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Supplemental Material

Internal Relative Potency Factors for the Risk Assessment of Mixtures of Per- and Polyfluoroalkyl Substances (PFAS) in Human Biomonitoring

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Figure S23. Simulation of a 28-day repeated dose experiment for PFDA. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Log_{10} PFDA serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.156 mg/kg bw/day; lower dashed line and triangles = 0.312 mg/kg bw/day; dotted line and plusses = 0.625 mg/kg bw/day; upper solid line and crosses = 1.25 mg/kg bw/day; upper dashed line and diamonds = 2.5 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Symbols are the measured concentrations from NTP. To distinguish measured points they have been shifted slightly.

Figure S24. Simulation of a 28-day repeated dose experiment for PFBS. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Log_{10} PFBS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 62.6 mg/kg bw/day; dashed line and triangles = 125 mg/kg bw/day; dotted line and plusses = 250 mg/kg bw/day; upper solid line and crosses = 500 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Symbols are the measured concentrations from NTP. To distinguish measured points they have been shifted slightly.

Figure S25. Simulation of a 28-day repeated dose experiment for PFBS for the last ~48 hours of the experiment. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Log_{10} PFBS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 62.6 mg/kg bw/day; dashed line and triangles = 125 mg/kg bw/day; dotted line and plusses = 250 mg/kg bw/day; upper solid line and crosses = 500 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Symbols are the measured concentrations from NTP. To distinguish measured points they have been shifted slightly.

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Figure S27. Simulation of a 28-day repeated dose experiment for PFOS. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Log_{10} PFOS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.312 mg/kg bw/day; lower dashed line and triangles = 0.625 mg/kg bw/day; dotted line and plusses = 1.25 mg/kg bw/day; upper solid line and crosses = 2.5 mg/kg bw/day; upper dashed line and diamonds = 5 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP. Symbols are the measured concentrations from NTP.

Figure S28. National Health and Nutrition Examination Survey (NHANES) perfluoroalkyl substance (PFAS) measurements in blood plasma presented as PFOA equivalents. Note: density plot of the sum PEQ concentration in serum (ng/mL) of all sexes and ages from the NHANES study population (*n*= 1929). The black line represents the sum PEQ of all PFAS included (perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), perfluoroocane sulfonic acid (PFOS), hexafluoropropylene oxide-dimer acid (HFPO-DA)) of which internal RPFs were derived.

Figure S29. Mean contribution of each PFAS to the individual's total PFOA equivalents (PEQs) concentration. Note: contribution (%) of each perfluoroalkyl substance (PFAS) (perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) as linear and branched forms combined, perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS) as linear and branched forms combined, and hexafluoropropylene oxide-dimer acid (HFPO-DA)) to the sum of PEQs based on the PFAS serum concentration data from the National Health and Nutrition Examination Survey (NHANES) 2017-2018 cycle (*n* = 1929).

References

Toxicokinetic model parameterization

The below text provides further explanation to footnotes c, d, e, i, j, and k of Table 1 of the main paper.

Tatum-Gibbs study on PFNA (*footnotes c and j*)

Tatum-Gibbs *et al.*[5] assume biphasic elimination, i.e. a 2-compartment model. The 2-compartment model is implemented with the in Tatum-Gibbs *et al.*[5] provided parameter values: $k10$ = 0.0015/h, $k01$ = 1/h, $k12$ = 0.00583691/h, $k21$ = 0.02708/h, bw = 0.5 kg, $V1$ = 0.139 L/kg ($k12$ and $k21$ obtained from $\alpha\_T1/2$ = 0.87 days and $\beta\_T1/2$ = 23.6 days according to footnote i).

Visually (Figure S2), this model does not seem to describe the serum concentration data for PFNA adequately: the data do not show biphasic elimination and the modelled elimination appears faster compared to the measured elimination. Therefore, a one-compartment model was preferred and the elimination rate was lowered ($k10$ = 0.00025/h) to obtain a more realistic description of the elimination phase (Figure S3). This fit slightly overestimated the data which was corrected by increasing $V1$ to 0.170 L/kg (Figure S4).

Kawabata study on PFDoDA *(footnote d)*

In Kawabata *et al.*[7], the distribution volume ($V1$) for PFDoDA was not provided. Therefore, $V1$ was estimated from the organ specific distributions. PFDoDA organ/serum partition coefficients were obtained by dividing the concentrations in the organs by that in the serum (Table S3). The $V1$ of PFDoDA was then estimated by summing products of the organ volume as a fraction of body weight and the partition coefficient of the organs (Eq. S6), following the theory below.

Given $F_{abs} \times D_0'$ as a continuous uptake rate in amount/day and one-compartmental kinetics the rate at which the amount in the whole body A_{body} changes is:

$$\frac{d}{dt}A_{body} = F_{abs} \times D_0' - k_{el} \times A_{body}(t), \qquad \text{(Eq. S1)}$$

with corresponding the time course of A_{body}:

$$A_{body}(t) = \frac{F_{abs} \times D_0'}{k_e} \times (1 - e^{-k_e \times t}), \quad \text{(Eq. S2)}$$

Furthermore:

$$A_{body}(t) = \sum_i V_i \times C_i(t) = \sum_i V_i \times p_i \times C_{plasma}(t) = C_{plasma}(t) \times \sum_i V_i \times p_i, \text{ (Eq. S3)}$$

Here V_i and p_i are the volume and the partition coefficient (relative to blood plasma) of the i^{th} organ. Defining the Volume of Distribution (*V1*, L) as scalor relating A_{body} to the plasma concentration $C_{plasma}(t)$:

$$A_{Body}(t) = V1 \times C_{Plasma}(t), \quad \text{(Eq. S4)}$$

then gives:

$$C_{plasma}(t) = \frac{A_{Body}(t)}{V1} = \frac{F_{abs} \times D_0'}{k_e \times V1} \times (1 - e^{-k_e \cdot t}), \quad \text{(Eq. S5)}$$

$$V1 = \sum_i V_i \times p_i, \quad \text{(Eq. S6)}$$

Here V_i reference values as proposed by Jongeneelen and ten Berge[8] (Table S4) were applied.

The volume of distribution was calculated using the serum, liver, kidney, adipose tissue (average of epididymal, mesenteric and subcutaneous), and remaining organs (average of lung, heart, spleen, brain and testis) partition coefficients (Table S3). As a default for the male rat, a body weight of 0.3 kg was used as proposed by Jongeneelen and ten Berge.[8] From this results that:

*V*1 = volume serum × rat bw × partition coefficient serum/serum +
volume liver × rat bw × partition coefficient liver/serum +
volume kidney × rat bw × partition coefficient kidney/serum +
volume adipose tissue × rat bw × partition coefficient adipose/serum +
volume remaining organs × rat bw × partition coefficient remaining organs/serum

*V*1 = 0.054 × 0.3 × 1 + 0.040 × 0.3 × 7.925 + 0.007 × 0.3 × 1.679 + 0.070 × 0.3 × 0.3933 + 0.787 × 0.3 × 0.3206 = 0.199 L, corresponding with **0.663 L (kg bw)$^{-1}$.**

Using the parameter values listed in Kawabata *et al.*[7] caused the model to overestimate the measured data with a factor of 3.8 (Figure S5). Adjusting the parameter values for $V1$ and F_{abs} such that the ratio $V1/F_{abs}$ equals approximately 1600, results in a good fit of the model. However, this may lead to unrealistic values for $V1$ or F_{abs}.

In another publication of Kawabata *et al.*[2], the serum concentrations on day 10 were reported after a single dose of 50 mg/kg PFOA, PFDA or PFDoDA in male rats. Fitting the models of PFOA and PFDA also showed that the models overestimate the measurements of Kawabata *et al.*[2] (Figures S6 and S7), while the data of Dzierlenga *et al.*[3] are fitted quite well (see Figures S11 and S13). From this we concluded that the measurements by Kawabata *et al.*[7] may have underestimated the actual serum concentration, and the PFDoDA model was parameterized as listed in Table 1 of the main paper.

Gannon[4,9] studies on HFPO-DA *(footnote e and k)*

The serum concentrations of animals at t = 12 h in Gannon[4], "Appendix A, 10 mg/kg results", are assumed to be transposed. For verification of the single-dose modelling, serum concentrations of 11300 ng/mL (animal 1), 2810 ng/mL (animal 2), and 1380 ng/mL (animal 3) were used at t = 12 h. After transposing the data, the data showed a more consistent decreasing trend over time.

Gannon *et al.*[9] provide a value for absorption rate ($k01$), alpha rate, beta rate and $V1$, but not for $k10$. Therefore, $k10$ was obtained by optimizing the ratio between the model and the measurements (the corrected serum concentrations at t = 12 were also used for this). The value for $k10$ always lies between the alpha and beta rate, i.e. it is constrained between 0.25 $hr^{-1}$and 0.0096 hr^{-1}. This resulted in an optimal $k10$ of 0.24 hr^{-1} (Figure S8).

Transfer rates between central and peripheral compartments ($k12$ and $k21$) *(footnote i)*

k12 and k21 can be derived from the alpha rate $\left(\frac{\ln(2)}{\alpha\, T1/2}\right)$ and beta rate $\left(\frac{\ln(2)}{\beta\, T1/2}\right)$, because -alpha and -beta are the roots of the quadratic equation:

$$x^2 + (k12 + k21 + k10)x + k21 \cdot k10 = 0$$ [10,11]

In this quadratic equation a = 1, which can therefore be omitted in this analysis,

$$b = k12 + k21 + k10, \qquad \text{(Eq. S7)}$$

and

$$c = k21 \times k10, \qquad \text{(Eq. S8)}$$

x can have two possible solutions, i.e. roots:

$$x = -alpha = \frac{-b+\sqrt{b^2-4c}}{2}, \qquad \text{(Eq. S9a)}$$

and

$$x = -beta = \frac{-b-\sqrt{b^2-4c}}{2}, \qquad \text{(Eq. S9b)}$$

At the vertex of this quadratic function, x is $\frac{-alpha+-beta}{2} = \frac{-b}{2}$.

