Caffeine PBBK model to predict MoIE for risk assessment

Contents

Part I. PBK model reporting template

A. Name of model

Caffeine PBBK model to predict MoIE for risk assessment

B. Model developer and contact details

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C. Summary of model characterisation, development, validation, and regulatory applicability

A PBBK model was developed to estimate blood concentrations following exposures to caffeine in experimental animals and humans and to obtain a point of departure as the Margin of Internal Exposure (MoIE).

The prediction are part of a risk assessment based on only in vitro in silico and historical in vivo data to demonstrate how read-across can be applied in order to fill data gaps in an assessment of the potential risk for the consumer from exposure to caffeine. For this purpose, in vivo data from structural analogues have been used while assuming that no in vivo repeated dose toxicity data were available for the target substance caffeine. The physiologically-based biokinetic (PBBK) model to estimate blood concentrations following exposures to caffeine in experimental animals and humans, a Margin of Internal Exposure (MoIE)

D. Model characterisation (Modelling workflow)

Step 1 – Scope and purpose of the model (problem formulation)

The physiological structure of PBBK models provides a particularly useful framework for conducting cross species extrapolations (Clewell and Andersen 1985). To apply a PBBK model for interspecies extrapolation, the model is first used to simulate the exposure of interest (dose, route, and duration) in the experimental species, and the internal dose metric (peak or average concentration) is calculated. The parameters in the PBBK model are then changed to those for the target species of concern and

the dose is adjusted until the same internal dose metric is achieved. The dose that produces the same internal dose metric (MoIE) is then considered the kinetically equivalent dose (Clewell et al. 2002).

Step 2 – Model conceptualisation (model structure, mathematical representation)

The PBBK model developed is structured with perfusion-limited compartments for skin, liver, fat, lung, kidney, blood, and lumped compartments for the remaining richly and slowly perfused tissues. Consistent with common practice, tissue:venous equilibration is assumed, and the tissues are assumed to be well-mixed reservoirs. Exposure is characterized in exposed skin (dermal) and gastrointestinal (GI, oral) compartments, and metabolism is described as a first-order clearance process in the liver. The model structure is shown schematically in Figure 1.

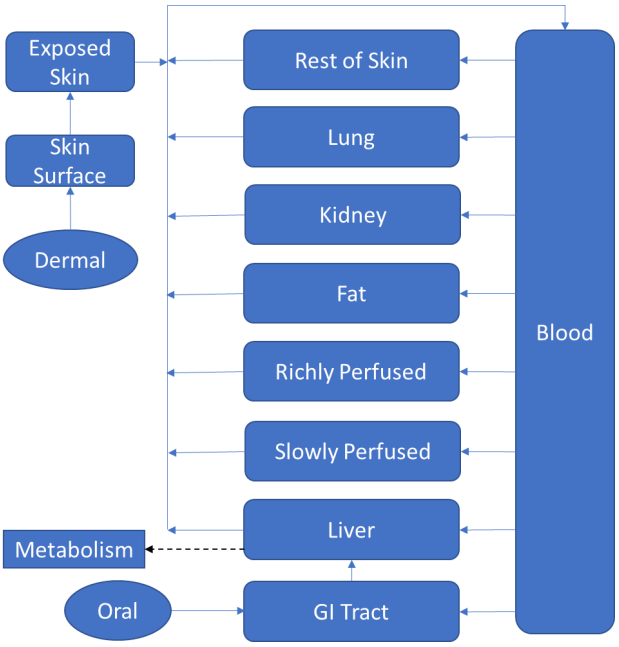

Figure 1. PBK model schematic for caffeine showing the representation of the main organs considered with various sub-compartments in the skin and GI tract for oral and dermal exposure

Caffeine by the GI tract or by the skin becomes systemically available, and is metabolized in the liver by CYP450 1A2 mainly to three metabolites, i.e. theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine), and paraxanthine (1, 7-dimethylxanthine) (see Figure 2), corresponding respectively to 4%, 12% and 84% of the parent caffeine (Puchem and DrugBank). Metabolism in the skin is regarded as negligible for caffeine since skin expresses little amount of CYP450 1A2 (Gajewska et al, 2014 & 2015, Genies et al 2019). Caffeine is metabolically converted into, theophylline, theobromine, and paraxanthine, primarily in the liver where ca. 90-99 % of the metabolism takes place.

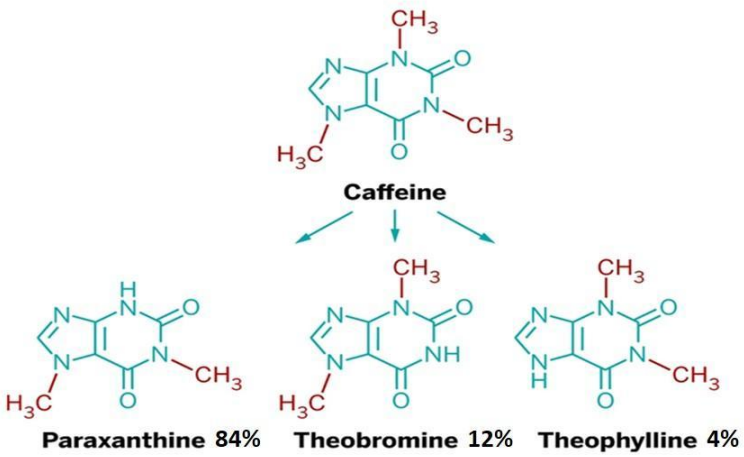

Figure 2. Caffeine metabolism.

Step 3. Model parameterisation (parameter estimation and analysis)

A list of physiological parameters is shown in Table 4, and chemical-specific parameters are shown in Table 5. Tissue blood flows and volumes are set to values in Brown et al. (1997, Tables 21 and 23), except for skin. Skin volume was calculated as the product of surface area and average skin thickness. Skin thickness was taken from Brown et al. (1997) and skin surface area was calculated using the following allometric relationship from Livingston and Lee (2001):

SA = 0.1173 * BW 0.6466 (m^2).

Blood flow to the exposed skin was calculated as the total skin blood flow adjusted by the ratio of volume exposed skin to volume of total skin. Exposed skin volume and blood flow was subtracted from the total skin to derive the parameters for the unexposed skin compartment.

Tissue:blood partition coefficients (PC) were estimated by Gajewska using the algorithm of Schmitt (2008), except for skin. Skin:blood PC was estimated as a weighted average of liver and fat PCs (i.e. 0.7*PL + 0.3*PF).

Oral exposure is modeled using a 1-compartment, first-order absorption model, with the gastrointestinal (GI) tract acting as a reservoir for the oral dose. All oral doses are simulated as bolus doses (i.e. all chemical ingested at once per dosing event). The blood flow from the GI enters the liver via the portal vein.

