Supporting Information

Development of a generic zebrafish embryo PBPK model and application to the developmental toxicity assessment of valproic acid analogs

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Table S1: Test concentrations used in the Fish Embryo Acute Toxicity (FET) test and measured concentration data.

Compound	**Nominal water concentration (µM)**	**Total water concentration (µM)**	**Total embryo[*] concentration (µM)**
Valproic acid	6.25, 12.5, 25, 50, 100, 200, 400	5, 9, 19, 51, 66, 168, 375**, 238	32, 56, 68, 120, 180, 327, 10387[**], 462
2,2-Dimethylvaleric acid	268, 312.5, 322, 386, 463, 500, 556, 667, 800	225, 298, 275, 416, 490, 556, 606, 713, 861	575, 647, 321, 919, 1697, 2148, 2424, 8746, 7053
2-Ethylbutyric acid	100, 200, 400, 800	88, 173, 431, 586	24, 92, 1446, 11142
2-Ethylhexanoic acid	12.5, 25, 50, 100, 200	13, 30, 58, 94, 168	53, 56, 75, 140, 667
2-Methylhexanoic acid	125, 250, 500	176, 320, 638	8, 12, 1775
2-Methylpentanoic acid	177.7, 266.6, 400, 600	287, 372, 614, 762	28, 72, 308, 3734
2-Propylheptanoic acid	12.5, 25, 50	14, 27, 52	32, 53, 52
4-eneValproic acid	200, 400	271, 414	666, 3594
4-Pentenoic acid	414, 500, 538, 700, 910, 1000	480, 481, 539, 775, 840, 860	99, ND[***], 222, 97, 6674, 12250
Hexanoic acid	512, 520, 563, 620, 625, 681	544, 559, 552, 637, 500, 666	ND, 28, 92, 6510, 10933, 11382

[*] Embryo and yolk included.

[**] Measurements at 72 hpf for a nominal water concentration at 400 µM.

[***] ND: below limit of detection.

Table S2: Mass spectrometric and chromatography conditions.

Mass Spectrometric Conditions

Ion Spray Voltage (V)	-4500
Temperature (°C)	50
Gas 1 (psi)	50
Gas 2 (psi)	50
Curtain Gas (psi)	40
CAD Gas (arb)	N/A
Detection	Hi Res
Mass detection range (amu)	50 - 800
Accumulation time (ms)	250

Chromatography Conditions

Column	Phenomenex Kinetex XB-C18 2.6µm 2.1x50mm
Column Temperature (°C)	60
Mobile Phase A	0.01% Formic acid (aq)
Mobile Phase B	MeOH

Time (min)	**Flow Rate (µL/min)**	**% A**	**% B**
0.00	800	98	2
1.50	800	5	95
1.90	800	5	95
2.00	800	98	2
2.50	800	98	2

Table S3: Physiological parameter values of the zebrafish embryo model.

Parameters	Symbols	Units	Values	References
Initial organ growth time				
Liver	τ_{liver}	minutes	960	Our data
Gut	τ_{gut}	minutes	600	Our data
Skeleton	$\tau_{skeleton}$	minutes	2880	Our data
Eyes	τ_{eyes}	minutes	660	Our data
Brain	τ_{brain}	minutes	540	Our data
Heart	τ_{heart}	minutes	1800	Our data
Skin	τ_{skin}	minutes	1440	Our data
Other tissues	τ_{others}	minutes	0	Our data
Organ growth rate				
Liver	$k_{g,\ liver}$	$minutes^{-1}$	5.30×10^{-6}	Estimated
Gut	$k_{g,\ gut}$	$minutes^{-1}$	2.28×10^{-5}	Estimated
Skeleton	$k_{g,\ skeleton}$	$minutes^{-1}$	1.82×10^{-5}	Estimated
Eyes	$k_{g,\ eyes}$	$minutes^{-1}$	1.20×10^{-5}	Estimated
Brain	$k_{g,\ brain}$	$minutes^{-1}$	2.22×10^{-5}	Estimated
Heart	$k_{g,\ heart}$	$minutes^{-1}$	3.01×10^{-5}	Estimated
Skin	$k_{g,\ skin}$	$minutes^{-1}$	1.37×10^{-5}	Estimated
Other tissues	$k_{g,\ others}$	$minutes^{-1}$	3.93×10^{-5}	Estimated
Muscle	$k_{g,\ muscle}$	$minutes^{-1}$	5.07×10^{-5}	Estimated
Yolk consumption rate	$K_{d,\ yolk}$	$minutes^{-1}$	3.13×10^{-4}	Estimated
Volume of culture well	V_{well}	L	0.0034	Busquet *et al.*[41]
Volume of culture medium	V_{medium}	L	0.001	Busquet *et al.*[41]
Volume of liver cell	$V_{cell\ liver}$	L	10^{-12}	Our data
Fraction lysosome in cells	f_{lyso}	-	0.01	Our data
Fraction mitochondria in cells	f_{mito}	-	0.1	Our data
Radius of embryo at initial time	$r_{embryo,0}$	mm	0.13	Kimmel *et al.*[36]
Radius of yolk at initial time	$r_{yolk,0}$	mm	0.4	Kimmel *et al.*[36]
Volume of embryo at final time	$V_{embryo\ (120hpf)}$	L	3.7×10^{-7}	Estimated
Organ vol. as fractions of total at 120 hpf				
Others	$sc_{others\ (120hpf)}$	-	1.99×10^{-1}	Estimated
Muscle	$sc_{muscle\ (120hpf)}$	-	2.00×10^{-1}	Estimated

Table S4: Zebrafish embryo-specific partition coefficient values predicted by the VIVD model, for VPA and some analogs.

Compound	**Fraction un-ionized in medium**	**Compartment to water partition coefficients**		**Medium unbound to compartment partition coefficients**											
		Plastic*	**Air**	**Yolk**	**Liver**	**Gut**	**Muscle**	**Skeleton**	**Eye**	**Brain**	**Heart**	**Skin**	**Others**	**Lysosomes**	**Mitochondria**
	fu_u	$P_{p:w}$	$P_{a:w}$	$P_{mu:yolk}$	$P_{mu:liver}$	$P_{mu:gut}$	$P_{mu:muscle}$	$P_{mu:os}$	$P_{mu:eye}$	$P_{mu:brain}$	$P_{mu:heart}$	$P_{mu:skin}$	$P_{mu:others}$	$P_{mu:lyso}$	$P_{mu:mito}$
Valproic acid	0.00627	5.20×10^{-4}	2.52	0.374	0.290	0.254	0.289	0.289	0.307	0.318	0.289	0.307	0.231	0.0510	0.486
2,2- Dimethylvaleric acid	0.00990	9.96×10^{-5}	1.84	0.245	0.297	0.272	0.303	0.303	0.332	0.332	0.303	0.332	0.235	0.0170	0.714
2-Ethylbutyric acid	0.00315	2.38×10^{-4}	5.84	0.167	0.207	0.189	0.211	0.211	0.231	0.231	0.211	0.231	0.164	0.0113	0.235
2-Ethylhexanoic acid	0.00627	3.56×10^{-4}	2.52	0.303	0.277	0.246	0.278	0.278	0.300	0.306	0.278	0.300	0.219	0.0349	0.483
2-Methylhexanoic acid	0.0124	1.49×10^{-4}	1.97	0.321	0.328	0.296	0.332	0.332	0.360	0.364	0.332	0.360	0.260	0.0310	0.856
2-Methylpentanoic acid	0.00990	4.56×10^{-5}	1.66	0.208	0.290	0.268	0.297	0.297	0.328	0.326	0.297	0.328	0.229	0.00908	0.712
2-Propylheptanoic acid	0.00627	5.19×10^{-3}	5.84	2.51	0.708	0.477	0.622	0.622	0.542	0.698	0.622	0.542	0.576	0.548	0.582
4-eneVPA	0.00315	2.38×10^{-4}	5.84	0.167	0.207	0.189	0.211	0.211	0.231	0.231	0.211	0.231	0.164	0.0113	0.235
4-Pentenoic acid	0.00990	1.02×10^{-5}	1.15	0.185	0.286	0.266	0.294	0.294	0.326	0.322	0.294	0.326	0.226	0.00410	0.710
Hexanoic acid	0.00788	8.15×10^{-5}	1.43	0.209	0.274	0.252	0.280	0.280	0.308	0.307	0.280	0.308	0.217	0.0113	0.588

*Unit: dm

Table S5: Physicochemical properties of compounds.

Substance	pKa	MW (g/mol)	logP	Henry's constant (Pa.m^3/mol)
Valproic acid	4.8	144.2	2.75	0.367
2,2-Dimethylvaleric acid	5.0	130.2	2.01	0.229
2-Ethylbutyric acid	4.8	116.2	1.68	0.184
2-Ethylhexanoic acid	4.8	144.2	2.58	0.367
2-Methylhexanoic acid	5.1	130.2	2.19	0.26
2-Methylpentanoic acid	5.0	116.2	1.66	0.184
2-Propylheptanoic acid	4.8	172.3	3.78	0.731
4-eneVPA	4.5	142.2	2.40	0.731
4-Pentenoic acid	4.9	100.0	0.99	0.0266
Hexanoic acid	4.9	116.2	1.92	0.119

Table S6: Estimation of mean, standard deviation (SD), 95% confidence intervals (IC95%) and maximum posterior (MP) value for the metabolic clearance, and residual uncertainty SD σ, for VPA and its analogs.

Compound	Cl_{met} (L/min)			σ		
	Mean ± SD	IC 95%	MP	Mean ± SD	IC 95%	MP
Valproic acid	$3.08\times10^{-11} \pm 2.98\times10^{-11}$	$[1.10\times10^{-12}; 1.10\times10^{-10}]$	2.26×10^{-13}	4.90 ± 0.728	[3.67;6.48]	4.49
2,2-Dimethylvaleric acid	$2.02\times10^{-11} \pm 1.85\times10^{-11}$	$[7.26\times10^{-13}; 6.86\times10^{-11}]$	1.66×10^{-13}	4.97 ± 0.755	[3.71;6.62]	4.64
2-Ethylbutyric acid	$6.30\times10^{-11} \pm 5.71\times10^{-11}$	$[1.65\times10^{-12}; 2.17\times10^{-10}]$	5.93×10^{-13}	4.53 ± 0.809	[3.25;6.22]	4.13
2-Ethylhexanoic acid	$3.37\times10^{-11} \pm 3.38\times10^{-11}$	$[7.58\times10^{-13}; 1.23\times10^{-10}]$	7.39×10^{-15}	4.19 ± 0.792	[2.97;6.12]	3.59
2-Methylhexanoic acid	$8.24\times10^{-11} \pm 6.55\times10^{-11}$	$[3.19\times10^{-12}; 2.48\times10^{-10}]$	3.47×10^{-13}	3.65 ± 0.737	[2.49;5.40]	3.18
2-Methylpentanoic acid	$4.61\times10^{-11} \pm 4.20\times10^{-11}$	$[1.25\times10^{-12}; 1.55\times10^{-10}]$	9.19×10^{-14}	3.54 ± 0.741	[2.36;5.33]	2.95
2-Propylheptanoic acid	$1.86\times10^{-11} \pm 1.90\times10^{-11}$	$[4.73\times10^{-13}; 7.39\times10^{-11}]$	9.10×10^{-14}	2.86 ± 0.727	[1.85;4.40]	2.25
4-eneVPA	$8.07\times10^{-11} \pm 7.34\times10^{-11}$	$[3.21\times10^{-12}; 2.82\times10^{-10}]$	1.98×10^{-13}	4.63 ± 0.834	[3.29;6.52]	4.11
4-Pentenoic acid	$4.23\times10^{-11} \pm 3.84\times10^{-11}$	$[1.14\times10^{-12}; 1.46\times10^{-10}]$	5.56×10^{-13}	4.14 ± 0.769	[2.95;5.96]	3.62
Hexanoic acid	$4.46\times10^{-11} \pm 4.18\times10^{-11}$	$[1.26\times10^{-12}; 1.56\times10^{-10}]$	1.64×10^{-13}	5.10 ± 0.781	[3.76;6.89]	4.72

Table S7: Estimation of mean, standard deviation (SD), 95% confidence intervals (IC95%) and maximum posterior value for the metabolic clearance, the coefficient partition correction factor, the air to water partition coefficient, and residual fit uncertainty σ, for VPA, its analogs and the 16 compounds studied by Brox *et al*.