This can be re-written to $b = alpha + beta,$ (Eq. S10)

Substituting equations 3 and 4 into $x^2 + bx + c = 0$ and gives:

$(-alpha)^2 + (alpha + beta) \times -alpha + c = 0$ and $(-beta)^2 + (alpha + beta) \times -beta + c = 0$

=>

$c = -(-alpha)^2 + (alpha + beta) \times alpha$ and $c = -(-beta)^2 + (alpha + beta) \times beta$

=>

$c = -(-alpha)^2 + (alpha)^2 + beta \times alpha$ and $c = -(-beta)^2 + (alpha \times beta) + beta^2$

=>

$c = alpha \times beta$ (in both cases), (Eq. S11)

From Eq. S8 and S11 follows that $k21 \times k10 = alpha \times beta$

Since $k10$, *alpha* and *beta* are known, we can derive $k21$:

$$k21 = \frac{alpha * beta}{k10}$$

From Eq. S7 and S10 follows that *k*12 + *k*21 + *k*10 = *alpha* + *beta*

Since *k*10, *k*21, *alpha* and *beta* are known, we can derive *k*12:

$$k12 = alpha + beta - k21 - k10$$

Table S1. Hepatoxicity data for perfluoroalkyl substances (PFAS). *Male rat dose-response data for 16 PFAS including full chemical name, chemical name abbreviation, CAS no. and reference. Database as presented in Bil et al.*[12]

PFAS (abbreviation, carbon chain length) [CAS no.][c]	Reference	Administration route	Strain	Exposure duration (days)	External dose (mg/kg bw/day)	Relative liver weight		
						Mean (g/100g bw)	SD	*n*
Perfluorobutanesulfonic acid (PFBS, C4)[a] [375-73-5]	Lieder *et al.*[13]	Oral gavage	Crl:CD(SD)IGS BR VAF/Plus (Sprague Dawley) rats	70	0	3.4	0.3	30
					30	3.5	0.4	30
					100	3.6	0.3	30
					300	3.8	0.3	30
					1000	4.1	0.4	30
Perfluorohexanesulfonic acid (PFHxS, C6)[a] [355-46-4]	Butenhoff *et al.*[14]	Oral gavage	Crl:CD(SD)IGS BR VAF/Plus (Sprague Dawley) rats	42	0	3.12	0.03	10
					0.3	3.20	0.23	10
					1.0	3.42	0.42	10
					3.0	3.73	0.23	10
					10.0	5.25	0.72	10
Perfluorooctanesulfonic acid (PFOS, C8)[a] [1763-23-1]	Seacat *et al.*[15]	Oral dietary	Crl:CD (Sprague Dawley) IGS BR rats	98	0	3.2	0.3	5
					0.03	3.2	0.2	5
					0.13	3.2	0.2	5
					0.34	3.6	0.3	5
					1.33	4.3	0.4	5
Perfluorobutanoic acid (PFBA, C4)[a] [375-22-4]	Butenhoff *et al.*[16]	Oral gavage	Sprague Dawley rats (Crl:CD Outbred, SPF quality)	90	0	2.1	0.23	20
					1.2	2.1	0.14	10
					6	2.2	0.27	10
					30	2.6	0.39	20
Perfluorohexanoic acid (PFHxA, C6)[a] [307-24-4]	Loveless *et al.*[17]	Oral gavage	Crl:CD(SD) rats	90	0	2.69	0.17	10
					20	2.70	0.26	10
					100	3.00	0.23	10
					500	4.38	0.49	10
Perfluorooctanoic acid (PFOA, C8) [335-67-1]	Perkins *et al.*[18]	Oral dietary	ChR-CD rats	91	0	3.24	0.28	15
					0.06	3.24	0.23	15
					0.64	3.69	0.32	15
					1.94	4.21	0.56	15
					6.50	5.50	0.84	15
Perfluorononanoic acid (PFNA, C9)[b] [375-95-1]	Mertens *et al.*[19]	Oral gavage	Crl:CD (SD)IGS BR rats	91	0	2.50	0.10	15
					0.025	2.63	0.19	10
					0.125	3.12	0.31	10

PFAS (abbreviation, carbon chain length) [CAS no.][c]	Reference	Administration route	Strain	Exposure duration (days)	External dose (mg/kg bw/day)	Relative liver weight		
						Mean (g/100g bw)	SD	*n*
					0.6	4.51	0.43	15
Perfluoroundecanoic acid (PFUnDA, C11) [2058-94-8]	Takahashi *et al.*[20]	Oral gavage	Crl:CD(SD) rats	42	0	2.88	0.27	5
					0.1	3.02	0.19	5
					0.3	3.39	0.16	5
					1.0	4.18	0.19	5
Perfluorododecanoic acid (PFDoDA, C12) [307-55-1][c]	Kato *et al.*[21]	Oral gavage	Crl:CD(SD) rats	42	0	2.51	0.14	5
					0.1	2.67	0.21	5
					0.5	3.00	0.30	5
					2.5	4.30	0.27	5
Perfluorotetradecanoic acid (PFTeDA, C14) [376-06-7]	Hirata-Koizumi *et al.*[22]	Oral gavage	Crl:CD(SD) rats	42	0	2.41	0.11	7
					1	2.49	0.12	7
					3	2.87	0.23	7
					10	3.25	0.07	7
Perfluorohexadecanoic acid (PFHxDA, C16) [67905-19-5]	Hirata-Koizumi *et al.*[22]	Oral gavage	Crl:CD(SD) rats	42	0	2.50	0.04	7
					4	2.45	0.10	7
					20	2.49	0.15	7
					100	3.26	0.07	7
Perfluorooctadecanoic acid (PFODA, C18) [16517-11-6]	Hirata-Koizumi *et al.*[23]	Oral gavage	Crl:CD(SD) rats	42	0	2.36	0.28	5
					40	2.48	0.25	5
					200	3.35	0.14	5
					1000	5.00	0.13	5
2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid (HFPO-DA)[a] [13252-13-6]	Haas [24]	Oral gavage	Crl:CD(SD) rats	90	0	2.716	0.1319	10
					0.1	2.727	0.2125	10
					10	3.556	0.4752	10
					100	4.535	0.5144	10
Ammonium 4,8-dioxa-3H-perfluorononanoate [a] (ADONA) [958445-44-8]	Gordon [25]	Oral gavage	Sprague-Dawley rats	90	0	2.29	0.09	10
					1	2.27	0.11	10
					3	2.26	0.14	10
					10	2.4	0.23	10
1H,1H,2H,2H,-perfluoro-1-octanol (6:2 FTOH) [647-42-7]	Serex *et al.*[26]	Oral gavage	Crl:CD(SD) rats	90	0	2.949	0.26	10
					5	3.035	0.15	10
					25	3.252	0.158	10
					125	3.942	0.172	10
					250	4.608	0.393	8
1H,1H,2H,2H,-perfluoro-1-decanol (8:2 FTOH) [678-39-7]	Ladics *et al.*[27]	Oral gavage	Sprague–Dawley rats	90	0	2.69	0.22	10
					1	2.61	0.14	10
					5	2.67	0.15	10
					25	3.05	0.12	10

PFAS (abbreviation, carbon chain length) [CAS no.][c]	Reference	Administration route	Strain	Exposure duration (days)	External dose (mg/kg bw/day)	Relative liver weight		
						Mean (g/100g bw)	SD	*n*
					125	4.1	0.41	10

Note: bw, body weight; *n*, number of animals; SD, standard deviation.
[a] Study was performed with the ammonium, sodium or potassium salt.
[b] Study was performed with the substance S-111—S-WB [72968-38-8], defined as a mixture of perfluoro fatty acid ammonium salts of different carbon length (C6-C13) with the major component being ammonium perfluorononanoic acid (PFNA).
[c] CAS no. of the acid (except ADONA and both telomers) as listed on the EPA Chemistry Dashboard: https://comptox.epa.gov/dashboard (accessed Jan. 30, 2020)

Table S2. Specifications of the perfluoroalkyl substances (PFAS) measured in human blood serum in the National Health and Nutrition Examination Survey (NHANES). *Chemical names, abbreviations and CAS numbers of PFAS measured in NHANES cycle 2017-2018.*[1]

Substance	**Abbreviation**	**CAS number**	**Lower limit of detection (ng/mL)**
Perfluorohexanoic acid	PFHxA	307-24-4	0.100
Perfluorooctanoic acid (linear and branched isomers)	PFOA	335-67-1	0.100
Perfluorononanoic acid	PFNA	375-95-1	0.100
Perfluorodecanoic acid	PFDA	335-76-2	0.100
Perfluoroundecanoic acid	PFUnDA	2058-94-8	0.100
Perfluorohexane sulphonic acid	PFHxS	355-46-4	0.100
Perfluoroheptane sulphonic acid	PFHpS	375-92-8	0.100
Perfluorooctane sulphonic acid (linear and branched isomers)	PFOS	1763-23-1	0.100
Ammonium salt of 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	HFPO-DA	62037-80-3	0.100
Ammonium salt of 4,8-dioxa-3H-perfluorononanoic acid	ADONA	958445-44-8	0.100
9-Chlorohexadecafluoro-3-oxanonane-1-sulphonic acid	6:2 Cl-PFESA	73606-19-6	0.100
2-(N-Methyl-perfluorooctane sulphonamido) acetic acid	Me-FOSAA	2355-31-9	0.100

Note: Description on the laboratory method, quality assurance and monitoring, analytical notes, and codebook and frequencies may be found here:
Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS_J): https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/PFAS_J.htm;
Perfluoroalkyl and Polyfluoroalkyl Substances (Surplus) (SSPFAS_J): https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/SSPFAS_J.htm

Table S3. Organ/tissue to serum partition coefficients for perfluorododecanoic acid (PFDoDA) to calculate the volume of distribution (V1). *The organ/tissue and serum concentrations reported in Kawabata et al.[7] were used to calculate organ/tissue to serum partition coefficients in order to estimate the volume of distribution (V1) for model parametrization.*

Organ/tissue to serum partition coefficients			
Liver/ Serum	Kidney/ Serum	Adipose tissue[a]/Serum	Remaining organs[b]/Serum
7.925	1.679	0.3933	0.3206

[a] Average concentration of epididymal, mesenteric, and subcutaneous adipose tissue used
[b] Average concentration of lung, heart, brain, spleen, and testis used

Table S4. Organ volume reference values to calculate the volume of distribution (V1) for perfluorododecanoic acid (PFDoDA). *Organ volume reference values for rats as proposed by Jongeneelen and ten Berge.[8]*

Organ	Volume as fraction of body weight (rat)
Whole blood	0.096
Serum/Plasma	0.054[a]
Liver	0.040
Adipose tissue	0.070
Kidneys	0.007
Remaining organs	0.787

[a] applying a 0.56 blood to plasma conversion while ignoring erythrocyte binding (in concordance with the PBK modelling in monkey and humans in Fàbrega *et al.*[28]

Table S5. Internal relative potency factors (RPFs) and lower and upper bounds of the 90%-confidence intervals for perfluoroalkyl substances (PFAS) based on relative liver weight increase in the male rat. *The confidence intervals do not include the uncertainty resulting from the external-to-internal dosing extrapolation, but solely the uncertainty in the toxicity data.*

