^{-3} / 0.25
Q_st2c	[heart] blood flow fraction	fraction of Q_cardiac	0.090697674	234 / 2580	234 / 2580
K_st2	[heart]:blood partition coefficient	none	0.0436	shown in Fig. 5b	0.0436
V_st3c	[brain] volume fraction	L/kg BW	N/A	N/A	N/A
Q_st3c	[brain] blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_st3	[brain]:blood partition coefficient	none	N/A	N/A	N/A
V_rbc	rest-of-body volume fraction	L/kg BW	0.536	(10 + 122 + 1.3 + 0.7) * 10^{-3} / 0.25	134 * 10^{-3} / 0.25
Q_rbc	rest-of-body blood flow fraction	fraction of Q_cardiac	0.204651163	(450 + 24 + 36 + 18) / 2580	528 / 2580
K_rb	rest-of-body:blood partition coefficient	none	0.0289	N/A	0.0289

PFOA PBPK model parameters for male rats given an IV dose of 0.041 mg/kg

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFOA PBPK model of Loccisano et al. (2012) for male rats from the study of Kudo et al. (2007) that were given a single small (0.041 mg/kg) IV dose of PFOA in the spreadsheet document "PFAS_template_parameters_PFOA.xlsx" on the worksheet "MKudo1BW". These values were taken from the paper (Loccisano et al., 2012) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the paper were used. (The source code provided by the authors in their supplemental material supplies logic (equations) for the model and *default* parameter values, but not the specific values used for particular simulations.) The body weight parameter was taken from the paper of Kudo et al. (2007). The general parameter values for simulations of these rats are provided in the table below.

Code	**Model Parameter**	**Units**	**Value**	**Value from Paper**	**Value from Source Code**
sim.time	End Time of Simulation	days	0.125	3 / 24	N/A
Q_cardiacc	Cardiac Output	L/h/BW^.75	7.56	14 * (1 – 0.46)	14 * (1 – 0.46)
hcrit	Hematocrit		0.46	0.46	0.46
BW	Body Weight	kg	0.29	0.28 to 0.30 (Kudo)	N/A
dose	Dose	ug/kg BW	41	0.041 * 10^3	N/A
F_free	Free Fraction (initial value)		0.006	0.006	0.022 (default)
delta	free fraction adjustment constant		N/A	N/A	N/A
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	N/A	N/A	N/A
F_unabs	Fraction Unabsorbed		N/A	N/A	N/A

T_mc	Transport Maximum	ug/h/BW^0.75	2.70×10^5	2.70×105 (typo "105" for "10^5")	120 (default)
K_t	Transport Affinity Constant	ug/L	6.70×10^4	6.70×104 (typo "104" for "10^4")	16.7 (default)
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	0.000954545	0.35 * 0.006 / 2.2 (Kbilec * Free / K_li)	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	0.1	0.1	0.002 (default)
K_f	Rate Constant to Fecal Storage from GI	1/h	N/A	N/A	N/A
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	0.6	0.6	0.008 (default)
K_abs	Oral Absorption Rate	1/h	31.3	31.3	25.1 (default)
K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	0.001	0.001	0 (default)
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	634.5	***	***
K_b	affinity constant for liver binding (saturable)	ug/L	N/A	N/A	N/A
Bmax	maximum binding capacity (liver, saturable)	ug/L	N/A	N/A	N/A
k_off	dissociation rate constant (liver)	1/h	N/A	N/A	N/A

*** Derivation of the value of K_ustc:

Consider the state equation for the urinary storage compartment. From the Loccisano et al. (2012) PFOA PBPK model, this equation is given as

$$dt(A\_stor) = Q\_fil*C\_fil - k\_urine*A\_stor$$

And from the Bernstein et al. (2021) PBPK model template this equation is given as

$$dt(A\_ust) = K\_ust*C\_fil*V\_fil - K\_u*A\_ust$$

To match in the input rate terms, we need to set K_ust such that

$$K\_ust = Q\_fil/V\_fil$$

The transport rate to the filtrate Q_fil is computed as

$$Q\_fil = Q\_filc*Q\_cardiac = Q\_filc*Q\_cardiacc*BW^{0.75}$$

And the filtrate volume is computed as

$$V\_fil = V\_filc*BW$$

Substituting these two expressions in the expression for K_ust gives

$$K\_ust = (Q\_filc*Q\_cardiacc*BW^{0.75})/(V\_filc*BW) = (Q\_filc*Q\_cardiacc/V\_filc)*BW^{-0.25}$$

In the template, K_ust is computed as

$$K\_ust = K\_ustc*BW^{-0.25}$$

So, in the input spreadsheet, we set

K_ustc = Q_filc*Q_cardiacc/V_filc

Note that the cardiac output constant of Loccisano et al. (2012) (QCC) needs to be multiplied by (1-hematocrit) for use in the template model, which has been done in the input spreadsheet for the value of Q_cardiacc. Then, using the values from Table 2 of Loccisano et al. (2012),

K_ustc = 0.0705*14*(1-0.46)/0.00084 = 634.5

In the input spreadsheet, this value is calculated using a formula referencing the values provided for the other parameters.

The compartment-specific values for simulations parameters for male rats are given in the table below.

Code	Model parameter	Units	Value	Value from Paper	Value from Source Code
V_blc	plasma volume fraction	L/kg BW	0.0312	0.0312	0.0312
V_gic	GI volume fraction	L/kg BW	N/A	N/A	N/A
Q_gic	GI blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_gi	GI:blood partition coefficient	none	N/A	N/A	N/A
V_lic	liver volume fraction	L/kg BW	0.035	0.035	0.035
Q_lic	liver blood flow fraction	fraction of Q_cardiac	0.183	0.183	0.183
K_li	liver:blood partition coefficient	none	2.2	2.2	3.72 (default)
V_kic	kidney volume fraction	L/kg BW	0.0084	0.0084	0.0084
Q_kic	kidney blood flow fraction	fraction of Q_cardiac	0.141	0.141	0.141
K_ki	kidney:blood partition coefficient	none	1.05	1.05	0.80 (default)
V_filc	filtrate volume fraction	L/kg BW	0.00084	0.00084	0.00084
Q_filc	filtrate blood flow fraction	fraction of Q_cardiac	0.0705	0.0705	0.0705
V_st1c	[lung] volume fraction	L/kg BW	N/A	N/A	N/A
Q_st1c	[lung] blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_st1	[lung]:blood partition coefficient	none	N/A	N/A	N/A
V_st2c	[heart] volume fraction	L/kg BW	N/A	N/A	N/A
Q_st2c	[heart] blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_st2	[heart]:blood partition coefficient	none	N/A	N/A	N/A
V_st3c	[brain] volume fraction	L/kg BW	N/A	N/A	N/A
Q_st3c	[brain] blood flow fraction	fraction of Q_cardiac	N/A	N/A	N/A
K_st3	[brain]:blood partition coefficient	none	N/A	N/A	N/A
V_rbc	rest-of-body volume fraction	L/kg BW	0.76456	N/A	0.84 – (0.0312 + 0.035 + 0.0084 + 0.00084) (No reference or justification for 84% total)