	Cl_{met} (L/min)			f_{pc}			$P_{a:w}$			σ		
	Mean ± SD	**IC 95%**	**MP**	**Mean ± SD**	**IC 95%**	**MP**	**Mean ± SD**	**IC 95%**	**MP**	**Mean ± SD**	**IC 95%**	**MP**
Valproic acid	$6.54\times10^{-12} \pm 4.89\times10^{-12}$	$[3.03\times10^{-13}; 1.82\times10^{-11}]$	7.80×10^{-14}	7.31 ± 0.919	[5.66;9.31]	6.87	4.10 ± 5.11	[0.293;18.7]	1.03	1.71 ± 0.163	[1.51;2.13]	1.56
2,2- dimethylvaleric acid	$1.78\times10^{-10} \pm 1.17\times10^{-11}$	$[1.07\times10^{-11}; 4.46\times10^{-10}]$	8.77×10^{-11}	0.432 ± 0.200	[0.141;0.919]	0.314	2.73 ± 3.28	[0.210;12.6]	0.614	2.16 ± 0.629	[1.52;3.84]	1.53
2-ethylbutyric acid	$5.47\times10^{-10} \pm 4.26\times10^{-10}$	$[2.25\times10^{-11}; 1.55\times10^{-9}]$	3.49×10^{-10}	0.259 ± 0.106	[0.0914;0.481]	0.201	9.23 ± 10.3	[0.672;39.4]	2.35	2.77 ± 0.644	[1.87;4.40]	2.26
2-ethylhexanoic acid	$1.43\times10^{-10} \pm 1.04\times10^{-10}$	$[7.11\times10^{-12}; 4.01\times10^{-10}]$	6.12×10^{-12}	0.332 ± 0.107	[0.186;0.604]	0.268	3.22 ± 3.72	[0.248;14.1]	0.710	1.75 ± 0.267	[1.51;2.48]	1.53
2-methylhexanoic acid	$2.95\times10^{-9} \pm 2.38\times10^{-9}$	$[1.66\times10^{-10}; 9.15\times10^{-9}]$	6.92×10^{-10}	0.0902 ± 0.0832	[0.0103;0.332]	0.033	4.34 ± 8.74	[0.265;23.8]	0.532	3.89 ± 0.797	[2.58;5.77]	3.45
2-methylpentanoic acid	$7.97\times10^{-10} \pm 6.91\times10^{-10}$	$[3.17\times10^{-11}; 2.57\times10^{-9}]$	8.18×10^{-12}	0.111 ± 0.0749	[0.0286;0.321]	0.0732	2.87 ± 5.87	[0.193;11.9]	0.441	3.25 ± 0.719	[2.22;4.94]	2.61
2-propylheptanoic acid	$2.63\times10^{-10} \pm 2.16 \times10^{-10}$	$[9.87\times10^{-12}; 7.69\times10^{-10}]$	1.25×10^{-11}	0.467 ± 0.177	[0.200;0.907]	0.34	8.59 ± 9.64	[0.629;33.6]	1.57	1.97 ± 0.538	[1.51;3.33]	1.51
4-ene-valproic acid	$1.07\times10^{-10} \pm 9.17\times10^{-11}$	$[4.97\times10^{-12}; 3.67\times10^{-10}]$	1.76×10^{-11}	1.42 ± 0.913	[0.346;4.06]	0.815	8.98 ± 10.1	[0.731;39.0]	1.50	2.45 ± 0.727	[1.55;4.28]	1.66
4-pentenoic acid	$1.65\times10^{-10} \pm 1.55\times10^{-10}$	$[8.87\times10^{-12}; 6.27\times10^{-10}]$	1.63×10^{-11}	0.740 ± 0.433	[0.150;1.80]	0.492	1.94 ± 2.60	[0.125;8.58]	0.353	2.31 ± 0.763	[1.52;4.22]	1.53
Hexanoic acid	$9.56\times10^{-10} \pm 8.57\times10^{-10}$	$[3.05\times10^{-11}; 3.44\times10^{-9}]$	3.13×10^{-10}	0.351 ± 0.368	[0.0286;1.48]	0.129	2.67 ± 3.39	[0.183;12.4]	0.490	4.20 ± 0.787	[2.86;5.95]	3.85
2,4-diclorophenoxy-acetic acid	$2.09\times10^{-11} \pm 1.80\times10^{-11}$	$[6.46\times10^{-13}; 6.88\times10^{-11}]$	1.27×10^{-12}	10.6 ± 1.84	[7.57;15.1]	9.45	1.73 ± 2.30	[0.149;8.92]	0.310	2.12 ± 0.258	[1.76;2.77]	1.92
Atropine	$1.35\times10^{-10} \pm 5.73\times10^{-11}$	$[2.28\times10^{-11}; 2.44\times10^{-10}]$	1.31×10^{-10}	0.0248 ± 0.00405	[0.0177;0.0341]	0.0238	2.17 ± 3.64	[2.17;3.64]	0.507	1.79 ± 0.186	[1.53;2.27]	1.68
Benzocaine	$1.54\times10^{-11} \pm 3.48\times10^{-12}$	$[8.57\times10^{-12}; 2.16\times10^{-11}]$	1.57×10^{-11}	2.04 ± 0.522	[1.43;3.59]	1.65	2.29 ± 2.84	[0.147;11.3]	0.322	1.59 ± 0.0798	[1.50;1.79]	1.52
Caffeine	$1.87\times10^{-11} \pm 6.14\times10^{-12}$	$[4.40\times10^{-12}; 2.77\times10^{-11}]$	1.84×10^{-11}	3.03 ± 0.605	[2.09;4.56]	2.58	1.92 ± 1.89	[0.133;7.26]	0.339	1.54 ± 0.0487	[1.50;1.66]	1.50
Chloramphenicol	$8.46\times10^{-10} \pm 2.84\times10^{-10}$	$[2.17\times10^{-10}; 1.37\times10^{-9}]$	8.41×10^{-10}	0.00164 ± 0.000336	[0.00111;0.00244]	0.00154	1.84 ± 2.41	[0.125;8.94]	0.364	1.96 ± 0.215	[1.64;2.45]	1.77
Cimetidine	$1.72\times10^{-10} \pm 1.23\times10^{-10}$	$[8.63\times10^{-12}; 4.70\times10^{-10}]$	6.11×10^{-11}	0.228 ± 0.0742	[0.129;0.432]	0.162	2.26 ± 2.76	[0.143;10.5]	0.298	2.66 ± 0.406	[2.04;3.65]	2.40
Clofibric acid	$1.11\times10^{-11} \pm 1.02\times10^{-11}$	$[3.00\times10^{-13}; 3.81\times10^{-11}]$	7.95×10^{-14}	15.1 ± 2.13	[11.1;19.2]	14.3	1.92 ± 2.65	[0.137;9.32]	0.481	2.01 ± 0.285	[1.63;2.68]	1.83
Colchicine	$2.71\times10^{-9} \pm 1.06\times10^{-9}$	$[4.98\times10^{-10}; 4.78\times10^{-9}]$	2.38×10^{-9}	0.0149 ± 0.00515	[0.00755;0.0284]	0.0117	2.26 ± 2.73	[0.145;10.1]	0.357	2.51 ± 0.385	[1.94;3.46]	2.24
Cyclophosphamide	$5.35\times10^{-10} \pm 1.67\times10^{-10}$	$[1.91\times10^{-10};8.41\times10^{-10}]$	5.96×10^{-10}	0.122 ± 0.0419	[0.0775;0.256]	0.0922	2.90 ± 3.70	[1.50;1.70]	0.288	1.55 ± 0.0507	[1.50;1.70]	1.50
Metoprolol	$1.99\times10^{-11} \pm 1.10\times10^{-11}$	$[1.15\times10^{-12}; 4.18\times10^{-11}]$	1.83×10^{-11}	0.0466 ± 0.00560	[0.0353;0.0575]	0.0448	1.83 ± 2.35	[0.141;8.58]	0.416	1.55 ± 0.0528	[1.50;1.69]	1.50
Metribuzin	$4.51\times10^{-12} \pm 3.78\times10^{-12}$	$[1.61\times10^{-13}; 1.42\times10^{-11}]$	2.53×10^{-13}	0.371 ± 0.0506	[0.291;0.486]	0.316	1.47 ± 1.31	[0.127;5.13]	0.310	1.56 ± 0.0579	[1.50;1.72]	1.50
Phenacetin	$1.63\times10^{-11} \pm 8.40\times10^{-12}$	$[1.68\times10^{-12}; 3.29\times10^{-11}]$	1.63×10^{-11}	0..788 ± 0.096	[0.623;0.978]	0.731	1.29 ± 1.06	[0.131;4.13]	0.293	1.54 ± 0.043	[1.50;1.66]	1.50
Phenytoin	$7.70\times10^{-12} \pm 6.82\times10^{-12}$	$[2.79\times10^{-13}; 2.61\times10^{-11}]$	5.22×10^{-13}	0.183 ± 0.0539	[0.124;0.352]	0.140	2.50 ± 3.25	[0.159;12.0]	0.316	1.94 ± 0.184	[1.66;2.37]	1.81
Sulfamethoxazole	$8.13\times10^{-11} \pm 4.56\times10^{-11}$	$[4.84\times10^{-12}; 1.78\times10^{-10}]$	7.20×10^{-11}	6.31 ± 1.04	[4.59;8.55]	5.98	1.73 ± 2.00	[0.160;7.88]	0.317	1.79 ± 0.176	[1.53;2.19]	1.64
Theophylline	$1.90\times10^{-11} \pm 1.00\times10^{-11}$	$[1.35\times10^{-12}; 3.89\times10^{-11}]$	1.33×10^{-11}	2.97 ± 1.08	[1.70;6.06]	2.17	2.42 ± 3.31	[0.135;12.9]	0.394	1.95 ± 0.262	[1.58;2.59]	1.76
Thiacloprid	$7.76\times10^{-12} \pm 7.00\times10^{-12}$	$[2.26\times10^{-13}; 2.61\times10^{-11}]$	1.05×10^{-13}	0.196 ± 0.081	[0.125;0.438]	0.138	3.14 ± 4.72	[0.124;20.0]	0.253	1.74 ± 0.163	[1.52;2.14]	1.57

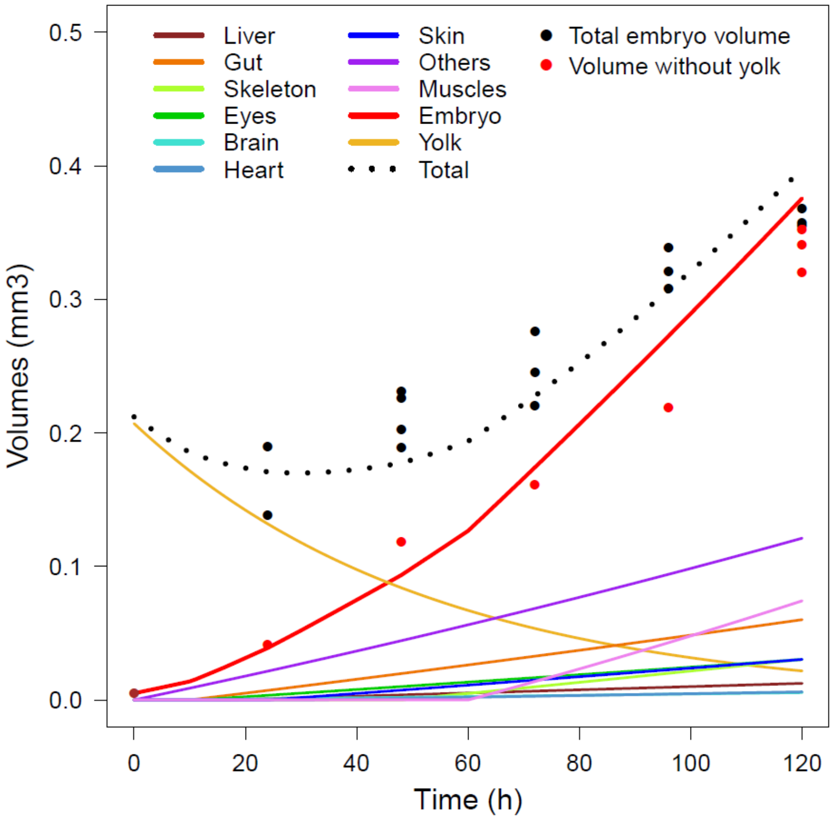

Figure S1: Observed (points) and modeled (lines) time course of the zebrafish embryo's total volume and individual organ volumes.