Compound	**Lower bound 90% CI**	**Upper bound 90% CI**	**Internal RPF**
PFBS	0.13	0.27	0.19
PFHxS	0.46	0.78	0.61
PFOS	2.2	5.0	3.5
PFBA	1.1	2.7	1.8
PFHxA	8.7	15	12
PFOA			1
PFNA	4.5	7.10	5.5
PFDoDA	8.3	14	11
HFPO-DA	6.5	12	8.8

Note: CI, confidence interval; HFPO-DA, hexafluoropropylene oxide-dimer acid; PFBA, perfluorobutanoic acid; PFBS, perfluorobutane sulfonic acid; PFDoDA, perfluorododecanoic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

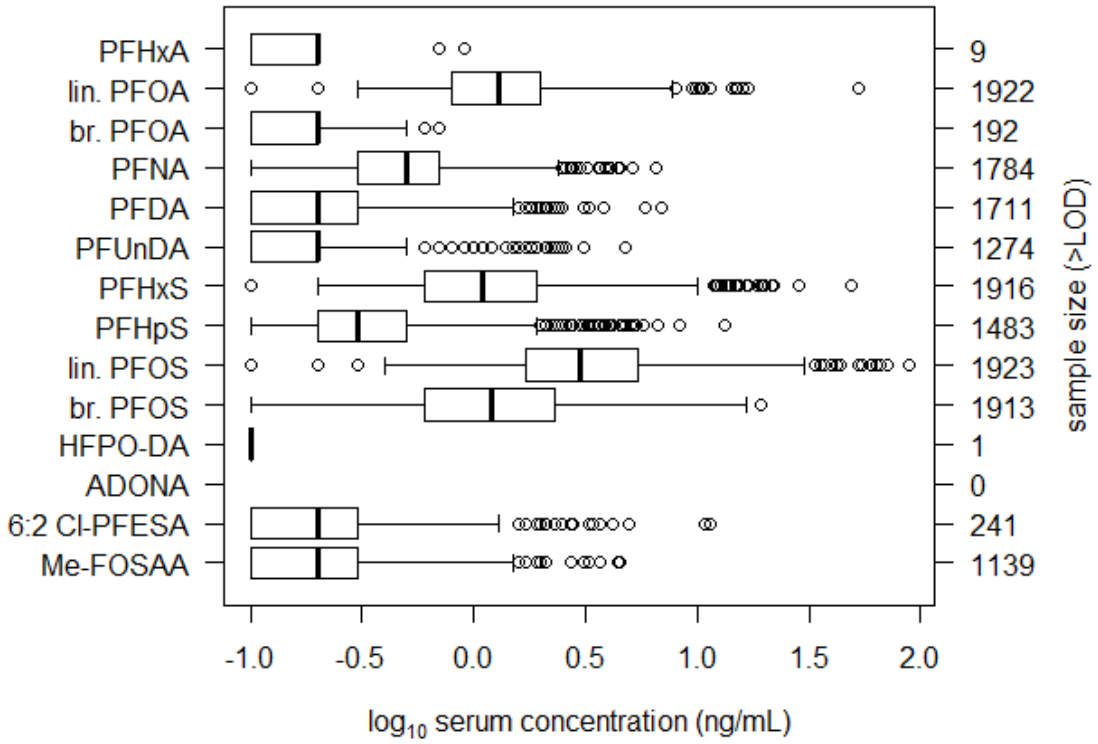

Figure S1. Overview of National Health and Nutrition Examination Survey (NHANES) results.[1] Note: serum concentrations of each perfluoroalkyl substance (PFAS) in the sampled NHANES study population (*n* = 1929) in the 2017-2018 cycle above lower limit of detection (LOD, 0.100 ng/mL) plotted on a $\log_{10}$-scale (*x*-axis). Values below lower LOD are not plotted. On the right *y*-axis are the number of samples above LOD. 6:2 Cl-PFESA, 9-chlorohexadecafluoro-3-oxanonane-1-sulphonic acid; ADONA, ammonium salt of 4,8-dioxa-3H-perfluorononanoic acid; br., branched; HFPO-DA, hexafluoropropylene oxide-dimer acid; lin., linear; LOD, lower limit of detection; Me-FOSAA, 2-(N-methyl-perfluorooctane sulphonamido) acetic acid; PFDA, perfluorodecanoic acid; PFHpS, perfluoroheptane sulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluoroocane sulfonic acid; PFUnDA, perfluoroundecanoic acid.

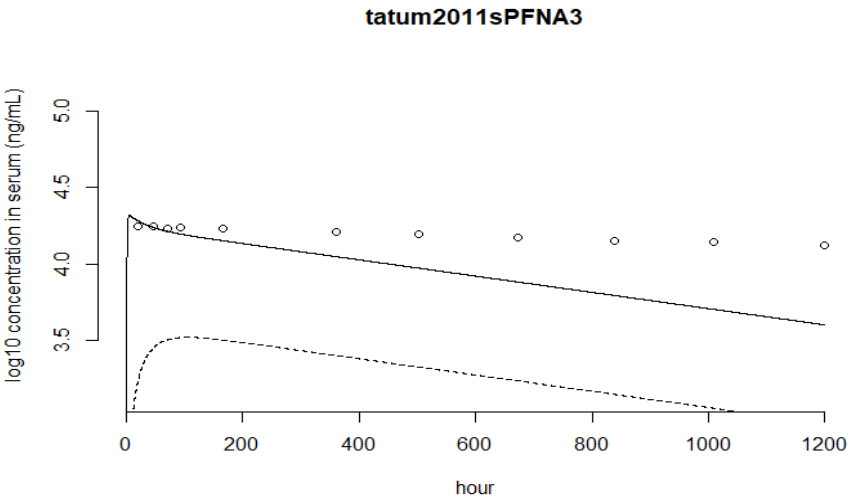

Figure S2. Simulation of a single dose experiment for PFNA with a two-compartment model fitted to measured serum concentrations for single oral exposure of male rats to 3 mg/kg PFNA. Note: modelling was based on the parameter values in Tatum-Gibbs *et al.*[5] and plotted together with the serum measurements reported in that study. The solid and dashed lines are the modelled concentrations in the first and second compartments respectively, the circles indicate the mean measured serum concentrations over time. Visually, this model does not seem to describe the data adequately; the data do not show biphasic elimination and the modelled elimination appears faster compared to the measured elimination.

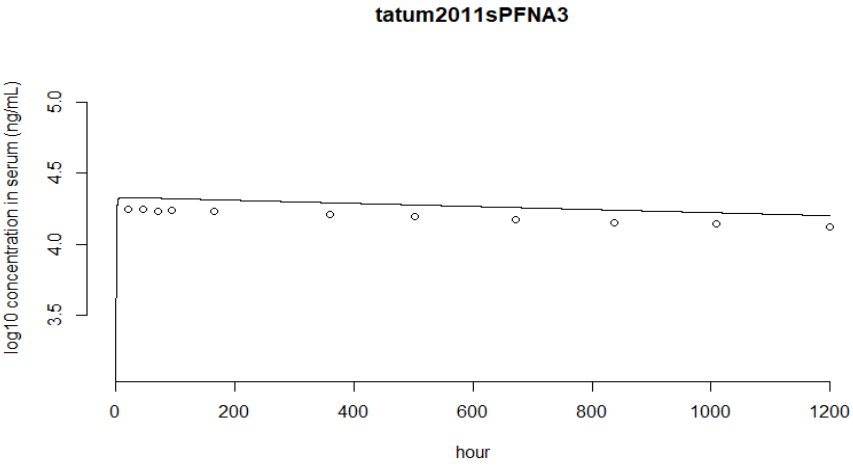

Figure S3. Simulation of a single dose experiment for PFNA with a one-compartment model fitted to measured serum concentrations for single oral exposure of male rats to 3 mg/kg PFNA with a lower elimination rate. Note: in this simulation, the elimination rate was lowered to obtain a more realistic description of the elimination phase. The solid line is the modelled concentration, the circles indicate the mean measured serum concentration over time. Visually, the fit slightly overestimated the serum concentration measurements reported in Tatum-Gibbs *et al.*[5] The parameter values used for the simulation were $k10$ (0.00025/h), $k01$ (1/h), bw (0.5 kg), $V1$ (0.139 L/kg).

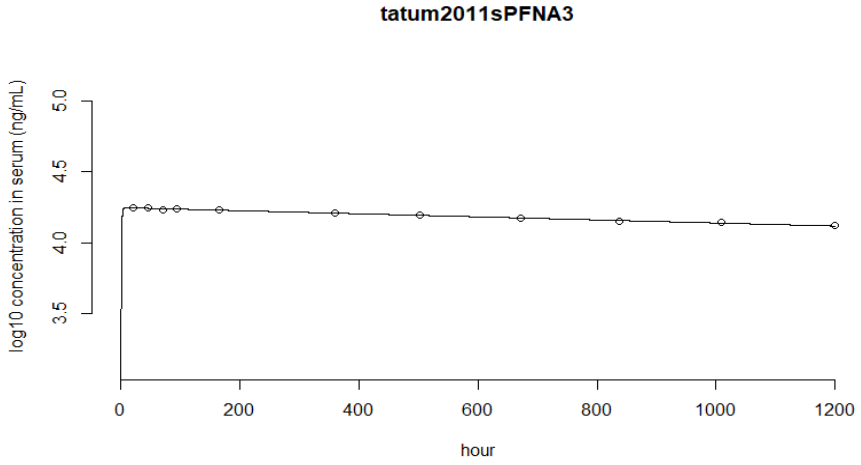

Figure S4. Simulation of a single dose experiment for PFNA with a one-compartment model fitted to measured serum concentrations for single oral exposure of male rats to 3 mg/kg PFNA with an increased volume of distribution. Note: in this simulation, the volume of distribution was increased. The solid line is the modelled concentration, the circles indicate the mean measured serum concentration over time. Visually, the simulation described the serum concentration measurements reported in Tatum-Gibbs *et al.*[5] The parameter values used for the simulation were $k10$ (0.00025/h), $k01$ (1/h), bw (0.5 kg), $V1$ (0.170 L/kg).

