**Supplemental Materials**

Table S1. Physiological parameter values for the rat and human physiologically based pharmacokinetic (PBPK) models. Variable names as they appear in the model are shown in italics.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Rat Values** | **Human Values** | **Sources** |
| Body weight (kg); *BW* | 0.25 | 80 (styrene) 70 (caffeine) | Default setting |
| Cardiac output (L/h/bw0.75); *QCc* | 15 | 15 | Brown et al., 1997 |
| Ventilation rate (L/h/bw0.75); *QPc* | 15 | 15 | Set to = *QCc* |
| Fractional venous blood volume; *VVBc* | 0.056A | 0.059 A | Brown et al., 1997 |
| Blood:Plasma Concentration Ratio; *RBP* | 1 | 1 (styrene)  0.71 (caffeine) | Default setting: 1  Caffeine: Lave et al. (1997) |
| Fractional liver volume; *VLc* | 0.04 | 0.026 | Brown et al., 1997 |
| Fractional rapidly perfused tissue volume; *VRc* | 0.06B | 0.05 B | Brown et al., 1997 |
| Fractional fat volume; *VFc* | 0.07 | 0.214 | Brown et al., 1997 |
| All Perfused Tissues; *VAll* | 0.865 | 0.878 | Brown et al., 1997 |
| Fractional slowly perfused tissue volume; *VSc* | 0.62 | 0.71 | Calculated from: *VAll* – (*VLc* + *VRc* + *VVc* + *VFc* + *VAc*) |
| Fractional liver blood flow; *QLc* | 0.18 | 0.227 | Brown et al., 1997 |
| Fractional rapidly perfused tissue blood flow; *QRc* | 0.58 | 0.333 | Brown et al., 1997 |
| Fractional fat blood flow; *QFc* | 0.07 | 0.052 | Brown et al., 1997 |
| Fractional slowly perfused tissue blood flow; *QSc* | 0.24 | 0.44 | Calculated from:  1 – (*QLc* + *QRc* + *QFc*) |
| **A**Fraction of venous blood was determined as the fraction of body that is blood multiplied by the proportion that is venous or arterial (75% or 25%, respectively; Brown et al., (1997)) | | | |
| B Rapidly perfused tissues: adrenals, brain, kidneys, large intestine, lungs, pancreas, stomach, thyroid, and spleen. | | | |

**Table S2**. Chemical specific parameter values for test cases using rat physiologically based pharmacokinetic (PBPK) models for hypothetical chemicals A, B, C and D

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Values** | **Sources** |
| **Hypothetical Chemical A** | | |
| Molecular weight (g/mole); *MW* | 98.96 |  |
| Partition coefficient, liver:blood; *PL* | 5.899 | HTTK 2.0.4 |
| Partition coefficient, rapidly perfused tissue:blood; *PR* | 5.899 | Set the same as liver:blood value |
| Partition coefficient, fat:blood; *PF* | 2.667 | HTTK 2.0.4 |
| Partition coefficient, slowly perfused tissue:blood; *PS* | 1.329 | HTTK 2.0.4; muscle:blood value |
| Oral absorption rate (1/h); *Ka* | 5 | Place holder value, fast absorption |
| Oral absorption fraction; *fa* | 1.0 | Assume 100% absorbed |
| Maximum reduction in oral absorption fraction; *fimax* | 0.0 | Assume no saturable oral absorption |
| ***Saturable metabolism of the parent*** | | |
| Maximum rate of metabolism of parent (µmol/hr); *Vmax* | 6.1 | IndusChemFate; set to zero when assuming first-order metabolism |
| Concentration associated with half-maximal metabolism of parent (µmol/hr); *Km* | 2.5 | IndusChemFate |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 0.0 | No first-order metabolism of the parent; set to (6.1/2.5) when assuming first-order metabolism |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 0.0 | No saturable urinary excretion of the parent |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 4.9 | (6.1/2.5)\*2, a larger value to distinguish the parent and the metabolite AUCs |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 0.0 | No first-order clearance of the metabolite |
| ***Saturable urinary excretion of the parent*** | | |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 6.1 | Assume to be the same as metabolism Vmax; set to zero when assuming first-order urinary excretion |
| Concentration associated with half-maximal urinary excretion of parent (µmol/hr); *KmU* | 2.5 | Assume to be the same as metabolism Km |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent; set to 6.1/2.5 when assuming first-order urinary excretion |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 2.4 | (6.1/2.5) |
| Maximum rate of Metabolism of parent (µmol/hr); *Vmax* | 0.0 | No saturable metabolism of the parent |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 4.9 | (6.1/2.5)\*2, a larger value to distinguish the parent and the metabolite AUCs |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 0.0 | No first-order clearance of the metabolite |
| ***Saturable urinary excretion of the metabolite*** | | |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 6.1 | Assume to be the same as metabolism Vmax; set to zero when assuming first-order clearance of the metabolite |
| Concentration associated with half-maximal urinary excretion of metabolite (µmol/hr); *KmUM* | 2.5 | Assume to be the same as metabolism Km |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 0.0 | No first-order urinary excretion of the metabolite; set to (6.1/2.5) when assuming first-order clearance of the metabolite |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 1.2 | (6.1/2.5)/2, a smaller value to distinguish the parent and the metabolite AUCs |
| Maximum rate of metabolism of parent (µmol/hr); *Vmax* | 0.0 | No saturable metabolism of the parent |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 0.0 | No saturable urinary excretion of the parent |
| ***Saturable metabolism of the parent and saturable clearance of metabolite*** | | |
| Maximum rate of metabolism of parent (µmol/hr); *Vmax* | 6.1 | IndusChemFate; set to zero when assuming first-order metabolism |
| Concentration associated with half-maximal metabolism of parent (µmol/hr); *Km* | 2.5 | IndusChemFate |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 0.0 | No first-order metabolism of the parent; set to (6.1/2.5) when assuming first-order metabolism |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 0.0 | No saturable urinary excretion of the parent |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 3 or 12 | (6.1/2) or (6.1\*2) to compare the metabolite AUCs when its clearance saturates before or after the parent metabolism saturates |
| Concentration associated with half-maximal urinary excretion of parent (µmol/hr); *KmU* | 2.5 | Set the same as the parent Km |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 0.0 | No first-order urinary excretion of the metabolite; set to (3/2.5) when assuming first-order clearance |
| **Hypothetical Chemical B** | | |
| Molecular weight (g/mole); *MW* | 93.13 |  |
| Partition coefficient, liver:blood; *PL* | 4.834 | HTTK |
| Partition coefficient, rapidly perfused tissue:blood; *PR* | 4.834 | Set the same as liver:blood value |
| Partition coefficient, fat:blood; *PF* | 1.32 | HTTK |
| Partition coefficient, slowly perfused tissue:blood; *PS* | 1.135 | HTTK, muscle:blood value |
| Oral absorption rate (1/h); *Ka* | 5 | Place holder value, fast absorption |
| First-order oral absorption fraction; *fa* | 1.0 | Assume 100% absorbed |
| Maximum reduction in oral absorption fraction; *fimax* | 0.0 | Assume no saturable oral absorption |
| ***Saturable metabolism of the parent*** | | |
| Maximum rate of metabolism of parent (µmol/hr); *Vmax* | 528 | IndusChemFate; set to zero when assuming first-order metabolism |
| Concentration associated with half-maximal metabolism of parent (µmol/L); *Km* | 180 | IndusChemFate |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 0.0 | No first-order metabolism of the parent; set to (528/180) when assuming first-order metabolism |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 0.0 | No saturable urinary excretion of the parent |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 5.9 | (528/180)\*2, a larger value to distinguish the parent and the metabolite AUCs |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 0.0 | No first-order clearance of the metabolite |
| ***Saturable urinary excretion of the parent*** | | |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 528 | Assume to be the same as metabolism Vmax; set to zero when assuming first-order urinary excretion |
| Concentration associated with half-maximal urinary excretion of parent (µmol/hr); *KmU* | 180 | Assume to be the same as metabolism Km |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent; set to (528/180) when assuming first-order urinary excretion |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 2.9 | (528/180) |
| Maximum rate of metabolism of parent (µmol/hr); *Vmax* | 0.0 | No saturable metabolism of the parent |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 5.9 | (528/180)\*2, a larger value to distinguish the parent and the metabolite AUCs |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 0.0 | No first-order clearance of the metabolite |
| ***Saturable urinary excretion of the metabolite*** | | |
| Maximum rate of urinary excretion of metabolite (µmol/hr); *VmaxUM* | 528 | Assume to be the same as metabolism Vmax; set to zero when assuming first-order clearance of the metabolite |
| Concentration associated with half-maximal urinary excretion of metabolite (µmol/hr); *KmUM* | 180 | Assume to be the same as metabolism Km |
| Rate of first-order urinary excretion of metabolite (L/hr); *CLUM* | 0.0 | No first-order urinary excretion of the metabolite; set to (528/180) when assuming first-order clearance of the metabolite |
| Rate of first-order metabolism of parent (L/hr); *CLH* | 1.47 | (528/180)/2, a smaller value to distinguish the parent and the metabolite AUCs |
| Maximum rate of metabolism of parent (µmol/hr); *Vmax* | 0.0 | No saturable metabolism of the parent |
| Rate of first-order urinary excretion of parent (L/hr); *CLU* | 0.0 | No first-order urinary excretion of the parent |
| Maximum rate of urinary excretion of parent (µmol/hr); *VmaxU* | 0.0 | No saturable urinary excretion of the parent |
| **Hypothetical Chemical C** |  |  |
| Molecular weight (g/mole); *MW* | 352.8 |  |
| Partition coefficient, liver:plasma; *PL* | 8.46 | HTTK 2.0.4 |
| Partition coefficient, fat:plasma; *PF* | 6.42 | HTTK 2.0.4 |
| Partition coefficient, rapidly perfused tissue:plasma; *PR* | 8.46 | HTTK 2.0.4 |
| Partition coefficient, slowly perfused tissue:plasma; *PS* | 1.90 | HTTK 2.0.4 |
| Hepatic clearance (L/h); *CLH* | 0.17 | ADMET predictor module v10.0 of GastroPlus |
| Urinary excretion of metabolite (L/h); *CLUM* | 0.34 | Place holder value, twice the hepatic clearance |
| Oral absorption rate (1/h); *Ka* | 5 | Place holder value, fast absorption |
| Maximum fractional reduction in relative bioavailability fraction; *fimax* | 0.97 | Fit to GastroPlus model results |
| First-order oral bioavailability fraction; *fa* | 0.97 | Fit to GastroPlus model results |
| Amount of additional dose above the *dose\_prop\_term* that is associated with half-maximal reduction in relative bioavailability; *f50* | 24.86 | Fit to GastroPlus model results |
| Dose at which the response is no longer dose proportional; *dose\_prop\_term* | 0.24 | Fit to GastroPlus model results |
| **Hypothetical Chemical D** |  |  |
| Molecular weight (g/mole); *MW* | 899.1 |  |
| Partition coefficient, liver:plasma; *PL* | 2.83 | HTTK 2.0.4 |
| Partition coefficient, fat:plasma; *PF* | 0.18 | HTTK 2.0.4 |
| Partition coefficient, rapidly perfused tissue:plasma; *PR* | 2.83 | HTTK 2.0.4 |
| Partition coefficient, slowly perfused tissue:plasma; *PS* | 0.87 | HTTK 2.0.4 |
| Hepatic clearance (L/h); *CLH* | 0.41 | ADMET predictor module v10.0 of GastroPlus |
| Urinary excretion of metabolite (L/h); *CLUM* | 0.8 | Place holder value, twice the hepatic clearance |
| Oral absorption rate (1/h); *Ka* | 5 | Place holder value, fast absorption |
| Maximum fractional reduction in relative bioavailability fraction; *fimax* | 1 | Fit to GastroPlus model results |
| First-order oral bioavailability fraction; *fa* | 0.48 | Fit to GastroPlus model results |
| Amount of additional dose above the *dose\_prop\_term* that is associated with half-maximal reduction in relative bioavailability; *f50* | 493 | Fit to GastroPlus model results |
| Dose at which the response is no longer dose proportional; *dose\_prop\_term* | 1E-6 | Fit to GastroPlus model results |