Q_rbc	rest-of-body blood flow fraction	fraction of Q_cardiac	0.6055	N/A	1 – (0.183 + 0.141 + 0.0705)
K_rb	rest-of-body:blood partition coefficient	none	0.11	0.11	0.22 (default)

PFOA PBPK model parameters for male rats given an IV dose of 16.56 mg/kg

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFOA PBPK model of Loccisano et al. (2012) for male rats from the study of Kudo et al. (2007) that were given a single large (16.56 mg/kg) IV dose of PFOA in the spreadsheet document "PFAS_template_parameters_PFOA.xlsx" on the worksheet "MKudo2BW". These values were taken from the paper (Loccisano et al., 2012) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the paper were used. (The source code provided by the authors in their supplemental material supplies logic (equations) for the model and *default* parameter values, but not the specific values used for particular simulations.) The body weight parameter was taken from the paper of Kudo et al. (2007). All parameters are identical to those listed above for *male rats given an IV dose of 0.0041 mg/kg*, except those listed in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code
sim.time	End Time of Simulation	days	0.104166667	2.5 / 24	N/A
dose	Dose	ug/kg BW	16560	16.56 * 10^3	N/A

PFOA PBPK model parameters for male rats given an oral dose of 25 mg/kg

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFOA PBPK model of Loccisano et al. (2012) for male rats from the study of Kemper (2003) that were given a single oral dose (25 mg/kg) of PFOA in the spreadsheet document "PFAS_template_parameters_PFOA.xlsx" on the worksheet "MKemperOral25BW". These values were taken from the paper (Loccisano et al., 2012) and/or source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the paper were used. (The source code provided by the authors in their supplemental material supplies logic (equations) for the model and *default* parameter values, but not the specific values used for particular simulations.) The body weight parameter was taken from the paper of Kudo et al. (2007). All parameters are identical to those listed above for *male rats given an IV dose of 0.0041 mg/kg*, except those listed in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code
sim.time	End Time of Simulation	days	25	600 / 24	N/A
dose	Dose	ug/kg BW	25000	25 * 10^3	N/A

PFOS PBPK model parameters for male rats given an oral dose of 15 mg/kg

Bernstein et al. (2021) list sources for parameter values used in their PBPK template version of the PFOS PBPK model of Loccisano et al. (2012) for male rats from the study of 3M that were given a single oral dose (15 mg/kg) of PFOS in the spreadsheet document "PFAS_template_parameters_PFOS.xlsx" on the worksheet "M3MOralBW". These values were taken from the paper (Loccisano et al., 2012) and/or

source code obtained from the authors, and in cases of discrepancies between these two sources, the values from the paper were used. (The source code provided by the authors in their supplemental material supplies logic (equations) for the model and *default* parameter values, but not the specific values used for particular simulations.) The body weight parameter was taken from a "table" in the source code that listed many values. The general parameter values for simulations of these rats are provided in the table below.

Code	Model Parameter	Units	Value	Value from Paper	Value from Source Code
sim.time	End Time of Simulation	days	120	120	N/A
Q_cardiacc	Cardiac Output	L/h/BW^.75	7.56	14 * (1 – 0.46)	14 * (1 – 0.46)
hcrit	Hematocrit		0.46	0.46	0.46
BW	Body Weight	kg	0.233 (non-constant)	N/A	0.233 (listed as one of many BW values in a table)
dose	Dose	ug/kg BW	15000	15 * 10^3	N/A
F_free	Free Fraction (initial value)		0.022	0.006	0.022 (default)
delta	free fraction adjustment constant		0.94	0.94	0.94 (default)
k_freec	rate constant for free fraction variation	1/h/BW^-0.25	0.035	0.035	0.035
F_unabs	Fraction Unabsorbed		N/A	N/A	N/A
T_mc	Transport Maximum	ug/h/BW^0.75	120	120	120 (default)
K_t	Transport Affinity Constant	ug/L	16.7	16.7	16.7 (default)
K_bilec	Biliary Excretion Rate	1/h/BW^-0.25	1	1	N/A
K_uc	Rate Constant to Urine	1/h/BW^-0.25	0.002	0.002	0.002 (default)
K_f	Rate Constant to Fecal Storage from GI	1/h	N/A	N/A	N/A
K_fstc	Rate Constant to Feces from Fecal Storage	1/h/BW^-0.25	0.008	0.008	0.008 (default)
K_abs	Oral Absorption Rate	1/h	25.1	25.1	25.1 (default)
K_unabs	Rate Unabsorbed fraction of Dose goes to Fecal Storage	1/h	0	0	0 (default)
K_ustc	Rate Constant to Urinary Storage	1/h/BW^-0.25	634.5	***	***
K_b	affinity constant for liver binding (saturable)	ug/L	0.0036	0.0036	0.0036
Bmax	maximum binding capacity (liver, saturable)	ug/L	6.5	6.5	6.5
k_off	dissociation rate constant (liver)	1/h	0.03	0.03	0.03

*** See derivation of K_ustc value of 634.5 above.

The compartment-specific values for simulations parameters for male rats given an oral dose of 15 mg/kg are identical to those listed above for *male rats given an IV dose of 0.0041 mg/kg*, except those listed in the table below.

Code	Model parameter	Units	Value	Value from Paper	Value from Source Code
K_li	liver:blood partition coefficient	none	3.72	3.72	3.72 (default)
K_ki	kidney:blood partition coefficient	none	0.8	0.8	0.80 (default)
K_rb	rest-of-body:blood partition coefficient	none	0.22	0.2	0.22 (default)

B2: Review, Verification, and Validation of Existing Computational PBPK/PK Models

B2.2: PBPK/PK Model Structure and Documentation (Criteria A)

Bernstein et al. (2021) provided a description of a PBPK model template in their manuscript. The PBPK model template structure is illustrated in Figure 1 of Bernstein et al. (2021) and is implemented in the MCSim model specification language (Bois, 2009) in the source code file "PFAS_template.model". The model template described in the manuscript is consistent with the model template implemented in the source code file.