Dermal exposure is modeled using a skin surface compartment to house the applied dose in terms of the volume and surface area, and a single skin compartment. Transfer to the systemic circulation occurs in the skin compartment. The skin is separated into exposed and unexposed compartments. Dermal absorption is driven by a permeability coefficient for uptake from the surface into the skin (units of cm/hr), the exposure area and volume of application, the amount of chemical applied, duration of application, and the fraction absorbed. Transfer from the skin to the blood is modeled assuming a well-mixed, blood flow-limited exposed skin compartment and a skin:blood partition coefficient.

The liver clearance was calculated by scaling the in vitro intrinsic clearance value to the whole body. The liver clearance rate is calculated as the uL/min/million cells, times hepatocellularity, times the volume of the liver:

$$CLint\ (L/h) = CLint\_invitro * hpgl * 10^{-6}\ (L/\mu L) * 60\ (min/h) * 10^{3}\ (g/kg) * VLc * BW\ (kg)$$

where CLint_invitro is the uL/min/million cells cleared in vitro, hpgl is the number of million hepatocytes per gram of liver, and VLc*BW is the volume of the liver.

Step 4 – Computer implementation (solving the equations)

The physiologically-based biokinetic (PBBK) model was developed in Berkeley Madonna software (version 8.3.18; University of California, Berkeley, CA; www.berkeleymadonna.com).

A typical equation for a perfusion-limited tissue describes the mass balance for the uptake and clearance of the chemical in the tissue, in this case the liver:

dALiver/dt = QL*(CArterial – CVenous) – ClLiver

This equation can be interpreted as: The rate of change in the mass of the chemical (ALiver) in the liver is equal to the liver blood flow (QL) multiplied by the difference between the concentrations in the blood entering and leaving the liver (CArterial – CVenous), minus the metabolic clearance of the chemical in the liver (ClLiver).

These models typically rely on three types of parameters; physiological (e.g. tissue volumes, blood flows), physicochemical (e.g. octanol:water portioning, vapor pressure, water solubility), and biochemical (e.g. absorption rates, metabolism, clearances). The particular parameters needed depend on factors such as the chemical properties and the purpose of the model. Various guidance documents for the application, use, and reporting of PBBK models have been published (WHO, 2010; USEPA, 2006; USFDA, 2018).

Step 5 – Model Performance

A qualitative evaluation of the agreement between experimental plasma concentration and simulations was conducted through visual inspection of the time-course concentration curves (USEPA, 2006). Good agreement, with model predictions generally within a factor of two of the data (WHO 2010) was obtained for both the oral and dermal route (figures 3, 4 and 5).

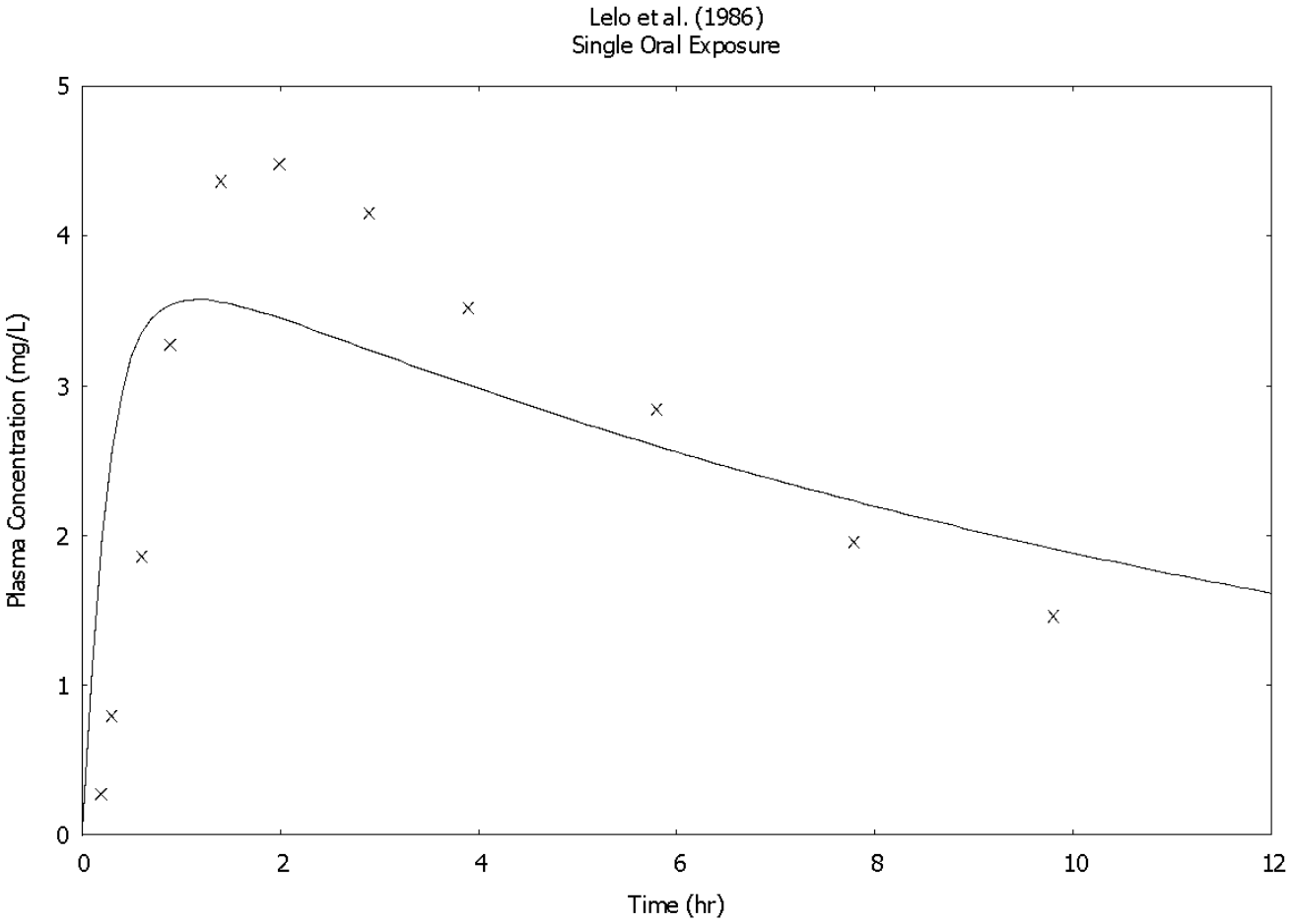

Figure 3. Caffeine PBBK simulations compared to oral exposure data of Lelo et al. (1986). Plasma concentration following ingestion of 3.25 mg/kg body weight caffeine in a gelatin capsule. Study subjects included 6 non-smoking, male volunteers aged 19-21 years and weighing 62-104 kg (average body weight of 83 kg used for the simulation).

