

Supplementary material to *Derivation of a Human In Vivo Benchmark Dose for Bisphenol A from ToxCast In Vitro Concentration Response Data Using a Computational Workflow for Probabilistic Quantitative In Vitro to In Vivo Extrapolation*

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PBPK MODEL

A human PBPK model was developed to study the fate of BPA following single oral doses. The initial model was based upon the PBPK model for the plasticizer DPHP described in McNally et al. (2012) with adaptations made as necessary. The final model described entry of BPA through ingestion with absorption of BPA from the gut and gastro-intestinal (GI) tract and a simple model of the lymphatic system describing uptake of BPA via the lacteals in the intestine and entering venous blood after bypassing the liver. The dose that entered the lymphatic system was coded as a fraction of the administered dose; a fraction of administered dose was coded as entering the liver via the portal vein; a fraction of the administered dose of BPA passed through the intestine without being absorbed and was coded as absorbed dose minus the lymphatic and hepatic dose components. The model described the metabolism of BPA to BPAG and BPAS in both liver and gut. Sub-models were included to describe the kinetics of BPAG and BPAS, with the models for BPA and the two metabolites connected via gut and liver. Binding of BPA, BPAG and BPAS was coded from arterial blood, with the consequence that only the unbound fraction in blood was available for distribution to organs and tissues, metabolism, and elimination.

The model structures for BPA and the two metabolites differed only in the coding of uptake required for BPA (including the simplified description of a lymphatic compartment). BPA and metabolite models had a stomach and GI tract draining into the liver. Adipose, blood, kidney, and slowly and rapidly perfused compartments were included. Elimination of BPA, BPAG and BPAS was coded through the kidney compartment, with first order elimination rates, proportional to kidney tissue concentration, coded in each case. Both BPA and metabolite models included the transport process of enterohepatic recirculation. Uptake of BPA and metabolites from the liver into bile was modelled as a first order uptake process with a delay of four hours (to represent transport in bile) before BPA (and metabolites) appeared in the gut and were available for reabsorption. First order elimination rates for each substance were coded to account for fractions of recirculated BPA and metabolites that were eliminated in faeces rather than reabsorbed from the small intestine. Because of coding enterohepatic recirculation, the PBPK model was solved as a system of delay differential equations (DDEs)

The final structure of the model described above followed the iterative model development process (incorporating uncertainty and sensitivity analysis) documented in McNally et al. (2021).

Default values for masses and flows, metabolism parameters, partition coefficients were based on literature sources and algorithms (Brown et al. 1997; ICRP 2002; Mazur et al. 2010; Schmitt 2008). Default values for uptake and elimination rates parameters were based on corresponding terms in McNally et al. (2021) and subsequently refined through tuning to available data. The default model assumed 80% of the administered BPA was absorbed through the hepatic route, with a further 5% absorbed through the lymphatic route with the complementary 15% passing through unabsorbed.

STATISTICAL ANALYSIS

Parameter Distributions

Probability distributions for tissue volumes, expressed as a fraction of body weight, and tissue blood flows, expressed as a fraction of cardiac output were estimated using a virtual population generated using PopGen (McNally et al. 2014). Specifically, a US population of 10,000 individuals comprised of 2500 Caucasian males; 2500 Caucasian females, 2500 African American males and 2500 African American females, aged 25-45 and with BMI ranging from 19-35 was generated (which captured the characteristics of the human volunteer study population). Probability distributions for tissue volumes and fractional blood flows were based upon statistical analysis of this model output. For other parameters, such as partition coefficients and rate parameters, uniform distributions were ascribed based upon author's judgement to represent conservative yet credible bounds and refined through the model development process. The probability distributions used in the reported uncertainty and sensitivity analyses and parameter calibrations are given in Table A1. The tabulated values are therefore based upon expert judgement and represent conservative yet credible bounding estimates.

Uncertainty Analysis

Uncertainty analysis was conducted throughout the model development process in order to identify coding errors, assess for deficiencies in the current version of the model, and to assess the bounding behaviour of the model as a function of its uncertain parameters; a formalised process for studying model behaviour and thus reducing the likelihood of coding errors is desirable for any PBPK model developed using a bespoke (open source) code (Loizou et al. 2021). An efficient approach using Latin Hypercube sampling was used throughout the model development process. For each new version of the PBPK model a 500-point maxi-min Latin Hypercube design was generated in order to efficiently cover the parameter space defined by the distributions ascribed to model parameter, the PBPK model was run for each of these design points, and various outputs from PBPK models were studied to assess the quantitative behaviour of the current version of the model, and the parameter limits in use at the time. The final uncertainty analysis reported on in this work was based on a 500-point maxi-min Latin Hypercube design and the parameter limits given in Table 2. This analysis used uniform distributions for all parameters, defined by upper and lower limits given in Table A1, with a total of 74 parameters studied. The outputs reported on in this work at the plasma concentrations of BPA, BPAG and BPAS (nM).

Sensitivity Analysis

Sensitivity analysis was conducted throughout the model development process in order to study the key model output sensitivities for each version of the model under development. A two-phased global sensitivity analysis comprising of elementary effects screening followed by a variance-based approach is generally desirable (Loizou et al. 2015; McNally et al. 2011), however during the model development and calibration phase of research, elementary effects screening alone was used to allow a more rapid analysis. In the final analysis reported on in this document the sensitivity analysis focussed on plasma concentrations of BPA, BPAG and BPAS (nM) with results at three time points: 0.5, 2- and 5-hours following ingestion of BPA.

The Morris Test computes two measures of sensitivity: μ^* is the average effect of a parameter on model output; σ is a measure of a non-linear effect of a parameter on the response under study and/or interactions between parameters. For each output under study and at each time point under study the maxima of each of the μ^* and σ over all parameters was determined, and all parameters where μ^* and

σ were within 5% of the maxima were recorded. Any parameter that BPA, BPAG or BPAS were sensitive to, at any time point and for either measure of sensitivity was considered as a variable for taking forward into calibration – parameters passing this initial filter were subsequently reviewed with decisions for inclusion/exclusion of parameters in the calibration model based on expert judgement. Parameters that did not show sensitivity based upon the within 5% criterion described above were fixed at central values during calibration. This analysis used uniform distributions for all parameters, defined by upper and lower limits given in Table A1, with 74 parameters studied. Five elementary effects were computed for each parameter, leading to a design of 375 model runs.

Calibration

Calibration of a subset of sensitive model parameters using the HBM data of Thayer et al. (2015) was attempted. A Bayesian approach (McNally et al., 2012) was followed. This requires the specification of a joint prior distribution for the parameters under study, which is refined through a comparison of PBPK model predictions and measurements within a statistical model. The resulting (refined) parameter space that is consistent with the prior specification and measurements is the posterior distribution.

In the first instance individual model calibrations were performed using the blood data from each of the 14 volunteers in turn. The final calibration model used in this work used data from all 14 individuals from the HBM study of Thayer et al. (2015) with data on three specific outputs, concentrations of BPA, BPAG and BPAS (nM) in plasma, formally compared within the calibration model.

The final form of the statistical calibration model is given in equations A1-A3, where $C_{Plasma\ BPA_{ij}}$, $C_{Plasma\ BPAG_{ij}}$ and $C_{Plasma\ BPAS_{ij}}$ denote measurement i (at time t_i) for individual j (for j in 1:14) for the three respective model outputs, and $\mu_{BPA}(\theta, \omega_j)_{ij}$, $\mu_{BPAG}(\theta, \omega_j)_{ij}$ and $\mu_{BPAS}(\theta, \omega_j)_{ij}$ denote the predictions from the PBPK model for these outputs corresponding to parameters (θ, ω_j) . The vectors θ and ω_j denote the global parameters common to all individuals (suitable for partition coefficients etc.), and participant specific parameters (suitable for accounting for variability in the physiology and modelling the participant specific uptake of BPA etc.). Prediction error for BPA, BPAG and BPAS was modelled through standard deviations σ_{BPA} , σ_{BPAG} and σ_{BPAS} respectively. Normal distributions, truncated at zero were assumed for all three relationships.

$$C_{Plasma\ BPA_{ij}} \sim N(\mu_{BPA}(\theta, \omega_j)_{ij}, \sigma_{BPA})[0, \infty] \quad (A1)$$

$$C_{Plasma\ BPAG_{ij}} \sim N(\mu_{BPAG}(\theta, \omega_j)_{ij}, \sigma_{BPAG})[0, \infty] \quad (A2)$$

$$C_{Plasma\ BPAS_{ij}} \sim N(\mu_{BPAS}(\theta, \omega_j)_{ij}, \sigma_{BPAS})[0, \infty] \quad (A3)$$

The prior distributions for model parameters are given in Table A1. Prior distributions for standard deviations were weakly informative half-normal distributions with standard deviations of 10, 250 and 25 for σ_{BPA} , σ_{BPAG} and σ_{BPAS} respectively.

Inference for the model parameters was made using Markov chain Monte Carlo (MCMC). Preliminary calibrations were undertaken using a single component Metropolis-Hastings algorithm. Inference in the final calibration model was made using Markov chain Monte Carlo (MCMC) with a sampling run of 100,000 iterations and every 50th retained for forward inference.

Software

The PBPK model was written using GNU MCSim and compiled. In uncertainty and sensitivity analysis and for runs to produce fits following calibrations the executable was run using scripts called from RStudio. MCMC runs were conducted using GNU MCSim called from a command line environment. The DiceDesign package of R was used for generating Latin Hypercube designs. GSA of model outputs (through elementary effects screening and eFAST) were conducted using the Sensitivity package of R. The reshape2 package of R was used for reshaping of data for plotting and other processing of results.

RESULTS

Assessment of model form

As described in the statistical analysis section an iterative process of model development was conducted with uncertainty and sensitivity analysis informing improvements to model structure. The final model was tested using a 500-point maxi-min Latin Hypercube design and the parameter limits given in Table S1. The simulations corresponding to these design points are shown for concentrations of BPA, BPAS and BPAG in blood (nM) over the period 0-48 hours. The broad range of the runs covered the HBM data reported in Thayer et al. (2015) with the model form considered suitable for the purposes of this work.

Sensitivity analysis

Results from elementary effects screening are reported for BPA, BPAS and BPAG in Tables S2 to S4 for three time points, 0.5, 2 and 5 hours following ingestion of BPA. The μ and σ measures at each time point are normalised with all results at the time point divided by the maximum value of μ or σ at that time point; values are therefore between 0 and 1, with 1 denoting the most important for a given measure at a particular time point. The parameters where any of the measures were within 5% of the maximum (i.e. any value above 0.05) are highlighted in bold and considered for inclusion in the calibration model. This analysis was conducted for BPA, BPAS and BPAG.

In total 29 global and a further 11 local (individual specific parameters) were taken forward to calibration.

Calibration

Results from calibration and our interpretation are provided in the main body of the paper, with the fit of the model demonstrated for a subset of 3 of the 14 volunteers (volunteers 1, 3 and 5). A similar comparison of model predictions (central estimate and a pointwise 95% credible interval) and measurements is made for the remaining 11 volunteers in Figures S2 to S12, thus demonstrating an excellent fit to all of the volunteers following calibration.

Table S1 Parameter distributions (and upper and lower bounds) used in Latin Hypercube sampling, Elementary effects screening sensitivity analysis and as prior distributions in calibration of sensitive parameters.

Parameter	Unit	Mean	SD	Lower bound	Upper bound	Distribution
BW	kg	4.36	0.313	3.747	4.973	Lognormal
VliC	L kg ⁻¹ BW	0.0307	0.00758	0.02	0.05	Normal
VstC	L kg ⁻¹ BW	0.0210	0.00069	0.021	0.0235	Normal
VguC	L kg ⁻¹ BW	0.0150	0.00234	0.008	0.0220	Normal
VkiC	L kg ⁻¹ BW	0.0038	0.00148	0.0012	0.005	Normal
VlymphC	L kg ⁻¹ BW	0.0036	0.0007	0.0022	0.0050	Normal
VfaC	L kg ⁻¹ BW	0.27	0.0600	0.1500	0.39	Normal
VspdC	L kg ⁻¹ BW	0.6050	0.1000	0.4500	0.7500	Normal
VrpdC	L kg ⁻¹ BW	0.0302	0.005	0.010	0.0454	Normal
VBldC	L kg ⁻¹ BW	0.060	0.01	0.04	0.09	Normal
QCC	L h ⁻¹ Kg ⁻¹ BW ^{0.75}	11	1	9	13	Normal
QhepartC	Unit less	0.0690	0.0060	0.03	0.12	Normal
QstC	Unit less	0.0110	0.0009	0.005	0.015	Normal
QguC	Unit less	0.1490	0.0130	0.09	0.25	Normal
QkiC	Unit less	0.20	0.05	0.10	0.30	Normal
QfaC	Unit less	0.0500	0.0050	0.0300	0.0699	Normal
QspdC	Unit less	0.2870	0.0221	0.2100	0.3600	Normal
QrpdC	Unit less	0.2100	0.0168	0.1600	0.2700	Normal
FracDOSEHep	Unit less	-	-	0.7	0.92	Uniform
FracDOSELymph	Unit less	-	-	0.02	0.08	Uniform
FB_BPA	Unit less	-	-	0	0.99	Uniform
FB_BPAG	Unit less	-	-	0.7	0.99	Uniform
FB_BPAS	Unit less	-	-	0.7	0.99	Uniform
MPY	mg/g	34	10	14	54	Normal
MPYgu	mg/g	3.9	0.8	2.3	5.5	Normal
BELLYPERM	h ⁻¹	-	-	0.1	10	Uniform
GIPERM	h ⁻¹	-	-	0.5	25	Uniform
BELLYPERMLymph	h ⁻¹	-	-	0.84	2.5	Uniform
GIPERMLymph	h ⁻¹	-	-	0.55	1.6	Uniform
KEMAX	h ⁻¹	-	-	0.1	25	Uniform
KEMIN	h ⁻¹	-	-	0.0025	0.0075	Uniform
K1_BPA_GUT	h ⁻¹	-	-	0.01	20	Uniform
K1_BPAG_GUT	h ⁻¹	-	-	0.01	20	Uniform
K1_BPAS_GUT	h ⁻¹	-	-	0.01	20	Uniform
K1_BPA_LIVER	h ⁻¹	-	-	0.55	1.6	Uniform
K1_BPAG_LIVER	h ⁻¹	-	-	0.005	0.015	Uniform
K1_BPAS_LIVER	h ⁻¹	-	-	0.005	0.015	Uniform
K1_BPA_Urine	h ⁻¹	-	-	0.0005	0.0015	Uniform
K1_BPAG_Urine	h ⁻¹	-	-	0.0005	0.0015	Uniform
K1_BPAS_Urine	h ⁻¹	-	-	0.0005	0.0015	Uniform
K1_BPA_REMOV	h ⁻¹	-	-	0.01	100	Uniform
ED_PLASMA	h ⁻¹	-	-	10	150	Uniform
K1_BPAG_REMO	h ⁻¹	-	-	0.01	100	Uniform
VED_PLASMA	h ⁻¹	-	-	0.25	0.75	Uniform
K1_Lymph	h ⁻¹	-	-	0.25	1.25	Uniform
Lymphlag	h ⁻¹	-	-	0.25	1.25	Uniform