Table S3. Chemical specific parameter values for test cases using physiologically based pharmacokinetic models for styrene and caffeine

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Values** | **Sources** |
| **Styrene** |  |  |
| Molecular weight (g/mole); *MW* | 104.16 |  |
| Partition coefficient, liver:plasma; *PL* | 2.7 | Ramsey and Anderson (1984) |
| Partition coefficient, fat:plasma; *PF* | 50 | Ramsey and Anderson (1984) |
| Partition coefficient, rapidly perfused tissue:plasma; *PR* | 5.7 | Ramsey and Anderson (1984) |
| Partition coefficient, slowly perfused tissue:plasma; *PS* | 1.0 | Ramsey and Anderson (1984) |
| Partition coefficient, blood:air; *PB* | 40 | Ramsey and Anderson (1984) |
| Maximum velocity of parent metabolism (μmol/h); *Vmax* | 51 (rat)  3852 (human) | Rat: Fit to data from Ramsey and Andersen (1984)  Human: allometrically scaled from rat value |
| Concentration associated with half-maximal metabolism (µmol/L); *Km* | 3.8 | Fit to rat data from Ramsey and Andersen (1984) |
| **Caffeine** |  |  |
| Molecular weight (g/mole); *MW* | 194.2 |  |
| Partition coefficient, liver:plasma; *PL* | 4.25 | OECD (2020) |
| Partition coefficient, fat:plasma; *PF* | 0.68 | OECD (2020) |
| Partition coefficient, rapidly perfused tissue:plasma; *PR* | 2.4 | OECD (2020) |
| Partition coefficient, slowly perfused tissue:plasma; *PS* | 0.995 | OECD (2020) |
| Partition coefficient, blood:air; *PB* | 1000 | Placeholder value, set to a large value to minimize clearance from exhalation |
| Maximum velocity of parent metabolism (μmol/h); *Vmax* | 735 | Estimated from hepatic clearance (OECD, 2020) |
| Concentration associated with half-maximal metabolism (µmol/L); *Km* | 100 | Placeholder value. |
| Oral absorption rate (1/h); *Ka* | 1.6 | OECD (2020) |

Table S4. Chemical specific parameter values for the test case using rat physiologically based pharmacokinetic (PBPK) modeling of 2,4-D. The table includes data optimized values for the maximum rate of parent urinary excretion (*VmaxU*), the concentration associated with half-maximal urinary excretion (*KmU*), and first-order urinary clearance (*CLU*).

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Values** | **Notes** |
| Molecular weight (g/mole); *MW* | 221.04 |  |
| Partition coefficient, liver:plasma; *PCL* | 13.57 | HTTK 2.0.4 |
| Partition coefficient, fat:plasma; *PCFat* | 14.07 | HTTK 2.0.4 |
| Partition coefficient, rapidly perfused tissue:plasma; *PCR* | 13.57 | HTTK 2.0.4 |
| Partition coefficient, slowly perfused tissue:plasma; *PCS* | 3.196 | HTTK 2.0.4 |
| Partition coefficient, blood:air; *PCB* | 1E6 | Assumed very high given low volatility of 2,4-D |
| Oral absorption rate (1/h); *Ka* | 5 | Assumed rapid oral absorption |
| Oral bioavailability fraction; *fa* | 1 | Assumed 100% of oral dose is absorbed |
| Maximal fractional reduction in relative bioavailability fraction; *fimax* | 0 | Set to 0 to ensure that oral bioavailability is first-order. |
| Maximum velocity of parent metabolism (μmol/h); *Vmax* | 0 | 2,4-D is thought to be virtually unmetabolized (Milby, 1980; Hayes and Wayland, 1982) |
| **Data Optimized Clearance Values** | | |
| Maximum rate of urinary excretion (*VmaxU*) in males; µmol/hr | 8.79 | Fit to male *in vivo* data (Saghir et al. 2013) |
| Dose associated with half-maximal urinary excretion (*KmU*) in males; µmol/L | 58.5 | Fit to female *in vivo* data (Saghir et al. 2013) |
| First-order urinary excretion rate (*CLU*) in males; L/hr | 0.0955 | Fit to male *in vivo* data (Saghir et al. 2013) |
| Maximum rate of urinary excretion (*VmaxU*) in females; µmol/hr | 2.91 | Fit to female *in vivo* data (Saghir et al. 2013) |
| Dose associated with half-maximal urinary excretion (*KmU*) in females; µmol/L | 58.5 | Fit to female *in vivo* data (Saghir et al. 2013) |
| First-order urinary excretion rate (CLU) in females; L/hr | 0.0186 | Fit to female *in vivo* data (Saghir et al. 2013) |

Figure S1. Comparison between GastroPlus predicted oral absorption fractions for hypothetical chemicals C (blue dots) and D (brown dots) and physiologically based pharmacokinetic (PBPK) model predicted oral absorption fractions for chemicals C (red curve) and D (blue curve)

Figure S2. Range of styrene concentrations in air (ppm) measured from personal air samplers in Occupational Safety and Health Administration inspections of occupational sites in the U.S.

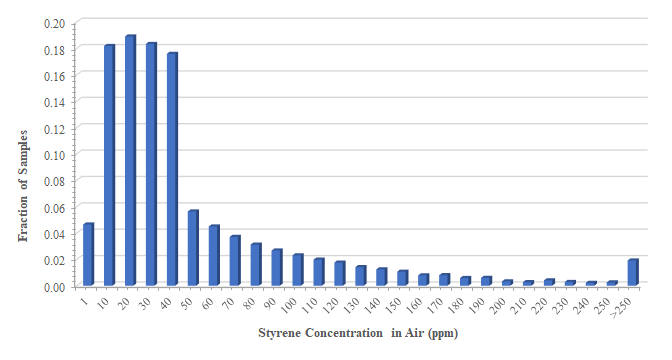


Figure S3. Comparison between physiologically based pharmacokinetic (PBPK) model-predicted styrene concentrations in plasma (curves) and observed data (symbols; from Young et al., 1979) in rats during and after a 6 hour inhalation exposure to styrene at concentrations of 80 ppm, 600 ppm, and 1200 ppm (Panel A). Comparison between PBPK model-predicted styrene concentrations in plasma (curve) and observed data (symbols; from Withey, 1976) in a rat given an oral dose of 9.3 mg/kg bw (panel B).

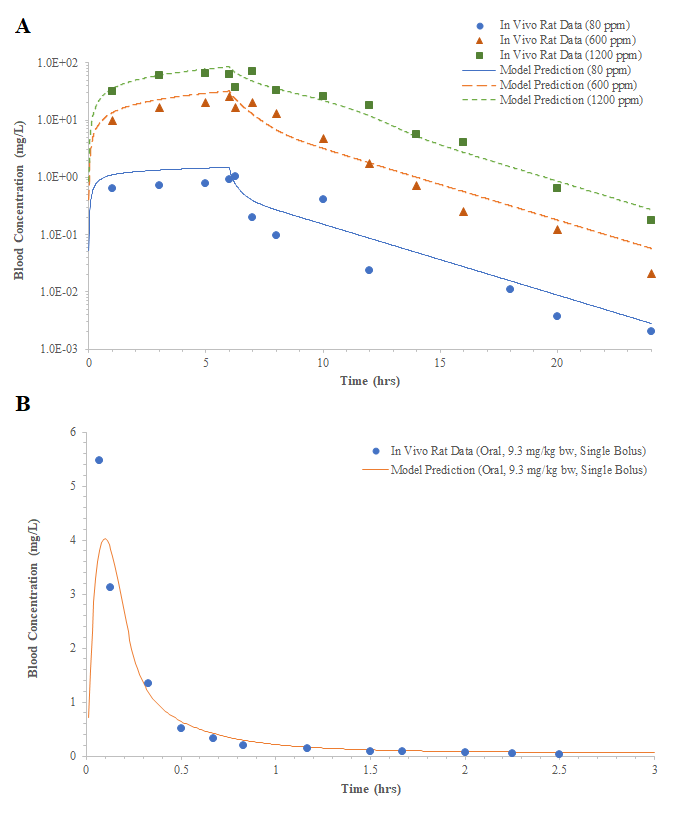


Figure S4. Comparing the total amount of metabolite generated based on the oral dosing in mg/kg bw/day and the inhalation exposure in ppm as an equivalent to mg/kg bw/day

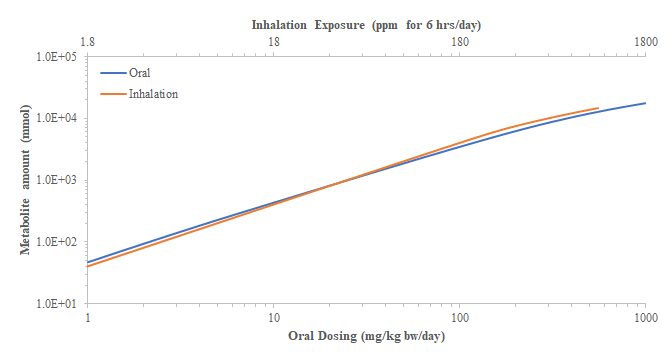
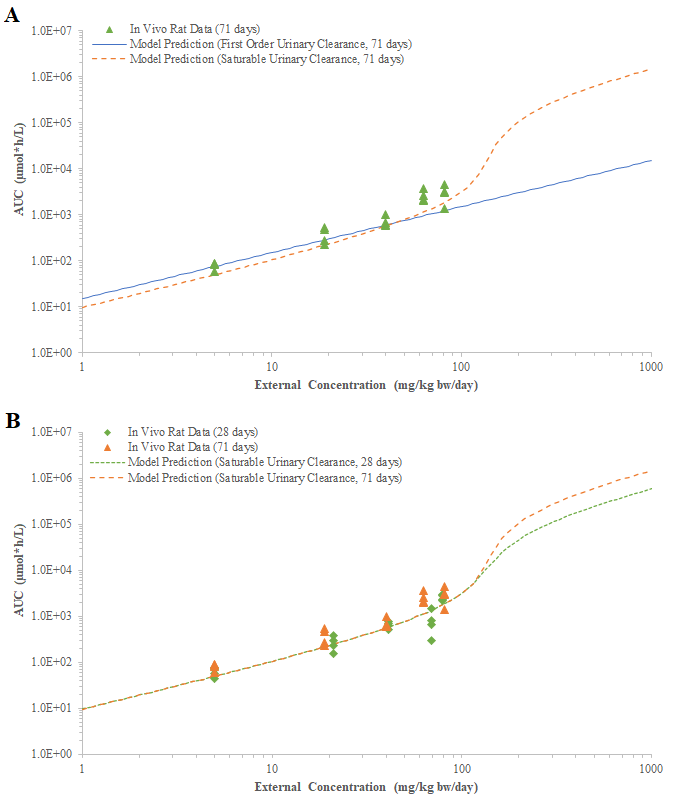


Figure S5: Simulated external concentrations (mg/kg bw/day) versus area under the curve of plasma concentrations during the last 24 hours (AUC; in µmole\*h/L) of 2,4-Dichlorophenoxyacetic acid (2,4-D) from dietary exposures of male rats are compared to *in vivo* data. Panel A shows plasma AUC data from a 71-day *in vivo* study (symbols), and simulated plasma AUC curves with saturable (VmaxU = 8.79; KmU = 58.5) or first-order urinary excretion (CLU = 0.0955) of 2,4-D. Panel B compares the plasma AUC data (symbols) and simulated plasma AUC curves with saturable urinary excretion (VmaxU = 8.79; KmU = 58.5) for both 28-day and 71-day exposures.