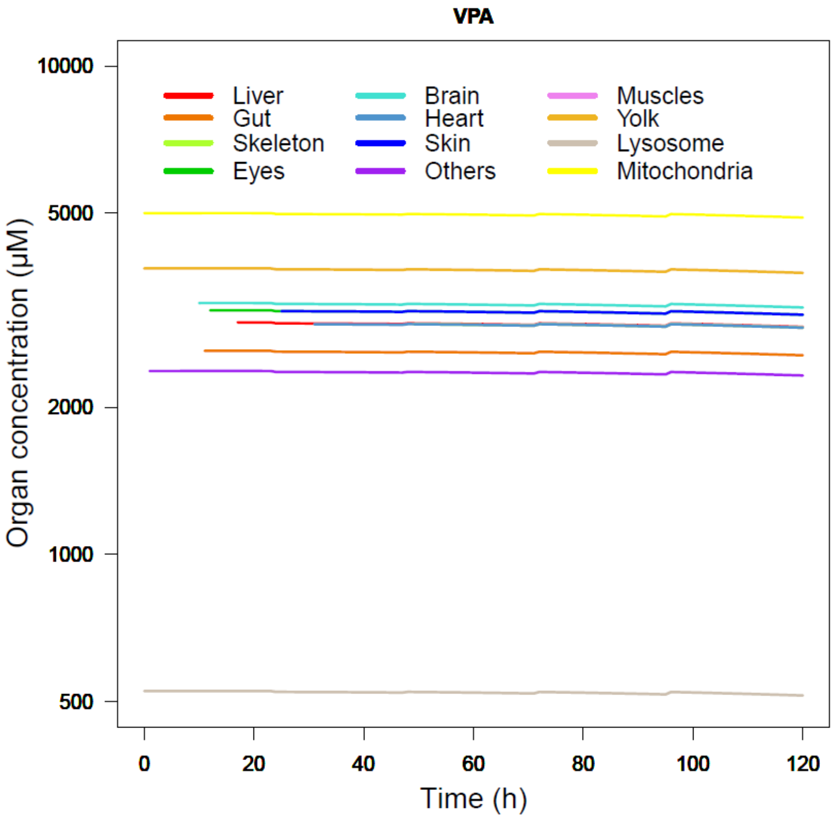

Figure S2: Organ concentrations (µM) as a function of time (h), after estimating Cl_{met} and f_{pc} for VPA.

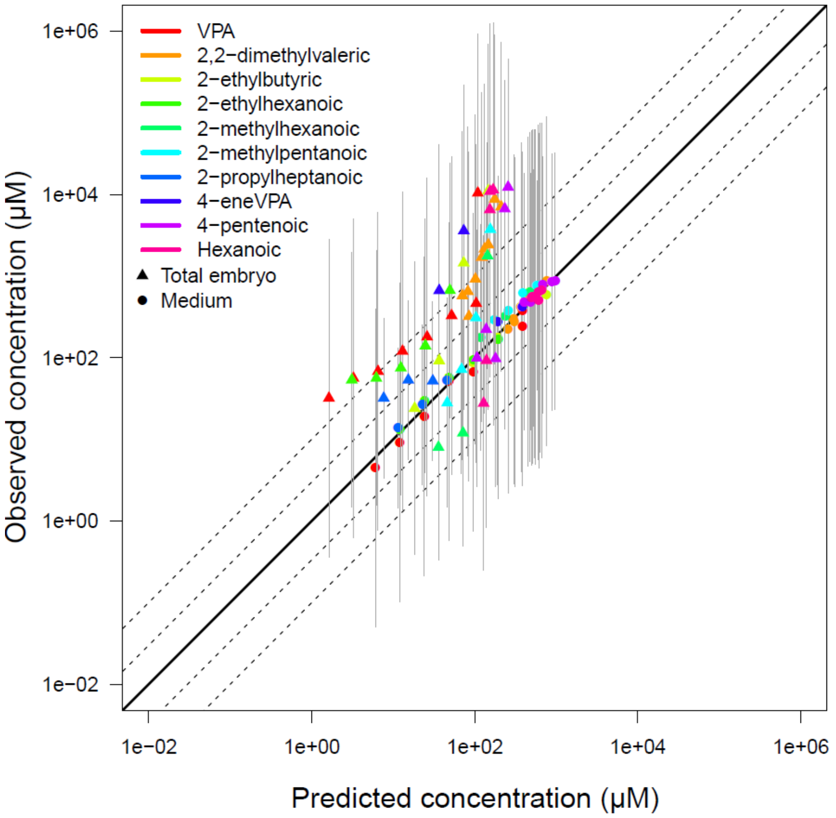

Figure S3: Observed versus predicted concentrations of VPA and nine analogs, in the zebrafish embryo and culture medium. This is the best fit obtained after Cl_{met} estimation by data fitting. The black line corresponds to perfect fit. Dashed lines correspond to the three- and ten-fold error intervals. The grey bars correspond to +/- one residual SD (σ).

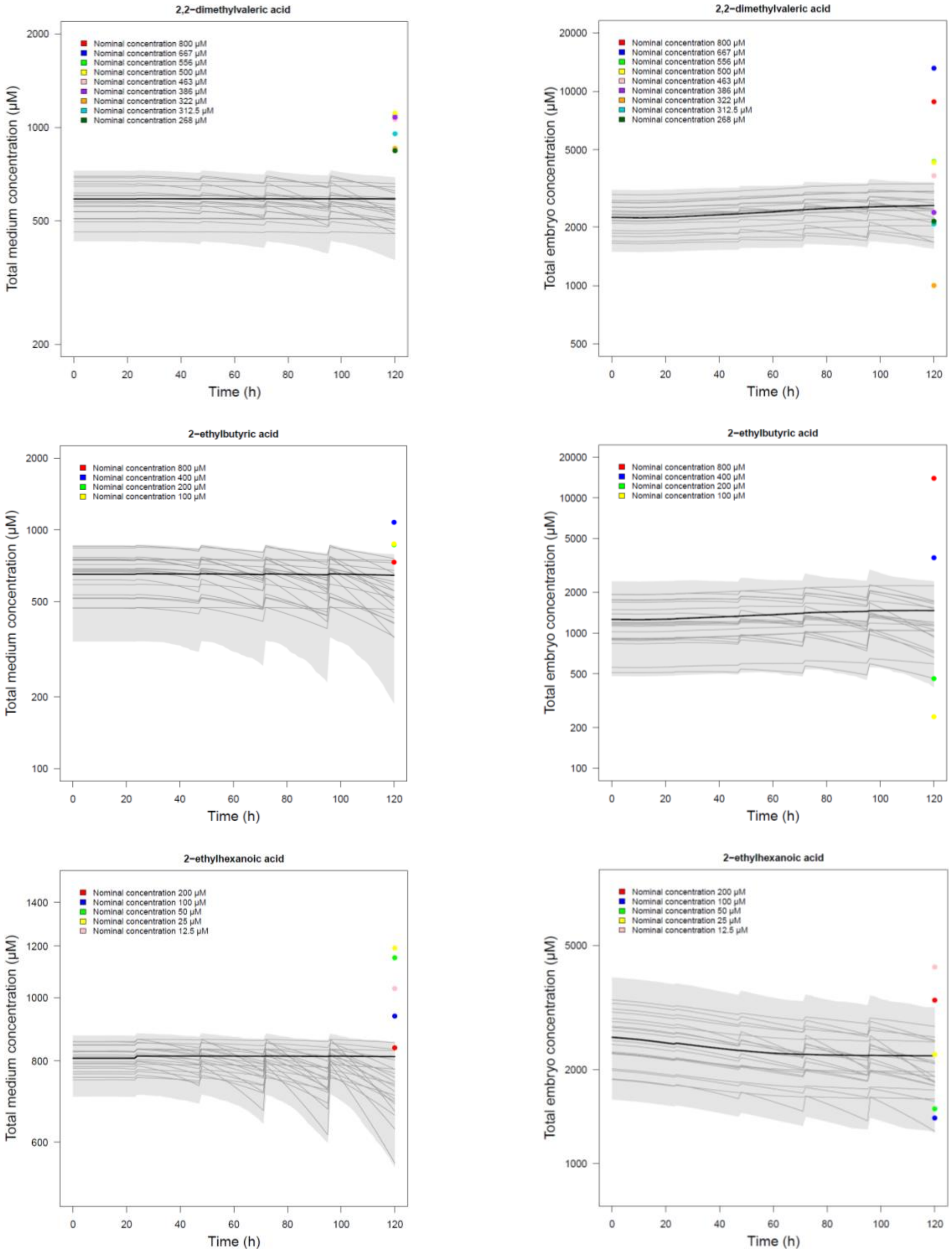

Figure S4: Predicted (lines) and observed (points) VPA analogs' concentrations in medium (left) and in total embryo (right) as a function of time, after estimating Cl_{met} and f_{pc}. All concentrations were normalized to a nominal dose of 1mM. The grey area defines the 95% confidence interval. The thick black line is the maximum posterior predicted concentration time-course. The thin lines are 20 predictions obtained using random parameter vectors drawn from their posterior distribution.