Vmax_liv_BPA_in_vitro	pmol/min/mg	4255	900	1000	8000	Normal
KM_liv_BPA_in_vitro	mg/L	1.118	0.20	0.1	2.5	Normal
Vmax_liv_BPAS_in_vitro	pmol/min/mg	80	30	13	133	Normal
KM_liv_BPAS_in_vitro	mg/L	3.114	0.6	1.5	6.5	Normal
Vmax_gut_BPAG_in_vitro	pmol/min/mg	487	100	244	974	Normal
KM_gut_BPAG_in_vitro	mg/L	18.29	4	9	37	Normal
Vmax_gut_BPAS_in_vitro	pmol/min/mg	73	30	13	133	Normal
KM_gut_BPAS_in_vitro	mg/L	3.114	0.6	1.5	6.5	Normal
Pbab	Unit less	-	-	0.36	1.1	Uniform
Plib	Unit less	-	-	0.36	1.1	Uniform
Pkib	Unit less	-	-	1.35	15	Uniform
Pfab	Unit less	-	-	1.35	15	Uniform
Pgub	Unit less	-	-	1.35	15	Uniform
Pstb	Unit less	-	-	1.35	15	Uniform
Prpdb	Unit less	-	-	1.4	4.2	Uniform
Pspdb	Unit less	-	-	1.4	4.2	Uniform
PbaG	Unit less	-	-	0.7	2.1	Uniform
PliG	Unit less	-	-	1.0	23	Uniform
PkiG	Unit less	-	-	1.0	23	Uniform
PfaG	Unit less	-	-	1.2	3.60	Uniform
PguG	Unit less	-	-	1.0	23	Uniform
PstG	Unit less	-	-	1.70	5.3	Uniform
PrpdG	Unit less	-	-	2.1	6.4	Uniform
PspdG	Unit less	-	-	1	3	Uniform
PbaS	Unit less	-	-	0.7	2.1	Uniform
PliS	Unit less	-	-	1.0	23	Uniform
PkiS	Unit less	-	-	1.0	23	Uniform
PfaS	Unit less	-	-	1.3	3.9	Uniform
PguS	Unit less	-	-	1.0	23	Uniform
PstS	Unit less	-	-	1.9	5.7	Uniform
PrpdS	Unit less	-	-	2.3	6.8	Uniform
PspdS	Unit less	-	-	1	3.1	Uniform

Table S2 Results (normalised mu and sigma) from elementary effects screening of BPA at three time points. Parameters satisfying the criteria for further consideration are highlighted in bold.

	BPA					
	mu 0.5	sigma 0.5	mu 2.0	sigma 2.0	mu 5.0	sigma 5.0
QCC	0.0553	0.0713	0.0314	0.0545	0.0353	0.0637
VfaC	0.1303	0.1427	0.0457	0.0564	0.0102	0.0142
VguC	0.0552	0.0414	0.0108	0.0186	0.0162	0.0287
VstC	0.0015	0.0026	0.0002	0.0002	0.0004	0.0005
VspdC	0.0473	0.0814	0.0277	0.0525	0.0167	0.0325
VrpdC	0.0096	0.0126	0.0026	0.0042	0.0064	0.007
VkiC	0.0067	0.0057	0.0304	0.0348	0.0931	0.1154
VliC	0.0314	0.0501	0.0042	0.0049	0.0067	0.0088
VlymphC	0.0002	0.0001	0	0	0	0
VBldC	0.2111	0.4171	0.0885	0.1684	0.0334	0.0649
QhepartC	0.0202	0.0311	0.0158	0.0242	0.0738	0.1209
QguC	0.0364	0.069	0.0078	0.0092	0.0417	0.0715
QstC	0.1056	0.2033	0.0044	0.0079	0.0085	0.0103
QspdC	0.0276	0.0246	0.0039	0.0029	0.0509	0.0907
QrpdC	0.09	0.169	0.0016	0.0006	0.0121	0.0198
QkiC	0.0069	0.0093	0.0281	0.0482	0.0478	0.0859
QfaC	0.0186	0.0165	0.0035	0.0045	0.0049	0.0081
FracDOSELymph	0.282	0.3197	0.3931	0.4466	0.538	0.5834
FracDOSEHep	0.0425	0.059	0.0092	0.0103	0.0122	0.0166
FB_BPA	1	1	1	1	1	1
FB_BPAG	0	0	0	0	0	0
FB_BPAS	0	0	0	0	0	0
BELLYPERM	0.3124	0.608	0.0229	0.0416	0.0948	0.185
GIPERM	0.2675	0.3855	0.008	0.0126	0.006	0.0071
KEMAX	0.1813	0.2626	0.0074	0.0113	0.0173	0.0276
KEMIN	0.0003	0.0005	0	0	0.0001	0.0002
MPY	0.1722	0.203	0.012	0.0159	0.0126	0.0168
MPYgu	0.0037	0.0065	0.0003	0.0003	0.0003	0.0003
K1_BPA_REMOVED_PLASMA	0.0146	0.0152	0.0408	0.0528	0.128	0.148
K1_BPAG_REMOVED_PLASMA	0	0	0	0	0	0
K1_BPAS_REMOVED_PLASMA	0	0	0	0	0	0
K1_BPA_GUT	0.0927	0.1095	0.0068	0.0075	0.0071	0.0076
K1_BPA_LIVER	0.0007	0.0014	0	0.0001	0.0001	0.0001
K1_BPAG_GUT	0	0	0	0	0	0
K1_BPAG_LIVER	0	0	0	0	0	0
K1_BPAS_GUT	0	0	0	0	0	0
K1_BPAS_LIVER	0	0	0	0	0	0
Pbab	0	0	0	0	0	0
Pfab	0.0006	0.0008	0.0056	0.0076	0.0517	0.0775
Pgub	0.0212	0.0211	0.0005	0.0007	0.0009	0.0013
Pstb	0.0487	0.0637	0.0079	0.0142	0.001	0.0008
Prpdb	0.0075	0.0048	0.0064	0.0106	0.0032	0.0042
Pspdb	0.0065	0.0068	0.0372	0.0351	0.1002	0.1401
Plib	0	0.0001	0	0	0	0
PbaG	0	0	0	0	0	0
PspdG	0	0	0	0	0	0
Plig	0	0	0	0	0	0
PrpdG	0	0	0	0	0	0
PfaG	0	0	0	0	0	0
PstG	0	0	0	0	0	0
PguG	0	0	0	0	0	0
PbaS	0	0	0	0	0	0
PspdS	0	0	0	0	0	0
Plis	0	0	0	0	0	0
PrpdS	0	0	0	0	0	0

PfaS	0	0	0	0	0	0
PstS	0	0	0	0	0	0
PguS	0	0	0	0	0	0
Pkib	0.0093	0.0096	0.0195	0.0223	0.0504	0.0576
PkiG	0	0	0	0	0	0
PkiS	0	0	0	0	0	0
Vmax_liv_BPA_in_vitro	0.5204	0.8879	0.1174	0.1563	0.2077	0.295
KM_liv_BPA_in_vitro	0.7788	0.6316	0.0854	0.0416	0.1178	0.0912
Vmax_gut_BPAG_in_vitro	0.0024	0.0022	0.0005	0.0005	0.0006	0.0006
KM_gut_BPAG_in_vitro	0.0054	0.0092	0.0008	0.0009	0.0007	0.0008
Vmax_liv_BPAS_in_vitro	0.0001	0.0001	0	0	0	0
KM_liv_BPAS_in_vitro	0	0	0	0	0	0
Vmax_gut_BPAS_in_vitro	0.0044	0.0076	0.0006	0.0011	0.0008	0.0013
KM_gut_BPAS_in_vitro	0.0008	0.0009	0.0001	0.0001	0.0001	0.0001
K1_BPA_Urine	0	0	0	0	0	0
K1_BPAG_Urine	0	0	0	0	0	0
K1_BPAS_Urine	0	0	0	0	0	0
Lymphlag	0.3377	0.2802	0.0161	0.0219	0.0316	0.0351
K1Lymph	0.2652	0.3801	0.1045	0.1857	0.0725	0.123

Table S3 Results (normalised mu and sigma) from elementary effects screening of BPAS at three time points. Parameters satisfying the criteria for further consideration are highlighted in bold.

	BPAS					
	mu 0.5	sigma 0.5	mu 2.0	sigma 2.0	mu 5.0	sigma 5.0
QCC	0.0513	0.0396	0.0372	0.0203	0.0411	0.0374
VfaC	0.0524	0.0198	0.0241	0.0226	0.0177	0.025
VguC	0.0252	0.0058	0.0146	0.0104	0.0055	0.0021
VstC	0.0009	0.0006	0.0004	0.0003	0.0002	0.0003
VspdC	0.1257	0.0558	0.0484	0.0375	0.0164	0.0202
VrpdC	0.0186	0.0116	0.0092	0.0064	0.0054	0.0069
VkiC	0.0043	0.0019	0.0144	0.0084	0.0473	0.057
VliC	0.0959	0.054	0.0394	0.0186	0.018	0.0168
VlymphC	0.0026	0.0017	0.0011	0.0006	0.0005	0.0004
VBldC	0.188	0.1233	0.1003	0.0897	0.0553	0.0794
QhepartC	0.0339	0.0188	0.016	0.0039	0.0087	0.0033
QguC	0.2096	0.1371	0.1087	0.088	0.1535	0.1598
QstC	0.0327	0.0305	0.003	0.0022	0.0028	0.0027
QspdC	0.0544	0.0218	0.0266	0.0151	0.0459	0.0404
QrpdC	0.0251	0.0133	0.0224	0.0137	0.0241	0.0266
QkiC	0.0661	0.0339	0.095	0.0776	0.1185	0.1361
QfaC	0.0114	0.005	0.004	0.002	0.0051	0.0041
FracDOSELymph	0	0	0.0015	0.0009	0.0032	0.0025
FracDOSEHep	0.0525	0.0184	0.0354	0.0147	0.0249	0.0179
FB_BPA	0.0002	0.0001	0.0026	0.0014	0.0009	0.0008
FB_BPAG	0	0	0	0	0	0
FB_BPAS	0.5243	0.2533	0.878	0.4307	1	1
BELLYPERM	0.0738	0.0454	0.1008	0.0887	0.0881	0.1281
GIPERM	0.8571	0.7807	0.5226	0.5389	0.203	0.3009
KEMAX	0.1951	0.0985	0.1508	0.1274	0.1346	0.1797
KEMIN	0.0001	0.0001	0	0	0	0
MPY	0.6095	0.5458	0.5632	0.5235	0.3948	0.5086
MPYgu	0.0073	0.0078	0.01	0.0088	0.0065	0.0067
K1_BPA_REMOVED_PLASMA	0	0	0.0004	0.0002	0.001	0.0007
K1_BPAG_REMOVED_PLASMA	0	0	0	0	0	0
K1_BPAS_REMOVED_PLASMA	0.0257	0.0236	0.0999	0.0732	0.162	0.1545
K1_BPA_GUT	0.2598	0.064	0.2382	0.1283	0.2026	0.1943
K1_BPA_LIVER	0.0002	0.0002	0.0001	0.0001	0	0