**Appendix S1. Berkeley Madonna code for the rat model – Chemical A**

{Solver settings}

method RK4 ;Runge-Kutta 4th-order solver, not stiff because of pulse oral repeated dose

starttime = 0 ;hours

stoptime = 480 ;hours

DT = 0.001 ;step size

dtout=0.01 ;communication interval (optional)

{RAT Physiological parameters}

BW = 0.25 ;Body weight- kg

QPC = 15 ;Alveolar ventilation rate - L/h

QCC = 15 ;Cardiac output - L/h

VPC = 0.86 ;Fraction all perfused tissue - kg/kg BW

VFC = 0.07 ;Fraction fat tissue - kg/kg BW

VLC = 0.04 ;Fraction liver tissue - kg/kg BW

VVBC = 0.045 ;Fraction venous blood - kg/kg BW

VRC = 0.125 ;Fraction rapidly perfused tissue - kg/kg BW

QFC = 0.07 ;Fractional blood flow to fat - (L/h)/QC

QLC = 0.20 ;Fractional blood flow to liver - (L/h)/QC

QRC = 0.5 ;Fractional blood flow to rapidly perfused - (L/h)/QC

VDMc = 1.0 ;Volume of distribution of metabolite, user defined

{Chemical-specific parameters for chemical A}

MW = 98.96 ;Molecular weight - g/mol

PL = 5.899 ;Liver/blood partition coefficient

PF = 2.667 ;Fat/blood partition coefficient

PS = 1.329 ;Slowly perfused tissue/blood partition

PR = 5.899 ;Rapidly perfused tissue/blood partition

PB = 1000 ;Blood/air partition coefficient

RBP = 1 ;Blood to plasma concentration ratio

{Parent liver metabolism parameters}

CLH = 0.0 ;L/h

Vmax = 6.1 ;Maximum velocity of metabolism - umol/h

Km = 2.5 ;Michaelis-Menten - umol/L

{Urinary Excretion Parent Compound}

CLU = 0.0 ;L/h

VmaxU = 0.0 ;umol/h

KmU = 2.5 ;umol/L

{Urinary Excretion Metabolite}

CLUM = 4.9 ;L/h; place holder value that is faster than parent clearance

VmaxUM = 0.0 ;umol/h

KmUM = 2.5 ;umol/L

{Calculated parameters}

VSC = VPC - VLC - VFC - VRC - VVBC ;Fraction slowly perfused tissue - kg/kg BW

VS = VSC\*BW ;Volume slowly perfused tissue - L

VF = VFC\*BW ;Volume fat tissue - L

VL = VLC\*BW ;Volume liver - L

VVB = VVBC\*BW ;Volume venous blood - L

VR = VRC\*BW ;Volume rapidly perfused tissue - L

QC = QCC\*BW\*\*0.75 ;Cardiac output - L/hr

QP = QPC\*BW\*\*0.75 ;Ventilation rate - L/hr

QF = QFC\*QC ;Blood flow to fat - L/h

QL = QLC\*QC ;Blood flow to liver - L/h

QR = QRC\*QC ;Blood flow to rapidly perfused tissue - L/h

QS = QC - QF - QL - QR ;Blood flow to slowly perfused tissue - L/h

VDM = VDMc\*BW ;Volume of distribution for metabolite - L

{Exposure parameters}

; Control all exposure

allstop = 1e6 ; hours

exposurezone = (Time <lt>= allstop) ; hours

;Inhalation Exposure

CONC = 0 ;inhaled concentration - ppm

LENGTH = 6 ;length of inhalation exposure - hr

DAY = 1 ;number of days - day

CI\_mgL = CONC\*MW/24450 ;inhaled concentration, mg/L

CI\_mol = CI\_mgL/MW\*1000 ;inhaled concentration, umol/L

CI\_total = CI\_mol\*SQUAREPULSE(0, DAY\*24) ;turn on inhalation exposure between 0 and defined days

CI = if MOD(time, 24) <lt>= LENGTH then CI\_total else 0 ;inhalation exposure between 0 and defined length

;Oral exposure

bolus = 0.06 ; mg/kg bw per day

ndose = 1 ; number of doses per day

start = 0 ; initial bolus time (hr)

ostop = 24 ; time to stop oral dosing (hr), single dose if <24, repeated dose if >=24

repeat = 24/ndose ; bolus repeat interval (hours)

days = STOPTIME/24 ; number of days

ondaily = if MOD(time-1, 24) <= ostop then 1 else 0 ; single dose if ostop <24, repeated dose if ostop >=24

GDOSE' = pulse(bolus/ndose, start, repeat) \* ondaily \* exposurezone ; mg/kg bw/h, oral bolus dose enter within DT

init GDOSE = 0 ; initial amount (mg/kg bw)

ODOSE = GDOSE/MW\*1000 ; umole/kg bw

DOSE = ODOSE\*BW ; umole

Ka = 5 ; oral absorption rate constant (1/hr), Ka=5 is fast absorption

fa = 1 ; first-order, maximum fraction absorbed (max = 1.0)

fimax = 0.0 ; maximum fractional reduction in relative bioavailability (max = 1.0; see Dolton and D'Argenio 2017)

f50 = 625 ; amount of additional dose above the *dose\_prop\_term* that is associated with half-maximal reduction in relative bioavailability (see Dolton and D'Argenio 2017)

dose\_prop\_term = 11 ; dose at which the response is no longer dose proportional (see Dolton and D'Argenio 2017)

fracs = IF (f50 < dose\_prop\_term) THEN fa ELSE fa\*(1-((fimax\*(bolus - dose\_prop\_term))/(f50+(bolus-dose\_prop\_term))))

frac = IF (fracs > fa) THEN fa ELSE (fracs) ; limit maximum absorption to fa

{Model Equations}

;Chemical in blood

CA = (QC\*CV+QP\*CI)/(QC+(QP/PB)) ;Arterial blood conc- umol/L

AVB' = QF\*CVF + QL\*CVL + QS\*CVS + QR\*CVR - QC\*CV - AU' ;Rate of change in venous blood amount - umol/h

init AVB = 0 ;initial amount in venous blood - umole

CV = AVB/VVB ;Venous blood conc - umol/L

;Exhaled Chemical

CX = CA/PB ;Exhaled conc - umol/L

CXppm = (0.7\*CX+0.3\*CI)\*24.450 ;Exhaled conc in air - ppm

;Chemical in rapidly perfused tissue compartment

AR' = QR\*(CA-CVR) ;rate of change in viscera amount - umol/h

init AR = 0. ;initial amount in viscera - umol

CR = AR/VR ;viscera concentration - umol/L

CVR = CR/PR ;concentration in viscera capillary blood - umol/L

;Chemical in slowly perfused tissue compartment

AS' = QS\*(CA-CVS) ;rate of change in slowly perfused amount - umol/h

init AS = 0. ;initial amount in slowly perfused tissue - umol

CS = AS/VS ;slowly perfused tissue concentration - umol/L

CVS = CS/PS ;concentration in SP tissue capillary blood - umol/L

;Chemical in fat compartment

AF' = QF\*(CA-CVF) ;rate of change in fat amount - umol/h

init AF = 0 ;initial amount in fat - umol

CF = AF/VF ;fat concentration - umol/L

CVF = CF/PF ;concentration in fat capillary blood - umol/L

;Oral exposure

AGI' = frac\*GDOSE'\*BW/MW\*1000 - Ka\*AGI ;rate of change in GI - umole/hr

Init AGI = 0 ;initial amount in GI - umole

RAO = KA\*AGI ;oral uptake rate (mg/h)

Absorb' = frac\*GDOSE'\*BW/MW\*1000 ;umole/hr, amount absorbed

Init Absorb = 0 ; umole

Absorb\_mgkg = Absorb\*MW/BW/1000 ; mg/kg bw

frac\_absorbed = Absorb\_mgkg/(bolus+1E-10) ; fraction absorbed

;Chemical in liver compartment

AL' = QL\*(CA-CVL) - RAM + RAO ;rate of change in liver amount - umol/h

init AL = 0 ;initial amount in liver - umol

CL = AL/VL ;liver concentration - umol/L

CVL = CL/PL ;concentration in liver capillary blood - umol/L

;Chemical metabolism

RAM = VMAX\*CVL/(KM+CVL) + CLH\*CVL ;rate of metabolism - umol/h

AM' = RAM

init AM = 0 ;initial amount metabolized - umol

; Metabolite blood concentration

AMET' = RAM - AUM' ;rate of metabolite in blood - umole/hr

init AMET = 0 ;initial amount in blood - umole

CMET = AMET/VDM ;concentration of metabolite in blood - umole/L

;Urine parent and metabolite

AU'=(VmaxU\*CV)/(KmU+CV) + CLU\*CV ;rate of parent change in urine - umole/hr

init AU = 0 ;initial amount in urine - umole

AUM'=(VmaxUM\*CMET)/(KmUM+CMET) + CLUM\*CMET ;rate of metabolite change in urine umole/hr

init AUM = 0 ;initial amount in urine - umole

{Dose metrics}

CVmMolar = CV/1000 ; blood conc in mmole/L

CVmgpl = CVmMolar\*MW ; blood conc in mg/:

