Supplemental Material for

Drug Metabolism and Disposition

An investigation into the prediction of the plasma concentration-time profile and its inter-individual variability for a range of flavin-containing monooxygenase substrates using a mechanistic physiologically based pharmacokinetic modelling approach

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Supplementary Table 1: Human *in vitro* data for FMO substrates (Jones et al., 2017)

	Human hepatocytes				Human liver microsomes				rFMO						Fm (% FMO) contribution
	Clint (µl/min/10^6 cells)	Fu inc[a]	Predicted Clint u (ml/min/kg)	Predicted Clearance (ml/min/kg)	Clint (µl/min/mg)	Fu mic	Predicted Clint u (ml/min/kg)	Predicted Clearance (ml/min/kg)	Clint (µl/min/pmol) with [ISEF]			Clint (µl/min/mg)[c]			
									FMO1	FMO3	FMO5	FMO1	FMO3	FMO5	
Benzydamine	9	0.74	104	11	18	0.35	148	13	0.435 [0.9]	0.288 [0.9]	0.001 [>100]	261	92	1	53
Imipramine	9	0.65	122	13	14	0.31	129	13	0.163 [1.6]	0.013 [13.6]	0.001 [>100]	98	4	2	21
Olanzapine	2	0.86	18	5	<3[b]	0.73	12	4	0.036 [1.8]	0.005 [9.4]	-	21	1	-	23[#]
Ranitidine	0.6*	0.98	5	4	<3[b]	0.97	9	6	0.011 [5.6]	0.003 [14.5]	0.028 [5]	7	1	47	26[#]
Moclobemide	3	0.87	30	11	3	0.98	9	5	0.018 [5.6]	0.031 [2.1]	0.003 [71]	11	10	5	38
Itopride	11	0.88	107	14	18	1.05	49	10	0.340 [1.4]	0.497 [0.6]	-	204	159	-	96
Clozapine	5	0.33	131	10	18	0.30	173	11	0.031 [9.9]	-	-	18	-	-	23
Tamoxifen	4	0.003	9706	3	4	0.02	662	0.2	0.083 [1.4]	-	-	50	-	-	28[#]
Tozasertib	29	0.21	1177	19	61	0.18	971	18	0.244 [5.6]	0.481 [1.9]	0.0004 [>100]	146	154	1	38

All data n = 3 except for data marked with * which is n = 2; [a] Data from rat hepatocytes; [b] Value of 3µl/min/mg used in calculations

**ISEF calculated using formula for each isoform= $ISEF = \frac{CLint,u\ in\ HLM\ (\frac{ul}{min}) \Big/ HLM\ FMO\ abundance\ (\frac{pmol}{mg})}{CLint,u\ in\ rFMO\ (\frac{ul}{min}/pmol)}$

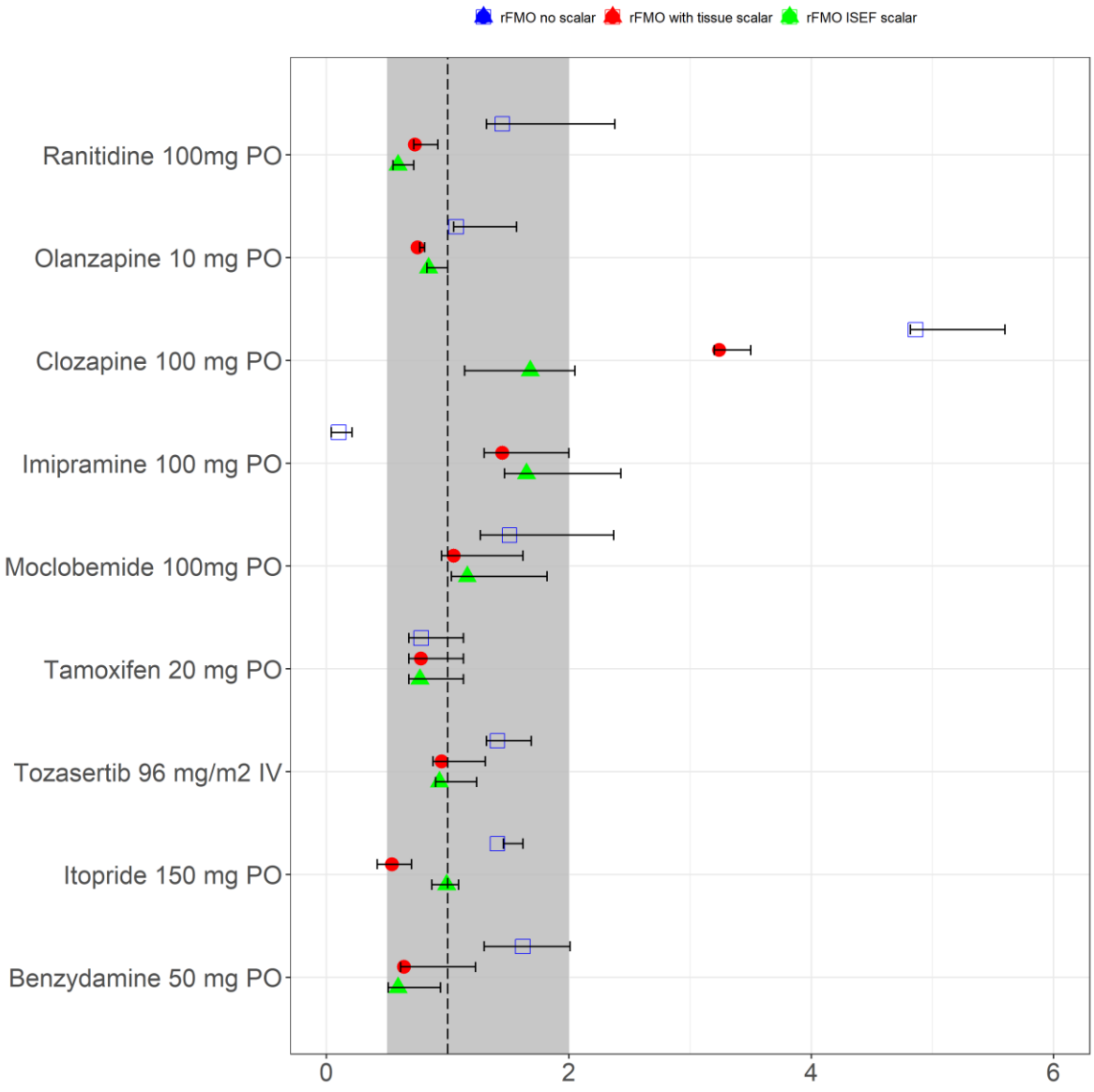
[#] It was not possible to determine the FMO contribution for ranitidine, olanzapine, and tamoxifen due to the low intrinsic clearance. However, an approximate FMO contribution was derived by fitting of the available rFMO CLint and FMO contribution data.