K1_BPAG_GUT	0	0	0	0	0	0
K1_BPAG_LIVER	0	0	0	0	0	0
K1_BPAS_GUT	0.004	0.0031	0.2118	0.0916	0.3252	0.2744
K1_BPAS_LIVER	0.0004	0.0002	0.0013	0.0004	0.0014	0.0013
Pbab	0	0	0	0	0	0
Pfab	0	0	0	0	0.0001	0
Pgub	0.1272	0.0466	0.2469	0.1654	0.2831	0.3199
Pstb	0.0083	0.0042	0.0037	0.0023	0.0006	0.0005
Prpdb	0	0	0.0001	0.0001	0	0
Pspdb	0	0	0.0002	0.0001	0.0004	0.0004
Plib	0	0	0	0	0	0
PbaG	0	0	0	0	0	0
PspdG	0	0	0	0	0	0
PliG	0	0	0	0	0	0
PrpdG	0	0	0	0	0	0
PfaG	0	0	0	0	0	0
PstG	0	0	0	0	0	0
PguG	0	0	0	0	0	0
PbaS	0	0	0	0	0	0
PspdS	0.0108	0.0075	0.0369	0.0168	0.0486	0.0683
PliS	0.3208	0.1836	0.0775	0.0576	0.024	0.0257
PrpdS	0.0057	0.0014	0.0026	0.0016	0.0007	0.0008
PfaS	0.0003	0.0001	0.0056	0.0021	0.0123	0.0106
PstS	0	0	0.0006	0.0001	0.0016	0.0014
PguS	0.4869	0.1888	0.2018	0.1327	0.1595	0.2231
Pkib	0	0	0.0002	0.0001	0.0003	0.0004
PkiG	0	0	0	0	0	0
PkiS	0.0305	0.0136	0.1012	0.0518	0.1572	0.1272
Vmax_liv_BPA_in_vitro	0.0463	0.0276	0.0182	0.006	0.0193	0.0094
KM_liv_BPA_in_vitro	1	1	1	1	0.5443	0.7893
Vmax_gut_BPAG_in_vitro	0.0041	0.0021	0.005	0.0016	0.0051	0.0039
KM_gut_BPAG_in_vitro	0.0047	0.002	0.006	0.0026	0.0062	0.0045
Vmax_liv_BPAS_in_vitro	0.0212	0.0067	0.0152	0.008	0.0158	0.013
KM_liv_BPAS_in_vitro	0.0036	0.0019	0.007	0.0028	0.0091	0.0052
Vmax_gut_BPAS_in_vitro	0.3133	0.1431	0.1265	0.0426	0.1053	0.0666
KM_gut_BPAS_in_vitro	0.1844	0.1108	0.4211	0.2849	0.5298	0.5886
K1_BPA_Urine	0	0	0	0	0	0
K1_BPAG_Urine	0	0	0	0	0	0
K1_BPAS_Urine	0	0	0	0	0	0
Lymphlag	0.0562	0.0572	0.0524	0.0462	0.0112	0.0107
K1Lymph	0.0001	0	0.0064	0.0037	0.0031	0.002

Table S4 Results (normalised mu and sigma values) from elementary effects screening of BPAG at three time points. Parameters satisfying the criteria for further consideration are highlighted in bold.

	BPAG					
	mu 0.5	sigma 0.5	mu 2.0	sigma 2.0	mu 5.0	sigma 5.0
QCC	0.0328	0.0395	0.0467	0.1349	0.0848	0.1359
VfaC	0.1433	0.1694	0.1199	0.0757	0.0744	0.098
VguC	0.0428	0.0252	0.0916	0.1012	0.1152	0.1069
VstC	0.0012	0.0013	0.0034	0.0017	0.0022	0.0036
VspdC	0.1578	0.125	0.0769	0.2857	0.0874	0.1029
VrpdC	0.0232	0.0305	0.0206	0.0584	0.013	0.0163
VkiC	0.0246	0.0386	0.1913	0.5072	0.2376	0.3121
VliC	0.079	0.1099	0.1362	0.3996	0.0528	0.1123
VlymphC	0.0031	0.0032	0.0032	0.0059	0.0016	0.0026
VBldC	0.0794	0.1035	0.0215	0.0726	0.0324	0.0409
QhepartC	0.0165	0.0197	0.0219	0.0495	0.0256	0.0448

QguC	0.1508	0.1242	0.0606	0.1793	0.1321	0.3144
QstC	0.0531	0.0568	0.0596	0.1378	0.0879	0.1688
QspdC	0.1078	0.0604	0.1269	0.3083	0.0901	0.0636
QrpdC	0.0336	0.0242	0.0312	0.0677	0.0337	0.0483
QkiC	0.0863	0.089	0.2057	0.4693	0.3035	0.5814
QfaC	0.0301	0.042	0.0645	0.1858	0.0341	0.0517
FracDOSELymph	0	0.0001	0.0251	0.0569	0.0399	0.0453
FracDOSEHep	0.0411	0.0134	0.2038	0.2595	0.2422	0.2436
FB_BPA	0.0019	0.0029	0.0187	0.0583	0.0053	0.013
FB_BPAG	0.5948	0.6454	1	0.9287	1	0.6251
FB_BPAS	0	0	0	0	0	0
BELLYPERM	0.1984	0.1959	0.1894	0.7073	0.2035	0.3176
GIPERM	1	1	0.2685	0.5515	0.2031	0.3273
KEMAX	0.2945	0.2712	0.2491	0.9648	0.2355	0.3533
KEMIN	0.0003	0.0005	0	0.0001	0.0001	0.0003
MPY	0.0241	0.0407	0.0179	0.0371	0.0184	0.0259
MPYgu	0.001	0.0016	0.0102	0.0393	0.0133	0.0346
K1_BPA_REMOVED_PLASMA	0.0001	0.0002	0.0034	0.0081	0.0065	0.0078
K1_BPAG_REMOVED_PLASMA	0.0216	0.0244	0.1907	0.2892	0.4365	0.557
K1_BPAS_REMOVED_PLASMA	0	0	0	0	0	0
K1_BPA_GUT	0.2879	0.1815	0.3078	0.8421	0.2872	0.6714
K1_BPA_LIVER	0.0002	0.0003	0.0008	0.0028	0.0005	0.001
K1_BPAG_GUT	0.0019	0.0025	0.3342	1	0.5865	1
K1_BPAG_LIVER	0.0009	0.0009	0.0027	0.0042	0.002	0.0036
K1_BPAS_GUT	0	0	0	0	0	0
K1_BPAS_LIVER	0	0	0	0	0	0
Pbab	0	0	0	0	0	0
Pfab	0	0	0.0002	0.0005	0.0023	0.0033
Pgub	0.1747	0.2164	0.2024	0.4539	0.1599	0.3026
Pstb	0.0321	0.0305	0.1258	0.2758	0.0272	0.0396
Prpdb	0	0	0.0007	0.0016	0.0002	0.0006
Pspdb	0	0	0.0045	0.0087	0.0094	0.0112
Plib	0.0002	0.0003	0.0003	0.0012	0.0002	0.0006
PbaG	0	0	0	0	0	0
PspdG	0.0215	0.027	0.1418	0.2366	0.0594	0.11
PlIG	0.5366	0.5089	0.1545	0.4385	0.1032	0.1742
PrpdG	0.0422	0.0475	0.0225	0.0722	0.023	0.0459
PfaG	0.0014	0.0014	0.0282	0.0411	0.0485	0.0569
PstG	0.0001	0.0001	0.0107	0.0181	0.0111	0.0164
PguG	0.0046	0.0039	0.0751	0.14	0.1471	0.2405
PbaS	0	0	0	0	0	0
PspdS	0	0	0	0	0	0
PlIS	0	0	0	0	0	0
PrpdS	0	0	0	0	0	0
PfaS	0	0	0	0	0	0
PstS	0	0	0	0	0	0
PguS	0	0	0	0	0	0
Pkib	0.0001	0.0002	0.0044	0.0209	0.0061	0.0166
PkiG	0.0248	0.0249	0.1144	0.1984	0.2379	0.2733
PkiS	0	0	0	0	0	0
Vmax_liv_BPA_in_vitro	0.0114	0.013	0.0226	0.0355	0.0186	0.0197
KM_liv_BPA_in_vitro	0.035	0.0452	0.0517	0.138	0.0383	0.0938
Vmax_gut_BPAG_in_vitro	0.0038	0.0076	0.0136	0.06	0.0188	0.0553
KM_gut_BPAG_in_vitro	0.0018	0.0028	0.0152	0.0671	0.0213	0.0616
Vmax_liv_BPAS_in_vitro	0.0009	0.0007	0.0013	0.0027	0.0014	0.0019
KM_liv_BPAS_in_vitro	0.0003	0.0003	0.0024	0.007	0.0028	0.005
Vmax_gut_BPAS_in_vitro	0.0349	0.0603	0.0393	0.073	0.0385	0.0438
KM_gut_BPAS_in_vitro	0.0058	0.0043	0.0239	0.069	0.0262	0.0588
K1_BPA_Urine	0	0	0	0	0	0
K1_BPAG_Urine	0	0	0	0	0	0

K1_BPAS_Urine	0	0	0	0	0	0
Lymphlag	0.0019	0.004	0.0058	0.0187	0.0021	0.002
K1Lymph	0.0002	0.0004	0.019	0.0469	0.0061	0.0154

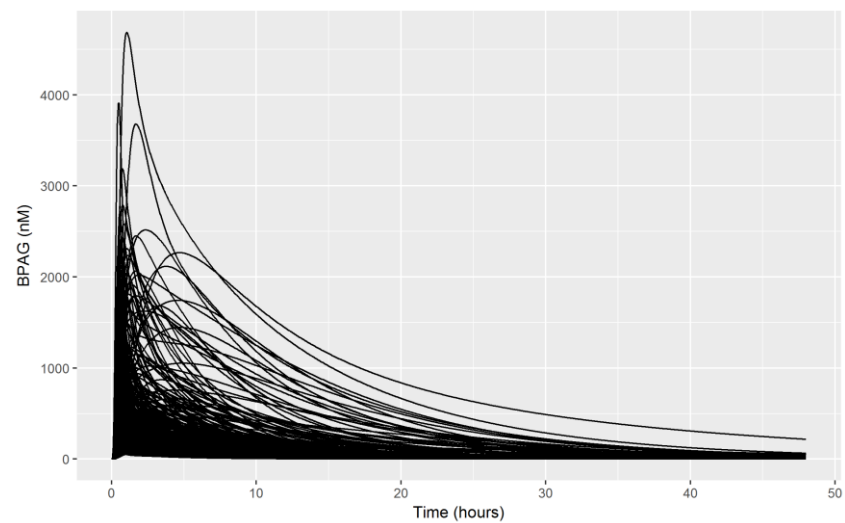
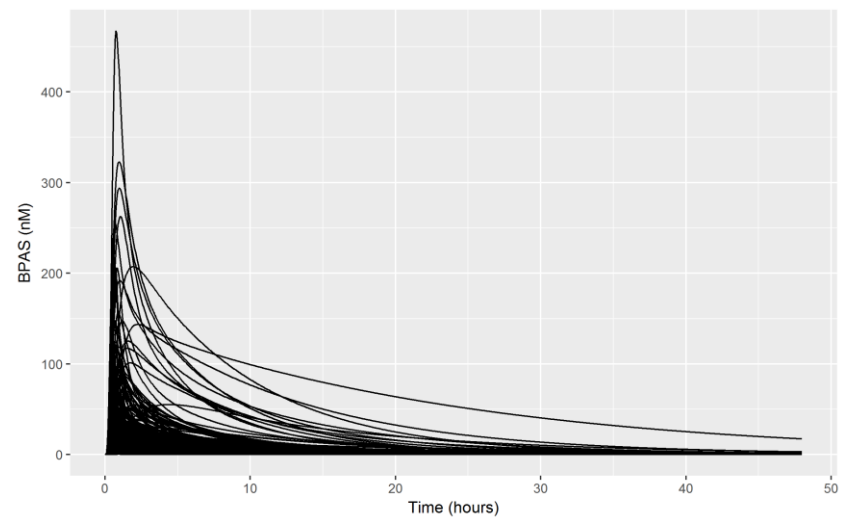
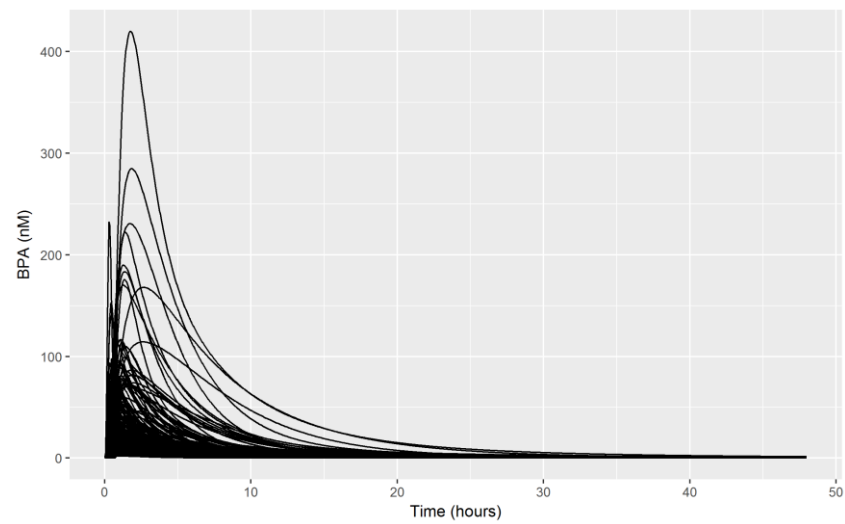