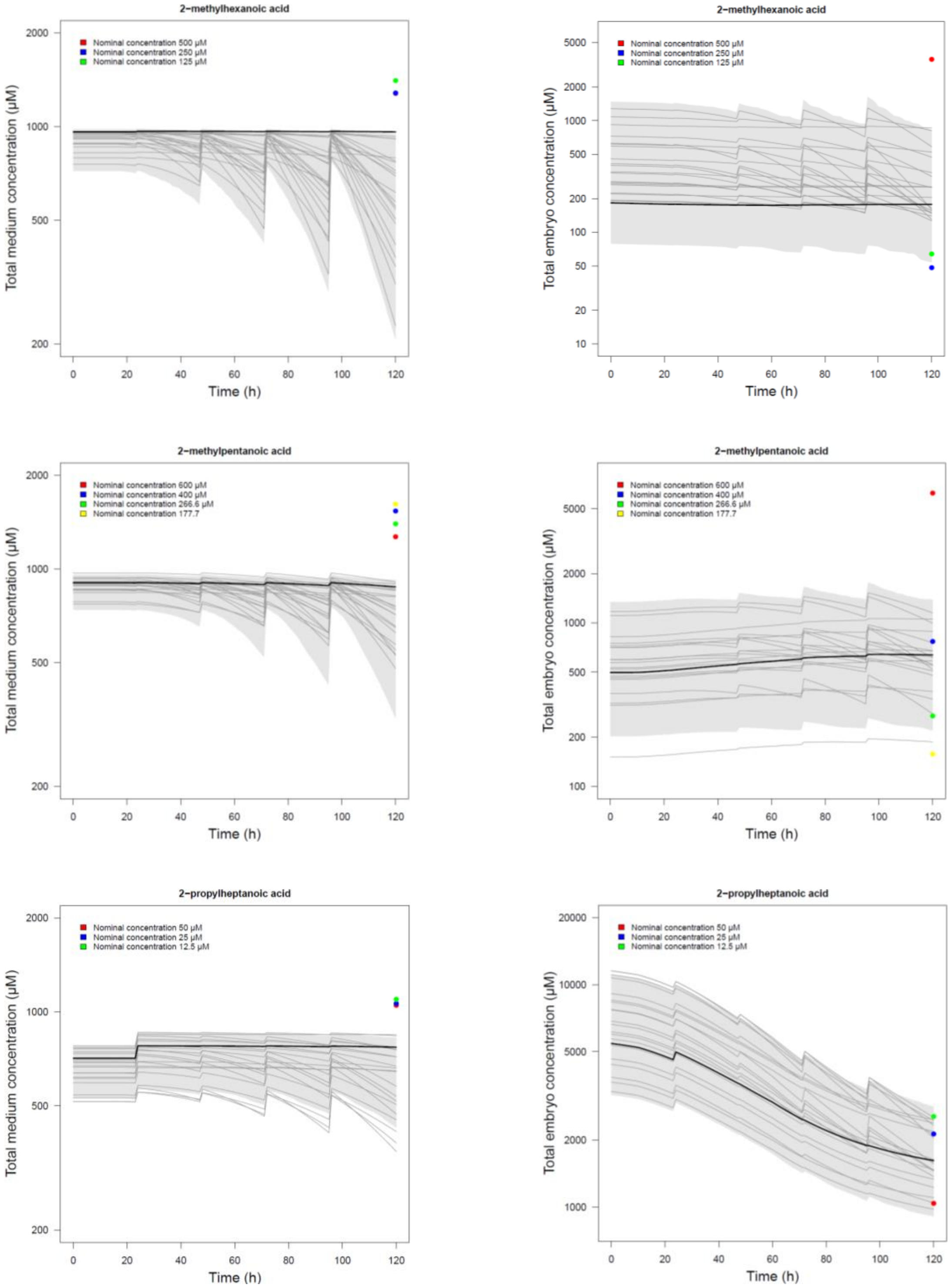

Figure S4 (followed).

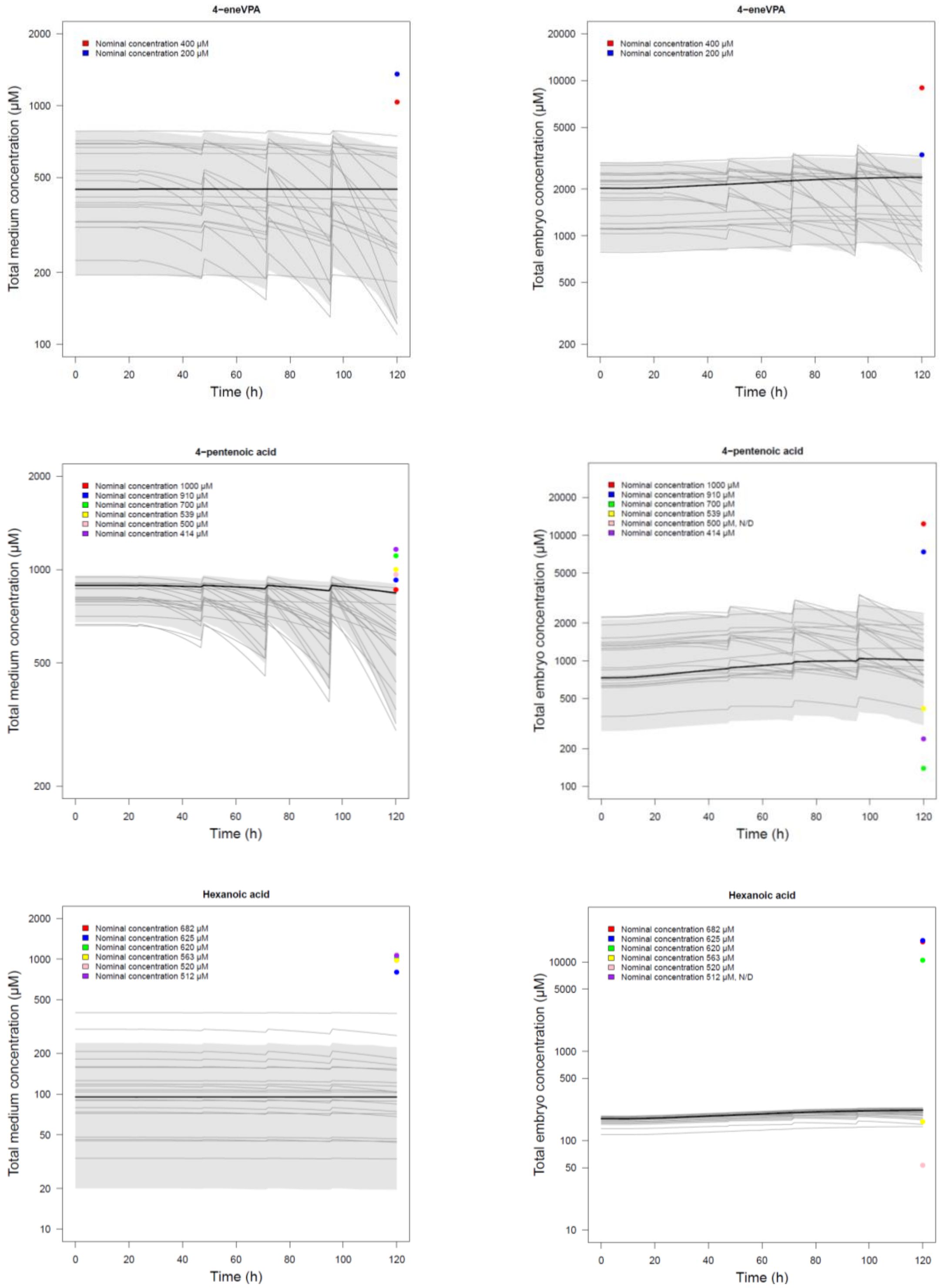

Figure S4 (followed).

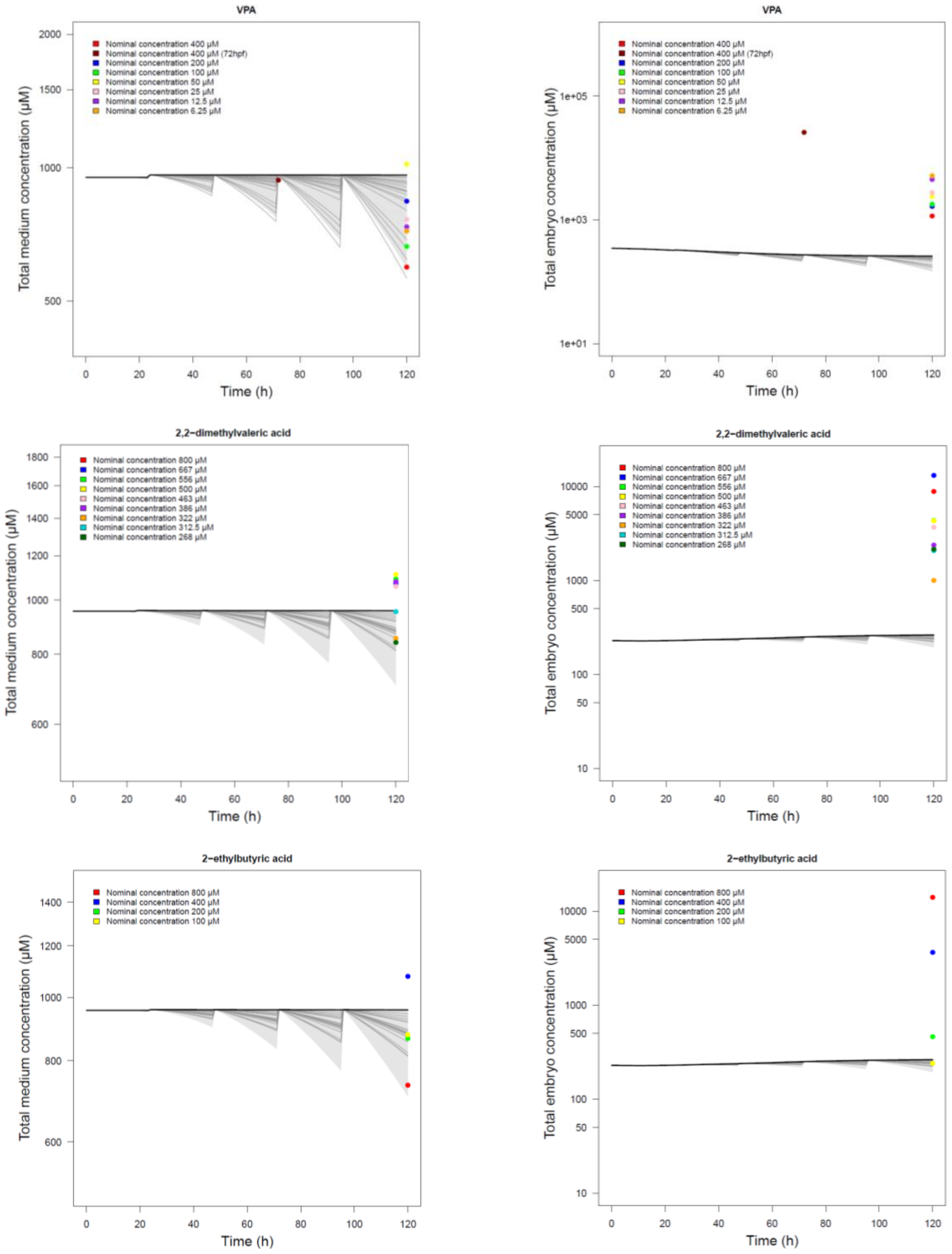

Figure S5: Predicted (lines) and observed (points) VPA and analogs' concentrations in medium (left) and in total embryo (right) as a function of time, after estimating Cl_{met} only. All concentrations were normalized to a nominal dose of 1mM. The grey area defines the 95% confidence interval. The thick black line is the maximum posterior predicted concentration time-course. The thin lines are 20 predictions obtained using random parameter vectors drawn from their posterior distribution.