CVugpl = CV\*MW ; blood conc in ug/L

CVPlas = CV/RBP ; plasma conc in umol/L

CVPlasmgpl = CVPlas\*MW ; plasma conc in ug/L

AUCV' = CV ; umole/L

init AUCV = 0 ; umole\*hr/L

AUCmghpl = AUCV\*MW/1000 ; mg\*hr/L

AUCVmmolar' = CVmMolar ; mmole/L

init AUCVmmolar = 0 ; mmole\*hr/L

AUCmgL = AUCVmmolar\*MW ; mg\*hr/L

; \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* Calculate last 24-hr AUC \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

init AUC24 = 0

AUC24' = IF (STOPTIME - time <lt> 24) THEN (CV) ELSE 0 ; umole\*h/L, last 24 hours for parent

init AUC\_M24 = 0

AUC\_M24' = IF (STOPTIME - time <lt> 24) THEN (CMET) ELSE 0 ; umole\*h/L, last 24 hours for metabolite

;========================================================

;Mass balance for Chemical

InhDOSE' = QP\*(CI-CX) ;Net inhalation absorption - mg/h

init InhDOSE = 0 ;initial inhalation dose - mg

TotalDOSE = InhDose + Absorb + AD ;total doses from all routes

MASS = AF + AR + AS + AL + AM + AVB + AGI +AU + ASFC + ASk + ASKe ;In tissues + metabolized and excreted - ug

MASSBAL = TotalDOSE-MASS ;Mass balance check for parent, should be ~0

MBALMET = AM - AMET – AUM ;Mass balance check for metabolite, should be ~0

**Appendix S2. Berkeley Madonna code for the human model – Caffeine**

{Solver settings}

method RK4 ;Runge-Kutta 4th-order solver

starttime = 0 ;Start time; hours

stoptime = 24 ;Stop time; hours

DT = 0.001 ;step size

dtout=0.01 ;communication interval (optional)

{HUMAN Physiological parameters}

BW = 70 ;Body weight; kg

QPC = 15 ;Alveolar ventilation rate - L/h/BW\*\*75; Brown et al. 1997

QCC = 15 ;Cardiac output - L/h/BW\*\*75; Travis, C. 1987

VPC = 0.878 ;Fraction all perfused tissue - kg/kg BW; Brown et al. 1997

VFC = 0.214 ;Fraction fat tissue - kg/kg BW; Brown et al. 1997

VLC = 0.026 ;Fraction liver tissue - kg/kg BW; Brown et al. 1997

VVBC = 0.059 ;Fraction venous blood - kg/kg BW; Brown et al. 1997

VRC = 0.05 ;Fraction rapidly perfused tissue - kg/kg BW; Brown et al. 1997

QFC = 0.052 ;Fractional blood flow to fat - (L/h)/QC; Brown et al. 1997

QLC = 0.227 ;Fractional blood flow to liver - (L/h)/QC; Brown et al. 1997

QRC = 0.333 ;Fractional blood flow to rapidly perfused - (L/h)/QC; Brown et al. 1997

VDMc = 1.0 ;Volume of distribution of metabolite, user defined

{Skin parameters}

SA = (0.1173\*BW\*\*0.6466)\*10E4 ;total body surface area - cm2 (Livingston and Lee, 2001)

AreaE = 25 ;surface area of skin exposed - cm2

SA\_exposed = min (AreaE, SA) ;limit exposed SA to <= total SA

Depth = 0.1 ;skin thickness - cm (Brown 1997)

Kp = 6E-4 ;skin permeability - cm/h

VSk = SA\*Depth/1000 ;skin volume – L

VSkC = VSk/BW ;fractional skin tissue - kg/kg BW

VSkE = SA\_exposed\*Depth/1000 ;exposed skin volume - L

QSkC = 0.058 ;fractional blood flow to skin - (L/h)/QC

QSKeC = QSkC \* (VSkE/VSk) ;fractional blood flow to exposed skin - (L/h)/QC

QSk = (QSkC-QSKeC)\*QC ;blood flow to skin - L/h

QSKe = QSKeC\*QC ;blood flow to exposed skin - L/h

{Chemical-specific parameters for caffeine}

MW = 194.2 ;Molecular weight - g/mol

PL = 4.25 ;Liver/blood partition coefficient

PF = 0.68 ;Fat/blood partition coefficient

PS = 0.995 ;Slowly perfused tissue/blood partition

PR = 2.4 ;Rapidly perfused tissue/blood partition

PB = 1000 ;Blood/air partition coefficient, no clearance from exhalation

RBP = 0.71 ;Blood to plasma concentration ratio

PSk = 0.7\*PL + 0.3\*PF ;Skin/blood partition coefficient; Campbell et al. (2015)

;doi:10.1016/j.tox.2015.03.010

{Parent liver metabolism parameters}

CLH = 0 ;L/h

Vmax = 735 ;Maximum velocity of metabolism - umol/h

Km = 100 ;Michaelis-Menten constant - umol/L

{parent urinary excretion}

CLU = 0.0 ;L/h

VmaxU = 0.0 ;umol/h

KmU = 2.5 ;umol/L

{metabolite urinary excretion}

CLUM = 1.0 ;L/h, place holder value

VmaxUM = 0.0 ;umol/h

KmUM = 2.5 ;umol/L

{Calculated parameters}

VSC = VPC - VLC - VFC - VRC - VVBC - VSkC ;Fraction slowly perfused tissue - kg/kg BW

VS = VSC\*BW ;Volume slowly perfused tissue - L

VF = VFC\*BW ;Volume fat tissue - L

VL = VLC\*BW ;Volume liver - L

VVB = VVBC\*BW ;Volume venous blood - L

VR = VRC\*BW ;Volume rapidly perfused tissue - L

QC = QCC\*BW\*\*0.75 ;Cardiac output - L/hr

QP = QPC\*BW\*\*0.75 ;Ventilation rate - L/hr

QF = QFC\*QC ;Blood flow to fat - L/h

QL = QLC\*QC ;Blood flow to liver - L/h

QR = QRC\*QC ;Blood flow to rapidly perfused tissue - L/h

QS = QC - QF - QL - QR - QSk - QSke ;Blood flow to slowly perfused tissue - L/h

VDM = VDMc\*BW ;Volume of distribution for metabolite - L

{Exposure parameters}

;Control all exposure

allstop = 1e6 ;hours

exposurezone = (Time <= allstop) ;hours

;Inhalation Exposure

CONC = 0 ;inhaled concentration - ppm

LENGTH = 6 ;length of inhalation exposure - hr

DAY = 1 ;number of days - day

CI\_mgL = CONC\*MW/24450 ;inhaled concentration, mg/L

CI\_mol = CI\_mgL/MW\*1000 ;inhaled concentration, umol/L

CI\_total = CI\_mol\*SQUAREPULSE(0, DAY\*24) ;turn on inhalation exposure between 0 and defined days

CI = if MOD(time, 24) <= LENGTH then CI\_total else 0 ;inhalation exposure for defined length (h)

;Oral exposure

bolus = 0.06 ;mg/kg bw per day

ndose = 1 ;number of doses per day

start = 0 ;initial bolus time (hr)

ostop = 24 ;time to stop oral dosing (hr), single dose if <24, repeated dose if >=24

repeat = 24/ndose ;bolus repeat interval (hours)

days = STOPTIME/24 ;number of days

ondaily = if MOD(time-1, 24) <= ostop then 1 else 0 ; single dose if ostop < 24, repeated dose if ostop>=24

GDOSE' = pulse(bolus/ndose, start, repeat) \* ondaily \* exposurezone ;mg/kg BW/day, oral bolus dose enter within DT

init GDOSE = 0 ;initial amount (mg/kg BW)

ODOSE = GDOSE/MW\*1000 ;umole/kg bw

DOSE = ODOSE\*BW ;umole

Ka = 1.6 ;oral absorption rate constant (1/hr), place holder value

fa = 1.0 ;first-order, maximum fraction absorbed (max = 1.0)

fimax = 0.0 ;maximum fractional reduction in relative bioavailability (max = 1.0; see Dolton and D'Argenio 2017)

f50 = 625 ; amount of additional dose above the *dose\_prop\_term* that is associated with half-maximal reduction in relative bioavailability (see Dolton and D'Argenio 2017)

dose\_prop\_term = 11 ;dose at which the response is no longer dose proportional (see Dolton and D'Argenio 2017)

fracs = IF (f50 < dose\_prop\_term) THEN fa ELSE fa\*(1-((fimax\*(bolus - dose\_prop\_term))/(f50+(bolus-dose\_prop\_term)))) ;ensure proper parameterization, else, default to fa

frac = IF (fracs > fa) THEN fa ELSE (fracs) ; limit maximum absorption to fa

;Dermal exposure

;Repeated dermal exposure is controlled by both DRepeat and DStop. For example one can apply a single dose (DStop = tinf < x < DRepeat),

;n evenly spaced doses (Dstop = (DRepeat\*n)), repeating doses at even intervals that stop after t hours (DStop = t),

;or evenly spaced doses throughout the modeled duration (DStop = STOPTIME)

;For instantaneous application of the full dose, set tinf = DT

DermalGdose = 1.25 ;mg/day

Dermaldose = DermalGdose/MW\*1000 ;umole

VolApp = 0.05 ;application volume - mL

tinf = 0.1 ;time to apply dermal dose - h

FracAvail = 0.67 ;Fraction of applied dose available for absorption

DRepeat = 24 ;time interval to repeat dermal exposure - h

DStop = STOPTIME ;time of last dermal exposure + DStop - h

DZone = if MOD(time, DRepeat) < tinf Then 1 ELSE 0 ;flag for dermal exposure on/off

AD' = DZone\*(Dermaldose\*FracAvail)/tinf \* (time <= DStop) \* exposurezone ;umole/h

init AD = 0.0 ;initial dermal dose, mg/day

{Model Equations}

;Chemical in blood

CA = (QC\*CV+QP\*CI)/(QC+(QP/PB)) ;Arterial blood conc- umol/L

AVB' = QF\*CVF + QL\*CVL + QS\*CVS + QR\*CVR +QSk\*CVSk + QSKe\*CVSke - QC\*CV - AU' ;Rate of change in venous blood amount - umol/h

init AVB = 0 ;Initial amount in venous blood - umol

CV = AVB/VVB ;Venous blood conc - umol/L

;Exhaled Chemical

CX = CA/PB ;Exhaled conc - umol/L

CXppm = (0.7\*CX+0.3\*CI)\*24.45 ;Exhaled conc in air - ppm

;Chemical in skin compartment

ASFC' = AD' - (Kp/VolApp \* SA\_exposed)\*ASFC ;Rate of absorption - umol/h

init ASFC = 0.0 ;Initial amount absorbed - umol

ASke' = (Kp\*SA\_exposed/VolApp)\*ASFC + QSke\*(CA - CVSke) ;rate of change in exposed skin - umol/h

init ASke = 0.0 ;initial amount in exposed skin - umol

CSke = ASke / (VSke + 1E-23) ;exposed skin concentration - umol/L

CVSke = CSke/PSk ;concentration in exposed skin blood - umol/L

ASK' = QSK\*(CA - CVSK) ;rate of change in unexposed skin - umol/h

init ASK = 0.0 ;initial amount in unexposed skin - umol

CSK = if SA\_exposed = SA then 0 else ASK/(VSK-VSKe+1E-23) ;unexposed skin concentration - umol/L