Figure S1 Uncertainty analysis results for BPA, BPAG and BPAS

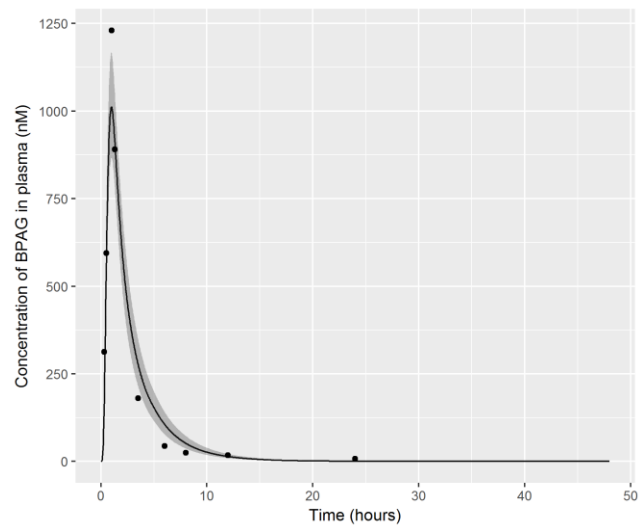
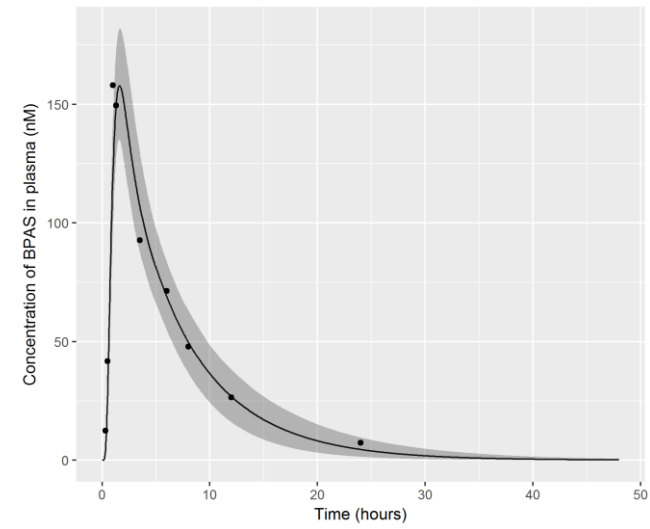
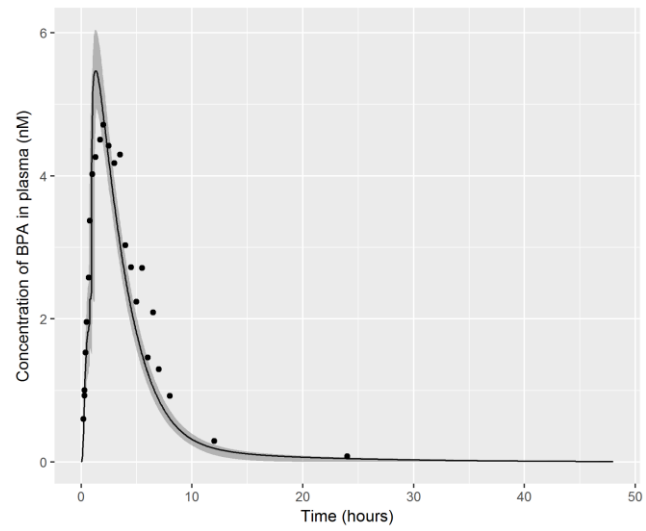


Figure S2 Individual 2

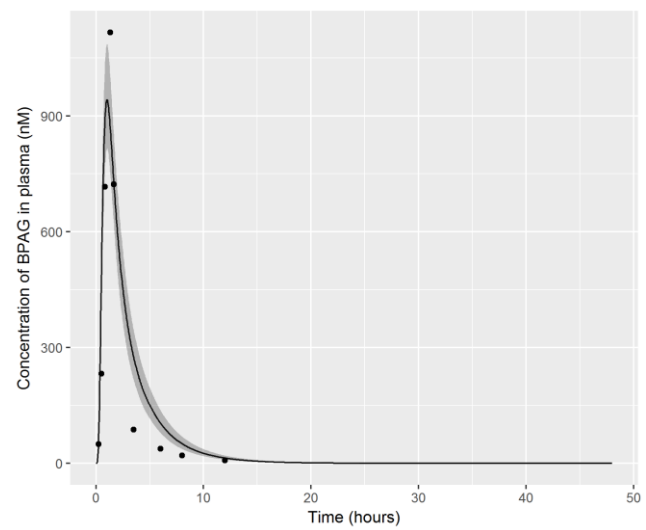
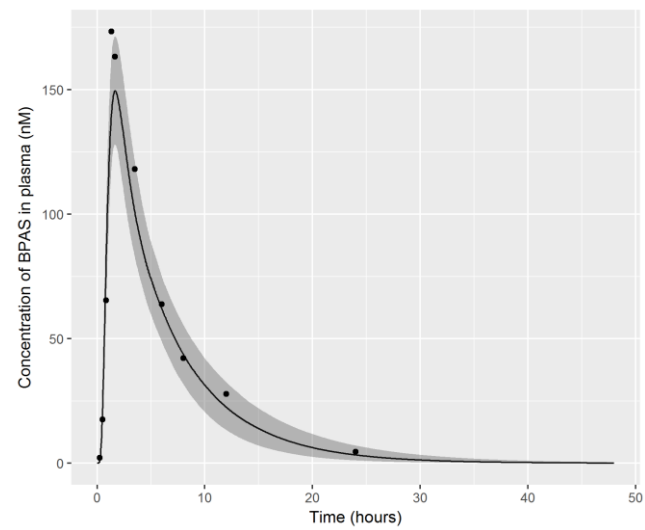
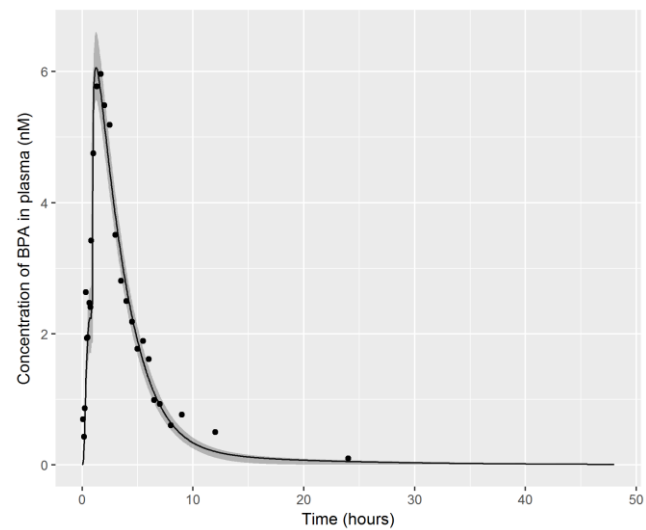


Figure S3 Individual 4

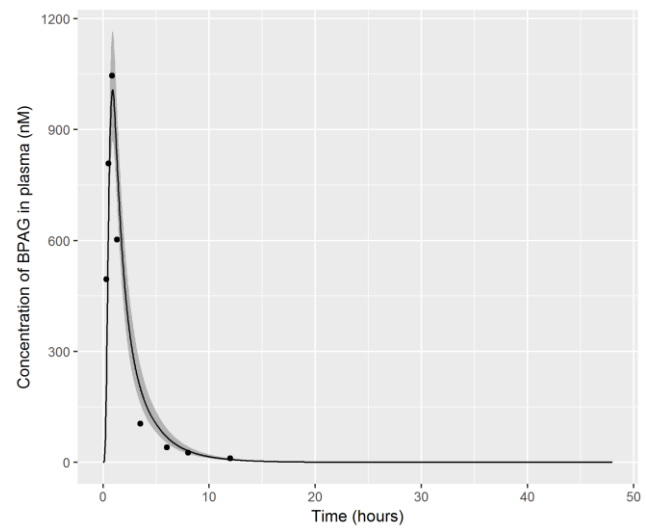
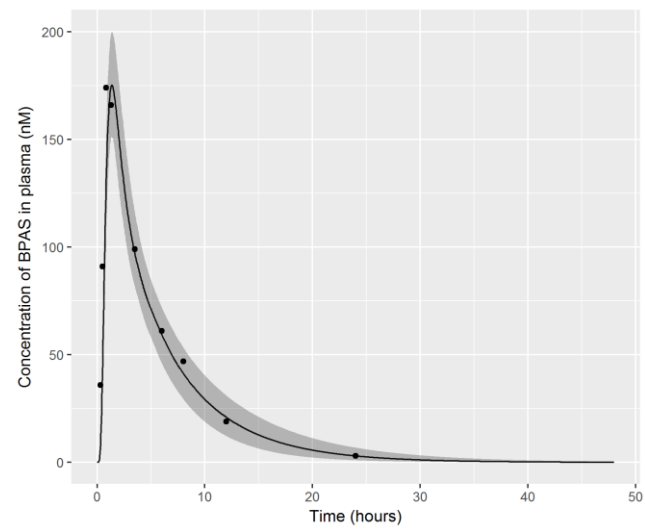
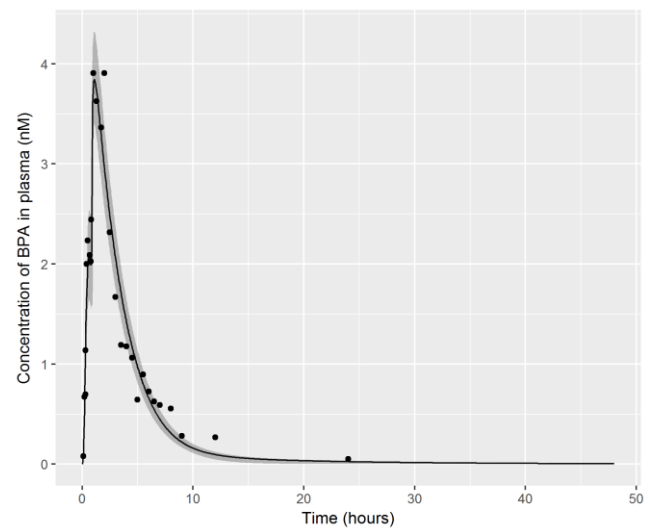


Figure S4 Individual 6

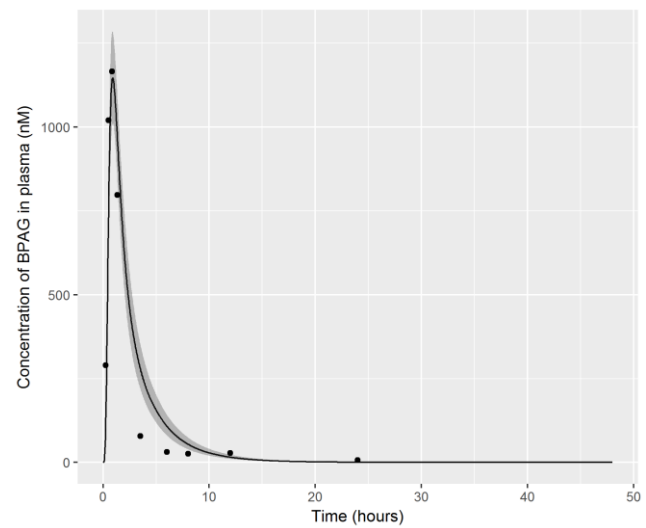
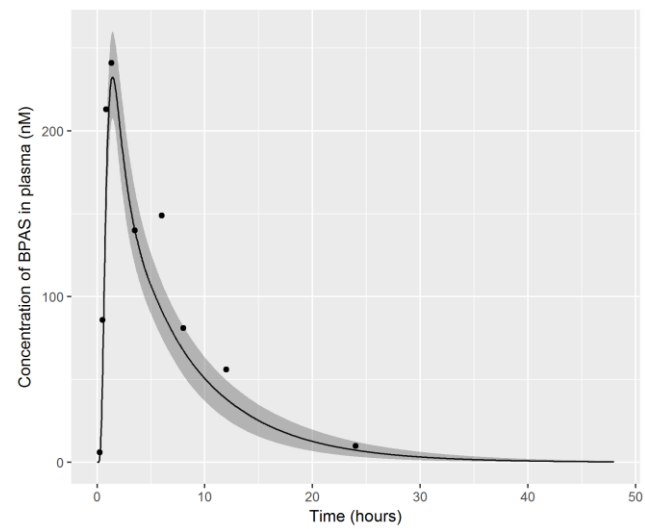
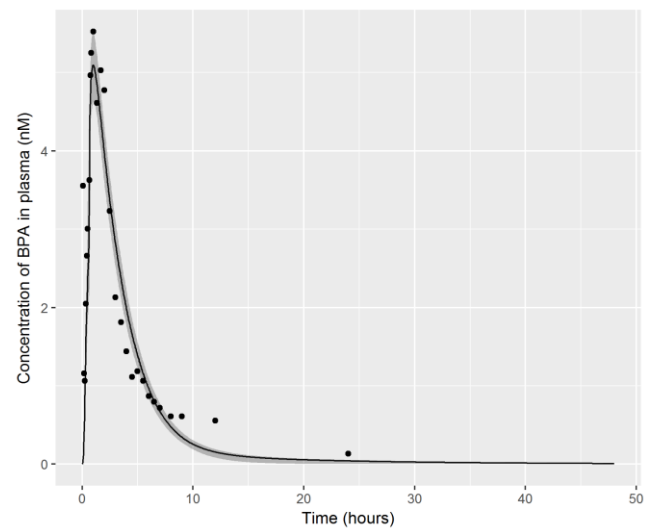