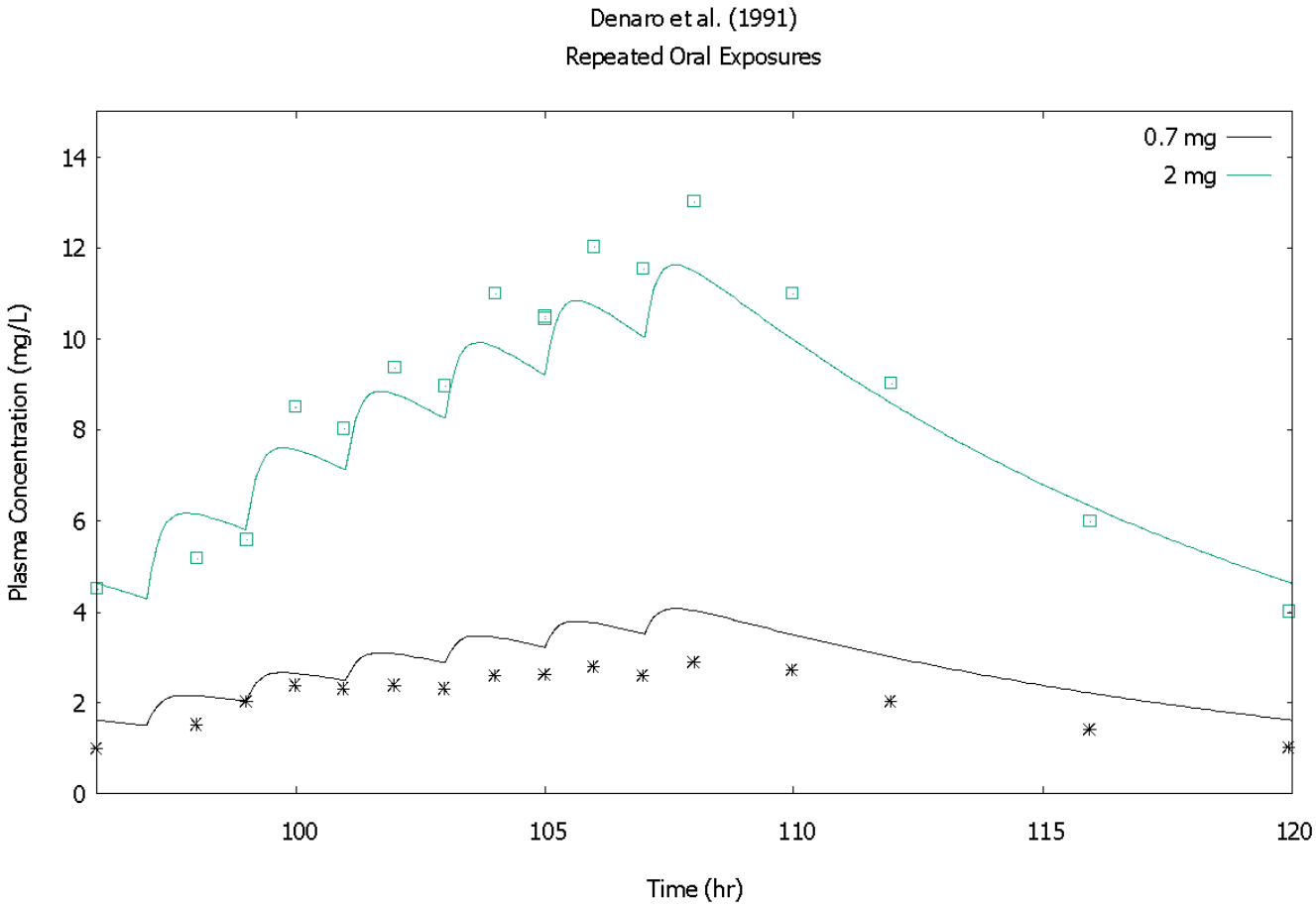

Figure 4. Caffeine PBBK simulations compared to repeated oral exposure data of Denaro et al. (1991). Nine healthy nonsmokers who regularly drank 4 or more cups of coffee were admitted for 16 days. There were 7 males and 2 females, with ages ranging from 19 to 55 years of age. No body weights were reported, and a value of 70 kg was assumed for the simulation. Each subject participated in 3 treatment blocks of a placebo, low and high dose of caffeine. Plasma concentration of caffeine was determined following ingestion of a cup of coffee (0.7 and 2 mg/kg caffeine) every 2 hours (a total of 6 cups of coffee per day) over 5 days.The last day of a low and high dose treatment block is shown in the figure.