The model template includes the following state variables:

- "A_bl", the amount (mg) of substance in the blood (or plasma);
- "A_gi", the amount (mg) of substance in the gastrointestinal (GI) tissue;
- "A_li", the amount (mg) of substance in the liver;
- "A_fst", the amount (mg) of substance in the fecal storage compartment;
- "A_ki", the amount (mg) of substance in the kidney;
- "A_fil", the amount (mg) of substance in the filtrate compartment;
- "A_ust", the amount (mg) of substance in the urinary storage compartment;
- "A_st1", the amount (mg) of substance in generic storage compartment 1;
- "A_st2", the amount (mg) of substance in generic storage compartment 2;
- "A_st3", the amount (mg) of substance in generic storage compartment 3;
- "A_st4", the amount (mg) of substance in generic storage compartment 4;
- "A_st5", the amount (mg) of substance in generic storage compartment 5;
- "A_rb", the amount (mg) of substance in the rest of body compartment;
- "A_glumen", the amount (mg) of substance in the gut lumen;
- "A_lib", the amount (mg) of substance bound in the liver;
- "iv_dose_cont", the continuous intravenous dose rate (mg/kg/d);
- "A_in", the total cumulative amount (mg) that has entered the system;
- "A_urine", the total cumulative amount (mg) that has been excreted in urine; and
- "A_fecal", the total cumulative amount (mg) that has been excreted in feces.

Each of these state variables has a corresponding differential equation in the source code file "PFAS_template.model" that describes its time rate of change. Each of these differential equations correctly describes the time rate of change of the appropriate state variable based on details and assumptions stated in the manuscript of Bernstein et al. (2021).

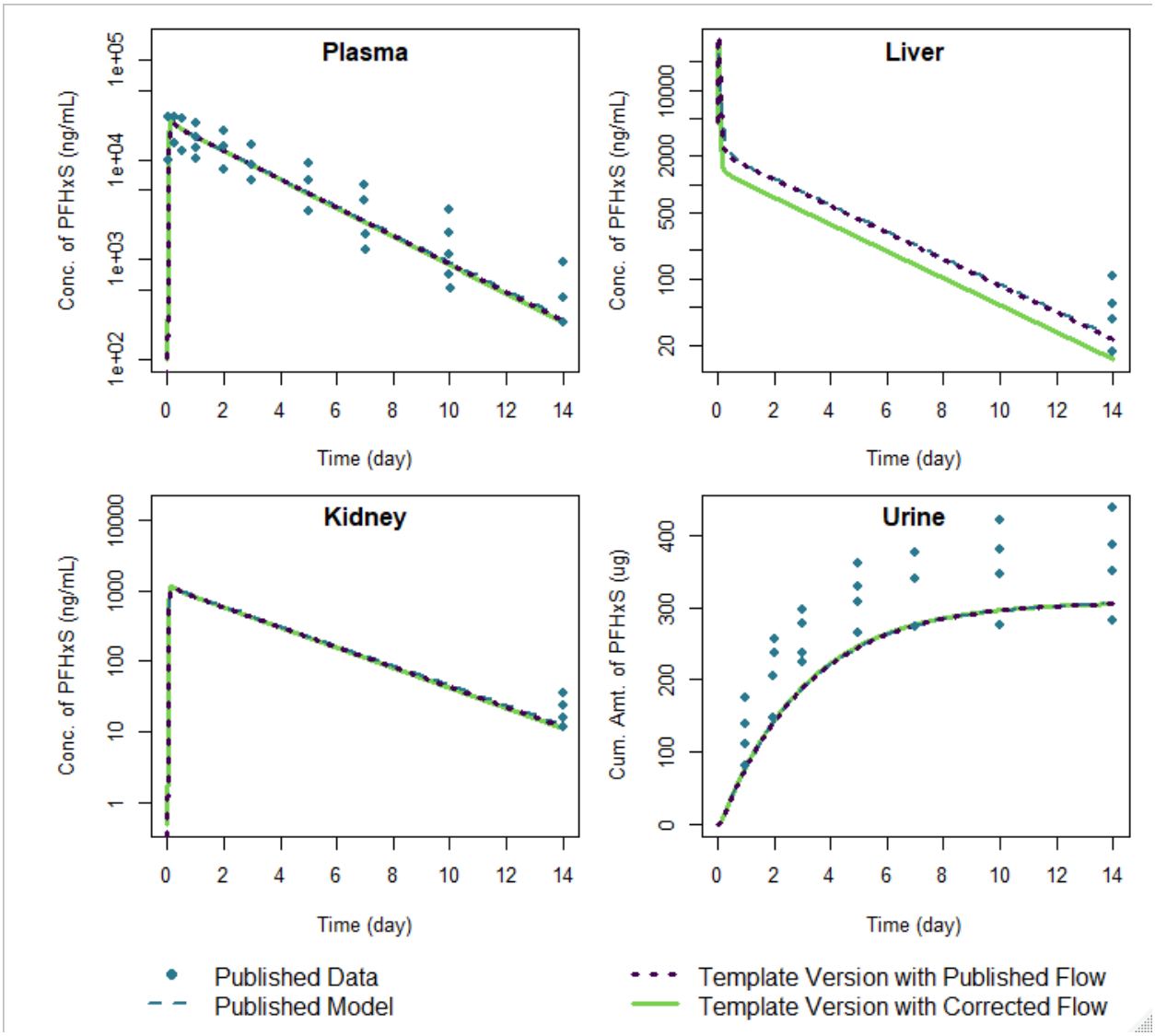
- The time rate of change of “A_bl”, the amount of substance in the blood, is correctly computed as the rate of flow of substance into the blood compartment (from the liver, kidney, rest of body, and generic storage compartments 1 through 5) **minus** the rate of flow of substance out of the blood compartment (to the liver, kidney, GI tissue, rest of body, and generic storage compartments 1 through 5) **minus** the rate of flow of substance into the renal filtrate compartment **plus** the rate of flow into the blood compartment due to continuous intravenous dosing.
- The time rate of change of “A_glumen”, the amount of substance in the gut lumen, is correctly computed as the negative of the rate of absorption (into the GI tissue compartment or the liver compartment) **minus** the rate of flow into the fecal storage compartment.
- The time rate of change of “A_gi”, the amount of substance in the GI tissue compartment, is correctly computed as the flow of substance into the GI tissue compartment (from the blood compartment) **minus** the rate of flow of substance out of the GI tissue compartment (to blood) **minus** the rate of transfer of substance from the GI tissue compartment to the fecal storage compartment **minus** the rate of transfer of the substance from the GI tissue compartment to the liver (if applicable).
- The time rate of change of “A_li”, the amount of substance in the liver, is correctly computed as the flow of substance into the liver (from the blood compartment and the blood leaving the GI tissue compartment) **minus** the rate of flow of substance out of the liver (to blood) **minus** the rate of transfer of substance from the liver to the fecal storage compartment (via biliary excretion) **plus** the rate of transfer from the gut lumen via absorption (if applicable) **minus** the rate of binding in the liver (the transfer rate to the “bound in liver tissue” compartment) **plus** the rate of unbinding in the liver (the transfer rate from the “bound in liver tissue” compartment).
- The time rate of change of “A_fst”, the amount of substance in the fecal storage compartment, is correctly computed as the rate of transfer from the liver **plus** the rate of transfer from the gut lumen **plus** the rate of transfer from the GI tissue compartment **minus** the rate of fecal elimination.
- The time rate of change of “A_fecal”, the cumulative amount of substance eliminated in feces, is correctly computed as the rate of fecal elimination (the transfer rate out of the fecal storage compartment).
- The time rate of change of “A_lib”, the amount of substance bound in the liver, is correctly computed as the rate of binding in the liver (the transfer rate from the liver tissue compartment) plus the rate of unbinding in the liver (the transfer rate to the liver tissue compartment).
- The time rate of change of “A_ki”, the amount of substance in the kidney, is correctly computed as the flow of substance into the kidney (from the blood compartment) **minus** the rate of flow of substance out of the liver (to blood) **plus** the rate of resorption from the renal filtrate compartment.
- The time rate of change of “A_fil”, the amount of substance in the renal filtrate compartment, is correctly computed as the rate of flow of substance into the filtrate compartment (from the blood compartment) **minus** the rate of flow to the kidney (via resorption) **minus** the rate of flow to the urinary storage compartment.