CVSK = CSK/PSk ;concentration in unexposed skin blood - umol/L

;Chemical in rapidly perfused tissue compartment

AR' = QR\*(CA-CVR) ;rate of change in rapidly perfused tissue amount - umol/h

init AR = 0. ;initial amount in rapidly perfused tissues - umol

CR = AR/VR ;rapidly perfused tissue concentration - umol/L

CVR = CR/PR ;concentration in rapidly perfused tissue blood - umol/L

;Chemical in slowly perfused tissue compartment

AS' = QS\*(CA-CVS) ;rate of change in slowly perfused tissue amount - umol/h

init AS = 0. ;initial amount in slowly perfused tissue - umol

CS = AS/VS ;slowly perfused tissue concentration - umol/L

CVS = CS/PS ;concentration in slowly perfused tissue blood - umol/L

;Chemical in fat compartment

AF' = QF\*(CA-CVF) ;rate of change in fat amount - umol/h

init AF = 0 ;initial amount in fat - umol

CF = AF/VF ;fat concentration - umol/L

CVF = CF/PF ;concentration in fat capillary blood - umol/L

;Oral exposure

AGI' = frac\*GDOSE'\*BW/MW\*1000 - Ka\*AGI ;rate of change in GI - umole/hr

Init AGI = 0 ;initial amount in GI - umole

RAO = KA\*AGI ;oral uptake rate (mg/h)

Absorb' = frac\*GDOSE'\*BW/MW\*1000 ;umole/hr, amount absorbed

Init Absorb = 0 ;umole

Absorb\_mgkg = Absorb\*MW/BW/1000 ; mg/kg bw

frac\_absorbed = Absorb\_mgkg/(bolus+1E-10) ; fraction absorbed

;Chemical in liver compartment

AL' = QL\*(CA-CVL) - RAM + RAO ;rate of change in liver amount - umol/h

init AL = 0 ;initial amount in liver - umol

CL = AL/VL ;liver concentration - umol/L

CVL = CL/PL ;concentration in liver capillary blood - umol/L

;Chemical metabolism

RAM = VMAX\*CVL/(KM+CVL) + CLH\*CVL ;rate of metabolism - umol/h

AM' = RAM

init AM = 0 ;initial amount metabolized - umol

; Metabolite blood concentration

AMET' = RAM - AUM' ;rate of metabolite in blood - umole/hr

init AMET = 0 ;initial amount in blood - umole

CMET = AMET/VDM ;concentration of metabolite in blood - umole/L

;Urine parent and metabolite

AU'=(VmaxU\*CV)/(KmU+CV) + CLU\*CV ;rate of parent change in urine - umole/hr

init AU = 0 ;initial amount in urine - umole

AUM'=(VmaxUM\*CMET)/(KmUM+CMET) + CLUM\*CMET ;rate of metabolite change in urine umole/hr

init AUM = 0 ;initial amount in urine - umole

{Dose metrics}

CVmMolar = CV/1000 ;blood conc in mmole/L

CVmgpl = CVmMolar\*MW ;blood conc in mg/L

CVugpl = CV\*MW ;blood conc in ug/L

CVPlas = CV/RBP ;plasma conc in umol/L

CVPlasmaugl = CVPlas\*MW ;plasma conc in ug/L

CVPlasmamgl = CVPlasmaugl/1000 ;plasma conc in mg/L

AUCV' = CV ;umole/L

init AUCV = 0 ;umole\*hr/L

AUCmghpl = AUCV\*MW/1000 ;mg\*hr/L

AUCVmmolar' = CVmMolar ;mmole/L

init AUCVmmolar = 0 ;mmole\*hr/L

AUCmgL = AUCVmmolar\*MW ;mg\*hr/L

; \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* Calculate last 24-hr AUC \*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*

init AUC24 = 0

AUC24' = IF (STOPTIME - time <lt> 24) THEN (CV) ELSE 0 ;umole\*h/L, last 24 hours for parent

init AUC\_M24 = 0

AUC\_M24' = IF (STOPTIME - time <lt> 24) THEN (CMET) ELSE 0 ;umole\*h/L, last 24 hours for metabolite

;========================================================

;Mass balance for Chemical

InhDOSE' = QP\*(CI-CX) ;Net inhalation absorption - mg/h

init InhDOSE = 0 ;initial inhalation dose - mg

TotalDOSE = InhDose + Absorb + AD ;total doses from all routes

MASS = AF + AR + AS + AL + AM + AVB + AGI +AU + ASFC + ASk + ASKe ;In tissues + metabolized and excreted - mg

MASSBAL = TotalDOSE-MASS ;Mass balance check for parent, should be ~0

MBALMET = AM - AMET – AUM ;Mass balance check for metabolite, should be ~0

**Appendix S3: R code for fitting saturable oral absorption parameters. There are two components to this code: the model and a scenario script that solves the model. The scenario calls the model file, solves the differential equations it contains based on the provided parameters, and plots the result.**

**Model:**

absorb\_model <- function(times,state,params){

with(

as.list(c(state,params)),{

###exposures###

#oral dosing

ROSWTICH <- 0

RGDOSE = (OSWITCH\*(bolus\*(1/DT)))

#oral uptake from the gut

if (f50 < dose\_prop\_term) {

frac = fa

} else {

frac = fa\*(1-((fimax\*(bolus - dose\_prop\_term))/(f50+(bolus-dose\_prop\_term))))

}

#ensure that the frac never exceeds fa

if (frac>fa) {

frac = fa

}

RABSORB = frac\*RGDOSE\*BW/MWC\*1000

Absorb\_mgkg = AABSORB\*MWC/BW/1000 #amount abosrbed in mg/kg

frac\_absorbed = Absorb\_mgkg/bolus #fraction absorbed

#return a list from the solver

list(#derivatives

c(RGDOSE,OSWITCH,

RABSORB

),

bolus = bolus,

Absorb\_mgkg = Absorb\_mgkg,

frac\_absorbed = frac\_absorbed

)

})

}

**Scenario to Solve the Oral Absorption Model**

#clear workspace

rm(list = ls())

#set the working directory to the location of these scripts

setwd(dirname(rstudioapi::getActiveDocumentContext()$path))

#suppress start-up messages from package loading (masking, etc.); Warnings will

#break through here still

shhh <- suppressPackageStartupMessages

#load the libraries

shhh(library(deSolve)) #solver

shhh(library(readxl)) #excel import

shhh(library(parallel)) #parallel computing

shhh(library(ggplot2)) #plotting

shhh(library(scales)) #plotting

shhh(library(reshape2)) #melting

shhh(library(pracma)) #logseq function lives here

# use parallel execution for speed (particularly for repeated runs)

# number of cores available

tot\_cores <- detectCores(all.tests = FALSE, logical = TRUE)

#because detectCores can produce an NAN, include a breakout to check for that and set it

#to 3 such that there is 1 core eventually allocated to the process.

if (is.na(tot\_cores)) {

tot\_cores <- 3

}

cores <- tot\_cores-2 #leave two cores for other system tasks

clust <- makeCluster(cores) #make the cluster for use in parallel execution

mwc = 228.291 #molecular weight; g/mole

fa = 0.97 #first-order bioavailability fraction

fimax = 0.97 #maximum fractional reduction in relative bioavailability fraction

f50 = 24.86 # amount of additional dose above the *dose\_prop\_term* that is associated with half-maximal reduction in relative bioavailability

dose\_prop\_term = 0.24 #dose at which the response is no longer dose proportional

#dosing

min\_dose = 0.01

max\_dose = 100000

n = 150

oral\_absorb\_mod <- function(bolus,mwc,f50,fimax,dose\_prop\_term) {

#load the libraries

library(deSolve) #solver

#load the model

source("absorb\_mod.R")

#parameterize the model here in the scenario for simplicity

#Parameter=value #Description, (units), and reference (where applicable)

params<-c(

#general model parameters

TSTART = 0, #time to start simulation (hours)

TSTOP = 24, #time to stop simulation (hours)

DT = 0.002, #timestep change (hours)

bolus = 0, #bolus dosing (mg/kg)

reps = 1, #doses per day

single\_bol = 1, #a single bolus at time zero, set this to "1", recurring everyday "0"

MWC = 0,

BW = 0.35,

#absorb parameters

fa = 0,

Ka = 0.5,

f50 = 0,

fimax = 0,

dose\_prop\_term = 0

)

#set any parameters that are to be manipulated (MWC, bolus, etc.)

params[["MWC"]] = mwc #molecular weight (g/mole)

params[["bolus"]] = bolus #bolus dosing (mg/kg)

params[[“fa”]] = fa #fa value (set above)

params[["f50"]] = f50 #f50 value (set above)

params[["fimax"]] = fimax #kfimax value (set above)

params[["dose\_prop\_term"]] = dose\_prop\_term #dose proportionality term (set above)

#set oral dosing interval/events

if (params[["bolus"]] > 0) {

#use the oral dose switch to turn on and off addition of new oral dosing to the

#GDOSE compartment

state\_1 = "OSWITCH"

state\_2 = "OSWITCH"

#switch ON value = 1, switch OFF value = 0

val\_1 = 1

val\_2 = 0

#replace the existing value with the new value (hence, "rep" in both spots)

op\_1 = "rep"

op\_2 = "rep"

#set the times for the events

#first, if there is only a single dose per day, that recurs every day...

if ((params[["reps"]] == 1) && (params[["single\_bol"]] == 0)) {

#set the "switch on" times at the first hour of each day

dose\_times <- head(seq(params[["TSTART"]],params[["TSTOP"]],24),-1)

#set the "switch off" times for the next time step after the switch on time

dose\_times\_2 <- dose\_times + params[["DT"]]

}

#second, if there is only a single dose per day, but it doesn't recur

else if ((params[["reps"]] == 1) && (params[["single\_bol"]] == 1)) {

#set the "switch on" times at the first hour of each day

dose\_times <- head(seq(params[["TSTART"]],params[["TSTOP"]],params[["TSTOP"]]),-1)

#set the "switch off" times for the next time step after the switch on time

dose\_times\_2 <- dose\_times + params[["DT"]]

}

#if there is more than one dose occurring per day...

else {

dose\_times <- unlist(

lapply(x = 1:(params[["TSTOP"]]/24),

#loop over the number of days in the simulation

func = function(x){

#build a list of events for each day, based on the dose interval set in the

#parameter list

head(seq(0,params[["dose\_int"]],1/params[["dose\_int"]],-1)) + (24\*(x-1))

}))

#as above, set the "switch off" times for the next time step after the switch on time

dose\_times\_2 <- dose\_times + params[["DT"]]

}

} else {

print("Bolus dosing set to zero; no exposure set.")