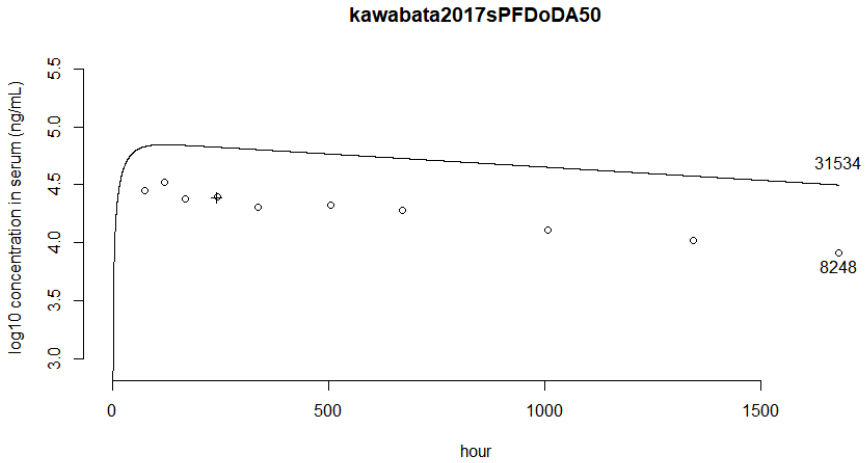

Figure S5. Simulation of a single dose experiment for PFDA with a one-compartment model fitted to measured serum concentrations for single oral exposure of male rats to 50 mg/kg PFDA. Note: modelling was based on the parameter values in Kawabata *et al.*[7], the volume of distribution of 0.663 L/kg calculated in this study, and plotted together with the serum measurements reported. The solid curve indicates the model estimate. Circles indicate the mean measured serum concentration data from Kawabata *et al.*[7] Plus sign at t = 240 hr indicates the measured serum concentration reported in Kawabata *et al.*[2] The two values on the right side of the plot indicate the measured and modelled serum concentrations at the end of the experiment. The fit overestimated the serum concentration measurement with a factor 3.8.

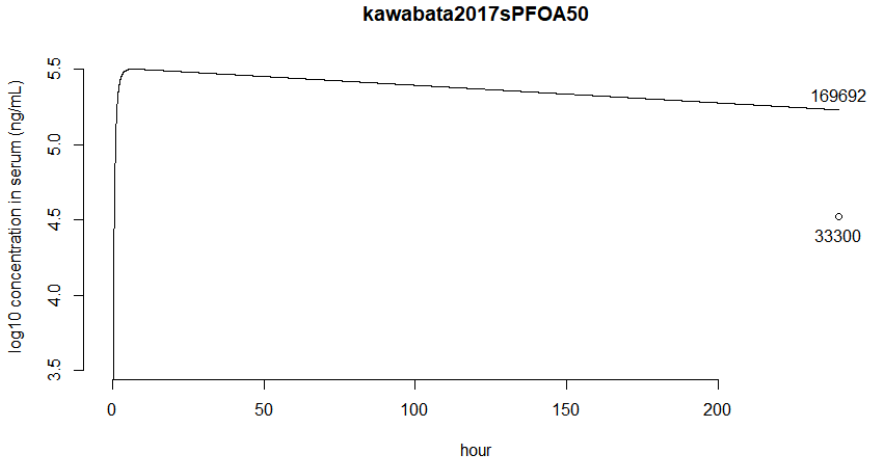

Figure S6. Simulation of a single dose experiment for PFOA with a one-compartment model according to the experimental conditions reported in Kawabata *et al.*[2], using the one-compartment model for PFOA parametrized based on Dzierlenga *et al.*[3] Note: the solid curve indicates the model estimate. Circle (at t = 240 hr) indicates the measured serum concentration reported in Kawabata *et al.*[2] The two values on the right side of the plot indicate the measured and modelled serum concentrations at the end of the experiment. Fitting the model of PFOA showed that the model overestimates the measurements of Kawabata *et al.*[2]

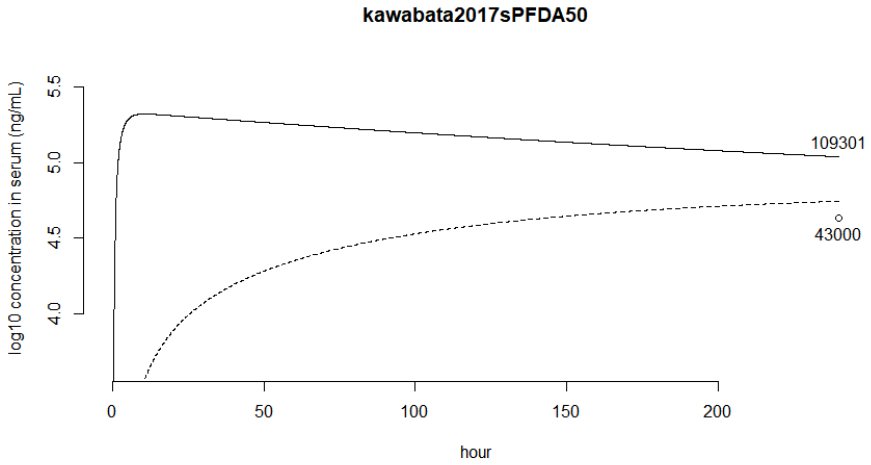

Figure S7. Simulation of a single dose experiment for PFDA with a one-compartment model according to the experimental conditions reported in Kawabata *et al.*[2] using the one-compartment model for PFDA based on parameters from Dzierlenga *et al.*[3] Note: the solid and dashed curves indicate the model estimates of the serum concentrations in the central an peripheral compartments respectively. Circle (at t = 240 hr) indicates the measured serum concentration reported in Kawabata *et al.*[2] The two values on the right side of the plot indicate the measured and modelled (central compartment) serum concentrations at the end of the experiment. Fitting the model of PFDA showed that the model overestimates the measurements of Kawabata *et al.*[2]

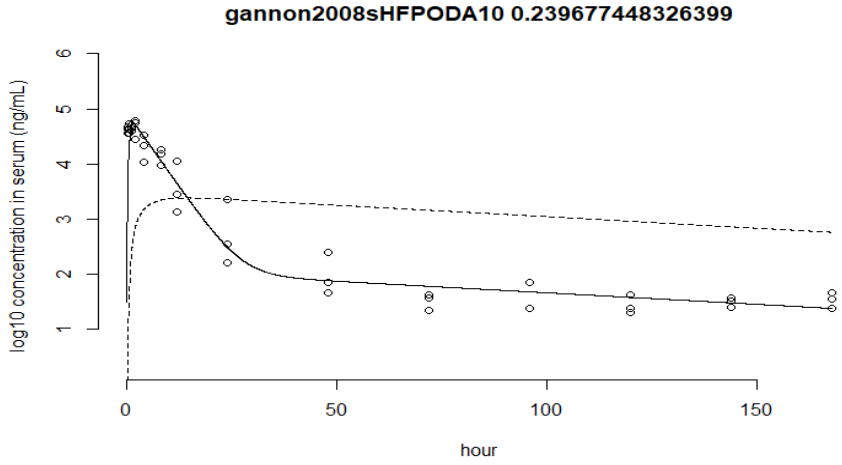

Figure S8. Simulation of a single dose experiment for HFPO-DA with a two-compartment model fitted to measured plasma concentrations for single oral exposure of male rats to 10 mg/kg HFPO-DA[4] to find the optimum elimination rate. Note: Gannon *et al.*[9] provide a value for absorption rate (*k*01), alpha rate, beta rate and the volume of distribution (*V*1), but not for the elimination rate (*k*10). Therefore, *k*10 was obtained by optimizing the ratio between the model and the plasma concentration measurements. Solid and dashed lines are the modelled concentrations in the first and second compartments respectively. Optimizing a two-compartment model to the measurements results in a value for *k*10 of 0.24/hr.

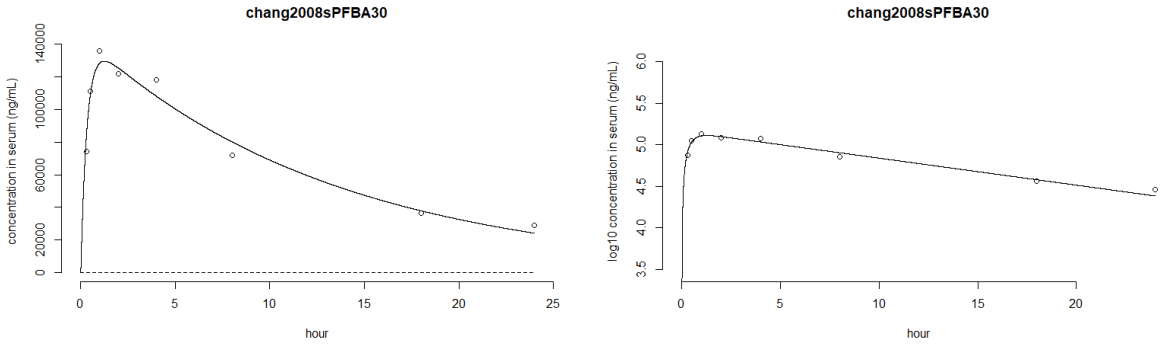

Figure S9. Simulation of a single dose experiment for PFBA based on the parameter values in Table 1 and an average assumed body weight of 0.400 kg. Note: PFBA serum concentration plotted against time (hr) after a single dose of 30 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. Circles are the mean measured concentrations from Chang *et al.*[29]

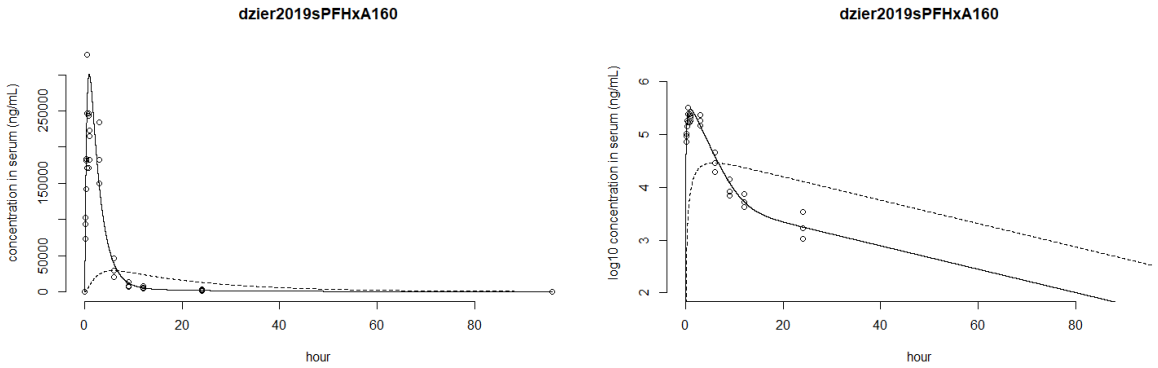

Figure S10. Simulation of a single dose experiment for PFHxA based on the parameter values in Table 1 and an average reported body weight of 0.223 kg in Dzierlenga *et al.*[3] Note: PFHxA serum concentration plotted against time (hr) after a single dose of 160 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. The dashed line indicates the concentration in the peripheral compartment. Circles are the individual measured concentrations from Dzierlenga *et al.*[3] Note: three serum concentrations at t = 96 hr are below LOQ, and not plotted on log *y*-axis.