- The time rate of change of “A_ust”, the amount of substance in the urinary storage compartment, is correctly computed as the rate of transfer from the filtrate compartment **minus** the rate of urinary excretion.
- The time rate of change of “A_urine”, the cumulative amount of substance excreted in urine, is correctly computed as the rate of urinary excretion.
- The time rate of change of “A_st1”, the amount of substance in generic storage compartment 1, is correctly computed as the flow of substance into the compartment (from the blood compartment) **minus** the rate of flow of substance out of compartment (to blood).
- The time rate of change of “A_st2”, the amount of substance in generic storage compartment 2, is correctly computed as the flow of substance into the compartment (from the blood compartment) **minus** the rate of flow of substance out of compartment (to blood).
- The time rate of change of “A_st3”, the amount of substance in generic storage compartment 3, is correctly computed as the flow of substance into the compartment (from the blood compartment) **minus** the rate of flow of substance out of compartment (to blood).
- The time rate of change of “A_st4”, the amount of substance in generic storage compartment 4, is correctly computed as the flow of substance into the compartment (from the blood compartment) **minus** the rate of flow of substance out of compartment (to blood).
- The time rate of change of “A_st5”, the amount of substance in generic storage compartment 5, is correctly computed as the flow of substance into the compartment (from the blood compartment) **minus** the rate of flow of substance out of compartment (to blood).
- The time rate of change of “A_rb”, the amount of substance in the rest of body compartment, is correctly computed as the flow of substance into the compartment (from the blood compartment) **minus** the rate of flow of substance out of compartment (to blood).
- The time rate of change of “iv_dose_cont”, the intravenous dose rate, is correctly computed as zero. This rate can undergo discrete changes by using deSolve “events” or by stopping the simulation and restarting it with a new intravenous dose rate at designated times.
- The time rate of change of “A_in”, the cumulative amount that has entered the organism, is correctly computed as the rate of intravenous dosing. This amount can undergo discrete changes by using deSolve “events” or by stopping the simulation and restarting it with a new amount to reflect bolus doses at designated times.

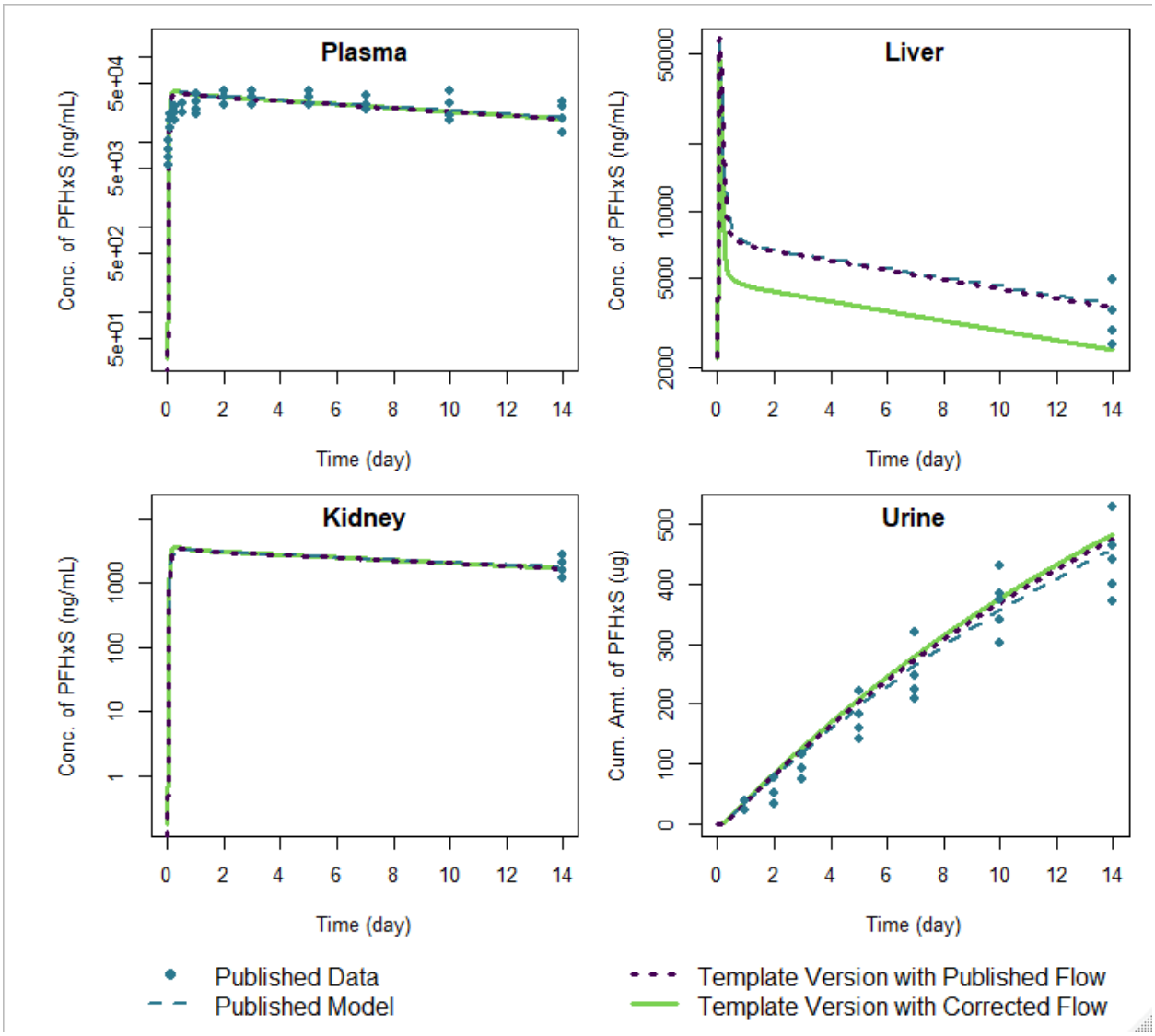
B2.3 PBPK/PK Model In-Depth Technical Evaluation (Criteria B)