}

#build a dataframe to contain the dose information assigned above

dose\_events <- data.frame(

var = c(rep(x = state\_1, each = length(dose\_times)),

rep(x = state\_2, each = length(dose\_times\_2))),

time = c(dose\_times, dose\_times\_2),

value = c(rep(x = val\_1, each = length(dose\_times)),

rep(x = val\_2, each = length(dose\_times\_2))),

method = c(rep(x = op\_1, each = length(dose\_times)),

rep(x = op\_2, each = length(dose\_times\_2)))

)

state = c(AGDOSE = 0, OSWITCH = 0, #dosing

AGI = 0, AABSORB = 0 #amount in various compartments

)

#build the sequence of times for model execution

times <- seq(params[["TSTART"]],params[["TSTOP"]],params[["DT"]])

#run the model and produce the corresponding output as modeloutput, currently using

#the rk4 method (as in Berkeley Madonna)

modeloutput <- ode(y = state, times = times, method = "rk4",

func = absorb\_model, parms = params,

events = list(data = dose\_events))

#store the model output as a dataframe and return that dataframe

result <- list()

#put the model output as a dataframe in there

result <- as.data.frame(modeloutput)

#then single out any specific parameters we want to look at individually

return(result)

}

#run the model with the parameters and produce a plot across the full

#dose space

model\_output <- parLapply(clust,logseq(min\_dose,max\_dose,n),oral\_absorb\_mod,

f50 = f50,fimax = fimax,

mwc = mwc, dose\_prop\_term = dose\_prop\_term)

#find the FINAL values and plot them

#generate an empty dataframe to store the desired output data

output\_df <- data.frame()

#cycle through the dataframes in the generated list, collect and bind all desired data.

#in this case, we're collecting the final time point (t = 480 hr) for all modeled doses.

for (frame in seq(1,length(model\_output),1)) {

output\_df <- rbind(output\_df,model\_output[[frame]][nrow(model\_output[[frame]]),])

row.names(output\_df) <- NULL #reset the index for the dataframe

}

#replace the ending dose with the 'initial' dose value (i.e., what was set)

output\_df[,'AGDOSE'] <- logseq(min\_dose,max\_dose,n)

#release the cores and stop the parallel operation

stopCluster(clust)

plot <- ggplot(data = output\_df) +

geom\_line(aes(x = AGDOSE, y = frac\_absorbed), size = 1) +

scale\_x\_continuous(trans = 'log10',

breaks = trans\_breaks('log10', function(x) 10^x),

labels = trans\_format('log10', math\_format(10^.x))) +

labs(title = sprintf("Absorption fitting test"),

subtitle = sprintf("Fitted with fa = %.4f, f50 = %.4f, fimax = %.4f, and dose\_prop = %.4f",

fa,f50,fimax,dose\_prop\_term),

x = "External Concentration (mg/kg)",

y = "Fraction Absorbed",

color = "")

#print plot in RStudio

print(plot)

**Appendix S4: R code for the generic rat model of oral exposure to 2,4-D. Like the oral absorption code in Appendix S3, there are multiple components to this code: the model, a set of parameters, and a scenario script that solves the model. The scenario calls the model file and solves the differential equations it contains based on the parameters provided in the parameter file. The model and parameters are designed to be generic; the scenario file will provide specific modifications to the parameter file to make it chemical specific prior to running the model.**

**Model:**

rat\_model <- function(times,state,params){

with(

as.list(c(state,params)),{

###concentrations###

#liver

CL = AL/VL #concentration of parent in the liver; umole/L

CVL = CL/PCL #concentration of parent in liver capillary blood; umole/L

#arterial blood

CA = AA/VA #concentration of parent in arterial blood; umole/L

#venous blood

CV = AV/VV #venous blood concentration of parent; umole/L

CVmMolar = CV/1000 #venous blood concentration of parent; mmole/L

CMET = AMET/VDM #concentration of metabolite in blood; umole/L

#C total

CTot = CV + CMET #total concentrattion; metabolite + parent; umol/L

#slowly perfused tissues

CS = AS/VS #concentration of pareint in slowly perfused tissues; umole/L

CVS = CS/PCS #concentration of parent in slowly perfused tissue capillary blood; umole/L

#richly perfused tissues

CR = AR/VR #concentration of pareint in richly perfused tissues; umole/L

CVR = CR/PCR #concentration of parent in richly perfused tissue capillary blood; umole/L

#fat tissues

CF = AFat/VF #concentration of parent in adipose tissues; umol/L

CVF = CF/PCFat #concentration of parent in adipose tissue capillary blood; umole/L

#exhalation loss

CX = CA/PCB #concentration of parent in alveolar spaces; umol/L

#dose metrics

CVmgpl = CV\*MWC #venous blood concentration of parent; mg/L

CVPlas = CV/RBP #plasma concentration of parent; umol/L

CVPlasmgpl = CVPlas\*MWC #plasma concentration of parent; ug/L

AUCmghpl = AUCV\*MWC/1000 #All time AUC in mg\*hr/L

AUCmgL = AUCVmmolar\*MWC #All time AUC in mg\*hr/L

###exposures###

#oral dosing

ROSWITCH <- 0

bolus = (bolus\*(1/DT))/reps #adjust the bolus dose so that the dose rate occurs as parameterized in Berkeley-Madonna

RGDOSE = OSWITCH\*bolus #pulsed dosing added by events; mg/kg/hr; the events add the dose to AGDOSE

ODOSE = AGDOSE/MWC\*1000 #umole/kg bw

DOSE = ODOSE\*BW #umole

#inhalation dosing

if (times < (24\*days\_exp)) {

CINH = (mod(times,24)<len)\*(inh\_conc) #concentration of chemical in inhaled air (ppm); controlled by mathematical logical switch

} else {

CINH = 0

}

#perform unit conversions for the inhaled dose.

CI\_mgL = CINH\*MWC/24450 #innhaled concentration in mg/L

CI\_mol = CI\_mgL/MWC\*1000 #inhaled concentration in umol/L

CI = CI\_mol #set CI to the molar concentration for unit consistency.

###model equations###

#oral uptake from the gut

RAGI = frac\*RGDOSE\*BW/MWC\*1000 - Ka\*AGI #rate of change in the gut; umol/hr

#oral absorption metrics

RABSORB = frac\*RGDOSE\*BW/MWC\*1000 #rate of absorption; umol/hr

Absorb\_mgkg = ABSORB\*MWC/BW/1000 #amount absorbed; mg/kg

frac\_absorbed = Absorb\_mgkg/(bolus + 1e-10) #fraction absorbed; 1E-10 added to the bolus to ensure a nonzero denominator

#liver equations

#metabolism

RAMTot = (Vmax\*CVL\*fub)/(Km + CVL) + CLH\*CVL\*fub #rate of metabolism; umol/hr

RAL = QL\*(CA-CVL) + (Ka\*AGI) - RAMTot #rate of change in the liver amount; umol/hr

#urine parent and metabolite

#parent

RAU = (VmaxU\*CV)/(KmU + CV) + CLU\*CV #rate of parent change in urine; umol/hr

#blood equations

#arterial blood

RAA = (QC\*CV + QP\*CI) - QP\*CX - QC\*CA #rate of change in arterial blood; umol/hr

#venous blood

RAV = (QL\*CVL + QS\*CVS + QR\*CVR + QF\*CVF) - QC\*CV - RAU #rate of change in venous blood; umol/hr

#slowly perfused tissue

RAS = QS\*(CA - CVS) #rate of change in slowly perfused tissues; umol/hr

#richly perfused tissues

RAR = QR\*(CA - CVR) #rate of change in richly perfused tissues; umol/hr

#fat tissues

RAFat = QF\*(CA - CVF) #rate of change in adipose tissues; umol/hr

#metabolite

RAUM = (VmaxUM\*CMET)/(KmUM + CMET) + CLUM\*CMET #rate of change of metabolite in urine; umol/hr

#metabolite blood concentration

#only one route will be active at a time to remove the metabolite

RAMET = RAMTot - RAUM #rate of change of metabolite in the blood; umol/hr

#dose metrics

RAUCV = CV #umol/L

RAUCVmmolar = CVmMolar #mmole/L

###Area under the curve

#calculate the last 24hr AUC if in the last 24 hrs of modeled time

if (TSTOP - times < 24) {

RAUC24 = CV

RAUC\_M24 = CMET

RAUC\_Tot24 = CTot

} else {

RAUC24 = 0

RAUC\_M24 = 0

RAUC\_Tot24 = 0

}

RINH = QP\*CI #rate of inhalation of chemical; umol/hr

REX = QP\*CX #rate of exhalation of chemical; umol/hr

###mass balance equations

input = DOSE + INH #chemical input; umole

stored = AFat + AS + AR + AL + AV + AA + AGI #stored chemical; umole

output = AMTot + AU + EX #output of chemical; umole

MASSBAL = input - stored - output #mass balance of parent; should be near 0; umole

MBALMET = AMTot - AMET - AUM #mass balance of metabolite; should be near 0; umole

#return a list from the ODE solver

list(#derivatives

c(RGDOSE, ROSWITCH, RINH, #dosing

RABSORB, #absorption

RAFat,RAGI,RAL,RAA,RAV,RAS,RAR,RAU,REX, #amount in various compartments

RAUM,RAMET,RAMTot, #metabolites

RAUCV,RAUCVmmolar,RAUC24,RAUC\_M24,RAUC\_Tot24 #AUC metrics

),

#test

RAU = RAU,

#exposure

DOSE = DOSE,

CI = CI,

CI\_mgL = CI\_mgL,

CI\_mol = CI\_mol,

CINH = CINH,

#absorption

Absorb\_mgkg = Absorb\_mgkg,

frac\_absorbed = frac\_absorbed,

#tissues/compartments

CL = CL,

CVL = CVL,

CA = CA,

CV = CV,

CVmMolar = CVmMolar,

CS = CS,

CVS = CVS,

CR = CR,

CVR = CVR,

CF = CF,

CVF = CVF,

#metabolites/losses

CMET = CMET,

CTot = CTot,

CX = CX,

#dose metrics

CVmgpl = CVmgpl,

CVPlas = CVPlas,

CVPlasmgpl = CVPlasmgpl,

AUCmghpl = AUCmghpl,

AUCmgL = AUCmgL,

#mass balance

input = input,

stored = stored,

output = output,

MASSBAL = MASSBAL,

MBALMET = MBALMET

)

})

}

**Parameters:**

params<-c(

#general model parameters

TSTART = 0, #time to start simulation (hours)

TSTOP = 24, #time to stop simulation (hours); set according to study in model

DT = 0.001, #timestep change (hours)

bolus = 0, #bolus dosing (mg/kg)

inh\_conc = 0, #inhaled concentration (ppm)

reps = 1, #number of bolus dose events per day

dose\_int = 0, #how frequently dosing occurs (hours; this is only needed if reps > 1; else 0)

#molecular weight and bodyweight

BW = 0.25, #rat body weight (kg); set in model

MWC = 221.04, #molecular weight (g/mole)

#blood flow scaling

QCc = 15, #cardiac output constant (L/hr/bw\*\*0.75); Brown et al. 1997

QPc = 15, #alveolar ventilation rate (L/hr/bw\*\*0.75); set equal to QCc

QC = 0, #calculated in the model (L/hr)

QP = 0, #calculated in the model (L/hr)