Figure S5 Individual 7

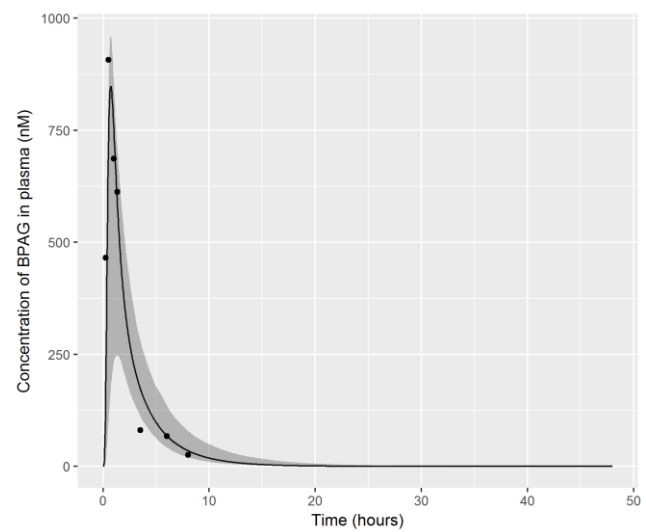
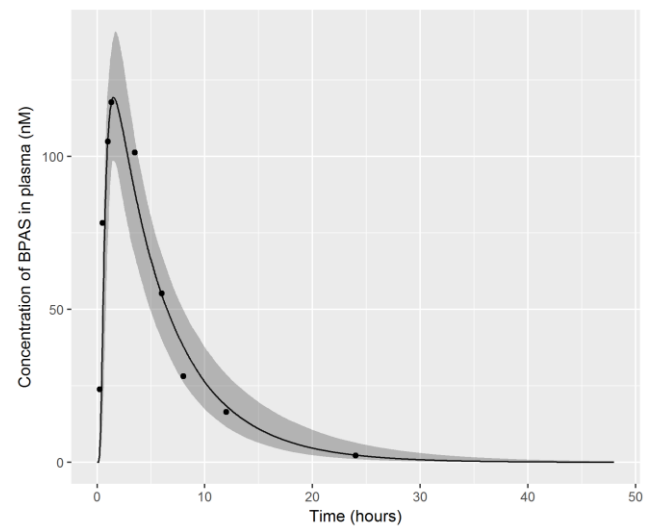
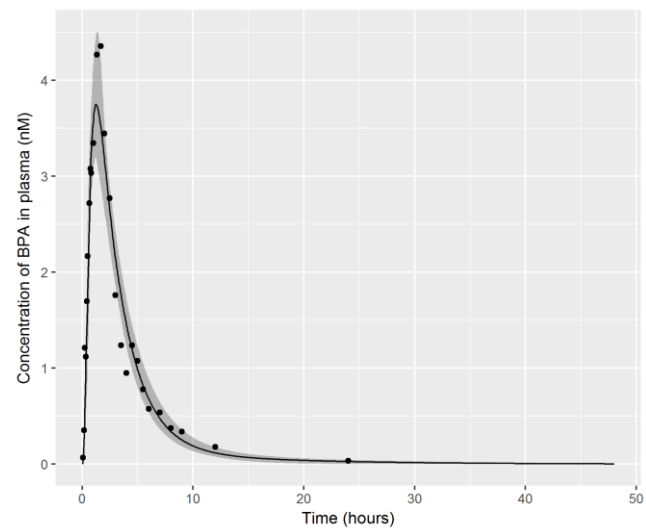


Figure S6 Individual 8

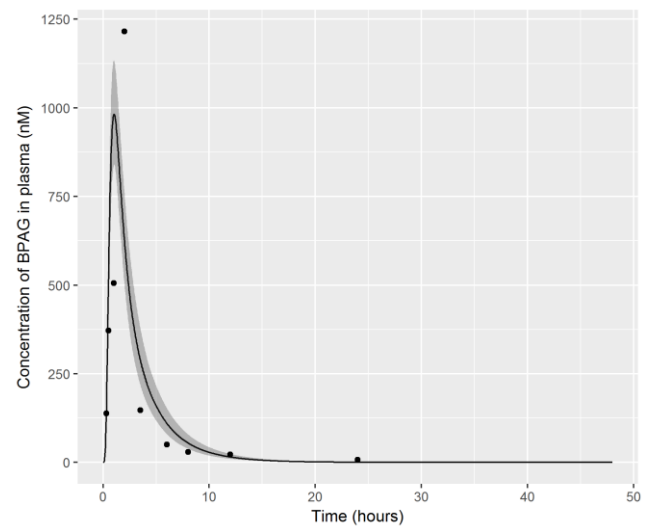
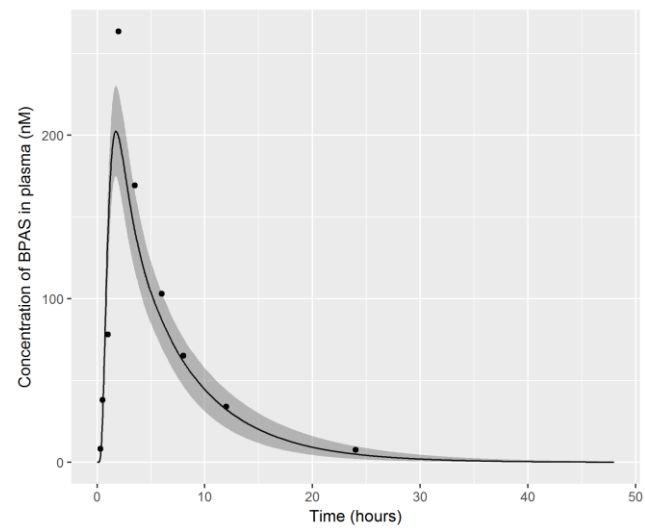
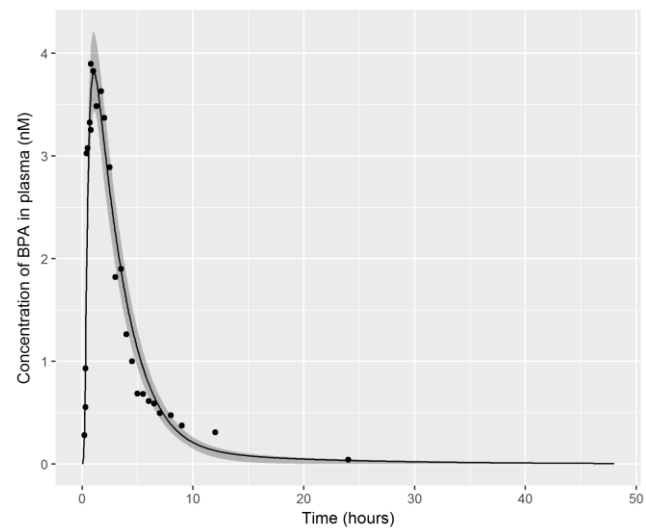


Figure S7 Individual 9

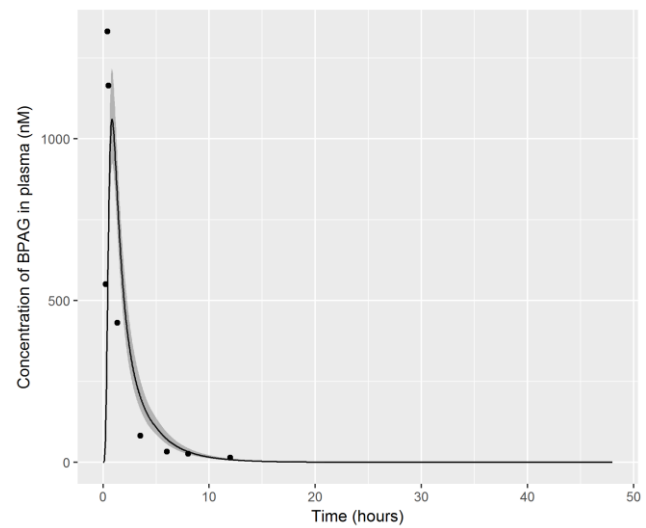
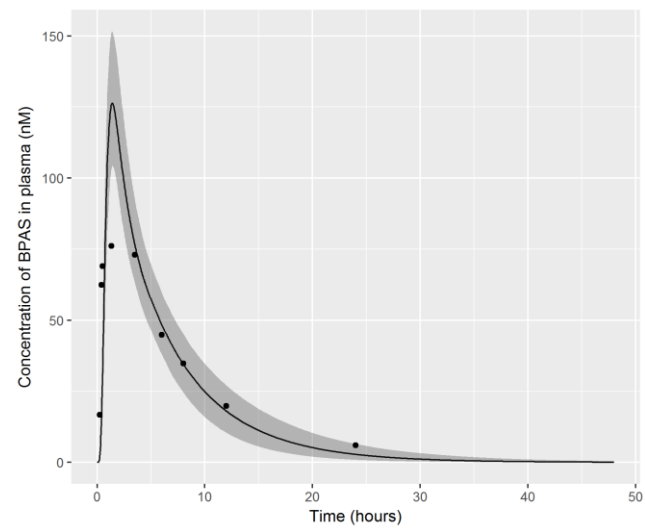
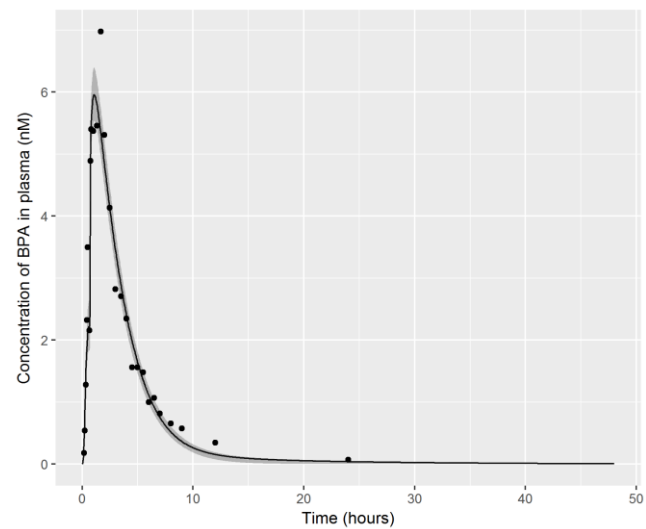


Figure S8 Individual 10

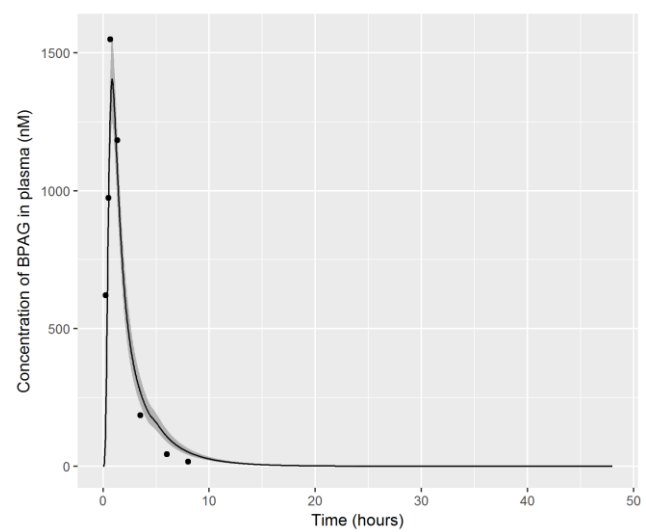
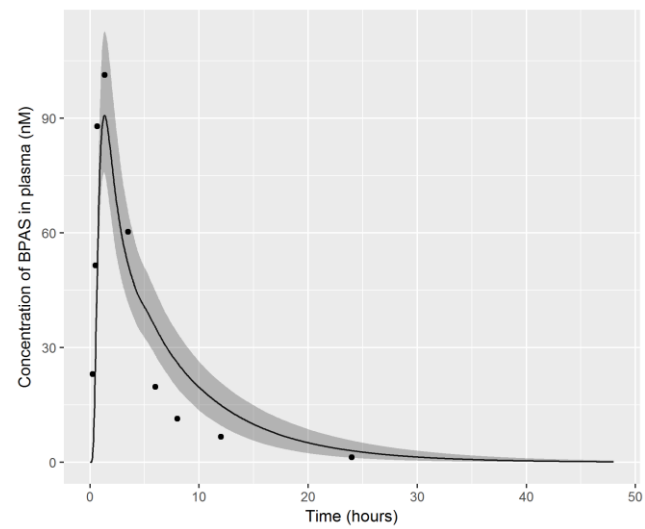
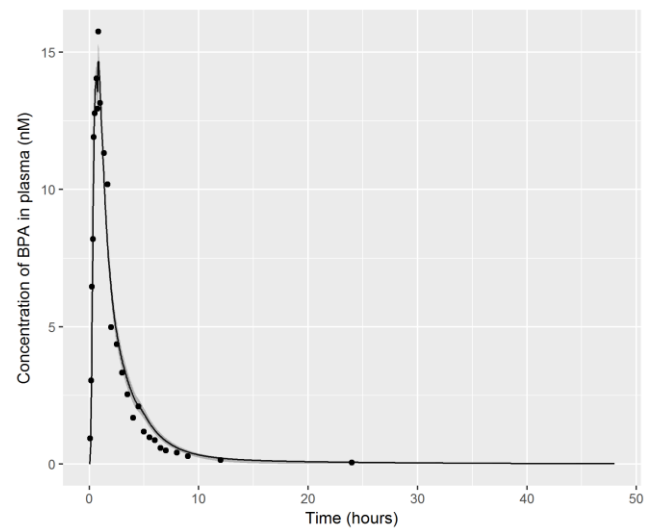