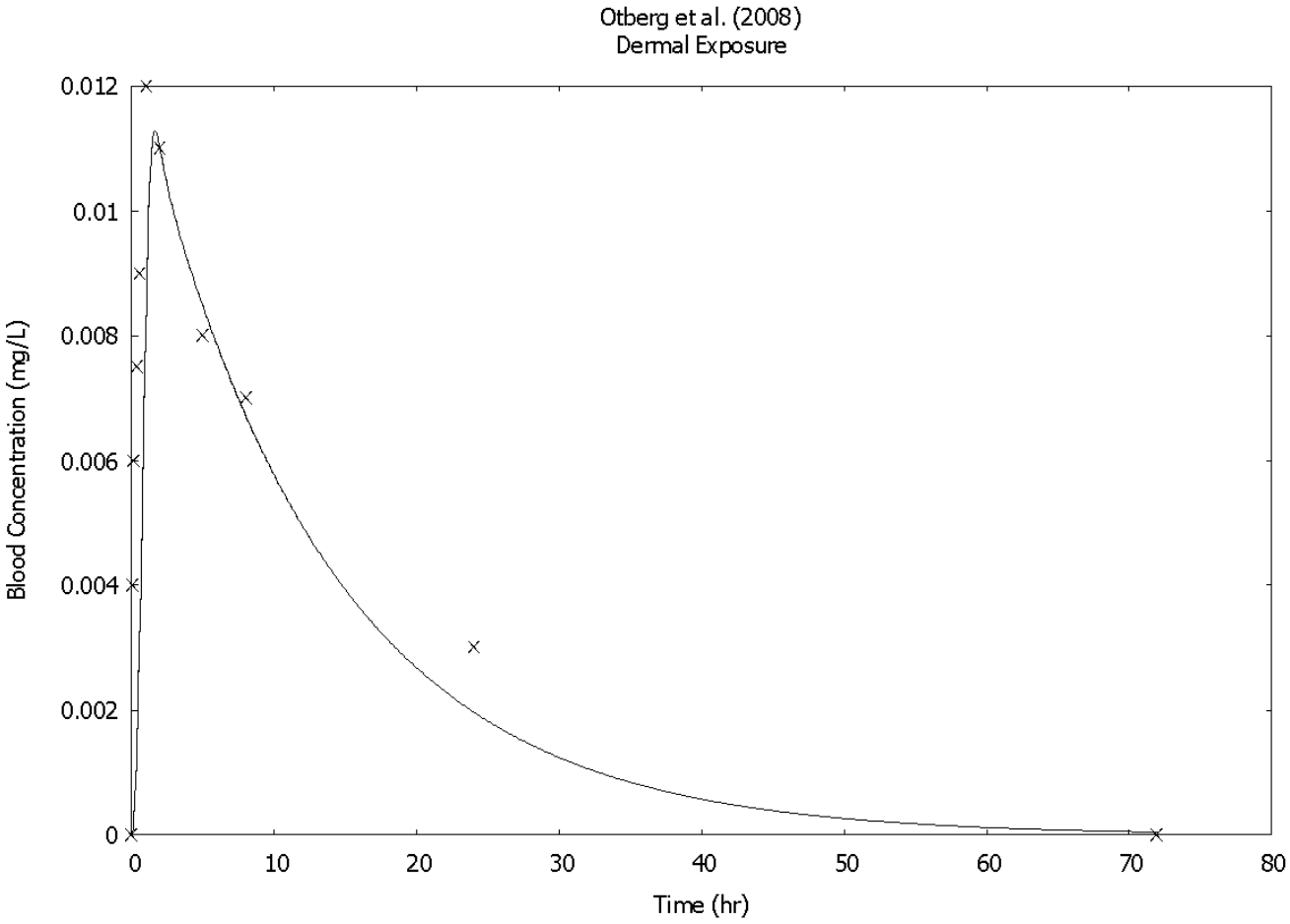

Figure 5. Caffeine PBBK simulations compared to dermal exposure data of Otberg et al. (2008). Plasma concentration was determined following application of 50 mg (0.05 mL) of ethanol/propylene glycol formulation containing 2.5% caffeine (1.25 mg caffeine) to 25 cm^2 of the chest of 6 healthy Caucasian male volunteers. Hair was clipped, and hair follicle density averaged between 20 and 32 follicles/cm^2. Volunteers were 26-39 years of age with a normal BMI between 21 and 24. Body weight was not reported, and default value of 70 kg used for the simulation.

A local sensitivity analysis for model parameters was conducted with the cosmetic exposure scenario using the built-in tool in the Berkeley Madonna software. The sensitivity coefficients were then normalized by the output and input parameter values according to the following equation:

$$Normalized\ Sensitivity\ Coefficient = \frac{\Delta Y/Y}{\Delta X/X}$$

where Y is the output (i.e., Cmax or AUC), X is the input parameter (e.g., Ka, Kp), ΔX is the change in the parameter value, and ΔY is the resulting change in the output value. Normalization of the sensitivity coefficients is necessary to make comparisons across parameters of different scales (Clewell et al. 1994).

Table 1. Summary of the largest normalized sensitivity coefficients. The sensitivity of the maximum plasma concentration (Cmax) and area under the plasma concentration curve (AUC) are shown.

Parameter	Symbol	Cmax Sensitivity	AUC Sensitivity
Fraction Absorbed	FracAvail	0.18	0.16
Body Weight	BW	-0.15	-0.16
Liver Volume	VLC	-0.68	-0.96
Cardiac Output	QCC	-0.06	-
Liver:Blood Partition	PCLiv	-0.08	-
Slowly-perfused:Blood Partition	PSC	-0.09	-
Oral Absorption Rate	Ka	0.11	-
Fraction Unbound in Blood	fub	-0.67	-0.96
Hepatic Intrinsic Clearance	hep_CLint_invitro	-0.67	-0.96
Hepatocellularity	hpgl	-0.67	-0.96

Step 6 – Model Documentation

The following PBBK model is also part of the IATA case study (2/2019, Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to Caffeine for repeated dose toxicity). And as peer review paper by Cosmetic Europe (in preparation).

E. Identification of uncertainties

Model structure

A PBBK model with relatively simple oral and dermal absorption models was developed. The model simulations agreed reasonably well across multiple oral and dermal exposure data sets using a common set of parameter values. The model did tend to underpredict the plasma concentration at the higher oral doses, though it was consistently within a factor of 2 from the data. This is likely due to saturation of oxidative metabolism, and there are also potential vehicle effects that are not being captured by the model (Lelo et al. used gelatin capsules, while Denaro et al. put anhydrous caffeine into decaffeinated coffee). A more complex description of the oral absorption model could potentially improve the accuracy of the simulations at high doses, though more data or assumptions would be required to parameterize the model, bringing with them uncertainties of their own.

Input parameters

The fraction available for dermal absorption was set to 50% based on extrapolation of the trend found using in vitro testing. It is noted, however, that the fraction absorbed has been observed as high as 67%, which would lead to higher predictions of Cmax and AUC. To estimate the impact of this assumption, the model was run again with 67% available for absorption. There is a 10% increase in Cmax, and approximately a 6% increase in AUC.

Table 2. Comparison of internal dose metrics with 67% dermal absorption vs 50%. The exposure scenario is the cosmetic use scenario described in Table 3.

Fraction Absorbed	50%	67%
Cmax (mg/L)	10	11
AUC (mg-h/L)	180	180

Model output

A simulation of the worst-case exposure estimated for caffeine via oral and dermal routes was conducted. Upper bound oral (13.1 mg/kg/d) and dermal (4.8 mg/kg/d) exposure estimates were determined and used as input to the model. Twice daily exposures were simulated, 12 hours apart, as bolus ingestions or applications. A 4 mL volume was used to simulate whole body exposure to lotion, as reported by Troutman et al. (2015). Caffeine concentration in the dermal formulation was assumed to be 2%, which corresponds to approximately 20 mg/mL caffeine. Using trends from in vitro dermal penetration data, 50% of the caffeine was assumed available for dermal absorption. A default body weight of 60 kg was also assumed.

The simulated internal dose metrics (Cmax and AUC) are shown in Table 3. The dosing simulation was run for 3 (simulated) days to achieve steady periodicity, as shown in Figure 6. The maximum concentration in blood (Cmax) was 10 mg/L, the daily area under the concentration curve (AUC, calculated over the time period from 48 hours to 72 hours) was 180 mg-h/L, and the average daily concentration (Cavg) was 7.3 mg/L.