Here we verify that figures produced by code of Bernstein et al. (2021) match those in the manuscript of Bernstein et al. (2021).

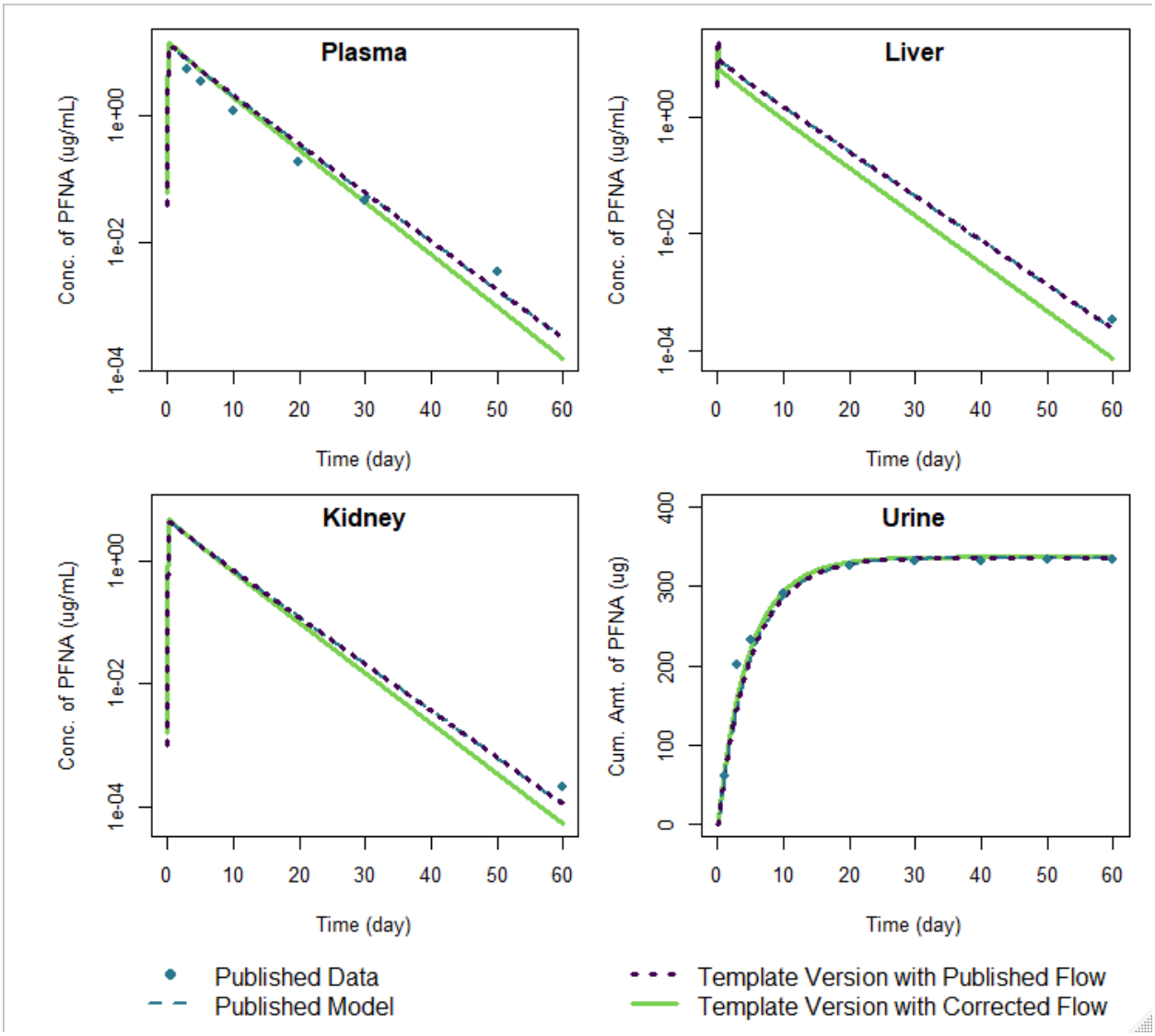
We were able to replicate Figure 3 of Bernstein et al. (2021) by running lines 9 through 12 of the source code file "PBPK_PFAS_manuscript_scripts.R". The result is shown below.



