Environ Health Perspect

DOI: 10.1289/EHP10009

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 S1. Overview of National Health and Nutrition Examination Survey (NHANES) results. 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*. 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*. 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*. 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*., 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*. Plus sign at t = 240 hr indicates the measured serum concentration reported in Kawabata *et al*. 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.

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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. 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. Symbols are the measured concentrations from NTP. 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. 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. Symbols are the measured concentrations from NTP. 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. 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. Symbols are the measured concentrations from NTP. 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. 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.

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. 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. Symbols are the measured concentrations from NTP. 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. 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