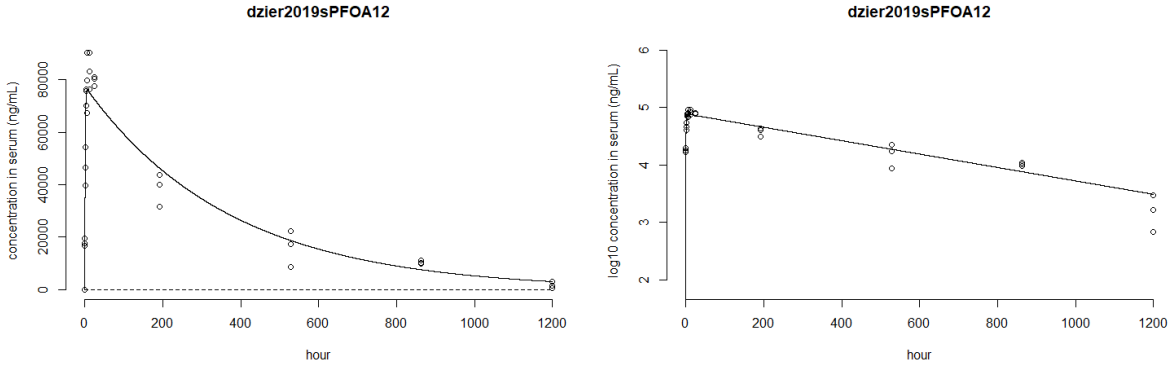

Figure S11. Simulation of a single dose experiment for PFOA based on the parameter values in Table 1 and an average reported body weight of 0.218 kg in Dzierlenga *et al.*[3] PFOA serum concentration plotted against time (hr) after a single dose of 12 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. Circles are the individual measured concentrations from Dzierlenga *et al.*[3]

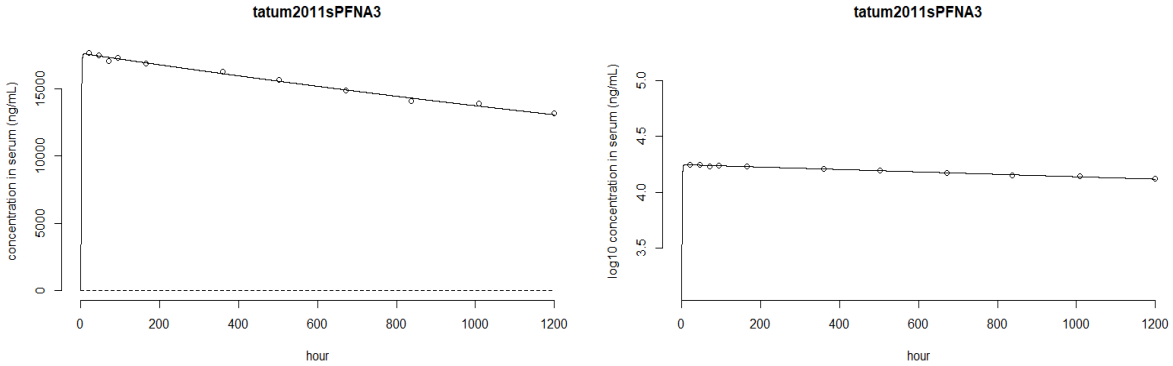

Figure S12. Simulation of a single dose experiment for PFNA based on the parameter values in Table 1 and an average assumed body weight of 0.500 kg in Tatum-Gibbs *et al.*[5] PFNA serum concentration plotted against time (hr) after a single dose of 3 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. Circles are the mean measured concentrations from Tatum-Gibbs *et al.*[5]

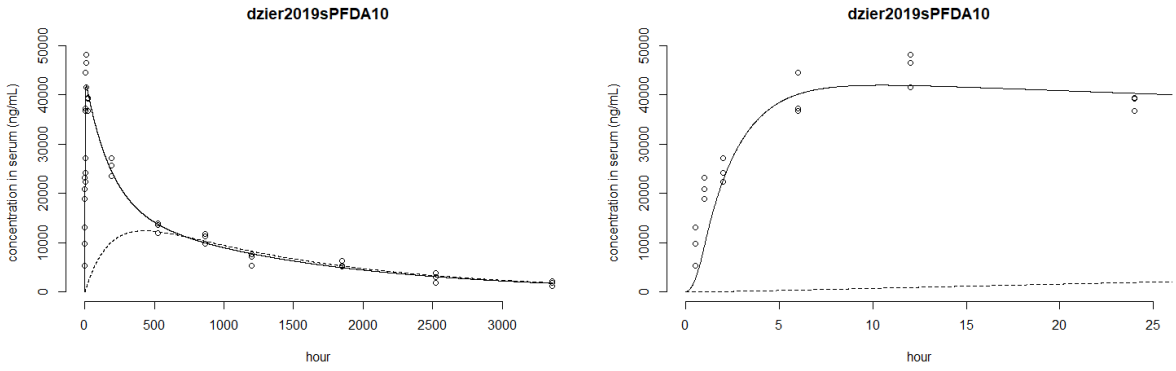

Figure S13. Simulation of a single dose experiment for PFDA based on the parameter values in Table 1 and an average reported body weight of 0.255 kg in Dzierlenga *et al.*[3] PFDA serum concentration plotted against time (hr) after a single dose of 10 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. The dashed line indicates the concentration in the peripheral compartment. Circles are the individual measured concentrations from Dzierlenga *et al.*[3]

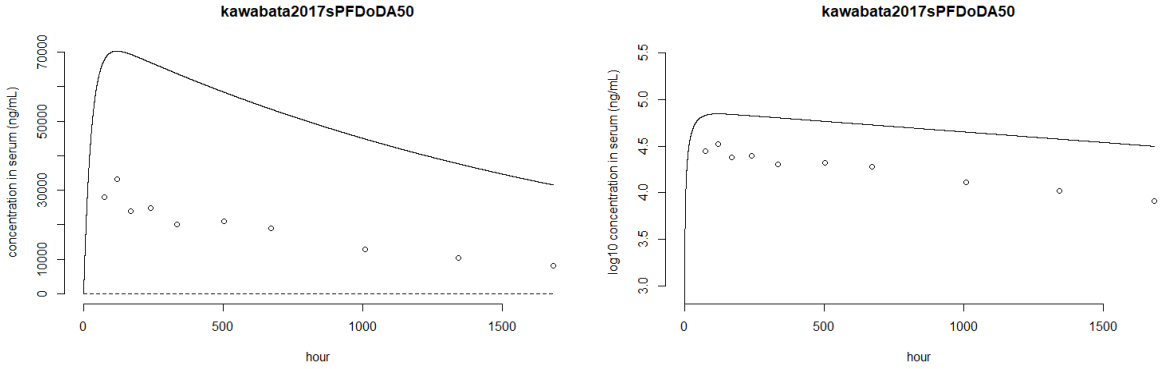

Figure S14. Simulation of a single dose experiment for PFDoDA based on the parameter values in Table 1 and an average assumed body weight of 0.400 kg. PFDoDA serum concentration plotted against time (hr) after a single dose of 50 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. Circles are the mean measured concentrations from Kawabata *et al.*[7]

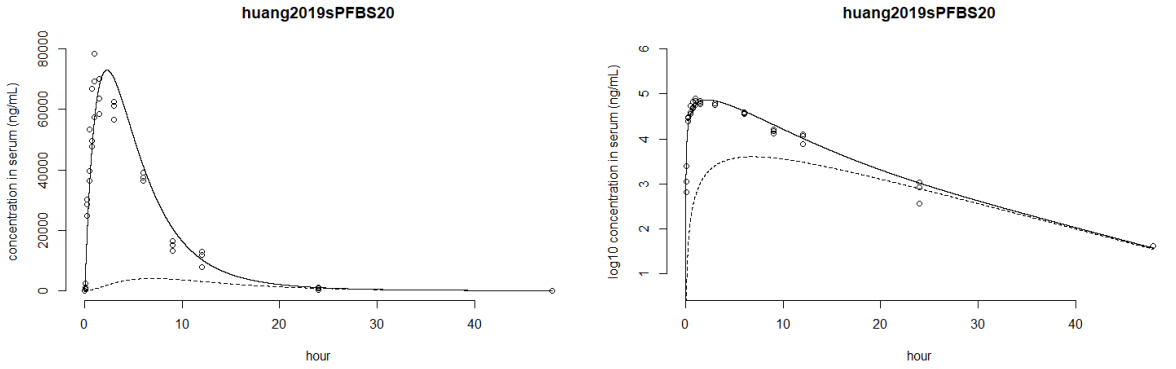

Figure S15. Simulation of a single dose experiment for PFBS based on the parameter values in Table 1 and an average reported body weight of 0.248 kg in Huang *et al.*[6] PFBS serum concentration plotted against time (hr) after a single dose of 20 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. The dashed line indicates the concentration in the peripheral compartment. Circles are the individual measured concentrations from Huang *et al.*[6]

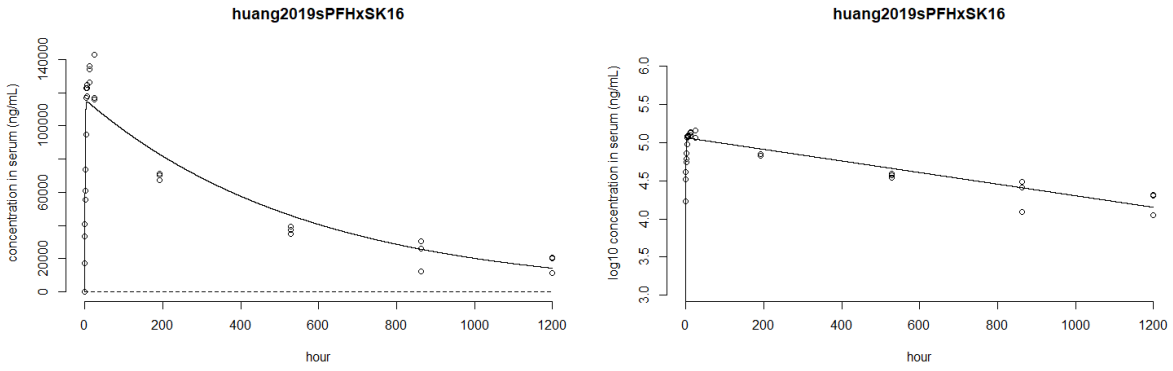

Figure S16. Simulation of a single dose experiment for PFHxS based on the parameter values in Table 1 and an average reported body weight of 0.247 kg in Huang *et al.*[6] PFHxS serum concentration plotted against time (hr) after a single dose of 16 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. Circles are the individual measured concentrations from Huang *et al.*[6]