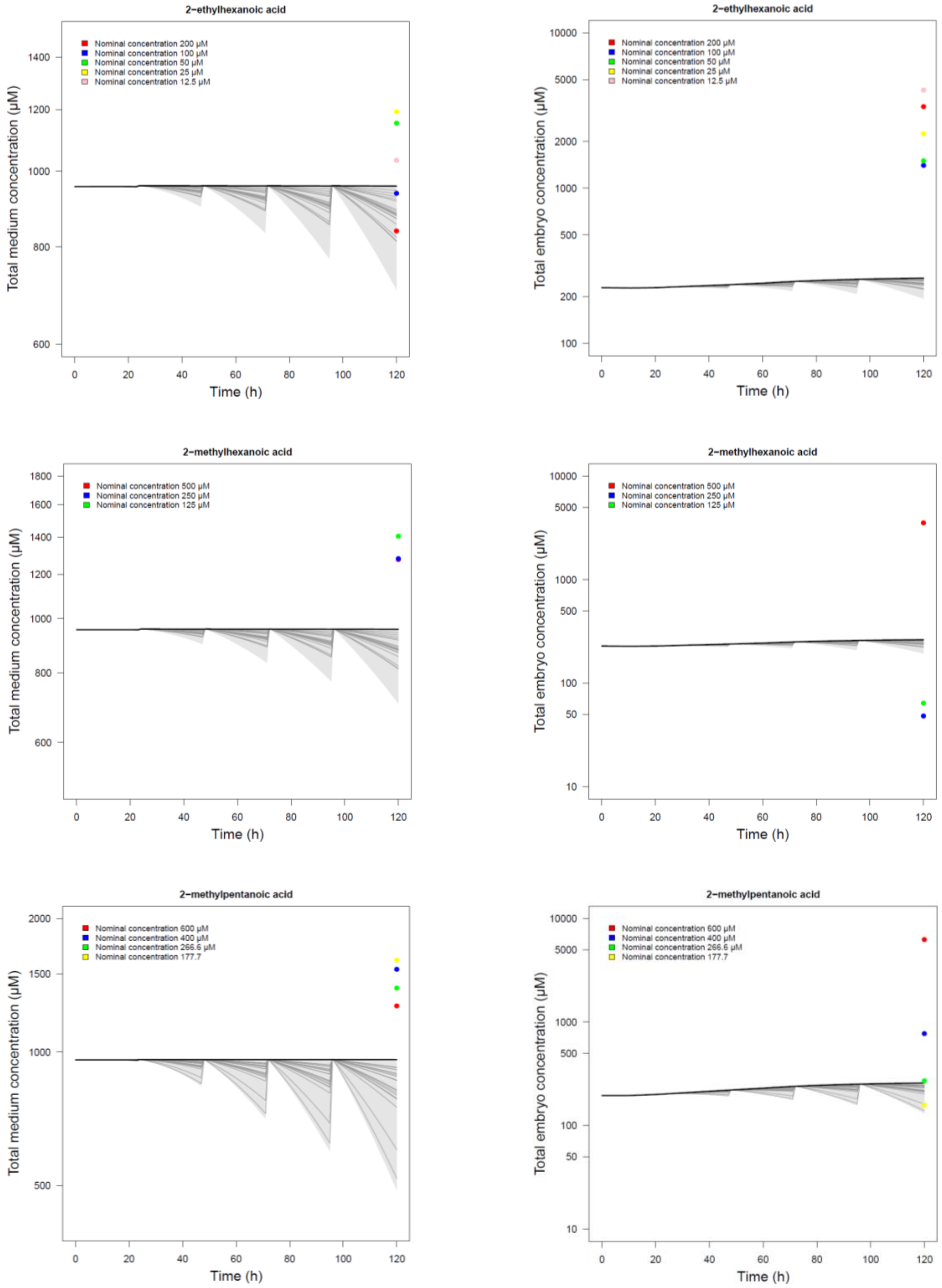

Figure S5 (followed).

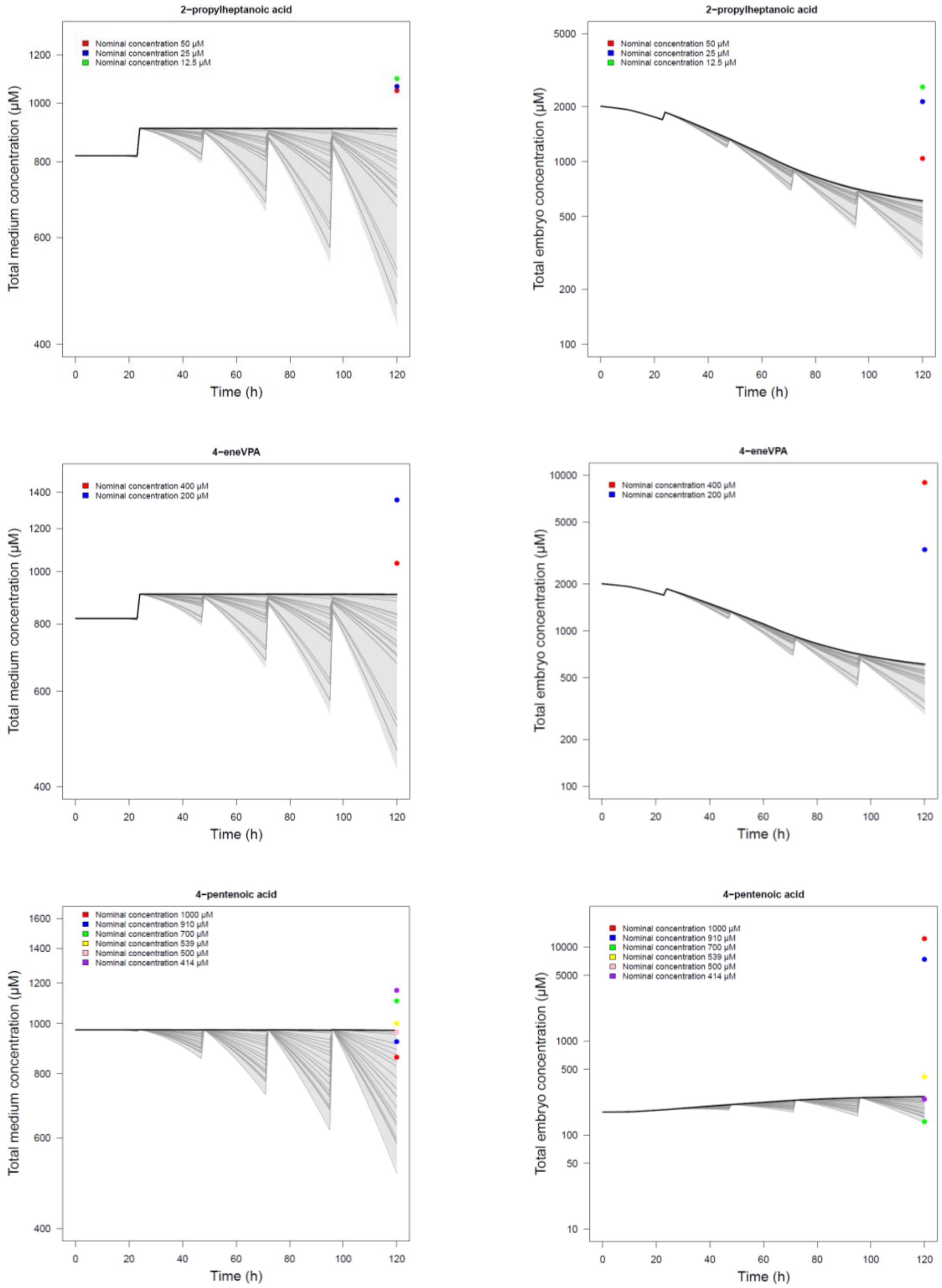

Figure S5 (followed).

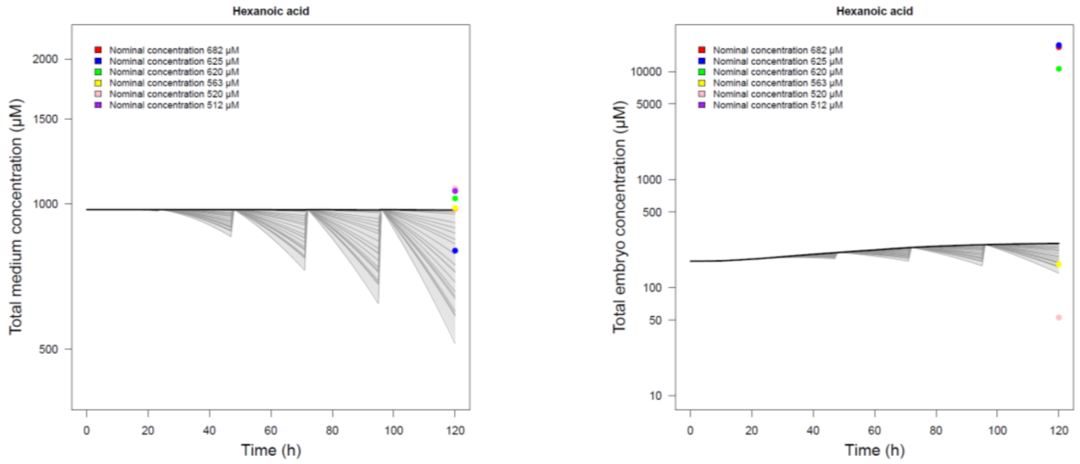

Figure S5 (followed).

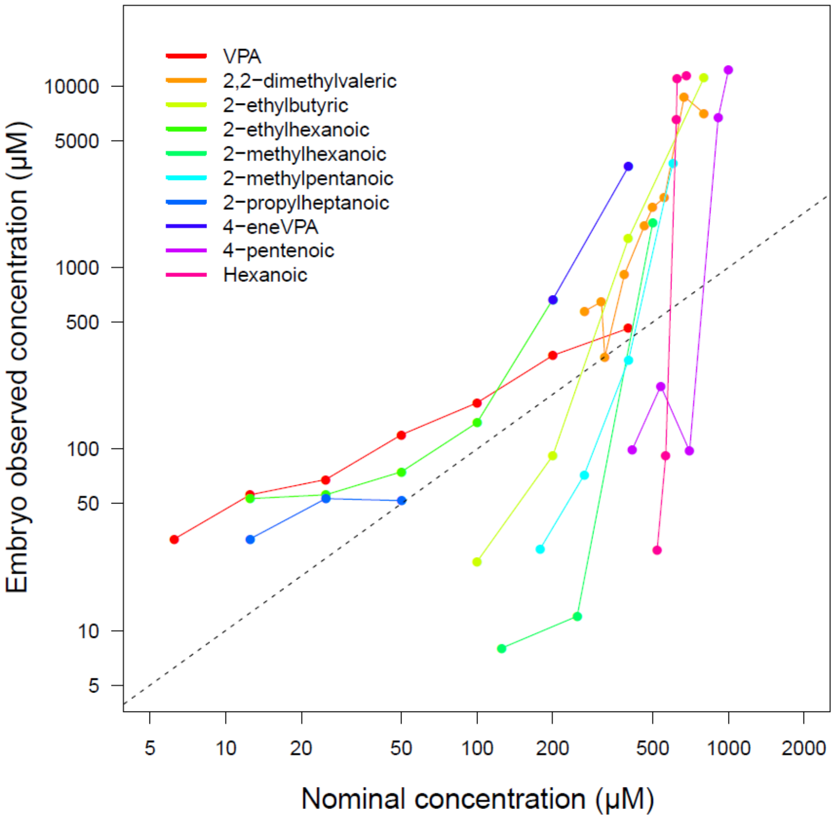

Figure S6: VPA and analogs observed concentration**s** in total embryo as a function of nominal concentration.