QFc = 0.07, #fraction of blood flow to fat tissue; Brown et al. 1997

QLc = 0.18, #fraction of blood flow to the liver; Brown et al. 1997

QRc = 0.58, #fraction of blood flow to richly perfused tissue; Brown et al. 1997

QSc = 0.17, #fraction of blood flow to slowly perfused tissue; Brown et al. 1997

QTot = 0,

#blood flows scaled to total cardiac output

QL = 0, #bloow flow to the liver (L/hr); calculated in the model

QR = 0, #blood flow to richly perfused tissues (L/hr); calculated in the model

QS = 0, #blood flow to slowly perfused tissues (L/hr); calculated in the model

QF = 0, #blood flow to fat tissues (L/hr); calculated in the model

#blood flow balance check - should equal 0

QBal = 0,

#tissue volume fractions (L or kg per kg body weight - assumes tissue density = 1 kg/L)

VLc = 0.04, #fraction liver tissue; Brown et al. 1997

VFc = 0.07, #fraction fat tissue; Brown et al. 1997

VSc = 0.62, #fraction of slowly perfused tissues; Brown et al. 1997

VVc = 0.056, #fraction venous blood (~0.074\*0.75); Brown et al. 1997

VAc = 0.019, #fraction arterial blood (~0.074\*0.25); Brown et al. 1997

VRc = 0.06, #fraction richly perfused tissue; Brown et al. 1997

VPc = 0.865, #fraction of bw that is perfused; Brown et al. 1997

VBAL = 0, #volume check to ensure it equals VPc

#volume of distribution of metabolite (L), user defined

VDMc = 1.0,

#allometric scaling

VL = 0, #Liver volume scaled to body weight (L); calculated in the model

VF = 0, #fat volume scaled to body weight (L); calculated in the model

VR = 0, #richly perfused tissue volume scaled to body weight (L); calculated in the model

VS = 0, #slowly perfused tissue volume scaled to body weight (L); calculated in the model

VV = 0, #venous blood volume scaled to body weight (L); calculated in the model

VDM = 0, #volume of distribution of metabolite scaled to body weight (L); calculated in the model

#oral uptake

Ka = 5, #first-order oral absorption rate constant (1/hr), Ka = 5 assumes fast absorption

fa = 1, #fraction absorbed; first-order oral absorption

frac = 1, #fraction absorbed for linear/non-linear absorption; calculated in the model

fimax = 0, #maximum fractional reduction in relative bioavailability (max = 1.0; see Dolton and D'Argenio 2017); 0 defaults to first-order

f50 = 10, # amount of additional dose above the *dose\_prop\_term* that is associated with half-maximal reduction in relative bioavailability (see Dolton and D'Argenio 2017); f50 must be larger than dose\_prop\_term

dose\_prop\_term = 5, #dose at which the response is no longer dose proportional (see Dolton and D'Argenio 2017); must be smaller than f50

OSWITCH = 0, #whether dosing occurs or not (1 if new dose applied, 0 if not)

#inhalation

len = 0, #length of exposure (hours per day); set in model

days\_exp = 0, #total number of days exposed (days); set in model

#partition coefficients

PCL = 13.57, #liver/blood partition coefficient; 2,4-D value from HTTK, Liver value

PCR = 13.57, #richly perfused tissue/blood partition coefficient; 2,4-D value from HTTK, Liver value

PCS = 3.196, #slowly perfused tissue/blood partition coefficient; 2,4-D value from HTTK, Muscle Value

PCFat = 14.07, #fat/blood partition coefficient; 2,4-D value from HTTK, Adipose Value

PCB = 1E6, #air/blood partition coefficient; 2,4-D value from HTTK; set arbitrarily high given low volatility of 2,4-D

#protein binding

fub = 1, #fraction unbound in blood

fup = 1, #fraction unbound in plasma

RBP = 0, #blood to plasma concentration ratio (assumes Cup = Cub); calculated in the model

#metabolism liver saturable and linear (not scaled from in vitro)

CLH = 0, #first-order hepatic clearance rate of parent (L/hr)

Vmax = 0.0, #maximum rate of hepatic metabolism of parent (umol/hr); set in model

Km = 0.0, #parent concentration at which the hepatic metabolism rate is half of Vmax (umol/L); set in model

#urinary excretion of the parent compound

CLU = 0, #first-order urinary excretion rate of parent (L/hr); set in model

VmaxU = 0.0, #maximum rate of urinary metabolism of parent (umol/hr); set in model

KmU = 2.5, #parent concentration at which the urinary metabolism rate is half of VmaxU (umol/L); set in model

#urinary excretion of the metabolite

CLUM = 0, #first-order urinary excretion rate of metabolite (L/hr); set in model

VmaxUM = 0.0, #maximum rate of urinary metabolism of metabolite (umol/hr); set in model

KmUM = 2.5 #metabolite concentration at which the urinary metabolism rate is half of VmaxU (umol/L); set in model

)

**Scenario:**

#clear workspace

rm(list = ls())

#set the working directory to the location of these scripts

setwd(dirname(rstudioapi::getActiveDocumentContext()$path))

#get the time and print it.

start\_time <- Sys.time()

print(start\_time)

#suppress start-up messages from package loading (masking, etc.); Warnings will

#breakthrough here still

shhh <- suppressPackageStartupMessages

#load the libraries

shhh(library(deSolve)) #solver

shhh(library(readxl)) #excel import

shhh(library(ggplot2)) #plotting

shhh(library(scales)) #plotting

shhh(library(reshape2)) #melting

shhh(library(parallel)) #parallel computing

shhh(library(pracma)) #logseq function lives here

shhh(library(lubridate)) #date/time for UTC filenaming

shhh(library(gsubfn)) #chaining gsub replacements

# use parallel execution for speed (particularly for repeated runs)

# number of cores available

tot\_cores <- detectCores(all.tests = FALSE, logical = TRUE)

#because detectCores can produce an NAN, include a breakout to check for that and set it

#to 3 such that there is 1 core eventually allocated to the process.

if (is.na(tot\_cores)) {

tot\_cores <- 3

}

cores <- tot\_cores-1 #leave one cores for other system tasks

clust <- makeCluster(cores) #make the cluster for use in parallel execution

#set the scenario based on the Saghir 2013 spreadsheet

#dose\_spacing\_24D\_saghir2013\_all.xlsx is an Excel spreadsheet, where each worksheet is a scenario #name from Saghir et al. 2013 (“male\_TD28”, “male\_TD71”, “female\_TD29”, and “female\_TD95”) and #contains the dose vs AUC data from that in vivo study

scen = "male\_TD71"

#Set the minimum, maximum, and number of doses to run

min\_dose <- 1

max\_dose <- 1000

n <- 100

#set the female metabolic parameters, these were fit to the in vivo data as described in the manuscript

female\_vmaxu <- 2.91262719010577

female\_kmu <- 58.454036230595

female\_clu <- 0.0186468749231713

#set the male metabolic parameters, these were fit to the in vivo data as described in the manuscript

male\_vmaxu <- 8.79249841087232

male\_kmu <- 58.454036230595

male\_clu <- 0.0954784852172845

##SAVING files; do you want to save? "Y" if yes, "N" if no

saving <- "N"

#set up a function that runs the parameterized model; used in later optimization or

#repeated runs/model executions with different parameters

oral\_dose\_mod <- function(bolus,vmax,km,clu,bw,dur) {

#put library, path, and source directories for parameter and main model in the repeated

#loop so that each core has access when they are assigned runs

library(deSolve) #solver

#path

this.path <- paste0(gsub("\\", "/", fileSnapshot()$path, fixed=TRUE),"/")

#get model file; if it can't be found, produce an error reflecting that

if (! file.exists("PBPKrat\_v2.R"))

stop(paste("PBPKrat\_v2.R", "not found"))

source(paste0(this.path,"/PBPKrat\_v2.R"))

#get parameter file; if it can't be found, produce an error reflecting that

if (! file.exists("kmd\_rat\_parameters\_v2.R"))

stop(paste("kmd\_rat\_parameters\_v2.R", "not found"))

source(paste0(this.path,"/kmd\_rat\_parameters\_v2.R"))

#set the bolus dosing parameters (dose and frequency), vmax, and km

#TSTART, TSTOP, MWC, and BW are set in the parameter files

#resetting some of these parameters for clarity and to ensure validity

params[["BW"]] = bw #body weight (kg)

params[["MWC"]] = 221.04 #molecular weight (g/mole)

params[["TSTART"]] = 0 #time to start simulation (hours)

params[["TSTOP"]] = dur #time to stop simulation (hours)

params[["DT"]] = 0.002 #time step change (hours)

params[["bolus"]] = bolus #bolus dosing (mg/kg/day)

params[["reps"]] = 24 #repetitions of dosing per day

params[["dose\_int"]] = 12 #how long to apply repeated dosing (h)

params[["Vmax"]] = 0 #vmax value (umol/hr); assuming no hepatic metabolism

params[["Km"]] = 2.5 #km value (umol/L); set to a value to ensure nonzero denominators

params[["VmaxU"]] = vmax #urinary vmax value (umol/hr)

params[["KmU"]] = km #urinary km value (umol/L)

params[["CLU"]] = clu #urinary excretion of parent (L/hr)

#partition coefficients for 2,4-D from HTTK

params[["PCL"]] = 13.57 #liver/blood partition coefficient; httk 2.0.4 (Kliver2pu)

params[["PCR"]] = 13.57 #richly perfused tissue/blood partition coefficient; httk 2.0.4 (liver value used; Kliver2pu)

params[["PCS"]] = 3.196 #slowly perfused tissue/blood partition coefficient; httk 2.0.4 (muscle value used; Kmuscle2pu)

params[["PCFat"]] = 14.07 #adipose tissue/blood partition coefficient; httk 2.0.4 (adipose value used; Kadipose2pu)

params[["PCF"]] = 1E6 #air/blood partition coefficient; assumed very high given low volatility of 2,4-D

#ensure inhalation dosing is turned off

params[["inh\_conc"]] = 0 #inhaled concentration (ppm)

params[["len"]] = 0 #length of inhalation exposure (hrs)

params[["days\_exp"]] = 0 #number of days exposed (days)

#set calculated parameters

initial\_params <- within(as.list(params),{

#scaling for flows

#calculations performed herein in case parameters are changed; allows

#recalculation based on local changes

QC = QCc\*(BW\*\*0.75) #CARDIAC OUTPUT (L/H) adjusted for plasma

QP = QPc\*(BW\*\*0.75)

QTot = QLc + QRc + QSc + QFc

QL = (QLc\*QC)/QTot #bloow flow to the liver (L/hr)

QR = (QRc\*QC)/QTot #blood flow to richly perfused tissues (L/hr)

QS = (QSc\*QC)/QTot #blood flow to slowly perfused tissues (L/hr)

QF = (QFc\*QC)/QTot #blood flow to fat tissues (L/hr)

#blood flow balance check - should equal 0; if not, print an error to the terminal

QBal = (QL + QR + QS + QF) - QC

if (QBal != 0) {print("Blood Flow Imbalance. Check.")}

#balance check of flows to ensure it equals 0.865; if not, print an error to the terminal

VBAL = VLc + VAc + VVc + VRc + VSc + VFc

if (VBAL != 0.865) {print("Tissue Volume Imbalance. Check.")}

#allometric scaling of the tissue volumes

VL = VLc\*BW\*(0.865/VBAL) #Liver volume scaled to body weight (L)

VR = VRc\*BW\*(0.865/VBAL) #richly perfused tissue volume scaled to body weight (L)

VS = VSc\*BW\*(0.865/VBAL) #slowly perfused tissue volume scaled to body weight (L)

VF = VFc\*BW\*(0.865/VBAL) #fat tissue volume scaled to bodyweight (L)

VV = VVc\*BW\*(0.865/VBAL) #venous blood volume scaled to body weight (L)

VA = VAc\*BW\*(0.865/VBAL) #arterial blood volume scaled to body weight (L)

VDM = VDMc\*BW #volume of distribution of metabolite scaled to body weight (L)

#determine the oral absorption fraction; assuming first-order for 2,4-D

if (f50 < dose\_prop\_term) {

frac = fa

} else {

frac = fa\*(1-((fimax\*(bolus - dose\_prop\_term))/(f50+(bolus-dose\_prop\_term))))

}

#ensure that the frac never exceeds fa

if (frac>fa) {

frac = fa

}

RBP = fup/fub #blood to plasma concentration ratio (assumes Cup = Cub)

})

#set oral dosing interval/events

if (params[["bolus"]] > 0) {

print("Oral Dosing Set.")