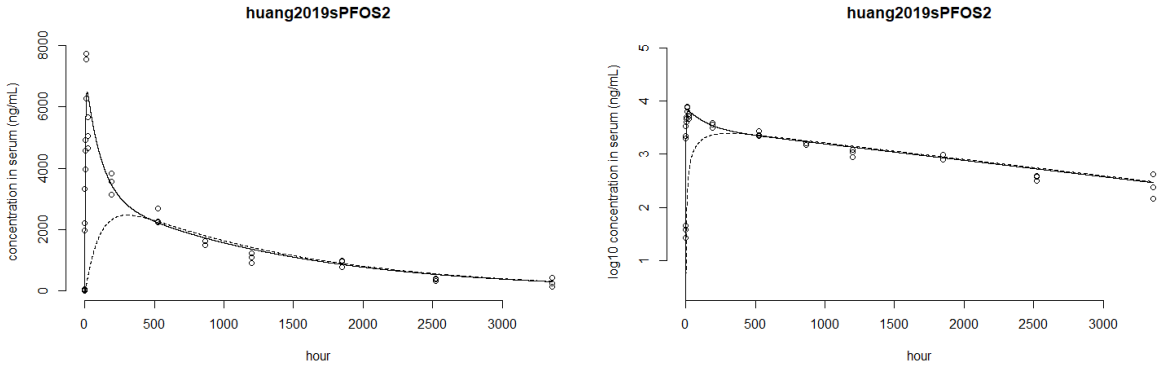

Figure S17. Simulation of a single dose experiment for PFOS based on the parameter values in Table 1 and an average reported body weight of 0.240 kg in Huang *et al.*[6] PFOS serum concentration plotted against time (hr) after a single dose of 2 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. The dashed line indicates the concentration in the peripheral compartment. Circles are the individual measured concentrations from Huang *et al.*[6]

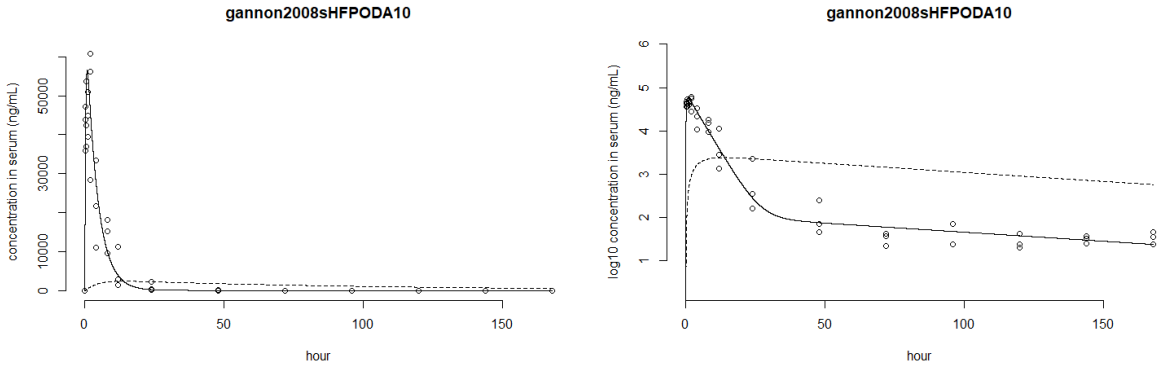

Figure S18. Simulation of single dose experiment for HFPO-DA based on the parameter values in Table 1 and an average assumed body weight of 0.400 kg. HFPO-DA serum concentration plotted against time (hr) after a single dose of 10 mg/kg. In the right panel serum concentrations are plotted on the $\log_{10}$ scale. The solid line is the modelled concentration. The dashed line indicates the concentration in the peripheral compartment. Circles are the individual measured concentrations from Gannon.[4]

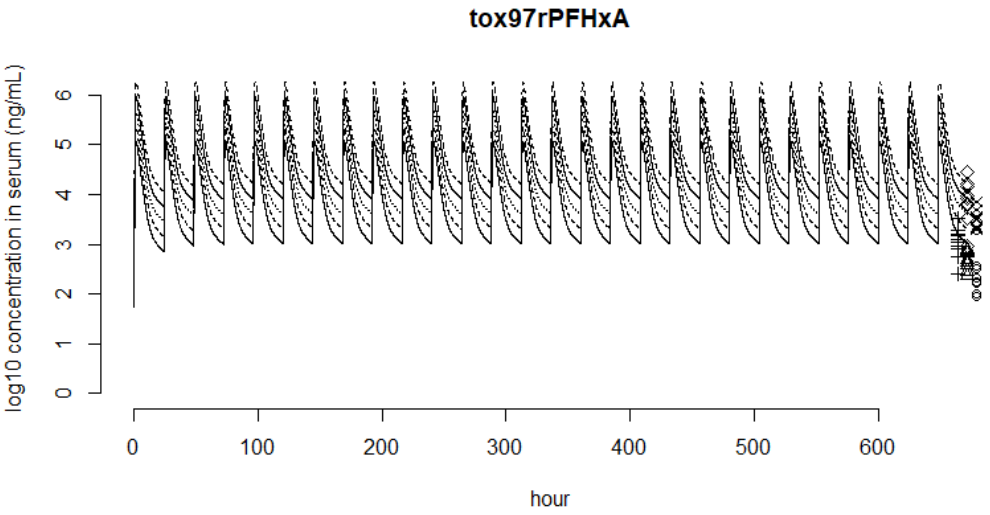

Figure S19. Simulation of a 28-day repeated dose experiment for PFHxA. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Log_{10} PFHxA serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 62.6 mg/kg bw/day; lower dashed line and triangles = 125 mg/kg bw/day; dotted line and plusses = 250 mg/kg bw/day; upper solid line and crosses = 500 mg/kg bw/day; upper dashed line and diamonds = 1000 mg/kg bw/day. The lines are the modeled concentrations using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Symbols are the measured concentrations from NTP [30]. To distinguish measured points they have been shifted slightly.

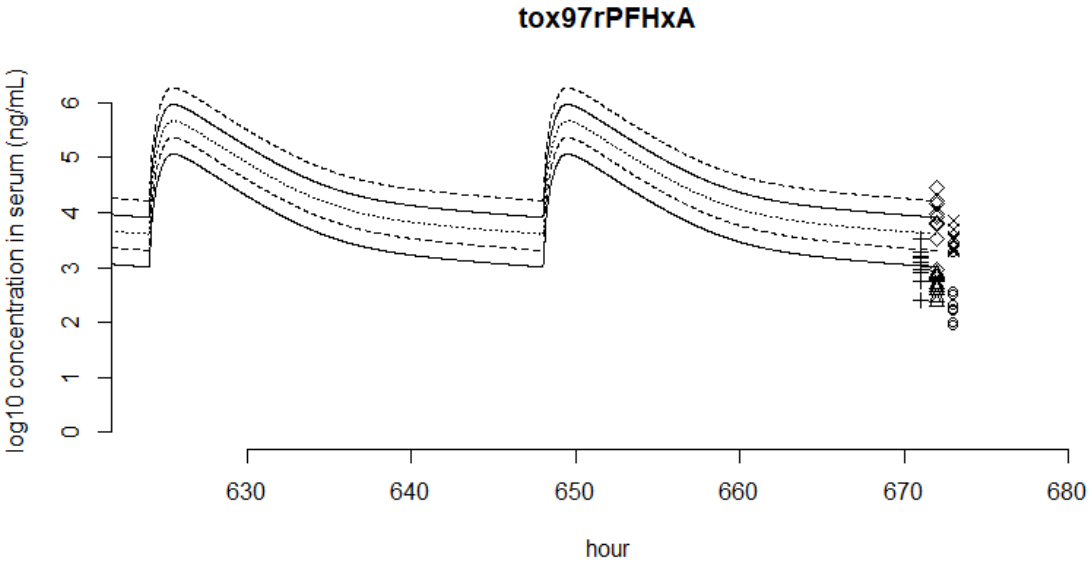

Figure S20. Simulation of a 28-day repeated dose experiment for PFHxA for the last ~48 hours of the experiment. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]**.** Log_{10} PFHxA serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 62.6 mg/kg bw/day; lower dashed line and triangles = 125 mg/kg bw/day; dotted line and plusses = 250 mg/kg bw/day; upper solid line and crosses = 500 mg/kg bw/day; upper dashed line and diamonds = 1000 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Symbols are the measured concentrations from NTP [30]. To distinguish measured points they have been shifted slightly.

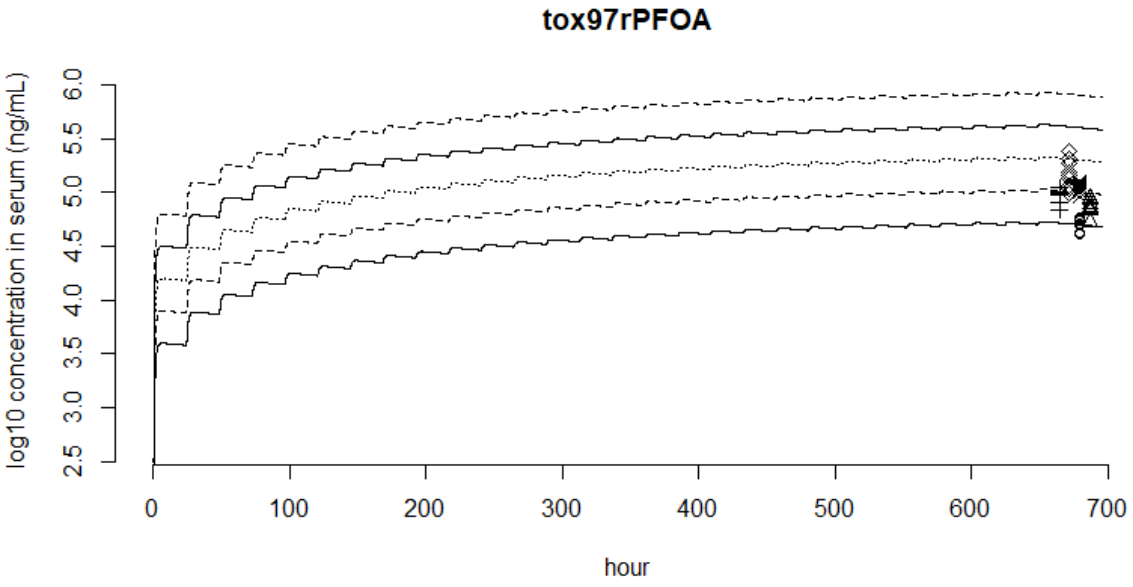

Figure S21. Simulation of a 28-day repeated dose experiment for PFOA. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Log_{10} PFOA serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.625 mg/kg bw/day; lower dashed line and triangles = 1.25 mg/kg bw/day; dotted line and plusses = 2.5 mg/kg bw/day; upper solid line and crosses = 5 mg/kg bw/day; upper dashed line and diamonds = 10 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Symbols are the measured concentrations from NTP [30]. To distinguish measured points they have been shifted slightly.

