Physiologically based pharmacokinetic modeling of human exposure to perfluorooctanoic acid suggests historical non drinking-water exposures are important for predicting current serum concentrations – SUPPLEMENTARY INFORMATION

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PBPK Model for Human Exposure to PFOA

Model code was written and simulations were performed using AcslX modeling software (AEgis Technologies, Huntsville, AL, version 3.1.4.2).

```
PROGRAM PBPK Model for PFOA in Adult Humans.
! Created by R. Worley scaled from PFOA Adult Rat Model
! Model includes compartments for GI Tract, Liver, Rest of Body(Tis), Kidney Blood, Proximal Tubule Cells,
Filtrate, and Plasma.
! Exposure route is oral ingestion (into gut compartment)
! Excretion pathways are feces and urine.
! Units of concentration in ug/L.

  INITIAL

CONSTANT tstart = 0.0

  ! Physiological Parameters
    CONSTANT BW = 82.3  !bodyweight (kg); EPA Factors Handbook, 2011

    ! Cardiac Output and Bloodflow (as fraction of cardiac output)
    CONSTANT QCC = 12.5 !cardiac output in L/h/kg^0.75; Brown 1997, Forsyth 1968
    CONSTANT QLC = 0.25 !fraction blood flow to liver; Brown 1997, Fisher 2000
    CONSTANT QKC = 0.175 !fraction blood flow to kidney; Brown 1997, Forsyth 1968
    CONSTANT Htc = 0.44 !hematocrit for the human; Davies and Morris 1993, Brown 1997

    ! Tissue Volumes
    CONSTANT VplasC = 0.0428 !fraction vol. of plasma (L/kg BW); Davies 1993
    CONSTANT VLC = 0.026 !fraction vol. of liver (L/kg BW); Brown 1997
    CONSTANT VKC = 0.004 !fraction vol. of kidney (L/kg BW); Brown 1997
    CONSTANT VfilC = 0.0004  !fraction vol. of filtrate (L/kg BW)
    CONSTANT VPTCC = 1.35e-4 !vol. of proximal tubule cells (L/g kidney) (60 million PTC cells/gram kidney, 1
PTC = 2250 um3) CHECK

  ! Chemical Specific Parameters
    CONSTANT MW = 414.07  !PFOA molecular mass (g/mol)
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    CONSTANT Free = 0.02 !free fraction in plasma; Loccisano, 2011

    !Kidney Transport Parameters
    CONSTANT Vmax_baso_invitro = 439.2 !Vmax of basolateral transporter (pmol/mg protein/min); averaged in
vitro value of OAT1 and OAT3 from Nakagawa, 2007
    CONSTANT Km_baso = 20100.0 !Km of basolateral transporter (ug/L; average of OAT1 and OAT3 from Nakagawa
et. al, 2007
    CONSTANT Vmax_apical_invitro = 37400.0 !Vmax of apical transporter (pmol/mg protein/min); invitro value
for OAT4 from Yang, 2010.
    CONSTANT Km_apical = 77500.0 !Km of apical transporter (ug/L); averaged in vitro value for OAT4 and URAT1
from Yang, 2010.
