SUPPLEMENTARY

When does the rate-determining step in the hepatic clearance of a drug switch from sinusoidal uptake to all hepatobiliary clearances? Implications for predicting drug-drug interactions

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SUPPLEMENTARY METHODS

Applying the RDS framework to drugs with published *in vitro* hepatobiliary clearances

Published hepatobiliary clearances (Camenisch and Umehara, 2012; Jones et al., 2012; Varma et al., 2014; Kunze et al., 2015; Riede et al., 2016) that were measured in vitro using HLMs (for CL_{met}) and hepatocytes (for CL^{s}_{in}, CL^{s}_{ef}, CL_{bile}) were scaled up to in vivo using IVIVE scaling factors (MPPGL, HPGL, liver weight, etc). For Varma et al., 2014, in vivo scale up was performed using the author's IVIVE scaling factors (118 10^6 hepatocytes/g liver, 39.8 mg microsomal protein/g liver, 24.5 g liver/kg body weight). Camenisch and Umehara, 2012, Jones et al., 2012, Kunze et al., 2015, and Riede et al., 2016 reported in vivo scaled up values. CL^{s}_{in} was quantified using SCHH in Varma et al,. 2014 and Jones et al., 2012 and suspended hepatocytes in Camenisch et al,. 2012, Kunze et al. 2015, and Riede et al. 2016. Furthermore, active versus passive contribution for sinusoidal uptake was determined in the presence and absence of rifamycin (OATP inhibitor) in Varma et al,. 2014 and Jones et al. 2012 and at 37°C vs 4°C in Camenisch et al,. 2012, Kunze et al. 2015, and Riede et al. 2016. CL^{s}_{ef} is assumed to be equal to sinusoidal membrane passive diffusion, except in Camenisch et al,. 2012 where CL^{s}_{ef} is back-calculated from total CL_{int} in SCHH. CL_{met} is quantified in pooled HLM's and CL_{bile} is quantified in SCHH using similar experimental procedures in all references. Fraction transported (ft) was calculated as active sinusoidal transport CL divided by total sinusoidal uptake CL. Tipping point was calculated by inputting $fu_b CL^{s}_{in} / Q_h$ into Eq. 2. Note that the fu_b as reported in each reference was used and this may differ for the same drug among the different reports. $PI_{met+bile}$ was calculated using Eq. 5 for drugs that had $CL_{met+bile}/CL^{s}_{ef}$ ratio greater than the tipping point (i.e. RDS_{uptake}). Classification of the RDS of drugs using the RDS framework presented (flowchart in Fig. 5) and via the Extended Clearance Classification System (ECCS) (Varma et al., 2015) or Extended Clearance Concept Classification System (ECCCS) (Camenisch and Umehara, 2012) is provided when available.

Supplementary Table 1. Applying the RDS framework to drugs with published *in vitro* hepatobiliary clearances

Drug	CL^{s}_{in} (ml/min/kg)	CL^{s}_{ef} (ml/min/kg)	CL_{met} (ml/min/kg)	CL_{bile} (ml/min/kg)	ft	$fu_b CL^{s}_{in}$ /Qh	$CL_{met+bile}$ /CL^{s}_{ef}	Tipping point	$PI_{met+bile}$	RDS	ECCS[e]	ECCCS[f]	REF
Aliskiren	58	134	89	31	0.56	1.95	0.90	1.37	–	all	n.d.	4	Camenisch and Umehara, 2012
	58	25	89	31	0.56	1.95	4.74	1.37	>71%	uptake		3	Riede et al,. 2017
Atorvastatin	61	25	58	4.3	0.59	0.12	2.52	3.59	–	all	1B	4	Varma et al,. 2014
	198	359	65	12	0.71	0.80	0.21	2.22	–	all			Camenisch and Umehara, 2012
	198	58	65	12	0.71	0.77	1.32	2.27	–	all			Kunze et al,. 2015
	1194[a]	25	58	4.3	0.98	2.27	2.52	1.22	>51%	uptake			Varma et al., 2014
	405[b]	25	58	4.3	0.94	0.77	2.52	2.26	>10%	uptake			Varma et al, 2014
Bosentan	132	29	20	5.8	0.78	0.36	0.87	2.95	–	all	1B	n.d.	Varma et al,. 2014
	35	12	n.d.	39	0.65	0.02	3.24	3.93	–	all			Jones et al., 2012
	142[a]	29	20	5.8	0.80	0.38	0.87	2.90	–	all			Varma et al,. 2014
	1117[b]	29	20	5.8	0.97	3.02	0.87	0.99	–	uptake			Varma et al,. 2014
	2035[c]	14	5.0[d]		0.99	1.09	0.36	1.91	–	all			Jones et al., 2012
Cerivastatin	99	51	31	0.6	0.49	0.09	0.63	3.67	–	all	1B	2	Varma et al,. 2014
	465	244	47	0.0	0.48	0.45	0.19	2.76	–	all			Kunze et al,. 2015
	87	63	n.d.	43	0.28	0.03	0.69	3.90	–	all			Jones et al., 2012
	658[a]	51	31	0.6	0.92	0.59	0.63	2.52	–	all			Varma et al,. 2014
	565[b]	51	31	0.6	0.91	0.50	0.63	2.66	–	all			Varma et al,. 2014
	3090[c]	36	13[d]		0.99	0.94	0.36	2.06	–	all			Jones et al., 2012
Cimetidine	6.6	3.6	529	0.2	0.45	0.27	147	3.16	>98%	uptake	n.d.	3	Camenisch and Umehara, 2012
Ciprofloxacin	30	14	22	0.0	0.23	0.99	1.57	2.01	–	all	3A	3	Camenisch and Umehara, 2012
	30	23	22	0.0	0.23	1.00	0.96	2.00	–	all		4	Riede et al,. 2017
Cyclosporine A	155	109	78	9.1	0.73	0.22	0.80	3.27	–	all	n.d.	4	Camenisch and Umehara, 2012