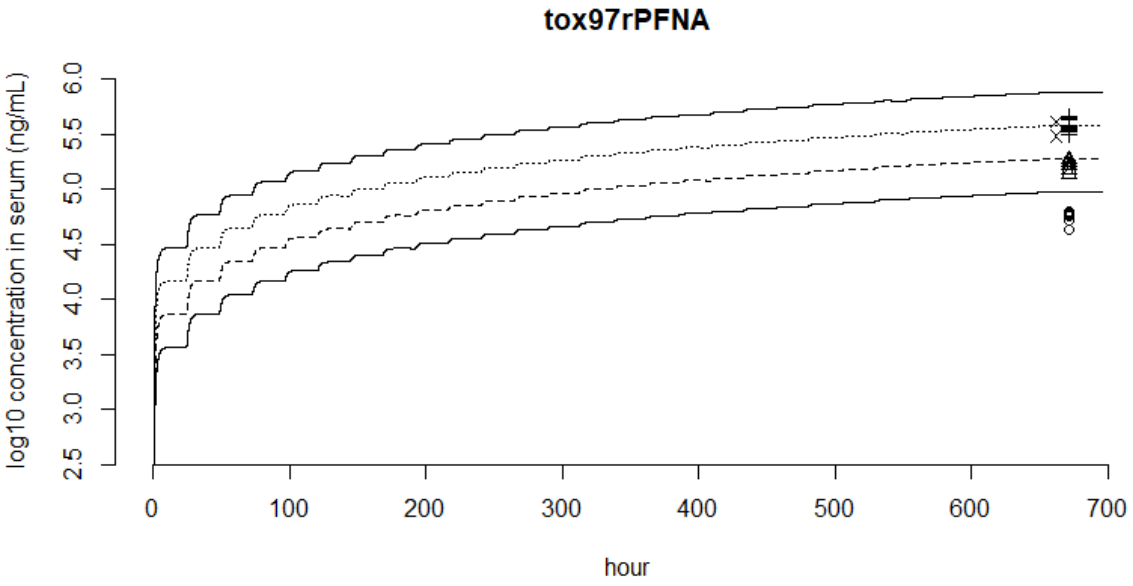

Figure S22. Simulation of a 28-day repeated dose experiment for PFNA. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Log_{10} PFNA serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.625 mg/kg bw/day; dashed line and triangles = 1.25 mg/kg bw/day; dotted line and plusses = 2.5 mg/kg bw/day; upper solid line and crosses = 5 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Symbols are the measured concentrations from NTP [30]. To distinguish measured points they have been shifted slightly. Only two animals in highest dose group survived. No animals survived in the 10 mg/kg bw/day dose group, therefore no curve and points are given.

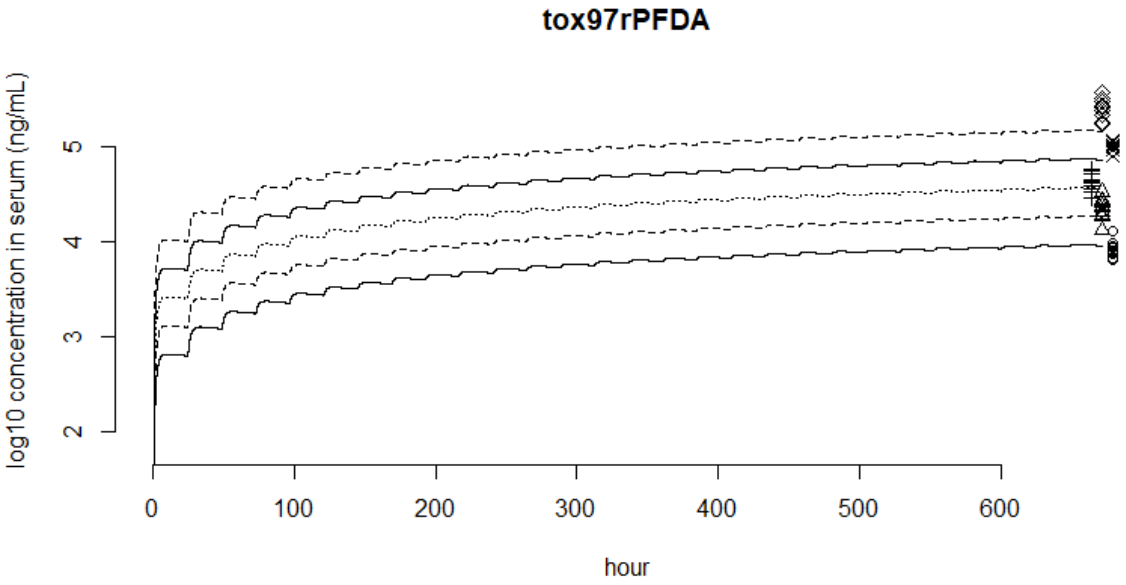

Figure S23. Simulation of a 28-day repeated dose experiment for PFDA. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Log_{10} PFDA serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.156 mg/kg bw/day; lower dashed line and triangles = 0.312 mg/kg bw/day; dotted line and plusses = 0.625 mg/kg bw/day; upper solid line and crosses = 1.25 mg/kg bw/day; upper dashed line and diamonds = 2.5 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [30]. Symbols are the measured concentrations from NTP [30]. To distinguish measured points they have been shifted slightly.

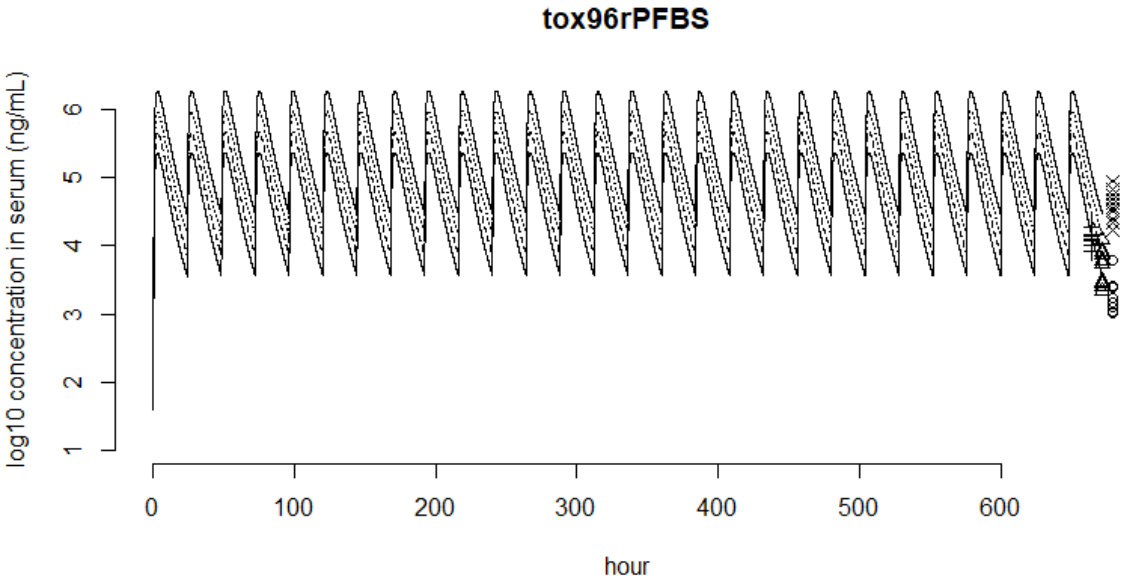

Figure S24. Simulation of a 28-day repeated dose experiment for PFBS. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Log_{10} PFBS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 62.6 mg/kg bw/day; dashed line and triangles = 125 mg/kg bw/day; dotted line and plusses = 250 mg/kg bw/day; upper solid line and crosses = 500 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Symbols are the measured concentrations from NTP [31]. To distinguish measured points they have been shifted slightly.

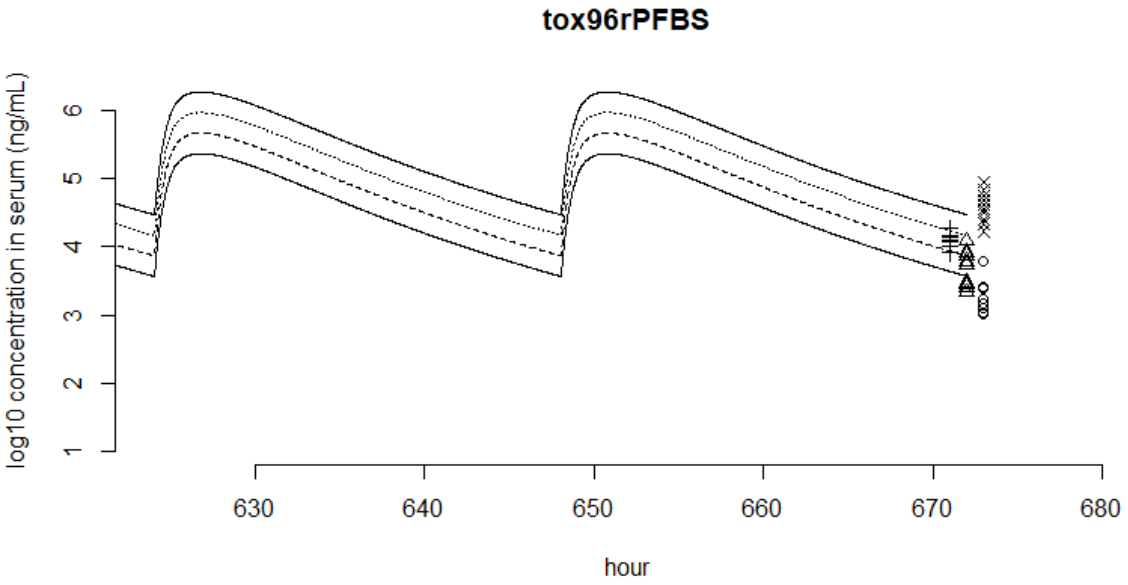

Figure S25. Simulation of a 28-day repeated dose experiment for PFBS for the last ~48 hours of the experiment. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Log_{10} PFBS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 62.6 mg/kg bw/day; dashed line and triangles = 125 mg/kg bw/day; dotted line and plusses = 250 mg/kg bw/day; upper solid line and crosses = 500 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Symbols are the measured concentrations from NTP [31]. To distinguish measured points they have been shifted slightly.