    CONSTANT RAFbaso = 1.0  !relative activity factor, basolateral transporters; fit to data
    CONSTANT RAFapi = 0.0007  !relative activity factor, apical transporters; fit to data
    CONSTANT protein = 2.0e-6  !amount of protein in proximal tubule cells (mg protein/proximal tubule cell);
Addis, 1936

    CONSTANT GFRC = 24.19  !glomerular filtration rate (L/hr/kg kidney); Corley, 2005

    !Partition Coefficients
    CONSTANT PL = 1.03 !liver:blood (from human cadaver data, Fabrega, 2014)
    CONSTANT PK = 1.17 !kidney:blood (from human cadaver data, Fabrega, 2014)
    CONSTANT PR = 0.11 !rest of body:blood (from rat tissue data, Kudo, 2007)

    !rate constants
    CONSTANT kdif = 0.001  !diffusion rate from proximal tubule cells (L/h)
    CONSTANT kabsc = 2.12  !rate of absorption of chemical from small intestine to liver (1/(h*BW^-0.25))(fit
to data)
    CONSTANT kunabsc = 7.06e-5  !rate of unabsorbed dose to appear in feces (1/(h*BW^-0.25)) (fit to data)
    CONSTANT GEC = 3.5 !gastric emptying time (1/(h*BW^-0.25)); from Yang, 2014
    CONSTANT k0C = 1.0  !rate of uptake from the stomach into the liver (1/(h*BW^-0.25))

    CONSTANT keffluxc = 0.1  !rate of clearance of PFOA from proximal tubule cells into blood (1/(h*BW^-
0.25))); fit to data
    CONSTANT kbilec = 0.0001 !biliary elimination rate ((male); liver to feces storage (1/(h*BW^-0.25)); fit
to data
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    CONSTANT kurinec = 0.063 !rate of urine elimination from urine storage (male) (1/(h*BW^-0.25))(fit to
data)
    CONSTANT kvoid = 0.06974  !daily urine volume rate (L/hr); Van Haarst, 2004

    ! Exposure Parameters
    countdw = 0.0
    hourofday = 0.0
    dayofweek = 0.0

  END ! INITIAL

  DYNAMIC

    ALGORITHM IALG = 2
    NSTEPS    NSTP = 10
    MAXTERVAL MAXT = 1.0e9
    MINTERVAL MINT = 1.0e-9
    CINTERVAL CINT = 100.0

     DERIVATIVE
   !Scaled Parameters
    !Cardiac output and blood flows
    QC = QCC*(BW**0.75)*(1-Htc)  !cardiac output in L/h; adjusted for plasma
    QK = (QKC*QC)  !plasma flow to kidney (L/h)
    QL = (QLC*QC)  !plasma flow to liver (L/h)
    QR = QC - QK - QL   !plasma flow to rest of body (L/h)
    QBal = QC - (QK + QL + QR) !Balance check of blood flows; should equal zero

    !Tissue Volumes
    VPlas = VplasC*BW   !volume of plasma (L)
    VK = VKC*BW   !volume of kidney (L)
    MK = VK*1.0*1000  !mass of kidney (g); based on density of kidney = 1.0 g/mL
    VKb = VK*0.16  !volume of blood in the kidney (L); fraction blood volume of kidney (0.16) from Brown,
1997
    Vfil = VfilC*BW  !volume of filtrate (L)
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    VL = VLC*BW  !volume of liver (L)
    ML = VL*1.05*1000  !mass of liver (g); based on density of liver = 1.05 g/mL, Overmeyer 1987
    VR = (0.93*BW) - VPlas - VPTC - Vfil - VL  !volume of remaining tissue (L)[Note: VKb not included as
already accounted for in VPlas];
    VBal = (0.93*BW) - (VR + VL + VPTC + VFil + VPlas)  !Balance check of tissue volumes; should equal zero

    !Kidney Parameters
    PTC =VKC*1000*6e7  !number of PTC (cells/kg BW) (based on 60 million PTC/gram kidney); assuming density
of 1 kg/L
    VPTC = VK*1000*VPTCC  !volume of proximal tubule cells (L)
    MPTC = VPTC*1000 !mass of the proximal tubule cells (g) (assuming density 1 kg/L)
    Vmax_basoC = (Vmax_baso_invitro*RAFbaso*PTC*protein*60*(MW/1e12)*1000000)!Vmax of basolateral
transporters (ug/h/kg BW ^0.75)
    Vmax_apicalC = (Vmax_apical_invitro*RAFapi*PTC*protein*60*(MW/1e12)*1000000) !Vmax of basolateral
transporters (ug/h/kg BW ^0.75)
    Vmax_baso = Vmax_basoC*BW**0.75  !(ug/h)
    VMax_apical = Vmax_apicalC*BW**0.75  !(ug/h)
    kbile = kbilec*BW**(-0.25)  !biliary elimination; liver to feces storage (/h)
    kurine = kurinec*BW**(-0.25)  ! urinary elimination, from filtrate (/h)
    kefflux = kefflux c*BW**(-0.25)  !efflux clearance rate, from PTC to blood (/h)
    GFR = GFRC*VK  !glomerular filtration rate, scaled to mass of kidney(in kg)(L/h)

    !GI Tract Parameters