Table 3. Summary of the worst-case cosmetic exposure scenario. Daily oral and dermal doses are split into 2 equal doses, administered at 12-h intervals.

Exposure	
Single Oral Dose (mg/kg)	6.55
Single Dermal Dose (mg/kg/d)	2.4
Dermal Exposure Area cm^2	16560 [a]
Doses/day	2
Daily Oral Dose (mg/kg/d)	13.1
Daily Dermal Dose (mg/d)	4.8
Internal Dose Metrics	
Cmax (mg/L)	10
AUC (mg-h/L)	180
Cavg (mg/L)	7.3

[a] Whole body surface area for a 60 kg human calculated using the equation of Livingston and Lee (2001)

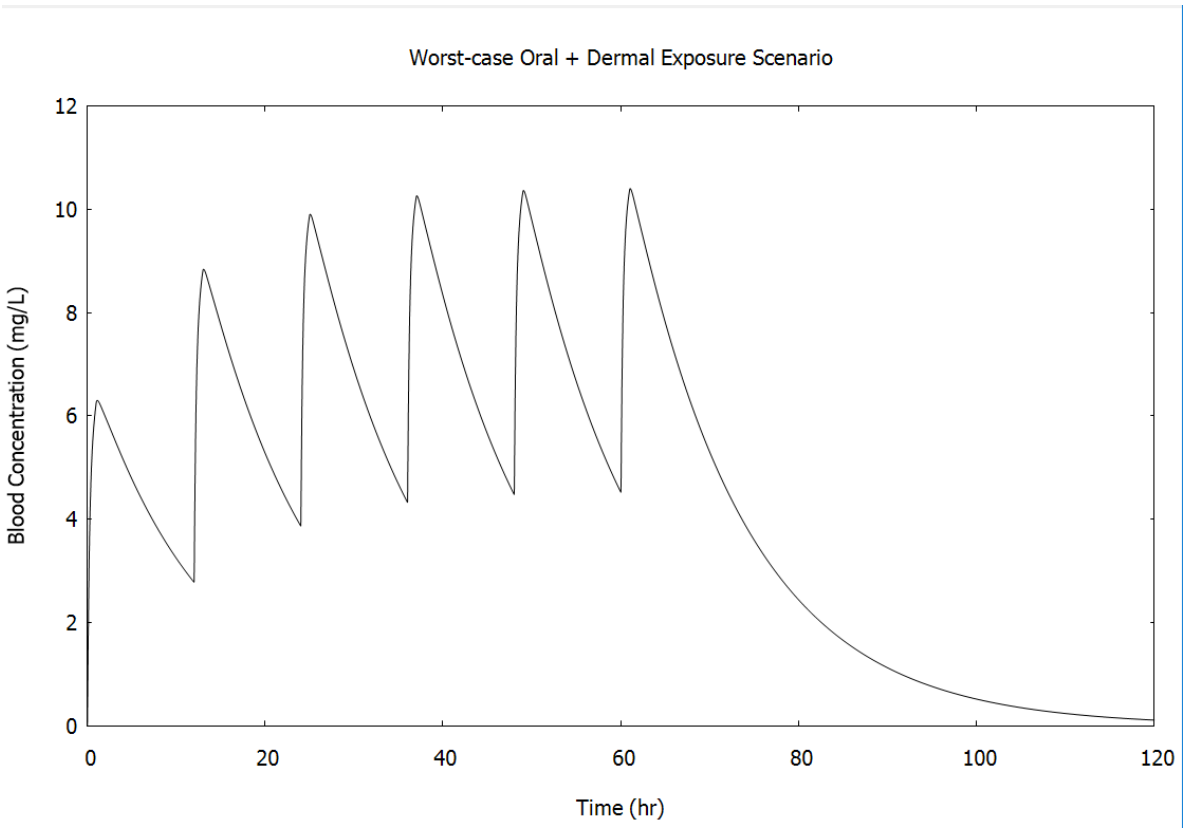

Figure 6. PBBK simulation of internal plasma concentration of caffeine following estimated oral and whole body dermal exposure in Table 3. Oral exposure = 13.1 mg/kg/d, whole body dermal exposure = 4.8 mg/kg/d in 4 mL of product, twice daily exposure. BW = 60 kg. Cmax = 10 mg/L, AUC = 180 mg-h/L, Cavg = AUC/24 = 7.3 mg/L.

The sensitivity analysis identified 6 parameters to which the AUC and Cmax were sensitive, and an additional 4 to which Cmax only was sensitive. The parameters driving absorption (FracAvail and Ka) had positive coefficients (i.e., increase parameter increase Cmax), while parameters increasing clearance (e.g., liver parameters and intrinsic clearance) had negative coefficients.

- other uncertainties (e.g. model developed for different substance and/or purpose)

Simulation of the product use scenario was conducted for adult consumers using parameters for an average individual. Previous evaluations of the impact of inter-individual variability in pharmacokinetics on PBK modeling of the relationship of internal dose to external exposure (Clewell and Andersen 1996) suggest that the resulting variability in internal dose is consistent with the recommended default factor of three recommended by IPCS (2005). Age-dependent variations in pharmacokinetics can also be expected. However, a study of the impact of age-dependence pharmacokinetics on internal dose (Clewell et al. 2004) found that, in general, predictions of average pharmacokinetic dose metrics for a chemical across life stages were within a factor of two, although larger transient variations were predicted, particularly during the neonatal period.

F. Model implementation details

- software (version no) : Berkeley Madonna software (version 8.3.18; University of California, Berkeley, CA; www.berkeleymadonna.com).
- availability of code **Yes**
- software verification / qualification not applicable

H. Peer engagement (input/review)

None

G. Parameter tables

Table 4. Human PBBK physiological parameter values for caffeine. Source for values is Brown et al. (1997) unless otherwise specified.

Parameter	Units	Symbol	Value
Body mass [d]	kg	BW	-
Skin thickness	cm	Depth	0.1
Blood Flows (Fraction of Cardiac Output) [a]			
Cardiac Output	$L/h/kg^{0.75}$	QCc	15
Fat	1	QFc	0.052
Kidney	1	QKc	0.175
Liver	1	QLc	0.227
Skin	1	QSkc	0.058
Lung	1	QLuc	0.025
Volumes (fraction of BW) [b]			
Fat	1	VFc	0.21
Kidney	1	VKc	0.004
Liver	1	VLc	0.026
Lung	1	VLuc	0.008
Blood [c]	1	VAc, VVc	0.079
Hepatocellularity [e]	millions of hepatocytes per gram liver	hpgl	99

[a] Richly perfused blood flow = 70% of QC minus liver, kidney, and lung volumes.