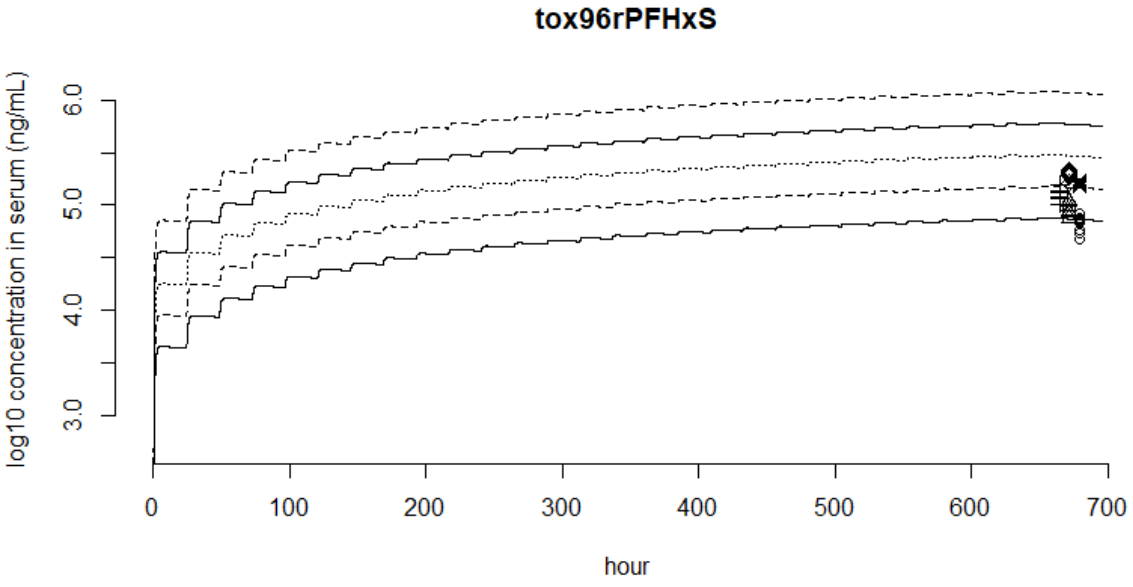

Figure S26. Simulation of a 28-day repeated dose experiment for PFHxS. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Log_{10} PFHxS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.625 mg/kg bw/day; lower dashed line and triangles = 1.25 mg/kg bw/day; dotted line and plusses = 2.5 mg/kg bw/day; upper solid line and crosses = 5 mg/kg bw/day; upper dashed line and diamonds = 10 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Symbols are the measured concentrations from NTP [31]. To distinguish measured points they have been shifted slightly.

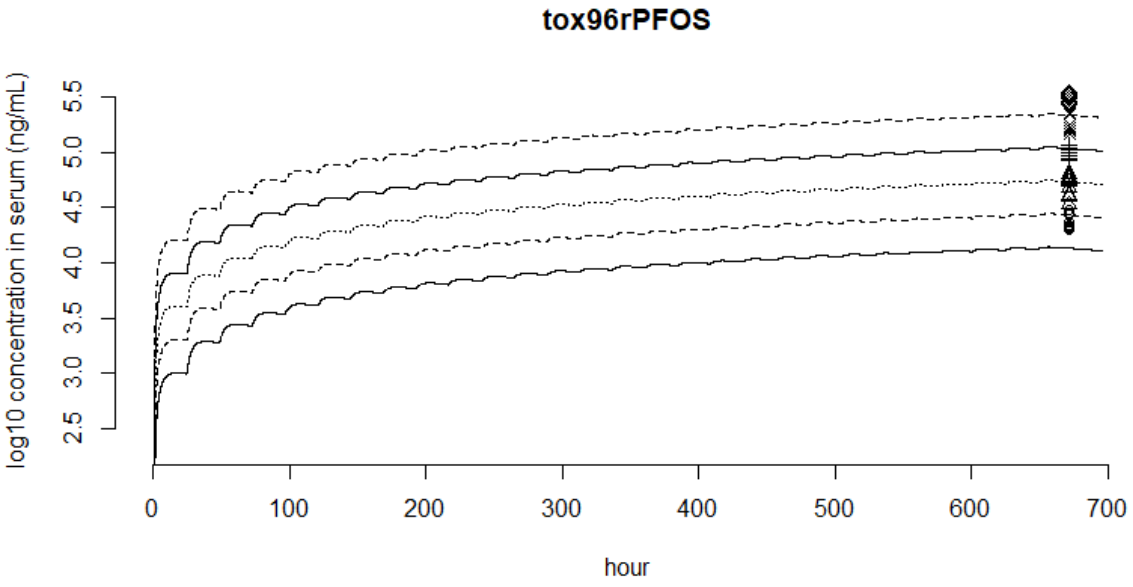

Figure S27. Simulation of a 28-day repeated dose experiment for PFOS. Note: for each PFAS their one- and two-compartment models were implemented using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Log_{10} PFOS serum concentration plotted against time (hr) after repeated doses of lower solid line and circles = 0.312 mg/kg bw/day; lower dashed line and triangles = 0.625 mg/kg bw/day; dotted line and plusses = 1.25 mg/kg bw/day; upper solid line and crosses = 2.5 mg/kg bw/day; upper dashed line and diamonds = 5 mg/kg bw/day. The lines are the modeled concentration using the parameter values listed in Table 1 and the exposure conditions as reported in NTP [31]. Symbols are the measured concentrations from NTP [31].

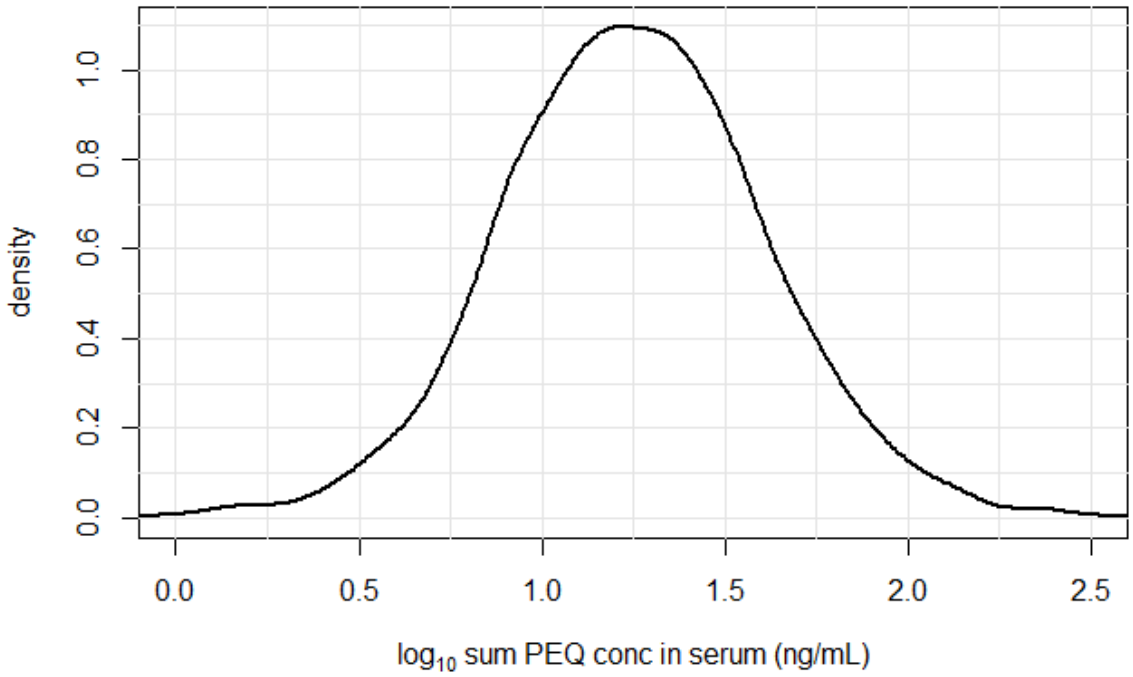

Figure S28. National Health and Nutrition Examination Survey (NHANES) perfluoroalkyl substance (PFAS) measurements in blood plasma [1] presented as PFOA equivalents. Note: density plot of the sum PEQ concentration in serum (ng/mL) of all sexes and ages from the NHANES study population (*n*= 1929). The black line represents the sum PEQ of all PFAS included (perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), perfluoroocane sulfonic acid (PFOS), hexafluoropropylene oxide-dimer acid (HFPO-DA)) of which internal RPFs were derived.

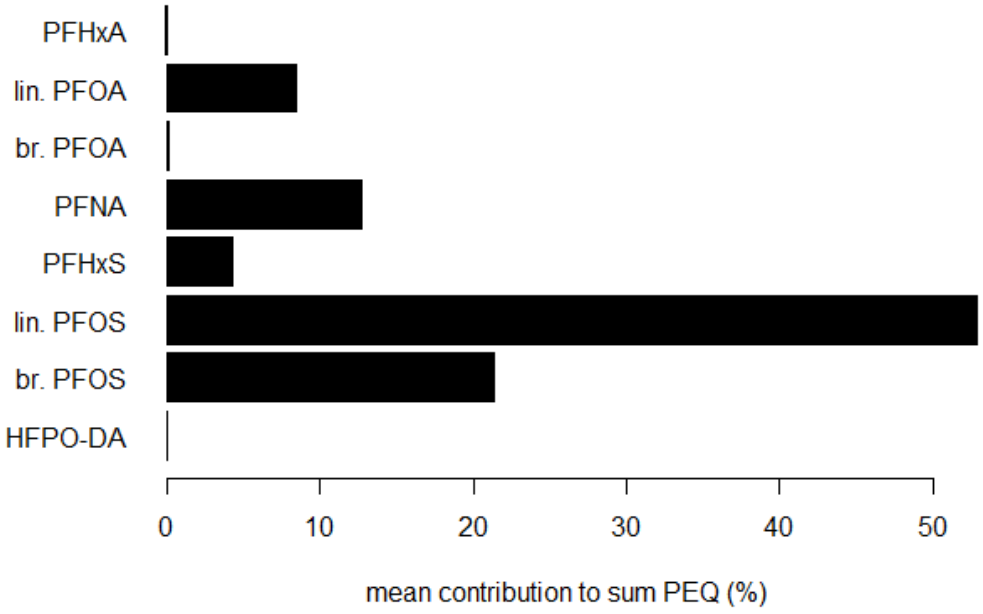

Figure S29. Mean contribution of each PFAS to the individual's total PFOA equivalents (PEQs) concentration. Note: contribution (%) of each perfluoroalkyl substance (PFAS) (perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) as linear and branched forms combined, perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS) as linear and branched forms combined, and hexafluoropropylene oxide-dimer acid (HFPO-DA)) to the sum of PEQs based on the PFAS serum concentration data from the National Health and Nutrition Examination Survey (NHANES) 2017-2018 cycle (*n* = 1929).

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