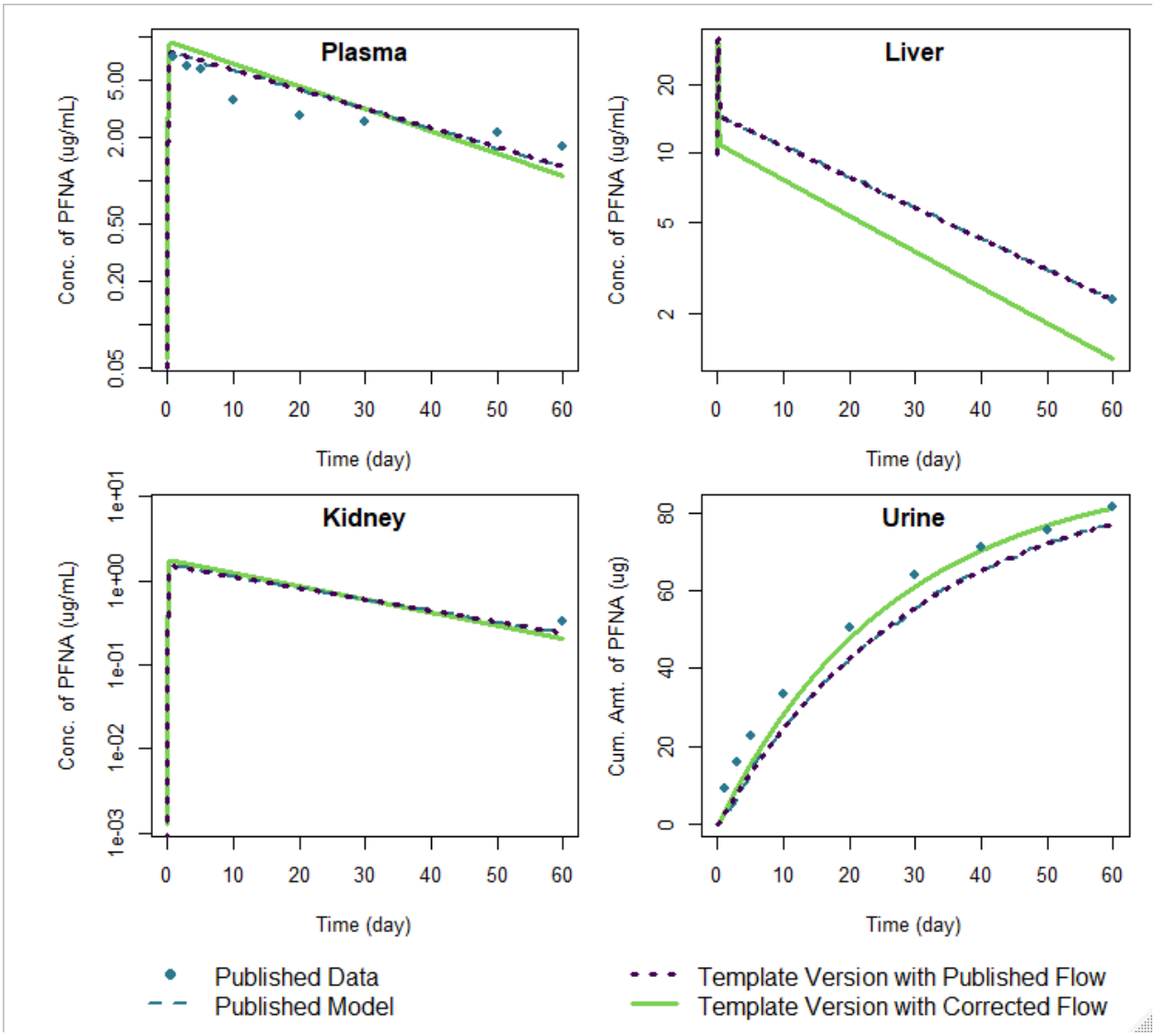
We were able to replicate Figure 4 of Bernstein et al. (2021) by running lines 28 through 31 of the source code file "PBPK_PFAS_manuscript_scripts.R". The result is shown below.



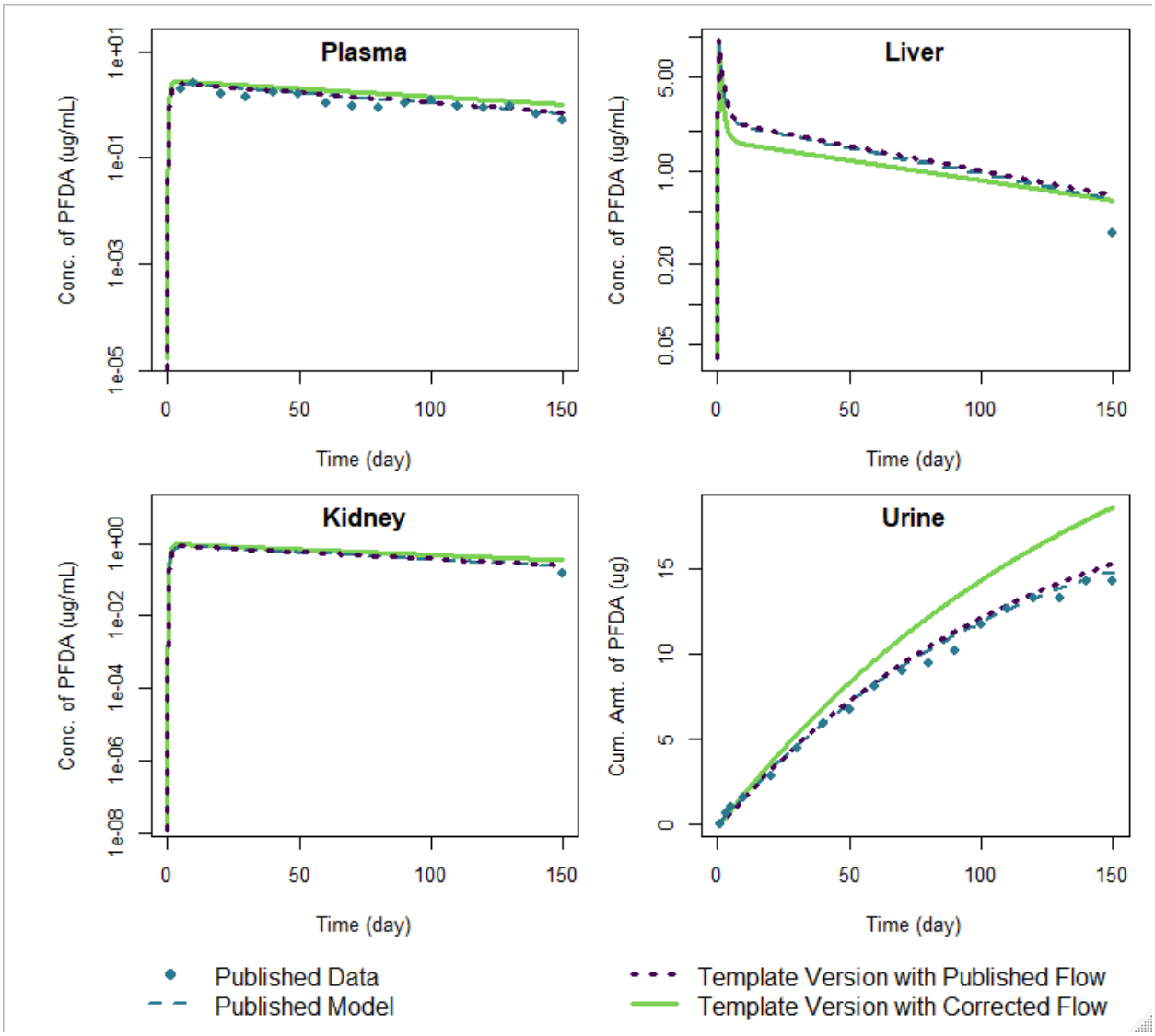
We were able to replicate Figure 5 of Bernstein et al. (2021) by running lines 47 through 50 of the source code file "PBPK_PFAS_manuscript_scripts.R". The result is shown below.