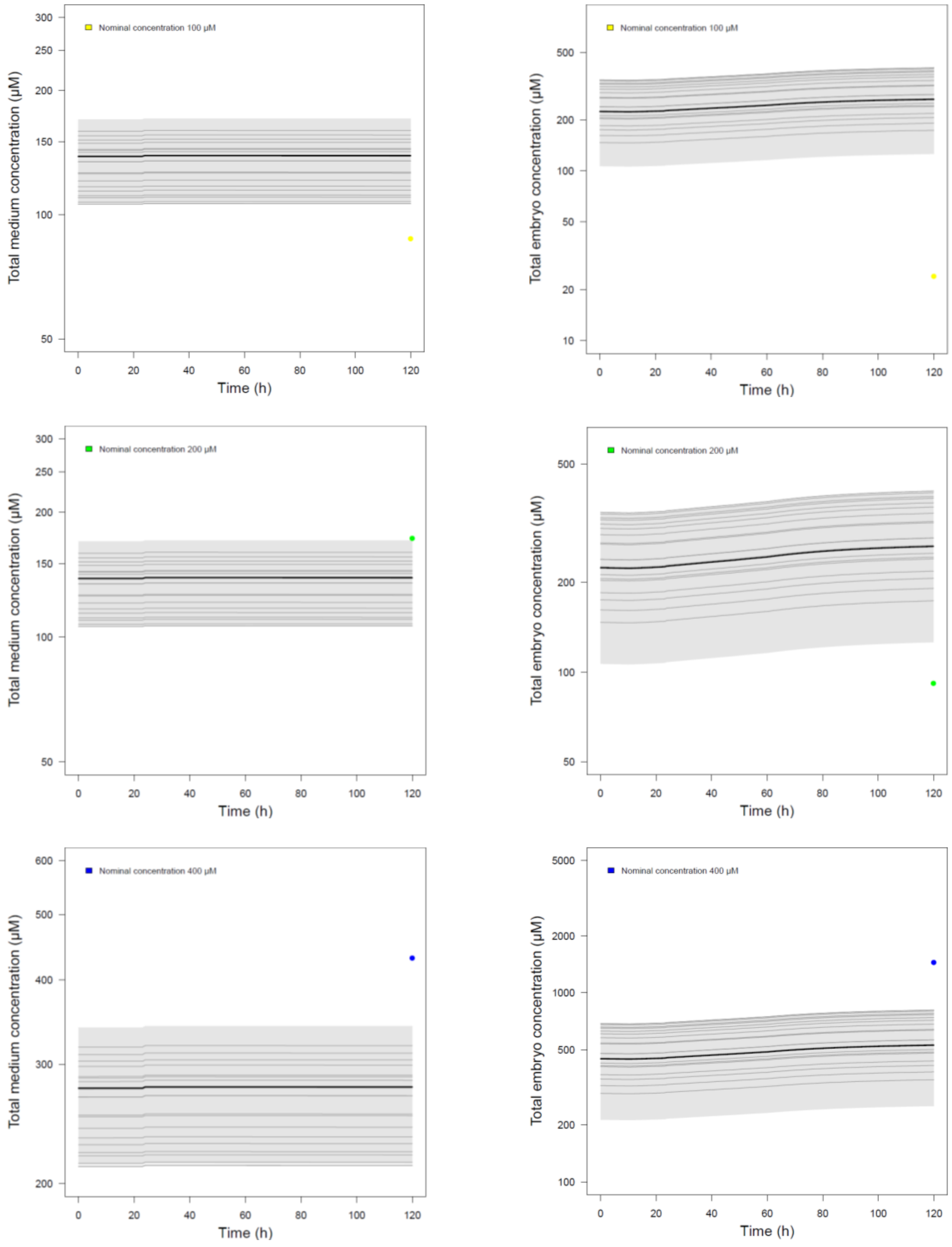

Figure S7: Predicted (lines) and observed (points) 2-ethylbutyric acid concentrations in medium (left) and in total embryo (right) as a function of time, after estimating V_{max}, K_m and f_{pc}, (*i.e*, using saturable Michaelis-Menten metabolism). Linearity is not guaranteed here, and there is one plot per nominal concentrations. The grey areas define the 95% confidence interval. The thick black lines are the maximum posterior predicted concentration time-courses. The thin lines are 20 predictions obtained using random parameter vectors drawn from their posterior distribution.

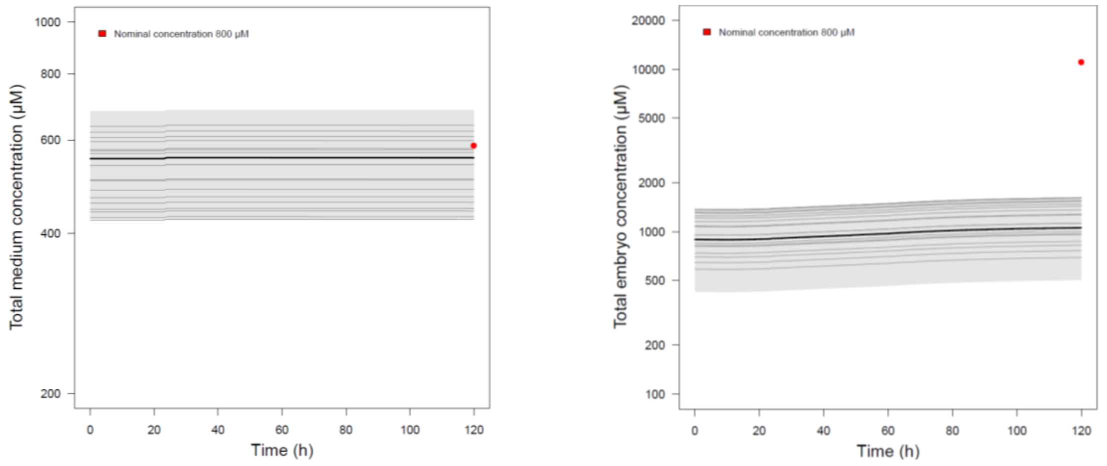

Figure S7 (followed).

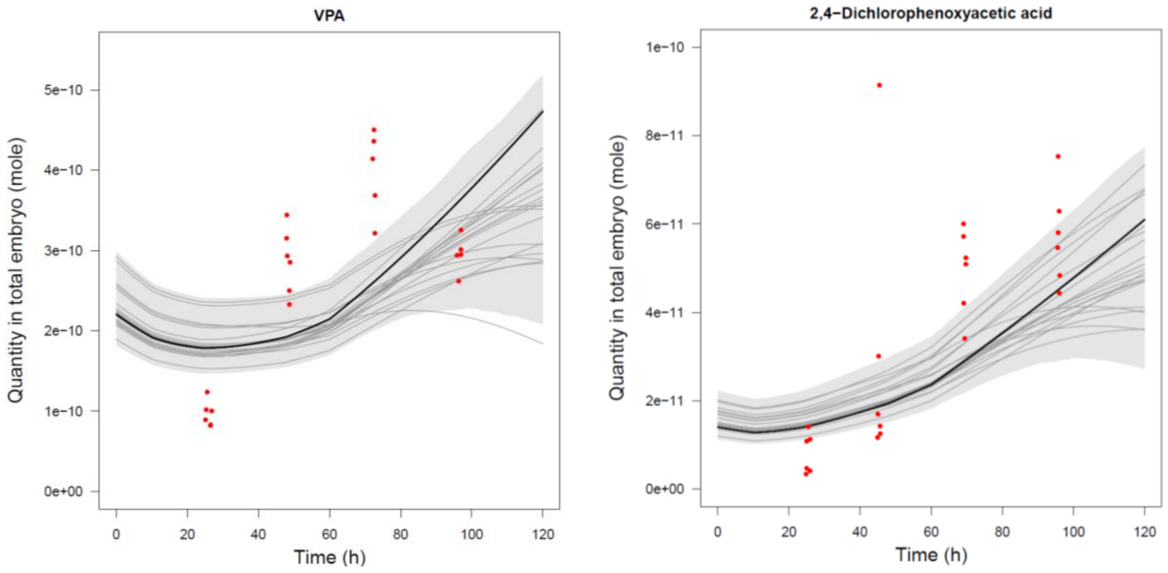

Figure S8: Predicted (lines) and observed (points) total quantities in embryo as a function of time for the 17 compounds studied by Brox *et al*. The grey area defines 95% confident interval. Bold black line is the maximum posterior predicted time-course quantities in the embryo and the other ones are 20 predicted quantity-time courses determined from different estimate parameter values randomly chosen from 1,000 iterations performed. The predictions are obtained with a simultaneous estimation of Cl_{met}, f_{pc} and $P_{a:w}$.

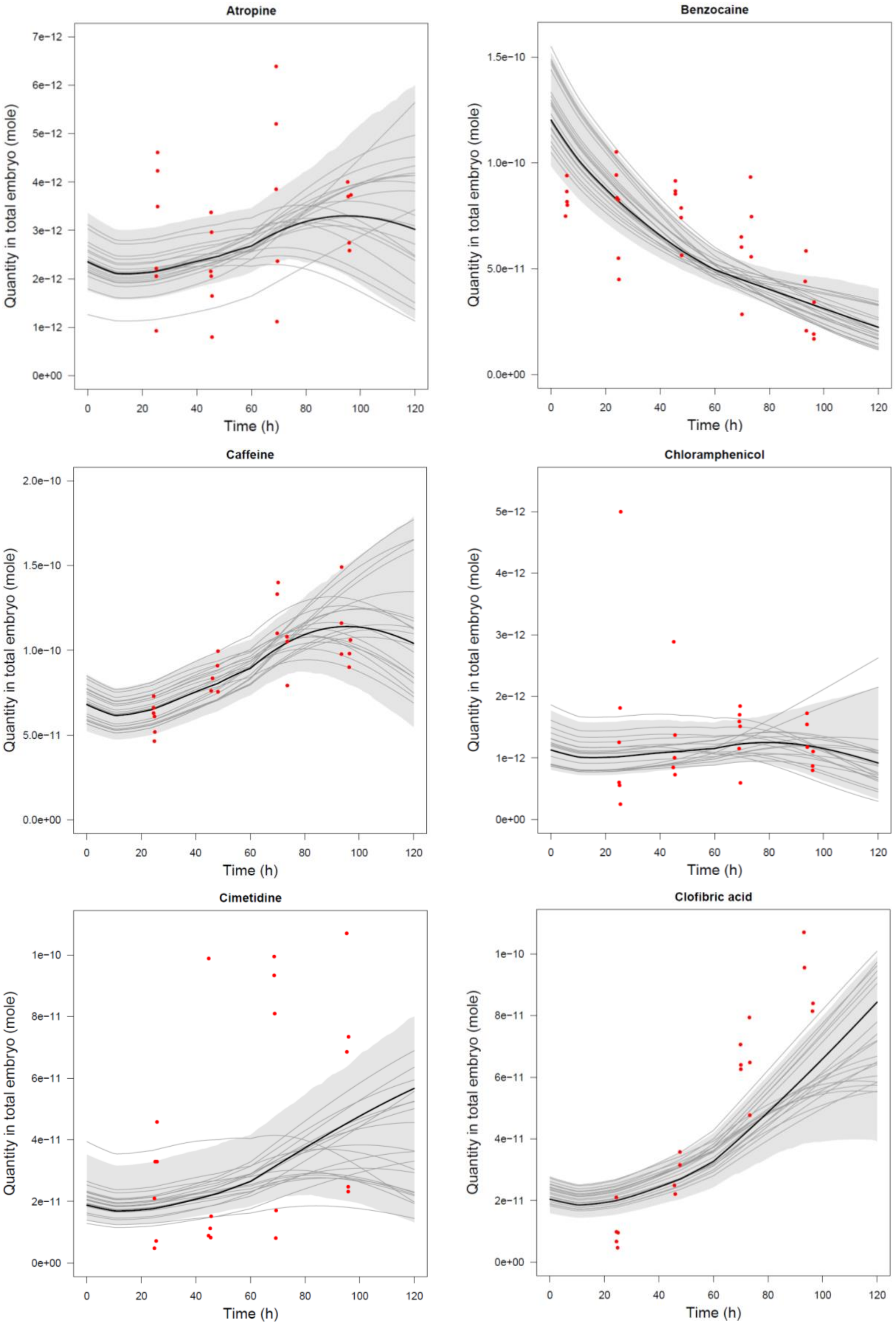

Figure S8 (followed).