[c] Data was corrected for in-vitro FAD content.

Supplementary Table 2: Options used to define the elimination within PBPK platform (Simcyp V16)

Elimination option	Hepatocyte Data	In-Vitro HLM Data	In-Vitro rFMO Data
Option used	Whole organ metabolic clearance	Whole organ metabolic clearance or enzyme kinetics via HLM option to take into account of non-cyp mediated pathways	Enzyme kinetics data of rFMO was used. For remaining metabolic CL was added as a HLM CL
FMO expression assigned	Default, not linked to specific enzyme	Unused UGT used as an user defined option within Simcyp	Unused UGT used as an user defined option within Simcyp (see Itopride workspace) Scalar value (ISEF or tissue specific value was optimized for rFMO data A tutorial video (entitled Elimination Route UGT- Part 2) in the E-learning tab of the Consortium Members' area facility of Certara shows the utilization of unused UGT enzyme and linking it to the abundance in population and tissue (https://members.simcyp.com/) https://members.simcyp.com/account/elearning/stream/?id=fd410b95-4fff-42e4-ac4b-e01f04a9b13a

Supplementary Figure 1: Forest plot showing the physiologically based pharmacokinetic modeling performance of FMO substrates using rFMO data with and without scalar (tissue specific or ISEF) in the model



Supplementary Table 3: Simulated mean (SD) PK parameters for FMO substrates using HLM or rFMO with ISEF or tissue specific scalar data

Drug	Route	Dose (mg)	HLM			rFMO tissue scalar			rFMO ISEF scalar		
			AUC [ng*h/ml]	Cmax [ng/ml]	CL [L/h]	AUC [ng*h/ml]	Cmax [ng/ml]	CL [L/h]	AUC [ng*h/ml]	Cmax [ng/ml]	CL [L/h]
Benzydamine	PO	50	5329 (2411)	416 (135)	13 (6)	3441 (2296)	378 (135)	14 (9)	2931 (1720)	345 (126)	17 (11)
Itopride	PO	150	5192 (1840)	1090 (194)	34 (15)	1161 (395)	828 (175)	129 (56)	2142 (553)	1112 (201)	70 (25)
Tozasertib	IV	96	88109 (2088)	3480 (798)	50 (11)	73764 (17263)	2948 (674)	57 (12)	73763 (15474)	2888 (612)	58 (13)
Tamoxifen	PO	30	2521 (1579)	116 (27)	9 (3)	1831 (436)	42 (11)	17 (5)	1788 (437)	43 (11)	17 (5)
Moclobemide	PO	100	1509 (497)	417 (140)	74 (28)	1653 (437)	478 (124)	61 (18)	1822 (468)	499 (130)	55 (16)
Imipramine	PO	100	2021 (740)	51 (21)	57 (24)	1433 (810)	44 (21)	97 (12)	1625 (957)	46 (22)	84 (51)
Clozapine	PO	100	6080 (2487)	566 (216)	19 (8)	12931 (5195)	672 (233)	9 (6)	6698 (3252)	572 (212)	20 (14)
Olanzapine	PO	10	819 (226)	22 (6)	13 (4)	615 (210)	20 (6)	19 (8)	691 (224)	21 (6)	16 (6)
Ranitidine	PO	100	1960 (711)	366 (103)	56 (18)	1265 (628)	238 (97)	79 (42)	1024 (485)	207 (93)	67 (74)