[a] Slowly perfused blood flow = 30% of QC minus fat, and skin volumes.

[b] Richly perfused tissue volume = 70% of BW minus liver, kidney, and lung volumes.

[b] Slowly perfused tissue volume = 83.6% of BW minus fat, blood, and skin volumes.

[c] Blood is divided into 3/4 arterial and 1/4 venous.

[d] Simulation specific

[e] Barter et al. (2007)

Table 5. Chemical-specific PBBK parameters for caffeine

Parameter	Units	Symbol	Value	Source
Molecular weight	g/mol	MWC	194.2	PubChem
Partition Coefficients				
Fat	1	PF	0.68	Schmitt (2008)
Kidney	1	PK	3.76	Schmitt (2008)
Liver	1	PL	4.25	Schmitt (2008)
Lung	1	PLu	1.23	Schmitt (2008)
Rich	1	PR	2.4	Schmitt (2008)
Slow	1	PS	0.995	Schmitt (2008)
Blood and Plasma				
Fraction unbound in blood	%	fub	96	Lave et al. (1997)
Fraction unbound in plasma	%	fup	68	Lelo et al. (1986)
Blood:plasma ratio	%	RBP	71	fup/fub
Oral absorption				
GI -> liver	1/h	Ka	1.6	fit
Dermal absorption				
Fraction available	%	FracAvail	50	Genies et al, Hewitt et al.
Permeability	cm/h	Kp	6E-3	Doucet et al. (1998)[1]
Metabolism				
Hepatocyte clearance	uL/min/million cells	hep_Clint_invitro	0.68	fit

[1] The value from Doucet et al. (1998) was 6E-4 cm/h. However, this value was adjusted upward an order of magnitude to obtain results matching the in human data of Otberg et al. (2008). Doucet used frozen female abdominal skin tissue, while Otberg exposed male chests with “pronounced terminal hair on the chest”. The adjustment was considered reasonable as there are known absorption differences and hair follicle densities across body locations.

J. References and background information

- Publications

OECD IATA, 2/2019, Case Study on the use of Integrated Approaches for Testing and Assessment for Systemic Toxicity Arising from Cosmetic Exposure to Caffeine for repeated dose toxicity.

Cosmetic Europe (in preparation).