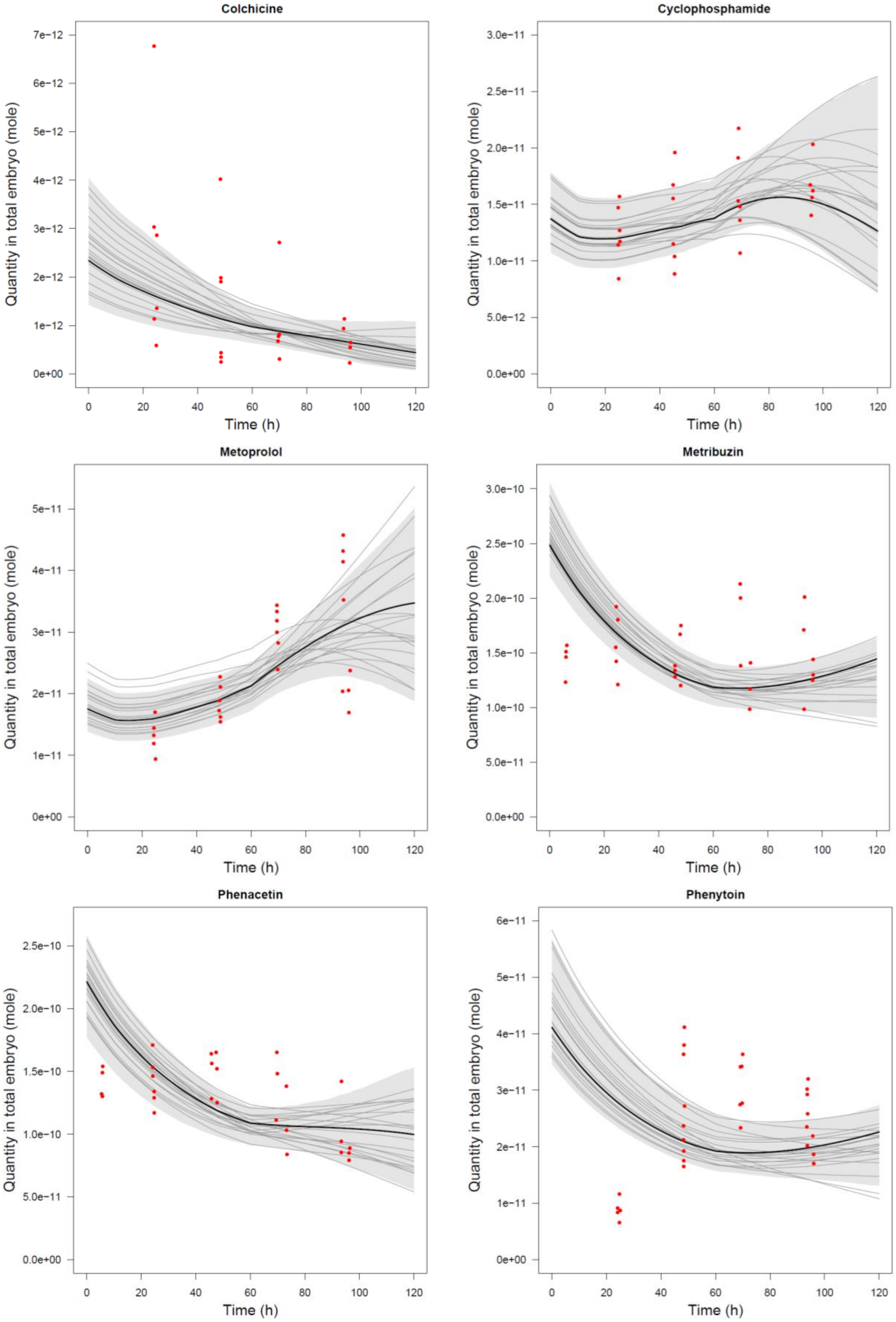

Figure S8 (followed).

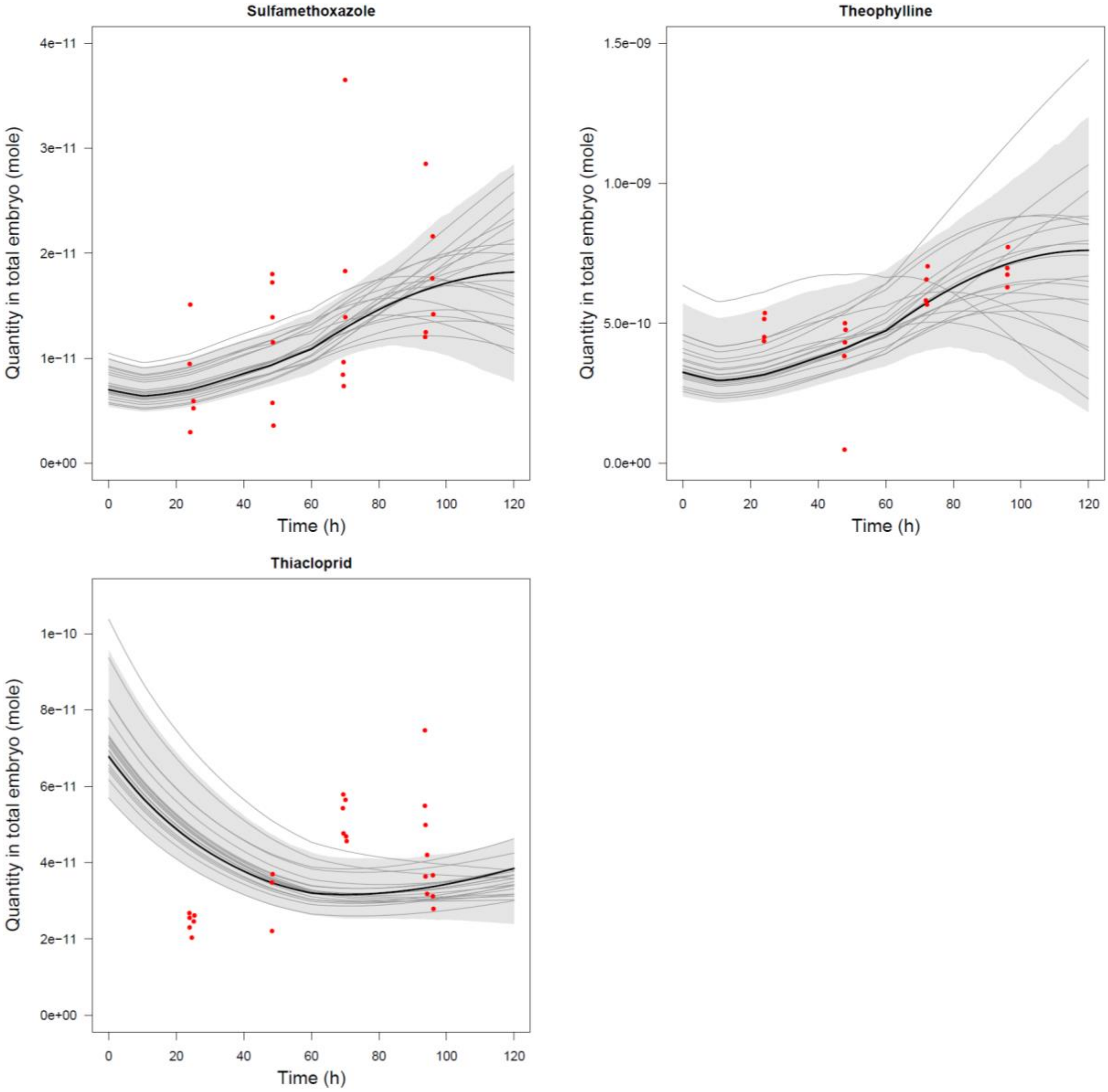

Figure S8 (followed).