*Cmax = conc. at end of infusion, [&] AUC (0-4hr) to match the reported AUC; PO: Per oral; IV Intravenous; SS: Steady-state; QD: once daily, BID: Twice daily; heps, hepatocytes; SD: standard deviation

Supplementary Table 4: Paediatrics simulations for Itopride using rFMO data

Age group	***FMO1 Expression****	***FMO3 Expression****	***Itopride dose, mg QD***	***Mean AUC (0-24), h·ng/mL***
Adults	1**	71±41 ***	150	2154
11–17 years	0.1±0.3	26.9±8.6	40	2435
10 months to 11 years	2.0±1.8	12.7±8.0	12	2281
3 weeks to <10 months	3.8±2.6	4.7±5.9	2.8	2006
0–3 weeks	7.8±5.3	1.1±3.3	1.4	2044

FMO expression data for age 0 to 17 years is from Koukouritaki et al., (Koukouritaki et al., 2002)

** (Cashman and Zhang, 2006)

*** (Haining et al., 1997; Overby et al., 1997)

Supplementary Table 5: Mass Spectrometer parameters for FMO substrates

Analyte	**MRM (Parent→Daughter) m/z**	**Dwell (s)**	**Cone voltage (V)**	**Collision energy (V)**
Tamoxifen	372.166→129.175	0.080	10	25
Moclobemide	269.053→182.073	0.080	10	15
Olanzapine	313.127→84.118	0.080	10	25
Imipramine	281.178→86.025	0.080	60	15
Tozasertib	465.195→190.215	0.080	20	40
Ranitidine	315.095→176.243	0.040	10	15
Benzydamine	310.173→86.065	0.080	60	30
Clozapine	327.14→270.16	0.080	40	20
Itopride	359.22→71.94	0.80	10	40
Verapamil (IS)	455.198→165.207	0.020	15	25

Supplementary Text:

LC-MS/MS analysis:

The concentration of all compounds in the incubations was determined by LC-MS/MS. An Acquity ultra performance liquid chromatography (UPLC) system, (Waters, UK) coupled to a triple-quadrupole mass spectrometer (Xevo TQ-S; Waters, Milford, MA) was used to carry out the sample analysis. The analytes were separated by reverse-phase liquid chromatography using a Waters Atlantis® T3, 3µm, 2.1X50mm column (Waters, UK). Mobile phases A and B consisted of water (containing 0.1% FA v/v) and ACN (containing 0.1% FA v/v), respectively. The flow rate was held constant at 0.73 ml/min throughout the gradient run. The initial mobile phase composition of 95% A and 5% B was held for 0.3 minutes. Mobile phase B was then increased linearly to 70% until 1.5 minutes, followed by further increase to 99% B until 1.59 minutes. At 1.6 minutes the composition of A and B was reversed to the initial 95% A and 5% B and was held until 2 minutes. Analyte quantitation was achieved by MS–MS detection in positive electrospray ionization mode. The MS operating conditions were as follows: the capillary voltage was 1.14 kV and source offset was 50 V. The desolvation temperature was set to 600 °C. Nitrogen was used as the desolvation gas (800 L/Hr) and cone gas (150 L/Hr). Argon was used as the collision gas at a flow rate of 0.15 ml/min. Detection of the ions was performed in the MRM mode using the transitions described in Supplementary Table 5. Peak integration and calibrations were performed using TargetLynx software (Version 4.1, Waters, Milford, MA).

References

Cashman JR and Zhang J (2006) Human flavin-containing monooxygenases. *Annu Rev Pharmacol Toxicol* **46:**65-100.

Haining RL, Hunter AP, Sadeque AJ, Philpot RM, and Rettie AE (1997) Baculovirus-mediated expression and purification of human FMO3: catalytic, immunochemical, and structural characterization. *Drug Metab Dispos* **25:**790-797.

Jones BC, Srivastava A, Colclough N, Wilson J, Reddy VP, Amberntsson S, and Li D (2017) An Investigation into the Prediction of in Vivo Clearance for a Range of Flavin-containing Monooxygenase Substrates. *Drug Metab Dispos* **45:**1060-1067.

Koukouritaki SB, Simpson P, Yeung CK, Rettie AE, and Hines RN (2002) Human hepatic flavin-containing monooxygenases 1 (FMO1) and 3 (FMO3) developmental expression. *Pediatr Res* **51:**236-243.

Overby LH, Carver GC, and Philpot RM (1997) Quantitation and kinetic properties of hepatic microsomal and recombinant flavin-containing monooxygenases 3 and 5 from humans. *Chem Biol Interact* **106:**29-45.