#use the oral dose switch to turn on and off addition of new oral dosing to the

#GDOSE compartment

state\_1 = "OSWITCH"

state\_2 = "OSWITCH"

#switch ON value = 1, switch OFF value = 0

val\_1 = 1

val\_2 = 0

#replace the existing value with the new value (hence, "rep" in both spots)

op\_1 = "rep"

op\_2 = "rep"

#set the times for the events

#first, if there is only a single dose per day...

if (params[["reps"]] == 1) {

#set the "switch on" times at the first hour of each day

dose\_times <- head(seq(params[["TSTART"]],params[["TSTOP"]],24),-1)

#set the "switch off" times for the next time step after the switch on time

dose\_times\_2 <- dose\_times + params[["DT"]]

}

#if there is more than one dose occurring per day...

else {

dose\_times <- unlist(

lapply(X = 1:(params[["TSTOP"]]/24),

#loop over the number of days in the simulation

FUN = function(x){

#build a list of events for each day, based on the dose interval set in the

#parameter list

head(seq(0,params[["dose\_int"]],params[["dose\_int"]]/params[["reps"]]),-1) + (24\*(x-1))

}))

#as above, set the "switch off" times for the next time step after the switch on time

dose\_times\_2 <- dose\_times + params[["DT"]]

}

} else if (params[["inh\_conc"]] > 0) {

print("Inhalation Dosing Set.")

} else {

print("Both oral and inhalation dosing set to zero; no exposure set.")

}

#build the sequence of times for model execution

times <- seq(initial\_params[["TSTART"]],initial\_params[["TSTOP"]],initial\_params[["DT"]])

max\_report <- 12000 #set a maximum number of time steps to report; saves memory and prevents failures because of low memory

# if the more than the above set timesteps, select evenly spaced number of them for reporting

reported\_points<-if(length(times)<= max\_report) times else

times[seq(1,length(times),by=round(length(times)/max\_report))]

#if dosing isn't controlled by events (i.e., inhalation dosing), run the model

#without building the 'events' dataframe.

if (!exists("dose\_times")) {

state = c(AGDOSE = 0, OSWITCH = 0, INH = 0, #dosing

ABSORB = 0, #absorption

AFat = 0, AGI = 0, AL = 0, AA = 0, AV = 0, AS = 0, AR = 0, AU = 0, EX = 0, #amount in various compartments

AUM = 0, AMET = 0, AMTot = 0, #metabolites

AUCV = 0, AUCVmmolar = 0, AUC24 = 0, AUC\_M24 = 0, AUC\_Tot24 = 0 #AUC values

)

modeloutput <- ode(y = state, times = times, method = "rk4",

func = rat\_model, parms = initial\_params)

}

#if dosing is 'event' driven, build the events dataframe and run the model

else {

#build a dataframe to contain the dose information assigned above

dose\_events <- data.frame(

var = c(rep(x = state\_1, each = length(dose\_times)),

rep(x = state\_2, each = length(dose\_times\_2))),

time = c(dose\_times, dose\_times\_2),

value = c(rep(x = val\_1, each = length(dose\_times)),

rep(x = val\_2, each = length(dose\_times\_2))),

method = c(rep(x = op\_1, each = length(dose\_times)),

rep(x = op\_2, each = length(dose\_times\_2)))

)

#ensure all events are in the times used for the model

dose\_events <- dose\_events[order(dose\_events$time),]

unique\_times <- cleanEventTimes(times, dose\_events$time)

times <- sort(c(unique\_times, dose\_events$time))

#state variable initialization

#set to zero at the beginning

state = c(AGDOSE = 0, OSWITCH = 0, INH = 0, #dosing

ABSORB = 0, #absorption

AFat = 0, AGI = 0, AL = 0, AA = 0, AV = 0, AS = 0, AR = 0, AU = 0, EX = 0, #amount in various compartments

AUM = 0, AMET = 0, AMTot = 0, #metabolites

AUCV = 0, AUCVmmolar = 0, AUC24 = 0, AUC\_M24 = 0, AUC\_Tot24 = 0 #AUC values

)

#run the model and produce the corresponding output as modeloutput, currently using

#the rk4 method

modeloutput <- ode(y = state, times = times, method = "rk4",

func = rat\_model, parms = initial\_params,

events = list(data = dose\_events))

}

#store the model output as a dataframe and return that dataframe

result <- as.data.frame(modeloutput)

#only keep the MAX number of time points as highlighted above

result <- result[result$time %in% reported\_points,]

rm(modeloutput)

result$log\_AUC24 <- log(result$AUC24)

return(result)

}

#read in the data for plotting

#dose\_spacing\_24D\_saghir2013\_all.xlsx is an Excel spreadsheet, where each worksheet is a scenario #name from Saghir et al. 2013 (“male\_TD28”, “male\_TD71”, “female\_TD29”, and “female\_TD95”) and #contains the dose vs AUC data from that in vivo study

fit\_data = read\_excel("dose\_spacing\_24D\_Saghir2013\_all.xlsx", sheet = scen)

#set the sex, bodyweight, and duration based on the scenario name

if (substr(scen,1,1) == 'f') {

bw = 0.22 #kg; Saghir 2013

vmax = female\_vmaxu

km = female\_kmu

clu = 0 #change to female\_clu and vmax to 0 for linear fit.

} else {

bw = 0.32 #kg; Saghir 2013

vmax = male\_vmaxu

km = male\_kmu

clu = 0 #change to male\_clu and vmax to 0 for linear fit.

}

#set the duration in hours based on the terminal day in the name

dur = strtoi(substr(scen,nchar(scen)-1,nchar(scen)))\*24

#execute the above function (oral\_dose\_mod) over log spaced doses (n doses between

#min and max) and store that output in a single large list (model\_output). This is

#done in parallel for speed using the above allocated cluster

model\_output <- parLapply(clust,logseq(min\_dose,max\_dose,n),oral\_dose\_mod,

vmax = vmax, km = km, clu = clu, bw = bw, dur = dur)

#generate an empty dataframe to store the desired output data

output\_df <- data.frame()

#cycle through the dataframes in the generated list, collect and bind all desired data.

#in this case, we're collecting the final time point (t = 480 hr) for all modeled doses.

for (frame in seq(1,length(model\_output),1)) {

data\_to\_append <- model\_output[[frame]][nrow(model\_output[[frame]]),]

data\_to\_append$cmax <- max(model\_output[[frame]][,"CVPlas"])

output\_df <- rbind(output\_df,data\_to\_append)

row.names(output\_df) <- NULL #reset the index for the dataframe

}

#replace the ending dose with the 'initial' dose value (i.e., what was set)

output\_df[,'AGDOSE'] <- logseq(min\_dose, max\_dose, n)

#append the vmax and km used in the model to the output data

output\_df$Vmax <- vmax

output\_df$Km <- km

output\_df$CLU <- clu

#plot the (final) AUC24 and AUC\_Tot24 for each of the modeled doses. Plot is performed

#on a log/log scale

cur\_time <- toString(now(tzone = "UTC")) #get time in UTC

cur\_time <- gsubfn(".",list("-" = "\_"," " = "\_",":" = "\_"),cur\_time) #replace spaces with underscores

plot <- ggplot(data = output\_df) +

geom\_line(aes(x = AGDOSE, y = AUC24), size = 1, color = 'turquoise3') +

geom\_point(data = fit\_data, aes(x = Dose, y = AUC)) +

theme(text = element\_text(size=20)) +

scale\_y\_continuous(trans = 'log10',

breaks = trans\_breaks('log10', function(x) 10^x),

labels = trans\_format('log10', math\_format(10^.x))) +

scale\_x\_continuous(trans = 'log10',

breaks = trans\_breaks('log10', function(x) 10^x),

labels = trans\_format('log10', math\_format(10^.x))) +

labs(title = "Generic Rat PBPK Model",

subtitle = sprintf("Run from %.2f to %.2f mg/kg with %i steps\nVmax = %.4f, Km = %.4f",

min\_dose,max\_dose,n,vmax,km),

x = "Dose (mg/kg/day)",

y = "AUC (\u03bcmole\*hr/L)", #includes unicode \u03bc for lower case mu

color = "")

#print plot in RStudio

print(plot)

#plot the (final) AUC24 and AUC\_Tot24 for each of the modeled doses. Plot is performed

#on a log/log scale

cur\_time <- toString(now(tzone = "UTC")) #get time in UTC

cur\_time <- gsubfn(".",list("-" = "\_"," " = "\_",":" = "\_"),cur\_time) #replace spaces with underscores

if (saving == "Y") {

#path for saving

this.path <- paste0(gsub("\\", "/", fileSnapshot()$path, fixed=TRUE),"/")

cur\_time <- toString(now(tzone = "UTC")) #get time in UTC

cur\_time <- gsubfn(".",list("-" = "\_"," " = "\_",":" = "\_"),cur\_time) #replace spaces with underscores

#save the plot

png(file.path(this.path,"Model\_Output",

paste0("24D\_rat\_model\_non\_linear\_",scen,"\_male\_fit\_",cur\_time,".png")),

width = 1200, height = 720, units = "px", pointsize = 12,

bg = "transparent")

print(plot)

dev.off()

#create a file name and directory (as needed) to save the data

if(!file.exists("Model\_Output")) dir.create("Model\_Output") #create a model output directory

fn <- paste0("/Model\_Output\\24D\_rat\_model\_non\_linear\_",scen,"\_male\_fit",cur\_time,".csv") #build filename

#save data file as a CSV

write.csv(output\_df,paste0(this.path,fn), row.names = FALSE)

#release the cores and stop the parallel operation

stopCluster(clust)

}

#calculate and print the time it took to execute the full model run

end\_time <- Sys.time()

print(end\_time - start\_time)