    kabs = kabsc*BW**(-0.25)  !rate of absorption of chemical from small intestine to liver (/h)
    kunabs = kunabsc*BW**(-0.25)  !rate of unabsorbed dose to appear in feces (/h)
    GE = GEC/BW**(-0.25)  !gastric emptying time (/h)
    k0 = K0C/BW**(-0.25)   !rate of uptake from the stomach into the liver (/h)

! Exposure Parameters
  day = t/24
  year = day/365

  !Drinking Water
  CONSTANT backgrounddw = 0.04 ! Drinking water concentration (ug/L or ppb)
  CONSTANT exposeddw = 0.04 !Contaminated drinking water concentration (ug/L or ppb)
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  CONSTANT dwtotal = 1.36 ! daily drinking water consumption (L), from the EPA Factors Handbook (2011)
  CONSTANT drinks = 4.0 !Total drinks per day (drink)
  CONSTANT tlendw = 0.25 !Length of time spent drinking each drinking event (hrs/drink)
  CONSTANT tbackground = 438000.0  !duration of exposure to background dw concentration (hrs)

  !Incidental Ingestion
  !CONSTANT ingest = 0.002   !incidental ingestion rate (ug/hr)
  CONSTANT ingest_past = 0.01
  CONSTANT ingest_current = 0.01

!!Ingestion Exposure
!DISCRETE IngestON
IF (T.LT.tbackground) THEN !if time is less that tbackground,
ingest = ingest_past  !ingestion exposure equals ingest_past
else !if time is less that tbackground,
ingest = ingest_current  !ingestion exposure equals ingest_past
ENDIF
aingestdose = integ(ingest, 0.)

!Drinking water exposure
DISCRETE Drinkint
SCHEDULE DrinkON .AT. tstart
END

DISCRETE DrinkON
IF (T.LT.tbackground) THEN !if time is less than tbackground,
INTERVAL C2 = 4.0 !simulate drinking exposure every four hours
IF (hourofday .LT. 16.0) THEN !if hour of day is less than 16 (8pm)
drinkdose = ((backgrounddw/tlendw)*(dwtotal/drinks)) !simulate drinking water (background
concentration)exposure at set interval(ug/hr)
countdw = countdw + 1 !increase drinking episode count by 1 following exposure
SCHEDULE DrinkOFF .AT. T+tlendw !turn off drinking water exposure after 15 minutes (tlen)
ENDIF

hourofday = hourofday + 4.0 !increase hour of day count by four hours following each exposure
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IF(hourofday .EQ. 24.0) dayofweek = dayofweek + 1.0 !when hour of day count reaches 24, increase day of week
count by 1
IF(hourofday .EQ. 24.0) hourofday = 0.0 !when hour of day count reaches 24, reset hour of day count to zero
IF (dayofweek .EQ. 7.0) dayofweek = 0.0 !when day of week count reaches 7, reset day of week count to zero
ENDIF

END
adrinkdose = integ(drinkdose, 0.)

DISCRETE DrinkON
IF (T.GT.tbackground) THEN !if time is greater than tbackground,
INTERVAL C2 = 4.0 !simulate drinking exposure every four hours
IF (hourofday .LT. 16.0) THEN !if hour of day is less than 16 (8pm)
drinkdose = ((exposeddw/tlendw)*(dwtotal/drinks)) !simulate drinking water (exposed concentration)exposure at
set interval(ug/hr)
countdw = countdw + 1.0 !increase drinking episode count by 1 following exposure
SCHEDULE DrinkOFF .AT. T+tlendw !turn off drinking water exposure after 15 minutes (tlen)
ENDIF

hourofday = hourofday + 4.0 !increase hour of day count by four hours following each exposure

IF(hourofday .EQ. 24.0) dayofweek = dayofweek + 1.0 !when hour of day count reaches 24, increase day of week
count by 1
IF(hourofday .EQ. 24.0) hourofday = 0.0 !when hour of day count reaches 24, reset hour of day count to zero
IF (dayofweek .EQ. 7.0) dayofweek = 0.0 !when day of week count reaches 7, reset day of week count to zero
ENDIF

END
adrinkdose2 = integ(drinkdose, 0.0)

DISCRETE DrinkOFF
Drinkdose = 0.0 !set drink dose to zero during non-exposure periods
END
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!Model Equations
    !Rest of Body (Tis)
    RR = QR*(CA-CVR)*Free  !rate of change in rest of body (ug/h)
    AR = integ(RR,0.0)  !amount in rest of body (ug)
    CR = AR/VR !concentration in rest of body (ug/L)
    CVR = CR/PR  !concentration in venous blood leaving the rest of the body (ug/L)

    !Kidney
      !Kidney Blood (Kb)
      RKb = QK*(CA-CVK)*Free - CA*GFR*Free - Rdif - RA_baso   !rate of change in kidney blood (ug/h).