- links to other resources

Model code

```
;Caffeine PBBK model
;Created for CosEu case study by Eric Hack, Alina Efremenko, Alicia Paini, and Harvey Clewell
;Model units
;time = hr
;volumes = L
;mass = mmole
{
Instructions for running the model simulations
Denaro repeated oral exposure simulation:
STOPTIME = 120
BW = 70
Bolus = 0.7 or 2
start = 1
ostop = 12
repeat = 2
allstop = 109

Lelo single oral exposure simulation:
STOPTIME = 24
BW = 83
Bolus = 3.25
start = 0
repeat > STOPTIME

Otberg dermal exposure simulation:
STOPTIME = 72
BW = 70
Bolus = 0
DermalGDose = 1.25
VolApp = 0.05
AreaE = 25
DRepeat > STOPTIME
tinf = 0.1

Cosmetic oral and dermal exposure simulation:
STOPTIME = 72
BW = 60
Bolus = 6.55
repeat = 12
ostop > STOPTIME
DermalGDose = 144
DRepeat = 12
DStop > STOPTIME
VolApp = 4
AreaE = 16560
tinf = 0.1
}
;-----------------------------------------------------------------------------------------------------------------------------------
; Solver settings
 METHOD RK4                    ; Runge-Kutta 4th-order solver (not using stiff system integrator because of
pulsed oral repeated dose)
 STARTTIME = 0     ; hours
 STOPTIME = 24     ; hours
```

```
; Molecular weight
 MWC = 194.2;                Molecular weight caffeine
;-----------------------------------------------------------------------------------------------------------------------------------
; Exposure calculations

; Control all exposure
 allstop = 1e6                                ; hours
 exposurezone = (Time <= allstop)   ; hours
;-----------------------------------------------------------------------------------------------------------------------------------
; Oral exposure - repeated bolus dose

 bolus = 6.55                        ; mg/kg per application
 start = 0                           ; initial bolus time (hr)
 ostop = 12                          ; time to stop oral dosing (hr)
 repeat = 1e6                        ; bolus repeat interval (hours)
 ondaily = if MOD(time-1, 24) <= ostop then 1 else 0

 GDOSE' = pulse(bolus, start, repeat) * ondaily * exposurezone        ; mg/kg/h
 init GDOSE = 0                      ; initial amount in stomach (mg/kg)
 ODOSE = GDOSE/MWC                   ; mmole/kg bw
 DOSE=ODOSE*BW                       ; mmole
;-----------------------------------------------------------------------------------------------------------------------------------
; Dermal exposure

 DermalGDose = 243.6                         ; mg
 VolApp = 0.1                                ; application volume (mL)
 DermalDose  = DermalGDose/MWC               ; mmole
 FracAvail = 0.67                            ; Fraction of applied dose available for absorption, from CE
                                             study
 tinf = 1.0                                  ; Time to apply dermal dose (h)
 DStart = 0                                  ; time to start dermal dosing (hr)
 DRepeat = 999                               ; time interval to repeat dermal exposure (hr)
 DStop = 1                                   ; time of last dermal exposure + delta t (hr) {e.g., DStop = 97 for
                                             last daily dose at 96 hr}
 DZone = IF MOD(TIME, DRepeat) < tinf THEN 1 ELSE 0          ; flag for dermal exposure on/off
 applddose' = DZone*(DermalDose*FracAvail)/tinf * (TIME <= DStop) * exposurezone; mmole/h
 INIT applddose = 0.0        ; initial dermal dose
;-----------------------------------------------------------------------------------------------------------------------------------
; Body weight
 BW = 60                     ; kg
;-----------------------------------------------------------------------------------------------------------------------------------
; Skin parameters
; Total Area of Skin (cm^2), Livingston and Lee (2001)

 SA = (0.1173*(BW)**0.6466) * 10**4          ; total body surface area, cm2 (the 10**4 converts m2 to cm2)
 AreaE = 25                          ; surface area of skin exposed cm2
 SA_exposed = min(AreaE, SA)         ; limit exposed SA to <= total SA
 Depth = 0.10                        ; skin thickness (cm), Brown 1997
 Kp = 6e-4                           ; skin permeability (cm/hr), Table III Doucet et al 1998, frozen human
                                     skin (female abdomen), simple O/W emulsion
 VSk = SA*Depth / 1000               ; total skin volume (L)
 VSkc = VSk/BW                       ; fractional skin volume (for slowly perfused volume calculation)
 VSkE = SA_exposed*Depth / 1000  ; exposed skin volume (L)
;-----------------------------------------------------------------------------------------------------------------------------------
; Other tissue volume fractions (L or kg per kg body weight)
 VLc = 0.026                         ; fraction of  liver tissue
 VKc = 0.004                         ; fraction of kidney tissue
```

```
 VLuc = 0.012                    ; fraction of lung tissue
 VFc = 0.214                     ; fraction of fat tissue
 VAc = 0.0185                    ; fraction of arterial blood  0.074*1/4
 VVc = 0.0555                    ; fraction of venous blood 0.074*3/4
 VRc = 0.08-VLc-VLuc-VKc         ; fraction of richly perfused tissue
 VSc = 0.836-VFc-VAc-VVc-VSkc    ; Fraction of blood flow to slowly perfused tissue
                                 ; total of fractions = 0.91
; Volume check = 0.91?
 VBal = VLc + VKc + VLuc + VFc + VSkc + VAc + VVc + VRc + VSc
;-----------------------------------------------------------------------------------------------------------------------------------
; Allometric scaling of tissue volumes (L or kg)
 VL = VLc*BW
 VLu= VLuc*BW
 VK = VKc*BW
 VF = VFc*BW
 VR = VRc*BW
 VS = VSc*BW
 VV = VVc*BW
 VA = VAc*BW
;-----------------------------------------------------------------------------------------------------------------------------------
; Blood flow rates

 QCc = 15
 QC = QCc*BW**0.74               ; Reference: Brown et al 1997, L/hr
 QLc = 0.259                     ; Fraction of blood flow to liver
 QKc = 0.19                      ; Fraction of blood flow to kidney
 QFc = 0.055                     ; Fraction of blood flow to fat
 QSKc = 0.058                    ; Fraction of blood flow to all skin (Brown)
 QSkeC = QSKc * (VSke/VSk)       ; Fraction blood flow to exposed skin
 QLuc = 0.025                    ; Fraction of blood flow to Lungs
 QRc = 0.70-QLc-QKc-QLuc         ; Fraction of blood flow to richly perfused tissue
 QSc = 0.30-QFc-QSKc             ; Fraction of blood flow to slowly perfused tissue
;-----------------------------------------------------------------------------------------------------------------------------------
; Scale blood flows by total cardiac output
 QL = QLc*QC                     {L/hr}
 QK = QKc*QC                     {L/hr}
 QF = QFc*QC                     {L/hr}
 QR = QRc*QC                     {L/hr}
 QS = QSc*QC                     {L/hr}
 QLu = QLuc*QC                   {L/hr}
 QSke = QSkeC*QC                 {L/hr}
 QSk = (QSkC - QSKeC)*QC         {L/hr}

; Blood flow balance check = 0?
 QBal = QL + QK + QF + QR + QS + QSK + QSke + QLu - QC
;-----------------------------------------------------------------------------------------------
; Partition Coefficients

 PCLiv = 4.25                    ; liver/blood partition coefficient based on Schmitt 2008
 PCLu = 1.23                     ; lung/blood partition coefficient
 PCK = 3.76                      ; kidney/blood partition coefficient
 PCF = 0.68                      ; fat/blood partition coefficient
 PCR = 2.4                       ; richly perfused tissues/blood partition coefficient
 PCS = 0.995                     ; slowly perfused tissues/blood partition coefficient
 PCSk = 0.7*PCLiv + 0.3*PCF      ; correlation from parabens model (See Campbell et al)

; Protein binding
```

```
 fub = 0.96                              ; fraction caffeine unbound in blood (Lave et al. 1997)
 fup = 0.68                              ; fraction unbound in plasma (Lelo et al 1986)
 RBP = fup/fub                           ; blood to plasma concentration ratio (assumes Cup = Cub)
;-----------------------------------------------------------------------------------------
; Linear hepatic in vitro clearance (uL/min/million hepatocytes)

 hep_CLint_invitro = 0                   ; ul/min/million hepatocytes
 hpgl = 99                       ; 10^6 Hepatocytes per gram liver (human value from PLETHEM)

 hep_CLint_invivo = hep_CLint_invitro * (10^-6) * 60 * hpgl * 1000     ; L/h/kg Liver
 CLintC = hep_CLint_invivo                       ; L/hr/kg liver
 CLint = CLintC*VL                               ; L/hr

;=========================================================================================
; Tissue compartment dynamic equations
;=========================================================================================
; Oral uptake from gut
 Ka = 5                                  ; 1st-order oral absorption rate constant (1/hr)
 AGI' = GDOSE'*BW/MWC - Ka*AGI; mmol/hr
 Init AGI = 0                    ; mmol
;-------------------------------------------------------
; Liver

; Linear Metabolism
 AMTot' = CLint * fub * CVL                      ; mmole/hr
 init AMTot = 0                                  ; mmole

 AL' = QL * (CA - CVL)  + Ka * AGI - AMTot'   ; mmole/hr
 init AL= 0                                      ; mmole
 CL = AL / VL                                    ; mmole/L
 CVL = CL / PCLiv                                ; mmole/L
;-------------------------------------------------------
; Skin

; Surface exposed (mmol)
; Rate absorbed (mmol/hr) = Permeability coeff (cm/hr) / Volume applied (cm3) * SA exposed (cm2) *
amount of surface (mmole)
 ASFC' =  applddose' - (Kp / VolApp * SA_exposed)*ASFC    ; mmole/hr
 INIT ASFC = 0.0                                          ; mmole

; Amount in Exposed Skin (mmole)
 ASke' = (Kp * SA_exposed / VolApp)*ASFC + (QSke * (CA - CVSke))          ; mmole/hr
 INIT ASke = 0.0                                                          ; mmole
 CSke = ASke / (VSke+1.0e-23)                                             ; mmole/L
 CVSke = CSke / PCSk                                                      ; mmole/L

; ASK = Amount in unexposed skin (mmole)
 ASK' = QSK*(CA - CVSK)                  ; mmole/hr
 INIT ASK = 0.0                          ; mmole
 CSK = ASK/(VSK-VSke+1.0e-23)            ; mmole/L
 CVSK = CSK/PCSk                         ; mmole/L
;-------------------------------------------------------
; Blood

; arterial blood
 AA' = QC*CV - QC*CA       ; mmole/hr
 Init AA = 0               ; mmole
```

```
 CA = AA/VA                    ; mmole/L
; venous blood
 AV' = ( QL*CVL + QS*CVS + QR*CVR + QF*CVF + QK*CVK + QLu*CVLu + QSK*CVSk + QSke*CVSke) -
QC*CV                          ; mmole/hr
 Init AV = 0                   ; mmole
 CV = AV/VV                    ; mmole/L
;-------------------------------------------------------
; Slowly perfused tissue
 AS' = QS * (CA - CVS)         ; mmole/h
 init AS=0                     ; mmole
 CS = AS / VS                  ; mmole/L
 CVS = CS/PCS                  ; mmole/L
;-------------------------------------------------------
; Richly perfused tissue
 AR' = QR * (CA - CVR)         ; mmole/hr
 init AR=0                     ; mmole
 CR = AR / VR                  ; mmole/L
 CVR = CR/PCR                  ; mmole/L
;-------------------------------------------------------
; Lung
 ALu' = QLu * (CA - CVLu)      ; mmole/hr
 init ALu=0                    ; mmole
 CLu = ALu / VLu               ; mmole/L
 CVLu = CLu / PCLu             ; mmole/L
;-------------------------------------------------------
; Fat
 AF' = QF * (CA - CVF)         ; mmole/hr
 init AF=0                     ; mmole
 CF = AF / VF                  ; mmole/L
 CVF = CF / PCF                ; mmole/L
;-------------------------------------------------------
; Kidney
 AK' = QK * (CA -CVK )         ; mmole/hr
 init AK=0                     ; mmole
 CK = AK / VK                  ; mmole/L
 CVK = CK / PCK                ; mmole/L
;=======================================================
; Dose metrics

 CVmgpl = CV*MWC               ; mg/L
 CVPlas = CV/RBP               ; mmol/L
 CVPlasmgpl = CVPlas*MWC       ; mg/L

 AUCV' = CV                    ; mmole/L
 init AUCV = 0                 ; mmole*hr/L
 AUCmghpl = AUCV*MWC           ; mg*hr/L
; ********************** Calculate last 24-hr AUC **************************
init AUC24 = 0
AUC24' = IF (STOPTIME - time < 24) THEN (CV*MWC) ELSE 0       ; mg*h/L
;=======================================================
; Mass balance

 In = DOSE + applddose         ; mmole
 Stored = AF + AS + AR + AL + AK + AV +AA + ALu +AGI + ASfc + ASk + ASke          ; mmole
 Out = AMTot                   ; mmole
 MASSBAL = In - Stored - Out   ; mmole
```