Figure S9 Individual 11

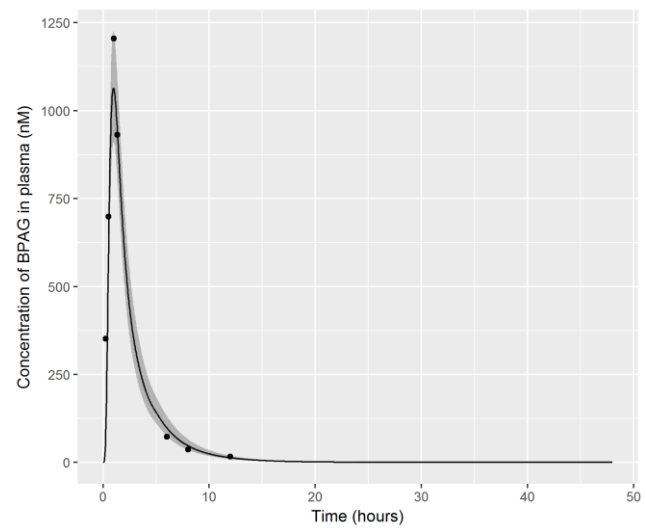
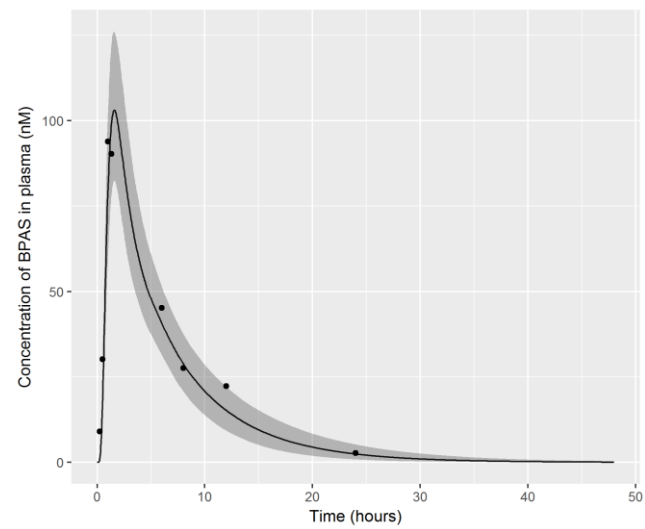
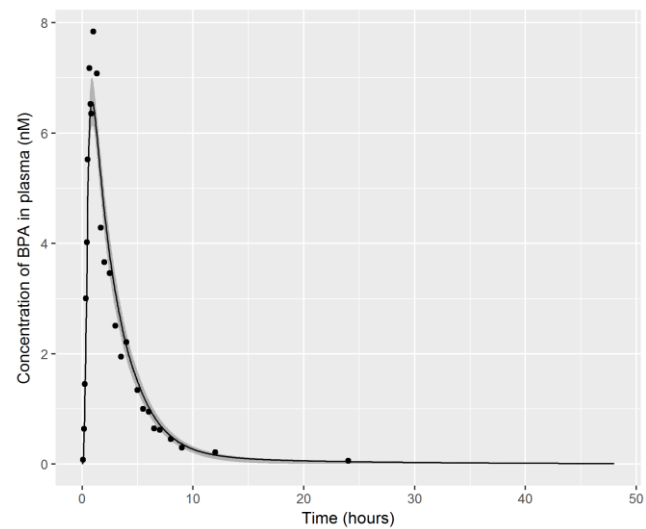


Figure S10 Individual 12

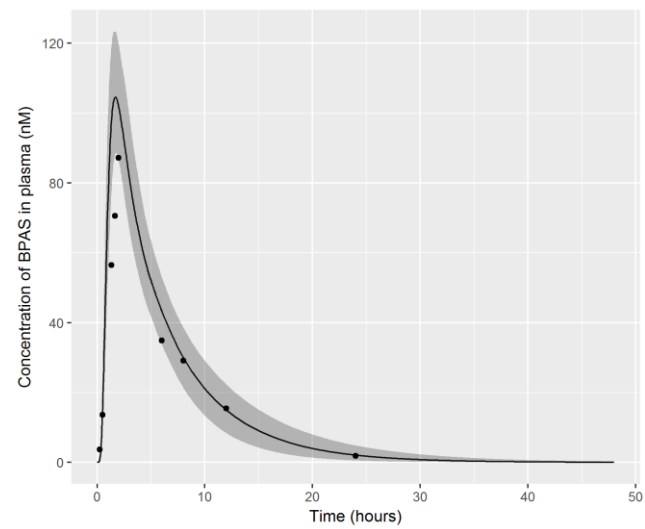
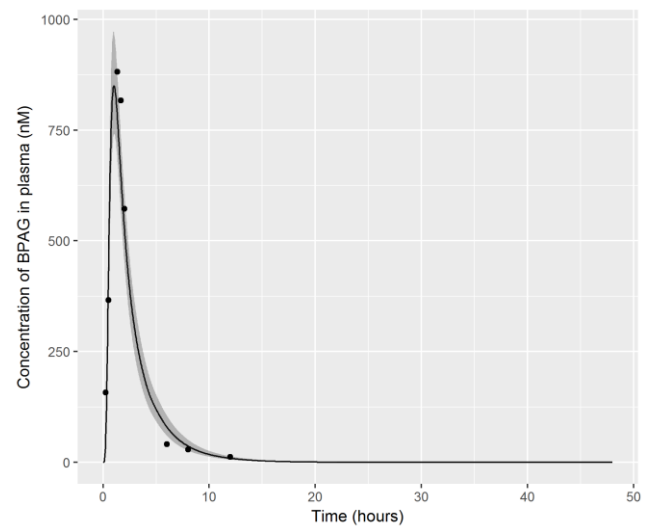
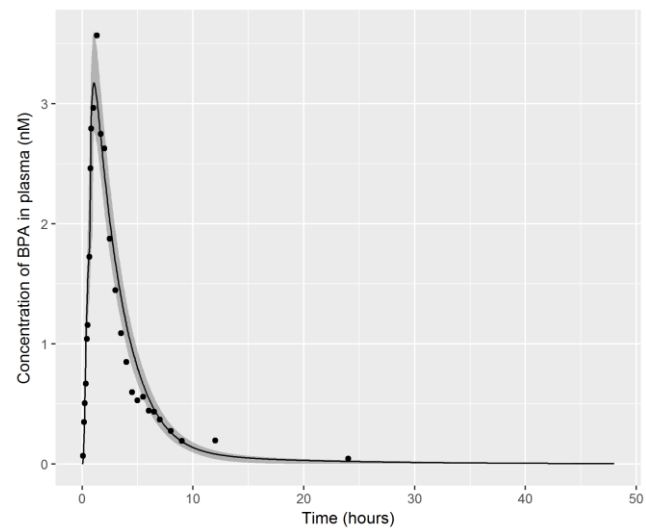


Figure S11 Individual 13

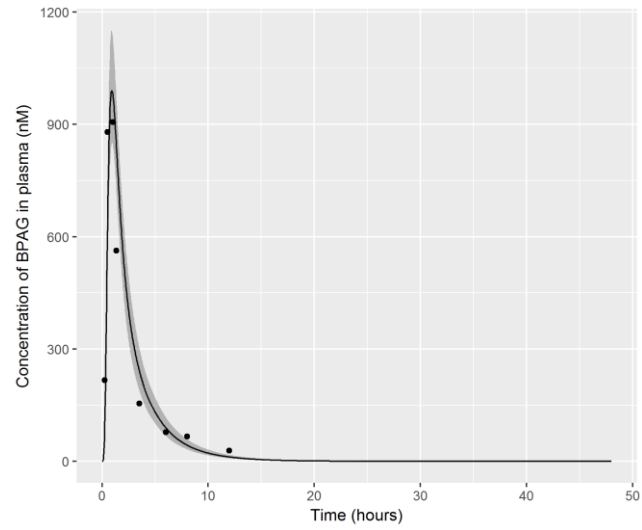
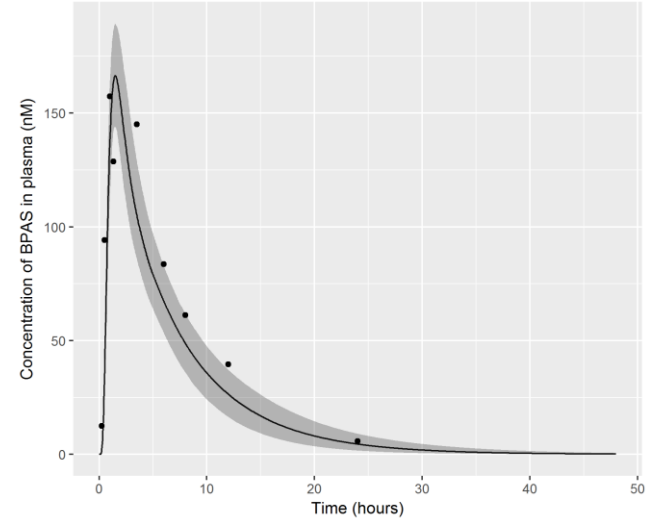
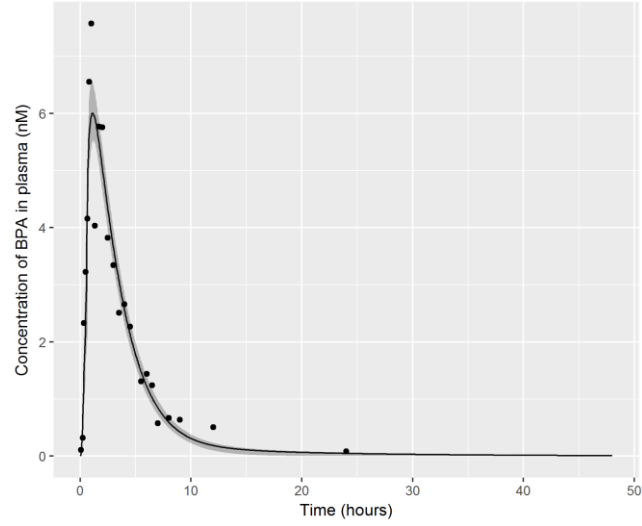


Figure S12 Individual 14

```

Model Code
# MCSim 5.x script

# *** BPA ***

# Di(2-propyl heptyl) phthalate
# George Loizou (HSL) 2020-01-16T00:00:00.0000000Z

# Compiled on: 2020-01-16T15:34:16.8794082Z

States =
{
  Afa,
  Agu1,
  Agu2,
  AMguBPAG,
  AMguBPAS,
  Ast,
  Arpd,
  Aspd,
  Ali,
  AMliBPAG,
  AMliBPAS,
  ABile,
  ALymph,
  Aplasm BPA,
  Aplasm BPAG,
  Aplasm BPAS,
  ABellyH,
  AGiTractH,
  ABowel,
  ABellylymph,
  AGiTractlymph,
  AfaG,
  AstG,
  AguG,
  AliG,
  ABileG,
  ABowelG,
  AspdG,
  ArpdG,
  AfaS,
  AstS,
  AguS,
  AliS,
  ABileS,
  ABowelS,
  AspdS,
  ArpdS,
  AMBPA REMOVED PLASMA,
  AMPAG REMOVED PLASMA,
  AMPAS REMOVED PLASMA,
  AMBPA Urine,
  AMPAG Urine,
  AMPAS Urine,

```

Gutswitch,
Lymphswitch,
DOSESTEP
 };

Outputs =

{
Cli,
Cfa,
Cgu1,
Cgu2,
Cst,
Clymph,
Cspd,
Crpd,
mass,
Uptake,
reloral,
CVfa,
CVgu1,
CVgu2,
CVst,
CVspd,
CVli,
CVrpd,
CV,
CVnmol,
CA_BPA,
CAT_BPA,
CV_total_nmol,
CVG,
CVGnmol,
CliG,
CVliG,
CguG,
CVguG,
CstG,
CVstG,
CfaG,
CVfaG,
CspdG,
CVspdG,
CrpdG,
CVrpdG,
CVS,
CVSnmol,
CliS,
CVliS,
CguS,
CVguS,
CstS,
CVstS,

CfaS,
CVfaS,
CspdS,
CVspdS,
CrpdS,
CVrpdS,
CA BPAG,
CAT BPAG,
CAT BPAS,
CA BPAS,
CV total BPAG nmol,
CV total BPAS nmol,
relBPAG,
massBPAG,
relBPAS,
massBPAS,
Curine BPA,
Curine BPAG,
Curine BPAS,
ORALDOSE,
ODOSE,
ODOSEliver,
ODOSElymph,
ODOSEbowel,
Vmax liv BPA,
Vmax gut BPAG,
Vmax gut BPAS,
Vmax liv BPAS,
Ali lag,
AliG lag,
Plasma BPA,
Plasma BPAG,
Plasma BPAS,
Urine BPA,
Urine BPAG,
Urine BPAS,
Aplasmub BPA,
Aplasmub BPAG,
Aplasmub BPAS

};

Inputs =

{
events Gutswitch,
events Lymphswitch,
events DOSESTEP,
events AMBPA Urine,
events AMBPAG Urine,
events AMBPAS Urine,

};