Model code

```
States = {Q_labile,    # Sum of Q in medium and air (moles)
          Q_fixed,     # Sum of Q in plastic and embryo (moles)
          Q_met};      # Q of metabolites in system (moles)

Inputs = {Event_labile}; # Dummy variables for discontinuities

Outputs = {C_medium_u,  # Concentration of free parent in medium (mol/L)
           C_medium,    # ~              of parent in medium
           C_yolk,      # ~                        in yolk
           C_liver,     # ~                        in liver
           C_gut,       # ~                        in digestive tract
           C_muscle,    # ~                        in muscles
           C_skeleton,  # ~                        in skeleton
           C_eyes,      # ~                        in eyes
           C_brain,     # ~                        in brain
           C_heart,     # ~                        in heart
           C_skin,      # ~                        in skin
           C_others,    # ~                        in other tissues
           C_embryo,    # ~                        in embryo, not including yolk
           C_embryo_total,  # ~                    in embryo, including yolk
           C_lyso,      # ~                        in lysosomes
           C_mito,      # ~                        in mitochondria
           C_air,       # ~                        in air
           C_plastic,   # ~                        on plastic (mol/dm2)
           Q_check,     # Mass balance checking
           Q_medium,    # Quantity in medium (mol)
           Q_yolk,      # ~        in yolk
           Q_liver,     # ~        in liver
           Q_gut,       # ~        in digestive tract
           Q_muscle,    # ~        in muscles
           Q_skeleton,  # ~        in skeleton
           Q_eyes,      # ~        in eyes
           Q_brain,     # ~        in brain
           Q_heart,     # ~        in heart
           Q_skin,      # ~        in skin pigmented cells
           Q_others,    # ~        in other tissues
           Q_embryo,    # ~        in embryo, not including yolk
           Q_embryo_total,  # ~    in embryo, including yolk
           Q_lyso,      # ~        in lysosomes
           Q_mito,      # ~        in mitchondria
           Q_air,       # ~        in air
           Q_plastic,   # ~        bound on plastic
           Q_parent,    # Sum of Q in medium, air, plastic and embryo
           L_embryo,    # Length of the embryo (dm)
           V_yolk,      # Volume of yolk (L)
           V_embryo,    # ~         embryo
           V_embryo_total,  # ~     total embryo
           V_liver,     # ~         liver
           V_gut,       # ~         gut
           V_muscle,    # ~         muscles
           V_skeleton,  # ~         skeleton
           V_eyes,      # ~         eyes
           V_brain,     # ~         brain
           V_heart,     # ~         heart
           V_skin,      # ~         skin
           V_others,    # ~         others embryonic tissues
           V_air,       # ~         air in the system
           F_labile,    # Labile fraction (in medium and air) of Q_parent
           S_ medium};  # Surface area of medium in contact with wall (dm2)

# ==========
# Dose of parent (mol)
Dose;

# Number of embryos per well
N_embryo = 1;

# Volume of a culture well (L)
V_well;

# Volume of culture medium at time 0 (L)
V_medium;

# Starting times for organ growth (minutes)
tau_liver = 16 * 60;
tau_gut  = 10 * 60;
tau_skeleton = 48 * 60;
tau_eyes = 11 * 60;
tau_brain = 9   * 60;
tau_heart = 30 * 60;
tau_skin = 24 * 60;
tau_others = 0   * 60;
tau_muscle = 60 * 60;
```

```
# Organ growth rates (1/min), adjustment from rescaled organ volumes
# as fractions of total embryo (without yolk) at 120 hpf
K_g_liver = 5.29509e-06;
K_g_gut = 2.2779e-05;
K_g_skeleton = 1.82294e-05;
K_g_eyes = 1.20414e-05;
K_g_brain = 2.22253e-06;
K_g_heart = 3.01003e-06;
K_g_skin = 1.3672e-05;
K_g_others = 3.92738e-05;
K_g_muscle = 5.07074e-05;

# Yolk consumption rate constant (1/min)
K_d_yolk = 0.000313;

# Yolk volume at time zero (L)
V_yolk_0 = 0.207E-6;

# Volume of the embryo without yolk at 0 and 120 hpf (L)
V_embryo_0 = 0.005E-6;
V_embryo_120 = 3.70597E-07;

# Flag for Michaelis-Menten vs linear metabolism (0 = linear)
Michaelis = 0;

# Metabolic clearance per liver cell, if linear metabolism, L/min
K_met;

# Maximum formation rate of metabolite, if saturable metabolism, mol/min
Vmax;

# Michaelis-Menten constant, if satutable metabolism, mol/L
Km;

# Diameter of a well (dm)
D_well;

# Preincubation to saturate plastic binding (yes=1, no=0)
preincubation = 0;

# Volume of a liver cell (L)
V_cell_liver = 1e-12;

# Fraction (v/v) lysosome in cells
f_lyso = 0.01;

# Fraction (v/v) mitochondria in cells
f_mito = 0.1;

# Fraction unbound in medium, corrected for serum dilution
fu_diluted;

# Fraction unionized in medium
fuu;

# Polymer / water partition coefficient (dm)
P_pw;

# Correction factor for partition coefficients
ffpc = 1;

# Statistical parameter
sigma;

# ==========
# Parameters recomputed in initialize section
# Air / water partition coefficient
P_aw;

# Medium unbound / lysosome partition coefficient
P_mu_ly;

# Medium unbound / mitochondria partition coefficient
P_mu_mi;

# Medium unbound / yolk partition coefficient
P_mu_yolk;

# Medium unbound / liver partition coefficient
P_mu_liver;

# For other organs
P_mu_gut;
P_mu_muscle;
P_mu_skeleton;
P_mu_eye;
P_mu_brain;
P_mu_heart;
```

```
P_mu_skin;
P_mu_others;

# ==========
# Parameters computed in initialize section
# Volume of well per embryo (L)
V_well_embryo;

# Volume of medium per embryo (L)
V_medium_embryo;

# Surface area of medium in contact with plastic without embryo in the
# well (dm2)
S_medium_0;

# Concentration unbound in medium without embryo in the well (M)
C_medium_u_0;

# ==========
Initialize {
  P_mu_yolk     = P_mu_yolk     * ffpc;
  P_mu_liver    = P_mu_liver    * ffpc;
  P_mu_gut      = P_mu_gut      * ffpc;
  P_mu_muscle   = P_mu_muscle   * ffpc;
  P_mu_skeleton = P_mu_skeleton * ffpc;
  P_mu_eye      = P_mu_eye      * ffpc;
  P_mu_brain    = P_mu_brain    * ffpc;
  P_mu_heart    = P_mu_heart    * ffpc;
  P_mu_skin     = P_mu_skin     * ffpc;
  P_mu_others   = P_mu_others   * ffpc;
  P_mu_ly       = P_mu_ly       * ffpc;
  P_mu_mi       = P_mu_mi       * ffpc;
  P_aw          = P_aw          * ffpc;

  V_well_embryo    = V_well   / N_embryo;
  V_medium_embryo  = V_medium / N_embryo;

  S_medium_0   = 4 * V_medium_embryo / D_well + 3.14159 * 0.25 * D_well * D_well;
  C_medium_u_0 = Dose / (V_medium_embryo / fu_diluted);
  C_plastic_0  = preincubation * P_pw * C_medium_u_0;
  Q_plastic_0  = C_plastic_0 * S_medium_0;

  Q_parent = (Dose / N_embryo) + Q_plastic_0;

  V_embryo_total = V_yolk_0 + V_embryo_0;
  V_content = V_medium_embryo + V_embryo_total;

  S_medium   = 4 * V_content / D_well + 3.14159 * 0.25 * D_well * D_well;
  V_air      = V_well_embryo - V_medium_embryo - V_embryo_total;
  C_medium_u = Q_parent / (V_medium_embryo / fu_diluted + P_aw * fuu * V_air +
               P_pw * S_medium + P_mu_yolk * V_yolk_0);

  C_air      = P_aw * C_medium_u * fuu;

  # Labile fraction (in medium and air) of Q_parent
  f_labile        = (C_medium_u / fu_diluted * V_medium_embryo +
                     C_air * V_air) / (Q_parent > 0 ? Q_parent : 1.0);
  Q_labile        = f_labile * Q_parent;
  Q_fixed         = (1 - f_labile) * Q_parent;
}

Dynamics {
  sc_liver    = (t < tau_liver    ?
                0 : exp(K_g_liver    * (t - tau_liver   )) - 1);
  sc_gut      = (t < tau_gut      ?
                0 : exp(K_g_gut      * (t - tau_gut     )) - 1);
  sc_skeleton = (t < tau_skeleton ?
                0 : exp(K_g_skeleton * (t - tau_skeleton)) - 1);
  sc_eyes     = (t < tau_eyes     ?
                0 : exp(K_g_eyes     * (t - tau_eyes    )) - 1);
  sc_brain    = (t < tau_brain    ?
                0 : exp(K_g_brain    * (t - tau_brain   )) - 1);
  sc_heart    = (t < tau_heart    ?
                0 : exp(K_g_heart    * (t - tau_heart   )) - 1);
  sc_skin     = (t < tau_skin     ?
                0 : exp(K_g_skin     * (t - tau_skin    )) - 1);
  sc_others   = (t < tau_others   ?
                0 : exp(K_g_others   * (t - tau_others  )) - 1);
  sc_muscle   = (t < tau_muscle   ?
                0 : exp(K_g_muscle   * (t - tau_muscle  )) - 1);

  # Organ volumes in L:
  V_liver    = V_embryo_120 * sc_liver;
  V_gut      = V_embryo_120 * sc_gut;
  V_skeleton = V_embryo_120 * sc_skeleton;
  V_eyes     = V_embryo_120 * sc_eyes;
  V_brain    = V_embryo_120 * sc_brain;
  V_heart    = V_embryo_120 * sc_heart;
```

```
  V_skin     = V_embryo_120 * sc_skin;
  V_others   = V_embryo_120 * sc_others;
  V_muscle   = V_embryo_120 * sc_muscle;

  # Volume of the embryo without yolk
  V_embryo = V_embryo_120 * (sc_liver + sc_gut + sc_skeleton + sc_eyes +
                             sc_brain + sc_heart + sc_skin + sc_others +
                             sc_muscle) + V_embryo_0;
  V_yolk   = V_yolk_0 * exp(-K_d_yolk * t);

  V_embryo_total = V_yolk + V_embryo;

  V_content = V_medium_embryo + V_embryo_total;

  S_medium = 4 * V_content / D_well;

  # Volume of air in head space (L)
  V_air = V_well_embryo - V_content;

  # Sum of Q in medium, air, plastic and embryo
  Q_parent = Q_labile + Q_fixed;

  # Concentration unbound in medium (mol/L)
  C_medium_u = Q_parent /
               (V_medium_embryo / fu_diluted +
                P_aw * fuu * V_air  +
                P_pw * S_medium  +
                P_mu_yolk     * V_yolk +
                P_mu_liver    * V_liver +
                P_mu_gut      * V_gut +
                P_mu_muscle   * V_muscle +
                P_mu_skeleton * V_skeleton +
                P_mu_eye      * V_eyes +
                P_mu_brain    * V_brain +
                P_mu_heart    * V_heart +
                P_mu_skin     * V_skin +
                P_mu_others   * V_others);

  # Concentration in liver (mol/L), null before organogenesis
  C_liver = (V_liver > 0 ? P_mu_liver * C_medium_u : 0);

  # Labile fraction (in medium and air) of Q_parent
  f_labile = (C_medium_u / fu_diluted * V_medium_embryo + C_air * V_air) /
             (Q_parent > 0 ? Q_parent : 1.0);

  # Linear metabolism in embryo, V_liver / V_cell_liver
  N_cells   = V_liver / V_cell_liver;
  dt(Q_met) = (Michaelis > 0.5 ?
               N_cells * C_liver * Vmax / (Km + C_liver) : # MM
               N_cells * C_liver * K_met); # linear

  dt(Q_labile) = - f_labile * dt(Q_met);
  dt(Q_fixed)  = - (1 - f_labile) * dt(Q_met);
}

CalcOutputs {
  # Concentrations (mol/L)
  C_air      = P_aw * C_medium_u * fuu;
  C_plastic  = P_pw * C_medium_u;
  C_medium   = C_medium_u / fu_diluted;
  C_yolk     = P_mu_yolk * C_medium_u;

  C_gut      = (V_gut      > 0 ? P_mu_gut      * C_medium_u : 0);
  C_muscle   = (V_muscle   > 0 ? P_mu_muscle   * C_medium_u : 0);
  C_skeleton = (V_skeleton > 0 ? P_mu_skeleton * C_medium_u : 0);
  C_eyes     = (V_eyes     > 0 ? P_mu_eye      * C_medium_u : 0);
  C_brain    = (V_brain    > 0 ? P_mu_brain    * C_medium_u : 0);
  C_heart    = (V_heart    > 0 ? P_mu_heart    * C_medium_u : 0);
  C_skin     = (V_skin     > 0 ? P_mu_skin     * C_medium_u : 0);
  C_others   = (V_others   > 0 ? P_mu_others   * C_medium_u : 0);
  C_lyso     = P_mu_ly * C_medium_u;
  C_mito     = P_mu_mi * C_medium_u;

  # Quantities (mol)
  Q_medium   = C_medium   * V_medium_embryo;
  Q_yolk     = C_yolk     * V_yolk;
  Q_liver    = C_liver    * V_liver;
  Q_gut      = C_gut      * V_gut;
  Q_muscle   = C_muscle   * V_muscle;
  Q_skeleton = C_skeleton * V_skeleton;
  Q_eyes     = C_eyes     * V_eyes;
  Q_brain    = C_brain    * V_brain;
  Q_heart    = C_heart    * V_heart;
  Q_skin     = C_skin     * V_skin;
  Q_others   = C_others   * V_others;
  Q_air      = C_air      * V_air;
  Q_plastic  = C_plastic  * S_medium;
  Q_lyso     = C_lyso     * V_embryo * f_lyso;
```

```
  Q_mito     = C_mito     * V_embryo * f_mito;
  Q_embryo   = Q_liver + Q_gut + Q_muscle + Q_skeleton + Q_eyes + Q_brain +
               Q_heart + Q_skin + Q_others;

  Q_embryo_total = Q_embryo + Q_yolk;
  C_embryo       = Q_embryo / V_embryo;
  C_embryo_total = Q_embryo_total / V_embryo_total;
  C_medium       = (C_medium       > 0 ? C_medium       : 1E-30);
  C_embryo_total = (C_embryo_total > 0 ? C_embryo_total : 1E-30);

  Q_parent       = Q_labile + Q_fixed;
  Q_check        = Q_medium + Q_embryo + Q_yolk + Q_air + Q_plastic + Q_met;

  # Length of the embryo (dm), calibrated with data from Kimmel 1995
  A = 2.6039428;
  B = 4.3973752;
  C = 26.9499832;
  D = 0.7551494;
  L_embryo = (A * pow(t/60, B) / (pow(C, B) + pow(t/60, B)) + D) / 100;
}

End.
```