Part II Checklist for model evaluation

PBK Model Evaluation Checklist	Checklist assessment	Comments
Name of the PBK model (as in the reporting template)		
Model developer and contact details		
Name of person reviewing and contact details		
Date of checklist assessment		
A. Context/Implementation		
A.1. Regulatory Purpose		
1. What is the acceptable degree of confidence/uncertainty (e.g. high, medium or low) for the envisaged application (e.g. priority setting, screening, full assessment?)		
2. Is the degree of confidence/uncertainty in application of the PBK model for the envisaged purpose greater or less than that for other assessment options (e.g. reliance on PBK model and *in vitro* data vs. no experimental data)?		
A.2. Documentation		
3. Is the model documentation adequate, i.e. does it address the essential content of model reporting template, including the following:		
● Clear indication of the chemical, or chemicals, to which the model is applicable?		
● Is the model being applied for the same scientific purpose as it was developed, or has it been repurposed somehow?		
● Model assumptions?		
● Graphical representation of the proposed mode of action, if known?		
● Graphical representation of the conceptual model?		
● Supporting tabulation for parameters (names, meanings, values, mean and standard deviations, units and sources)?		
● Relevance and reliability of model parameters?		
● Uncertainty and sensitivity analysis?		
● Mathematical equations?		
● PBK model code?		
● Software algorithm to run the PBK model code?		
● Qualification of PBK software platform?		
A.3 Software Implementation and Verification		
4. Does the model code express the mathematical model?		
5. Is the model code devoid of syntactic and mathematical errors?		

6. Are the units of input and output parameters correct?		
7. Is the chemical mass balance respected at all times?		
8. Is the cardiac output equal to the sum of blood flow rates to the tissue compartments?		
9. Is the sum total of tissue volumes equal to total body volume?		
10. Is the mathematical solver a well-established algorithm?		
11. Does the mathematical solver converge on a solution without numerical error?		
12. Has the PBK modelling platform been subjected to a verification process (for a different use, for instance, in the pharmaceutical domain)?		
A.4 Peer engagement (input/review)		
13. Has the model been used previously for a regulatory purpose?		
● Is prior peer engagement in the development and review of the model sufficient to support the envisaged application?		
● Is additional review required? Peer engagement includes input/review by experts on specific aspects of model development, individual reviews of the model by experts, or collective reviews by peer review panels. Availability of the comments and tracking of revisions to the model in response to peer input contributes to increased confidence in the model for potential application.		
B. Assessment of Model Validity		
B.1 Biological Basis (Model Structure and Parameters)		
14. Is the model consistent with known biology?		
● Is the biological basis for the model structure provided?		
● Is the complexity of the model structure appropriate to address the regulatory application?		
● Are assumptions concerning the model structure and parameters clearly stated and justified?		
● Is the choice of values for physiological parameters justified?		
● Is the choice of methods used to estimate chemical-specific ADME parameters justified?		
● Saturable Kinetics		
B.2 Theoretical Basis of Model Equations		
15. Are the underlying equations based on established theories, .e.g. Michaelis-Menten kinetics, Fick's laws of diffusion?		
● In the case of PBK models for particles, does the model take into consideration the properties of particles, e.g.		

particle size ranges, (poor) solubility, aggregation, partitioning and diffusion/sedimentation behaviour?		
B.3. Reliability of input parameters		
16. Has the uncertainty (individual variability, experimental reproducibility and reliability) in the input parameters been characterised?		
B.4. Uncertainty and Sensitivity Analysis		
17. Has the impact of uncertainty (individual variability, experimental reproducibility and reliability) in the parameters on the chosen dose metric been estimated?		
● Local sensitivity analysis?		
● Global sensitivity analysis?		
18. Is confidence in influential input parameter estimates (i.e., based on comparison of uncertainty and sensitivity) reasonable (within expected values; similar to those of analogues) in view of the intended application?		
B.5. Goodness-of-Fit and Predictivity		
19. For PBK models for which there are sufficient *in vivo* data for the chemical of interest:		
● Suitability as analogue (chemical and biological similarity) been assessed?		
● Reliable estimation of chosen dose metric for analogue?		
● In general is the biological Variability of *in vivo* reference data (from analogue) established?		

NA= Not applicable; NR = Not Reported

Part III Final Evaluation

Overall Conclusion on mode evaluation for the intended application