Parameters

=====
#

BW = 89; # body mass (kg)
MWBPA = 228.291; # BPA molecular mass (g/mol)
MWBPAG = 404.41; # BPAG molecular mass (g/mol)
MWBPAS = 308.4; # OH-BPAS molecular mass (g/mol)
CAE = 0.75; # cardiac allometric exponent
QCC = 11.22; # cardiac allometric constant (L/h/kg^{CAE})

VT = 0.95; # proportion of vascularised tissue
VfaC = 0.195; # fractional volume
VguC = 0.067; # fractional volume
VstC = 0.0158; # fractional volume
VspdC = 0.4714; # fractional volume poorly perfused
VrpdC = 0.033; # fractional volume richly perfused
VliC = 0.0203; # fractional volume
VlymphC = 0.0036; # lymph system fractional volume
VBldC = 0.05; # blood fractional volume

QhepartC = 0.06; # hepatic artery fractional blood flow
QguC = 0.17; # fractional blood flow
QstC = 0.01; # fractional blood flow
QspdC = 0.27; # overall fractional blood flow to slowly perfused tissue
QrpdC = 0.42; # overall fractional blood flow to rapidly perfused tissue
QfaC = 0.05; # fractional blood flow

FracDOSELymph = 0.05; # Fraction of dose taken into lymph
FracDOSEHep = 0.8; # Fraction of dose taken into hepatic

FB BPA = 0.1; # Fraction of BPA bound to plasma proteins 0.9998752
FB BPAG = 0.1; # Fraction of BPAG bound to plasma proteins 0.9854
FB BPAS = 0.1; # Fraction of BPAG bound to plasma proteins 0.9854
FracMetabG = 0.8; # Fraction of metabolism BPA -> BPAG
FracMetabS = 0.1; # Fraction of metabolism BPA -> BPAS

PORALDOSE = 0.1; # oral dose [mg/kg]
DRINKTIME = 0.25; # Drink time [h]
BELLYPERM = 0.685; # [h]
GIPERM1 = 5.1; # [h]
GIPERM2 = 5.1; # [h]
BELLYPERMlymph = 0.685; # [h]
GIPERMlymph = 5.1; # [h]
KEMAX = 10.2; # [Maximum emptying rate /h]
KEMIN = 0.005; # [Minimum emptying rate /h]
Lymphswitch = 1;
Gutswitch = 1;

MPY = 34; # microsomal protein yield [mg microsomal protein/g liver]
MPYgu = 3.9; # microsomal protein yield [mg microsomal protein/g gut]

Vmax liv BPAS in vitro = 73; # BPA -> BPAS hepatic female Vmax (pmol/min/mg prot) Kurebayashi et al (2010)
KM liv BPAS in vitro = 3.114; # BPA -> BPAG hepatic female KM (mg/L) (10.1 micromoles * MWBPA) Kurebayashi et al (2010)

Vmax gut BPAS in vitro = 73; # BPA -> BPAS hepatic female Vmax (pmol/min/mg prot) Kurebayashi et al (2010) scaled with gut MPYgu
KM gut BPAS in vitro = 3.114; # BPA -> BPAG hepatic female KM (mg/L) (10.1 micromoles * MWBPA) Kurebayashi et al (2010)

#BPAS invit gut Clint = 1.0; # BPA -> BPAS GUT in vitro clearance (ul/min/mg prot)

Vmax liv BPA in vitro = 4255; # BPA -> BPAG hepatic female Vmax (pmol/min/mg prot) Mazur et al (2010)
KM liv BPA in vitro = 1.118; # BPA -> BPAG hepatic female KM (mg/L) (4.9 micromoles * MWBPA) Mazur et al (2010)
Vmax gut BPAG in vitro = 487; # BPA -> BPAG Gut mixed Vmax (pmol/min/mg prot) Mazur et al (2010)
KM gut BPAG in vitro = 18.29; # BPA -> BPAG Gut mixed KM (mg/L) (80.1 micromoles * MWBPA) Mazur et al (2010)

K1 BPA REMOVED PLASMA = 0.5; # BPA First-order elimination rate from blood [/h]
K1 BPAG REMOVED PLASMA = 1; # BPAG First-order elimination rate from blood [/h]
K1 BPAS REMOVED PLASMA = 1; # BPAS First-order elimination rate from blood [/h]

K1 BPA Urine = 0.1; # BPA First-order elimination rate from bladder[/h]
K1 BPAG Urine = 0.1; # BPAG First-order elimination rate from bladder [/h]
K1 BPAS Urine = 0.1; # BPAS First-order elimination rate from bladder [/h]

K1 BPA GUT = 0.1; # First-order elimination rate of BPA from gut into bowel [/h]
K1 BPA LIVER = 10; # First-order elimination rate of BPA from liver into bile [/h]

K1 BPAG GUT = 0.1; # First-order elimination rate of BPAG from gut into bowel [/h]
K1 BPAG LIVER = 1; # First-order elimination rate of BPAG from liver into bile [/h]

K1 BPAS GUT = 0.1; # First-order elimination rate of BPAG from gut into bowel [/h]
K1 BPAS LIVER = 1; # First-order elimination rate of BPAG from liver into bile [/h]

K1Lymph = 0.2; # First-order elimination rate from Lymph into blood [/h]
Lymphlag = 0.01; # Lag between uptake into Lymph and emptying into blood [h]
Gutlag = 0.01; # Lag between uptake into GItract and emptying into gut [h]

Pbab = 0.73; # BPA blood:air partition coefficient
Pfab = 5.0; # BPA tissue:blood partition coefficient
Pgub = 2.8; # BPA tissue:blood partition coefficient
Pstb = 2.8; # BPA tissue:blood partition coefficient
Prpdb = 2.8; # BPA tissue:blood partition coefficient
Pspdb = 2.7; # BPA tissue:blood partition coefficient
Plib = 0.73; # BPA tissue:blood partition coefficient

PbaG = 1.39; # BPAG Red blood cells:plasma partition coefficient

PspdG = 2.00; # BPAG Slowly perfused tissue:air partition coefficient
PliG = 11.5; # BPAG tissue:air partition coefficient
PrpdG = 4.29; # BPAG Richly tissue:air partition coefficient
PfaG = 2.38; # BPAG Fat tissue:air partition coefficient
PstG = 3.52; # BPAG Stomach tissue:air partition coefficient
PguG = 3.52; # BPAG GI Tract tissue:blood partition coefficient

PbaS = 1.52; # BPAS Red blood cells:plasma partition coefficient
PspdS = 2.08; # BPAS Slowly perfused tissue:air partition coefficient
PliS = 12.20; # BPAS tissue:air partition coefficient
PrpdS = 4.56; # BPAS Richly tissue:air partition coefficient
PfaS = 2.63; # BPAS Fat tissue:air partition coefficient
PstS = 3.80; # BPAS Stomach tissue:air partition coefficient
PguS = 3.80; # BPAS GI Tract tissue:blood partition coefficient

tau = 4; # the required delay

Vfa = 0;
Vgu = 0;
Vst = 0;
Vspd = 0;
Vrpd = 0;
Vli = 0;
Vlymph = 0;
Qfa = 0;
Qgu = 0;
Qst = 0;
Qrpd = 0;
Qspd = 0;
Qli = 0;
QCMC = 0;
ODOSEliver = 0;
ODOSElymph = 0;
ODOSEbowel = 0;
Uptake = 0;
Vplas = 0;
VRB = 0;
Vbld = 0;
Qhepart = 0;
CA_BPA = 0;
CAT_BPA = 0;
CA_CRBC = 0;
CVnmol = 0;
CV_total_nmol = 0;
CVG = 0;
CA_BPAG = 0;
CAT_BPAG = 0;
CV_total_BPAG_nmol = 0;

SD terms for MCMC

=====

#

Sigma1 = 0.1;

Sigma2 = 0.1;

Sigma3 = 0.1;

Sigma4 = 0.1;

Initialize

{

BWc = pow(BW, CAE); # cardiac scaling output factor (kg)

VplasC = 0.55 * VBldC; # plasma fractional volume

HEME = 1 - (VplasC / VBldC); # Volume of Haeme

VRBC = HEME * VBldC; # Volume of red blood cells

Gelman reparameterisations

Qcci = QrpdC + QspdC + QhepartC + QfaC + QstC + QguC;

Qrpdci = QrpdC / Qcci;

Qspdc = QspdC / Qcci;

Qhepartci = QhepartC / Qcci;

Qfaci = QfaC / Qcci;

Qstci = QstC / Qcci;

Qguci = QguC / Qcci;

Vti =

(1 - VT) + Vrpdc + Vspdc + Vlic +

Vfac + VstC + VguC + VplasC +

VRBC + VlymphC;

Vguci = VguC / Vti;

Vstci = VstC / Vti;

Vfaci = VfaC / Vti;

Vlici = VliC / Vti;

Vspdc = VspdC / Vti;

Vrpdc = Vrpdc / Vti;

VBldci = VBldC / Vti;

Vplasci = VplasC / Vti;

VRBCci = VRBC / Vti;

Vlymphci = VlymphC / Vti;

Volumes scaled to actual volumes

Vfa = Vfaci * BW; # scaled fractional volume

Vgu = Vguci * BW; # scaled fractional volume

Vst = Vstci * BW; # scaled fractional volume

Vspd = Vspdc * BW; # scaled fractional volume

Vli = Vlici * BW; # scaled fractional volume

Vrpd = Vrpdc * BW; # scaled fractional volume

Vlymph = Vlymphci * BW; # scaled fractional volume

VRB = VRBCci * BW; # scaled red blood cell fractional volume

VBld = VBldci * BW; # Whole blood fractional volume

Vplas = Vplasci * BW; # plasma fractional volume

Calculate actual blood flows from total flow and percent flows

QC = QCC * BWc; # cardiac output (L/h)

Qfa = Qfaci * QC; # scaled fractional blood flow

Qgu = Qguci * QC; # scaled fractional blood flow

```

    Qst = Qstci * QC;                # scaled fractional blood flow
    Qrpd = Qrpdci * QC;              # scaled fractional blood flow
    Qspd = Qspdci * QC;              # scaled fractional blood flow
    Qhepart = Qhepartci * QC;        # scaled hepatic artery fractional blood flow
    Qli = Qhepart + Qst + Qgu;        # scaled fractional blood flow
    QCMC = Qhepart + Qgu + Qst + Qfa + Qrpd + Qspd;

} # End of model initialization

Dynamics
{
    Ali_lag = CalcDelay(Ali, tau);
    AliG_lag = CalcDelay(AliG, tau);
    AliS_lag = CalcDelay(AliS, tau);

    ORALDOSE = PORALDOSE * BW;        # scaled oral dose (mg/day)
    DOSEFLOW = ORALDOSE / DRINKTIME;  # zero order uptake rate constant
    ODOSE = DOSEFLOW * DOSESTEP;      # amount absorbed (mg)
    ODOSEliver = ODOSE * FracDOSEHep;
    ODOSElymph = ODOSE * FracDOSElymph;
    ODOSEbowel = ODOSE * (1 - FracDOSEHep - FracDOSElymph);

    #ClintBPASgu = (BPAS_invit_gut_Clint/1E6) * MPYgu * Vgu * 1000 * 60;          #
    Clearance (L/h whole liver)

    #Vmax_liv_BPAG = (Vmax_liv_BPAG_in_vitro * MPY * Vli * 60 * MWBPA)/1000;      #
    Vmax scaled (mg/h whole liver)
    #Vmax_gut_BPAG = (Vmax_gut_BPAG_in_vitro * MPYgu * Vgu * 60 * MWBPA)/1000;
    # Vmax scaled (mg/h whole gut)

    Vmax_liv_BPA = ((Vmax_liv_BPA_in_vitro/1E9) * MPY * Vli * 1000 * 60 * MWBPA);  #
    Vmax scaled (mg/h whole liver)
    Vmax_gut_BPAG = ((Vmax_gut_BPAG_in_vitro/1E9) * MPYgu * Vgu * 1000 * 60 * MWBPA);
    # Vmax scaled (mg/h whole gut)

    Vmax_liv_BPAS = ((Vmax_liv_BPAS_in_vitro/1E9) * MPY * Vli * 1000 * 60 * MWBPA);
    # Vmax scaled (mg/h whole liver)
    Vmax_gut_BPAS = ((Vmax_gut_BPAS_in_vitro/1E9) * MPY * Vgu * 1000 * 60 * MWBPA);
    # Vmax scaled (mg/h whole liver)

    ****!
    #          BPA  Concentrations in Compartments

    ****!

    # cellular concentrations (mg/L)
    Cfa = Afa / Vfa;
    Cgu1 = Agu1 / Vgu;
    Cgu2 = Agu2 / Vgu;
    Cst = Ast / Vst;