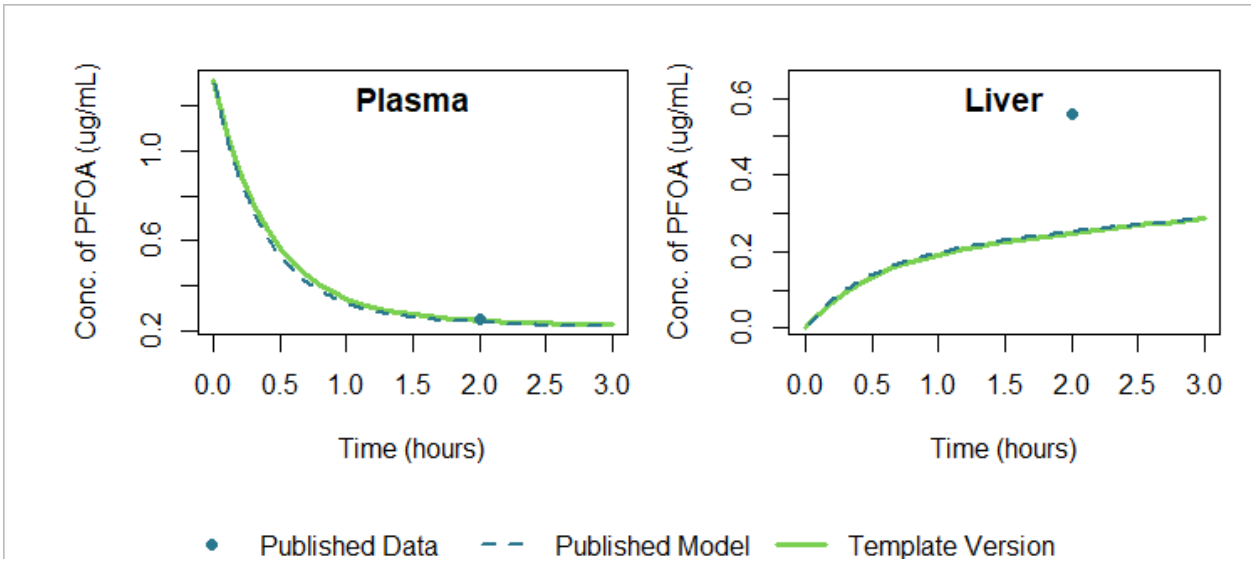
We were able to replicate Figure 6 of Bernstein et al. (2021) by running lines 66 through 69 of the source code file "PBPK_PFAS_manuscript_scripts.R". The result is shown below.



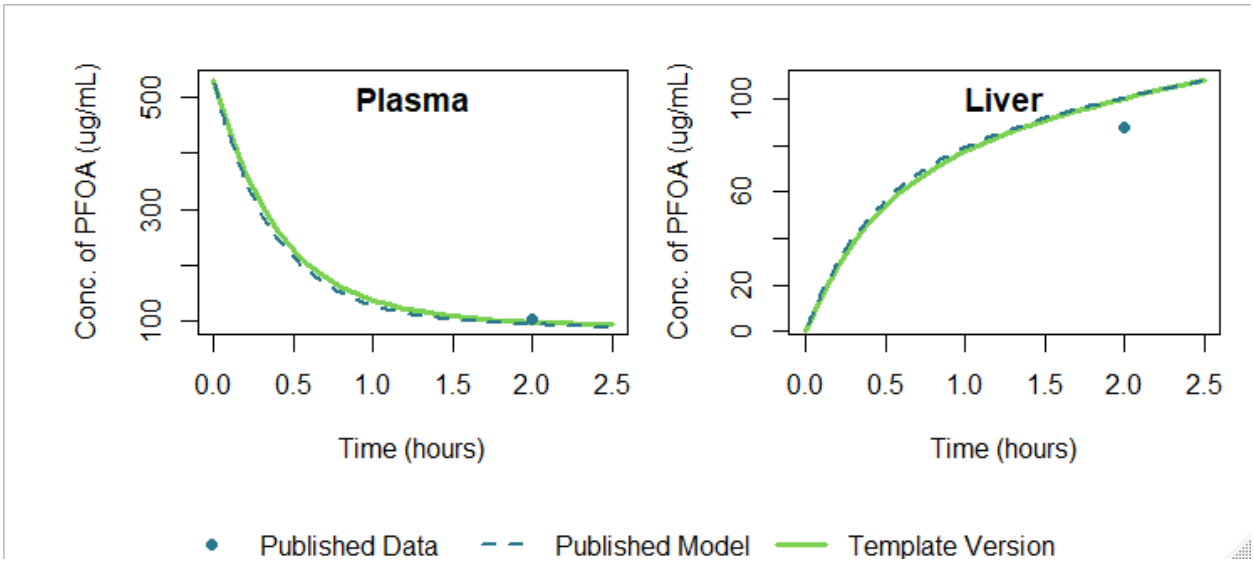
We were able to replicate Figure 7 of Bernstein et al. (2021) by running lines 85 through 88 of the source code file "PBPK_PFAS_manuscript_scripts.R". The result is shown below.