      AKb = integ(RKb, 0.0) !amount in kidney blood (ug)
      CKb = AKb/VKb  !concentration in kidney blodd (ug/L)
      CVK = CKb !/PK  !concentration in venous blood leaving kidney (ug/L)

      RCl = CA*GFR*Free  !rate of clearance via glomerular filtration (ug/h)
      ACl = integ(RCl, 0.0)    !amount moved via glomerular filtration (ug)

      Rdif = Kdif*(CKb - CPTC)  !rate of diffusion from into the PTC (ug/hr)
      Adif = integ(Rdif, 0.0)  !amount diffused into the PTC (ug)

      RA_baso = (Vmax_baso*CKb)/(Km_baso + CKb)  !rate of transport through basolateral transporters (ug/h)
      A_baso = integ(RA_baso, 0.0)    !amount transported through basolateral transporters (ug)

      !Proximal Tubule Cells (PTC)
      RPTC =  Rdif + RA_apical + RA_baso - RAefflux !rate of change in PTC(ug/h)
      APTC = integ(RPTC, 0.0)  !amount in proximal tubule cells (ug)
      CPTC = APTC/VPTC  !concentration in PTC (ug/L)

      RA_apical = (Vmax_apical*Cfil)/(Km_apical + Cfil)     !rate of transport through apical transporters
(ug/h)
      A_apical = integ(RA_apical, 0.0)!amount transported through apical transporters (ug)

      RAefflux = kefflux*APTC    !rate of efflux from proximal tubule cells into circulation (ug/h)
      Aefflux = integ(RAefflux, 0.0)  !amount effluxed from proximal tubule cells into circulation (ug)
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```
  !Filtrate (Fil)
  Rfil = CA*GFR*Free - RA_apical - Afil*kurine  !rate of change in filtrate (ug/h)
  Afil = integ(Rfil, 0.0)  !amount in filtrate (ug)
  Cfil = Afil/Vfil  !concentration in filtrate (ug/L)

  !Urinary elimination
  Rurine = kurine*Afil  !rate of change in urine (ug/h)
  Aurine = integ(Rurine, 0.0)  !amount in urine (ug)
  Curine = Rurine/kvoid  !concentration in urine (ug/L)

!GI Tract (Absorption site of oral dose)
  !Stomach
  RST= ingest + drinkdose - k0*AST - GE*AST  !rate of change in the stomach (ug/h)
  AST = integ(RST, 0.0)  !amount in the stomach (ug)

  RabsST = k0*AST  !rate of absorption in the stomach (ug/h)
  AabsST = integ(RabsST, 0.0)  !amount absorbed in the stomach (ug)

  !Small Intestine
  RSI = GE*AST - kabs*ASI - kunabs*ASI  !rate of change in the small intestine (ug/hr)
  ASI = integ(RSI, 0.0)  !amount in the small intestine (ug)

  RabsSI = kabs*ASI  !rate of absorption in the small intestine (ug/hr)
  AabsSI = integ(RabsSI, 0.0)  !amount absorbed in the small intestine (ug)

  total_oral_uptake = AabsSI + AabsST  !total oral uptake in the GI tract (ug)

  !Feces compartment
  Rfeces = kbile*AL + kunabs*ASI !rate of change in the feces compartment (ug/h)
  Afeces = integ(Rfeces, 0.0)  !amount in the feces compartment (ug)

!Liver
RL = QL*(CA-CVL)*Free - kbile*AL + kabs*ASI + k0*AST !rate of change in the liver (ug/h)
AL = integ(RL, 0.0) !amount in liver (ug)
CL = AL/VL  !concentration in the liver (ug/L)
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    CVL = CL/PL  !concentration in the venous blood leaving the liver (ug/L)