```

```

Cspd = Aspd / Vspd;
Crpd = Arpd / Vrpd;
Cli = Ali / Vli;
Clymph = Alymph / Vlymph;

# venous organ concentration (mg/L)
CVfa = Cfa / Pfab;
CVgu1 = Cgu1 / Pgub;
CVgu2 = Cgu2 / Pgub;
CVst = Cst / Pstb;
CVspd = Cspd / Pspdb;
CVli = Cli / Plib;
CVrpd = Crpd / Prpdb;

GPER = KEMAX / (1 + KEMIN * Cst);

# venous concentration (mg/L)
CV =
((CVfa * Qfa) +
(CVrpd * Qrpd) +
(CVspd * Qspd) +
(CVli * Qli)) / QCMC;

# BPA Venous concentration (nmoles/L)
CVnmol = (CV / MWBPA) * 1000000;

# Fraction unbound
Aplasmub BPA = Aplasm BPA * (1 - FB BPA);

# mass in system (kg)
mass =
Aplasm BPA + AMliBPAG + AMliBPAS + Ali + AMguBPAG + AMguBPAS + ABellyH + AGiTractH +
Ast + Agu1 + Agu2 + ABowel + ABellylymph + AGiTractlymph + Alymph + Afa + Arpd +
Aspd + ABile + AMBPA REMOVED PLASMA;

Uptake = ODOSEliver + ODOSElymph + ODOSEbowel;

# mass balance
reloral = ((t>0) ? mass / (ORALDOSE + 1e-10) : 1);
#reloral = ((t>0) ? mass / (Uptake + 1e-10) : 1);

CA BPA = Aplasmub BPA / (Vplas); # Arterial unbound concentration (ng/L)

#*****
***!
# BPAG Concentrations in Compartments

#*****
***!

# cellular concentrations (mg/L)
CguG = AguG / Vgu;

```

CstG = AstG / Vst;
CfaG = AfaG / Vfa;
CliG = AliG / Vli;
CspdG = AspdG / Vspd;
CrpdG = ArpdG / Vrpd;

venous organ concentration (mg/L)
CVguG = CguG / PguG;
CVstG = CstG / PstG;
CVfaG = CfaG / PfaG;
CVliG = CliG / PliG;
CVspdG = CspdG / PspdG;
CVrpdG = CrpdG / PrpdG;

CVG = ((CVliG * Qli) + (CVfaG * Qfa) + (CVspdG * Qspd) + (CVrpdG * Qrpd)) / QCMC;

BPAG Venous concentration (nmoles/L)
CVGnmol = (CVG / MWBPAG) * 1000000;

#unbound model
Aplasmub BPAG = Aplasm BPAG * (1 - FB BPAG);
CA BPAG = Aplasmub BPAG / (Vplas);

mass in system (kg)
massBPAG = AliG + AguG + AstG + AfaG + AspdG + ArpdG + ABileG + Aplasm BPAG + ABowelG +
AMBPAG REMOVED PLASMA;

BPAG mass balance
relBPAG = ((t>0) ? massBPAG / (AMliBPAG + AMguBPAG + 1e-10) : 1);
#relBPAG = ((t>0) ? massBPAG / (AMguBPAG + 1e-10) : 1);
#relBPAG = ((t>0) ? massBPAG / (AMliBPAG + 1e-10) : 1);

 ***!
 # BPAS Concentrations in Compartments

 ***!

cellular concentrations (mg/L)
CguS = AguS / Vgu;
CstS = AstS / Vst;
CfaS = AfaS / Vfa;
Clis = AliS / Vli;
CspdS = AspdS / Vspd;
CrpdS = ArpdS / Vrpd;

venous organ concentration (mg/L)
CVguS = CguS / PguS;
CVstS = CstS / PstS;
CVfaS = CfaS / PfaS;

CVliS = CliS / Plis;
CVspdS = CspdS / PspdS;
CVrpdS = CrpdS / PrpdS;

CVS = ((CVliS * Qli) + (CVfaS * Qfa) + (CVspdS * Qspd) + (CVrpdS * Qrpd)) / QCMC;
BPAS Venous concentration (nmoles/L)
CVSnmol = (CVS / MWBPAS) * 1000000;

#unbound model
Aplasm BPAS = Aplasm BPAS * (1 - FB BPAS);
CA BPAS = Aplasm BPAS / (Vplas);

mass in system (kg)
massBPAS = AliS + AguS + AstS + AfaS + AspdS + ArpdS + ABileS + Aplasm BPAS + ABowelS +
AMBPAS REMOVED PLASMA;

BPAS mass balance
relBPAS = ((t>0) ? massBPAS / (AMliBPAS + AMguBPAS + 1e-10) : 1);

***!
BPA Differential Equations

***!

dt (Gutswitch) = 0;
dt (Lymphswitch) = 0;
dt (DOSESTEP) = 0;

dt (Aplasm BPA) = QCMC * (CV - CA BPA) +
Lymphswitch * Alymph * K1Lymph
- (K1 BPA REMOVED PLASMA * Aplasm BPA); # Amount in plasma (mg)

#dt (AMliBPAG) = ((CVli * Vmax liv BPA) / (CVli + KM liv BPA in vitro)) * FracMetabG;
Amount of hepatic metabolism BPA -> BPAG (mg/h/kg)

#dt (AMliBPAS) = ((CVli * Vmax liv BPA) / (CVli + KM liv BPA in vitro)) * (1 - (FracMetabG));
Amount of hepatic metabolism BPA -> BPAS (mg/h/kg)

dt (AMliBPAG) = ((CVli * Vmax liv BPA) / (CVli + KM liv BPA in vitro)); #
Amount of hepatic metabolism BPA -> BPAG (mg/h/kg)

dt (AMliBPAS) = ((CVli * Vmax liv BPAS in vitro) / (CVli + KM liv BPAS in vitro));
Amount of hepatic metabolism BPA -> BPAS (mg/h/kg)

dt (Ali) = (Qhepart * CA BPA) + (Qst * CVst) + (Qgu * CVgu2) - (Qli * CVli) -
(dt (AMliBPAG) + dt (AMliBPAS)) -

$(K1_BPA_LIVER * Ali);$ # Amount in liver (mg)

$dt (ABile) = K1_BPA_LIVER * (Ali - Ali_lag);$ # Amount in Bile (mg)

$dt (AMguBPAG) = ((CVgu1 * Vmax_gut_BPAG) / (CVgu1 + KM_gut_BPAG_in_vitro));$
 # Amount of gut metabolism BPA -> BPAG (mg/h/kg)

$dt (AMguBPAS) = ((CVgu1 * Vmax_gut_BPAS) / (CVgu1 + KM_gut_BPAS_in_vitro));$
 # Amount of gut metabolism BPA -> BPAS (mg/h/kg)

$\#dt (AMguBPAS) = ((Qgu * ClintBPASgu) / (Qgu + ClintBPASgu / Phab)) * CVgu1;$
 # Amount of gut metabolism BPA -> BPAS (mg/h/kg)

$dt (ABellyH) = (ODOSEliver) - (GPER * ABellyH) - (BELLYPERM * ABellyH);$
 # Amount of in stomach compartment (mg/h/kg)

$dt (AGiTractH) = (GPER * ABellyH) - (GIPERM1 * AGiTractH);$ #
 Amount of in GI Tract compartment (mg/h/kg)

$dt (Ast) = Qst * (CA_BPA - CVst) + BELLYPERM * ABellyH;$ #
 Amount of in STOMACH compartment (mg/h/kg)

$dt (Agu1) = (GIPERM1 * AGiTractH) -$
 $(dt (AMguBPAG) + dt (AMguBPAS)) -$
 $(Gutswitch * GIPERM2 * Agu1);$

$dt (Agu2) = Qgu * (CA_BPA - CVgu2) +$
 $(Gutswitch * GIPERM2 * Agu1) -$
 $(Lymphswitch * K1_BPA_GUT * Agu2) +$
 $(K1_BPA_LIVER * Ali_lag);$

$dt (ABowel) = ODOSEbowel + (Lymphswitch * K1_BPA_GUT * Agu2);$ #
 Elimination rate from gut into faeces (mg)

$dt (ABellylymph) = (ODOSElymph) - (GPER * ABellylymph) - (BELLYPERMlymph * ABellylymph);$

$dt (AGiTractlymph) = (GPER * ABellylymph) - (GIPERMlymph * AGiTractlymph);$
 # BPA rate of uptake in lymph compartment (mg/h/kg)

$dt (Alymph) =$ # Amount in lymph
 $(BELLYPERMlymph * ABellylymph) +$
 $(GIPERMlymph * AGiTractlymph) -$
 $Lymphswitch * Alymph * K1Lymph;$

$dt (Afa) = Qfa * (CA_BPA - CVfa);$ # cellular compartment derivative (mg/h/kg)

$dt (Arpd) = Qrpd * (CA_BPA - CVrpd);$ # cellular compartment derivative (mg/h/kg)

$dt (Aspd) = Qspd * (CA_BPA - CVspd);$ # cellular compartment derivative (mg/h/kg)

 ***!

```

# BPAG Differential Equations

#####
***!

dt (AliG) =
(Qhepart * CA_BPAG) +
(Qst * CVstG) +
(Qgu * CVguG) -
(Qli * CVliG) +
dt (AMliBPAG) -
(K1_BPAG_LIVER * AliG);

dt (ABileG) = K1_BPAG_LIVER * (AliG - AliG_lag);
dt (AstG) = Qst * (CA_BPAG - CVstG);

dt (AguG) =
Qgu * (CA_BPAG - CVguG) +
dt (AMguBPAG) -
(Lymphswitch * K1_BPAG_GUT * AguG) +
(K1_BPAG_LIVER * AliG_lag);

dt (ABowelG) = K1_BPAG_GUT * AguG;

dt (AfaG) = Qfa * (CA_BPAG - CVfaG);
dt (AspdG) = Qspd * (CA_BPAG - CVspdG);
dt (ArpdG) = Qrpd * (CA_BPAG - CVrpdG);

#unbound model

dt (Aplasm_BPAG) = QCMC * (CVG - CA_BPAG) - (K1_BPAG_REMOVED_PLASMA * Aplasm_BPAG);

#####
***!
# BPAS Differential Equations

#####
***!

dt (AliS) =
(Qhepart * CA_BPAS) +
(Qst * CVstS) +
(Qgu * CVguS) -
(Qli * CVliS) +
dt (AMliBPAS) -
(K1_BPAS_LIVER * AliS);

dt (ABileS) = K1_BPAS_LIVER * (AliS - AliS_lag);
dt (AstS) = Qst * (CA_BPAS - CVstS);

```

```

dt (AguS) =
  Qgu * (CA_BPAS - CVguS) +
  dt (AMguBPAS) -
  (Lymphswitch * K1_BPAS_GUT * AguS) +
  (K1_BPAS_LIVER * AliS_lag);

dt (ABowelS) = K1_BPAS_GUT * AguS;

dt (AfaS) = Qfa * (CA_BPAS - CVfaS);
dt (AspdS) = Qspd * (CA_BPAS - CVspdS);
dt (ArpdS) = Qrpd * (CA_BPAS - CVrpdS);

#unbound model

dt (Aplasm_BPAS) = QCMC * (CVS - CA_BPAS) - (K1_BPAS_REMOVED_PLASMA*Aplasm_BPAS);

#####
***!
# BPAG Urinary excretion

#####
***!

dt (AMBPA_REMOVED_PLASMA) = K1_BPA_REMOVED_PLASMA*Aplasm_BPA; # Removal of
BPA from liver
dt (AMPAG_REMOVED_PLASMA) = K1_BPAG_REMOVED_PLASMA*Aplasm_BPAG; # Removal of
BPAG from liver
dt (AMPAS_REMOVED_PLASMA) = K1_BPAS_REMOVED_PLASMA*Aplasm_BPAS; # Removal of
BPAS from liver

dt (AMBPA_Urine) = dt (AMBPA_REMOVED_PLASMA) - K1_BPA_Urine * AMBPA_Urine; #Rate
of change in bladder
dt (AMPAG_Urine) = dt (AMPAG_REMOVED_PLASMA) - K1_BPAG_Urine * AMPAG_Urine;
#Rate of change in bladder
dt (AMPAS_Urine) = dt (AMPAS_REMOVED_PLASMA) - K1_BPAS_Urine * AMPAS_Urine; #
Rate of change in bladder

Curine_BPA = (AMBPA_Urine/MWBPA*1e6); # Concentration of BPA in urine
(nmol)

Curine_BPAG = (AMPAG_Urine/MWBPAG*1e6)*(MWBPAG/MWBPA); # Concentration
of BPA in urine (nmol)

Curine_BPAS = (AMPAS_Urine/MWBPAS*1e6)*(MWBPAS/MWBPA); # Concentration
of BPA in urine (nmol)

}

#These are new addition. Also specified in final four lines of 'outputs'
CalcOutputs
{

```

CAT_BPA = Aplasm_BPA/(MWBPA*Vplas)*1e6; # Total concentration in plasma (nmol/l)
CV_total_nmol = (Aplasm_BPA)/(MWBPA*Vbld)*1e6; # Total concentration in blood (nmol/l)

#CAT_BPAG = Aplasm_BPAG/(MWBPA*Vplas)*1e6;
CAT_BPAG = Aplasm_BPAG/(MWBPA*Vplas)*1e6*(MWBPA/MWBPA);

CV_total_BPAG_nmol = (Aplasm_BPAG)/(MWBPA*Vbld)*1e6*(MWBPA/MWBPA);

CAT_BPAS = Aplasm_BPAS/(MWBPA*Vplas)*1e6*(MWBPA/MWBPA);
CV_total_BPAS_nmol = (Aplasm_BPAS)/(MWBPA*Vbld)*1e6*(MWBPA/MWBPA);

Plasma_BPA = (Aplasm_BPA)/(Vbld);
Plasma_BPAG = (Aplasm_BPAG)/(Vbld);
Plasma_BPAS = (Aplasm_BPAS)/(Vbld);

Urine_BPA = Curine_BPA;
Urine_BPAG = Curine_BPAG;
Urine_BPAS = Curine_BPAS;
 }

End.

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