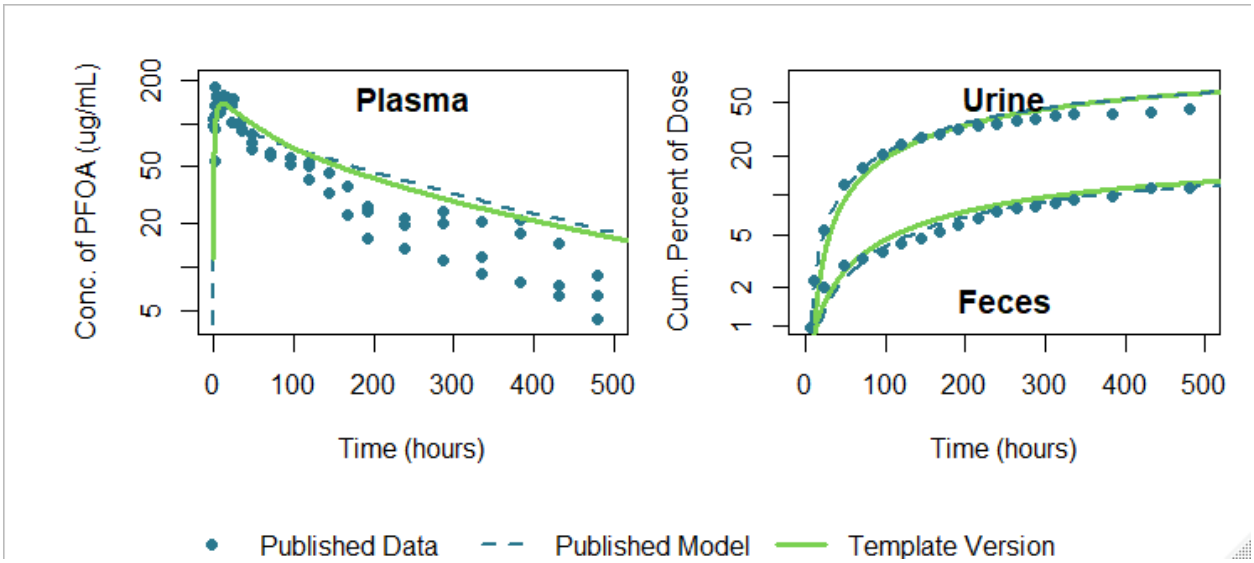
We were able to replicate Figure 8 of Bernstein et al. (2021) by running lines 104 through 106 of the source code file “PBPK_PFAS_manuscript_scripts.R”. The result is shown below.



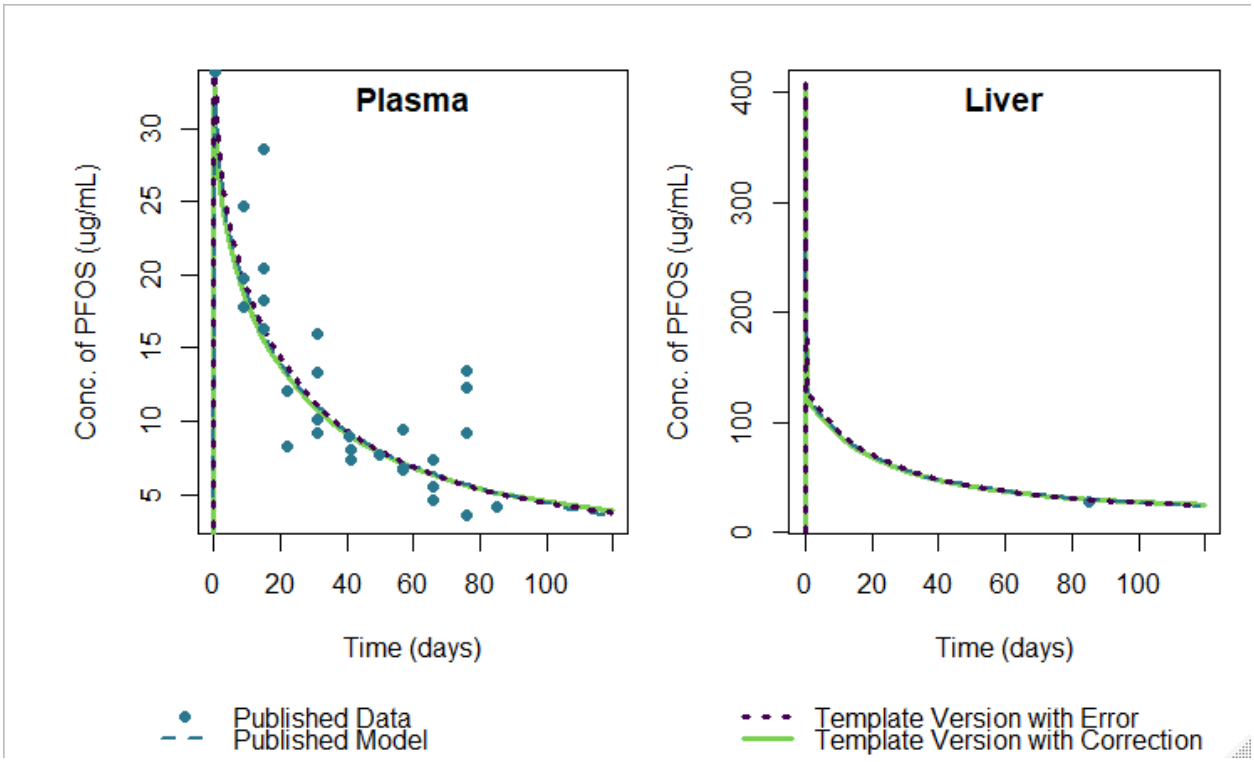
We were able to replicate Figure 9 of Bernstein et al. (2021) by running lines 123 through 125 of the source code file “PBPK_PFAS_manuscript_scripts.R”. The result is shown below.



We were able to replicate Figure 10 of Bernstein et al. (2021) by running lines 142 through 144 of the source code file “PBPK_PFAS_manuscript_scripts.R”. The result is shown below.



We were able to replicate Figure 11 of Bernstein et al. (2021) by running lines 161 through 164 of the source code file “PBPK_PFAS_manuscript_scripts.R”. The result is shown below.



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