    CLiver = AL/ML  !concentration in liver (ug/g)

    Abile = kbile*AL !amount in the bile (ug)

    !Plasma compartment
    RPlas_free = (QR*CVR*Free) + (QK*CVK*Free) + (QL*CVL*Free) - (QC*CA*Free) + RAefflux  !rate of change in
the plasma (ug/h)
    Aplas_free = integ(RPlas_free, 0.0)  !amount in the plasma (ug)
    CA_free = APlas_free/VPlas    !concentration in plasma (ug)
    CA = CA_free/Free  !concentration of total PFOA in plasma (ug/L)

    !Mass Balance Check
    Atissue = APlas_free + AR + AKb + AFil + APTC + AL + AST + ASI   !sum of mass in all compartments (ug)
    Aloss = Aurine + Afeces   !sum of mass lost through urinary and fecal excretion (ug)
    totaldose = Adrinkdose + Aingestdose
           Atotal = Atissue + Aloss   !total mass; should equal total dose

    END ! DERIVATIVE

    CONSTANT TSTOP = 24.0 !hours
    TERMT (T .GE. TSTOP, 'checked on communication interval: REACHED TSTOP')

  END ! DYNAMIC

  TERMINAL
    ! code that is executed once at the end of a simulation run goes here


  END ! TERMINAL

END ! PROGRAM
```

Additional Sensitivity Analysis Information

Sensitivity coefficients were determined for the serum concentration resulting from a 1% change in the value of each parameter value using the forward difference method. Sensitivity analysis was conducted for simulations with drinking water concentration set to high (3.55 µg/L) and low (0.04 µg/L) concentrations. Positive sensitivity coefficients indicate a direct association between the model output and the corresponding parameter. Negative sensitivity coefficients indicate an inverse correlation between the model output and the corresponding parameter. Parameters with absolute sensitivity coefficients greater than 0.1 were identified as sensitive.

Table S1. *Normalized sensitivity coefficients for sensitivity analysis of the PBPK model for PFOA in the adult male human.*

PARAMETER	SENSITIVITY COEFFICIENT (DW CONC = 3.55 µg/L)	SENSITIVITY COEFFICIENT (DW CONC = 0.04 µg/L)
BW	**-0.839478**	**-0.839478**
DRINKS	**-0.937227**	**-0.937227**
DWTOTAL	**0.946598**	**0.946598**
EXPOSEDDW	**0.946599**	**0.946593**
FREE	**-0.387236**	**-0.387236**
GEC	0.000064	0.000064
GFRC	**-0.388368**	**-0.388368**
HTC	-0.000895	-0.000895
INGEST	0.000000	0.000000
K0C	-0.000091	-0.000091
KABSC	-0.000399	-0.000399
KBILEC	**-0.611969**	**-0.611969**
KDIF	0.000005	0.000005
KEFFLUXC	0.000025	0.000025
KM_APICAL	**-0.383738**	**-0.383738**
KM_BASO	0.000008	0.000008
KUNABSC	-0.000026	-0.000026
KURINEC	**-0.3852010**	**-0.3851814**
KVOID	0.000000	0.000000
PK	0.000000	0.000000
PL	**-0.607463**	**-0.607463**
PR	-0.000075	-0.000075
PROTEIN	**0.382970**	**0.382970**
QCC	0.001118	0.001118
QKC	-0.000235	-0.000235

QLC	0.000253	0.000253
RAFAPI	**0.382978**	**0.382978**
RAFBASO	-0.000008	-0.000008
TLENDW	0.000046	0.000046
VFILC	**-0.383738**	**-0.383738**
VKC	-0.004561	-0.004561
VLC	**-0.607460**	**-0.607460**
VMAX_APICAL_INVITRO	**0.382978**	**0.382978**
VMAX_BASO_INVITRO	-0.000008	-0.000008
VPLASC	0.000015	0.000015
VPTCC	-0.000005	-0.000005