	155	42	78	9.1	0.73	0.22	2.07	3.27	–	all			Riede et al,. 2017
Digoxin	27	102	24	18	0.74	1.07	0.42	1.94	–	all	n.d.	4	Camenisch and Umehara, 2012
	27	6.9	24	18	0.74	1.07	6.17	1.94	>69%	uptake		3	Riede et al,. 2017
Fluvastatin	133	44	29	8.4	0.67	0.09	0.84	3.67	–	all	1B	2	Varma et al,. 2014
	544	326	147	0.0	0.40	1.05	0.45	1.95	–	all			Kunze et al,. 2015
	163	50	n.d.	115	0.70	0.06	2.33	3.79	–	all			Jones et al., 2012
	9079[a]	44	29	8.4	1.00	6.16	0.84	0.56	>33%	uptake			Varma et al,. 2014
	985[b]	44	29	8.4	0.96	0.67	0.84	2.40	–	all			Varma et al,. 2014
	18252[c]	35		20[d]	1.00	6.34	0.59	0.54	>7%	uptake			Jones et al., 2012
Furosemide	35	78	19	1.2	0.32	0.05	0.26	3.79	–	all	3A	4	Camenisch and Umehara, 2012
	35	24	19	1.2	0.32	0.05	0.85	3.81	–	all			Riede et al,. 2017
Glyburide	61	15	52	0.0	0.75	0.11	3.37	3.61	–	all	1B	n.d.	Varma et al,. 2014
	500 [b]	15	52	0.0	0.97	0.87	3.37	2.13	>37%	uptake			Varma et al,. 2014
Ketoconazole	1569	2576	97	30	0.00	1.21	0.05	1.81	–	all	n.d.	2	Camenisch and Umehara, 2012
	1569	1569	97	30	0.00	1.52	0.08	1.59	–	all			Riede et al,. 2017
Lovastatin Acid	311	146	459	0.0	0.53	1.20	3.15	1.82	>42%	uptake	n.d.	1	Kunze et al,. 2015
NVS 1	332	332	524	n.d.	0.00	0.80	1.58	2.22	–	all	n.d.	2	Riede et al,. 2017
NVS 2	115	115	30	n.d.	0.00	0.39	0.26	2.88	–	all	n.d.	2	Riede et al,. 2017
NVS 3	457	457	112	n.d.	0.00	0.44	0.24	2.77	–	all	n.d.	2	Riede et al,. 2017
NVS 4	407	407	236	n.d.	0.00	0.39	0.58	2.87	–	all	n.d.	2	Riede et al,. 2017
NVS 5	294	154	36	n.d.	0.48	4.27	0.23	0.76	–	all	n.d.	2	Riede et al,. 2017
NVS 6	300	300	82	3.2	0.00	1.16	0.28	1.85	–	all	n.d.	2	Riede et al,. 2017
NVS 7	94	94	207	n.d.	0.00	0.23	2.20	3.26	–	all	n.d.	3	Riede et al,. 2017
NVS 8	84	28	1.7	945	0.67	0.81	33.8	2.21	>93%	uptake	n.d.	3	Riede et al,. 2017

NVS 9	88	88	42	n.d.	0.00	0.09	0.48	3.69	–	all	n.d.	4	Riede et al,. 2017
NVS 10	4.5	2.0	0.7	n.d.	0.56	0.02	0.35	3.91	–	all	n.d.	4	Riede et al,. 2017
Pitavastatin	133	32	15	2.0	0.76	0.28	0.52	3.13	–	all	1B	n.d.	Varma et al,. 2014
	623	259	18	0	0.58	2.11	0.07	1.29	–	all			Kunze et al,. 2015
	1270[a]	32	15	2.0	0.97	2.64	0.52	1.10	–	all			Varma et al,. 2014
	1099[b]	32	15	2.0	0.97	2.29	0.52	1.22	–	all			Varma et al,. 2014
Pravastatin	5.2	1.2	0.0	1.2	0.78	0.21	1.00	3.30	–	all	3B	4	Varma et al,. 2014
	94	16	0.9	2.2	0.62	4.41	0.19	0.74	–	all			Camenisch and Umehara, 2012
	94	36	0.9	2.2	0.62	4.40	0.09	0.74	–	all			Kunze et al,. 2015
	4.8	0.3	n.d.	2.9	0.95	0.18	10.9	3.39	>69%	uptake			Jones et al., 2012
	80[a]	1.2	0.0	1.2	0.99	3.23	1.00	0.95	>5%	uptake			Varma et al,. 2014
	44[b]	1.2	0.0	1.2	0.97	1.79	1.00	1.44	–	all			Varma et al,. 2014
	98[c]	1.0	n.d.	0.4	0.99	3.69	0.36	0.85	–	all			Jones et al., 2012
Propranolol	577	194	111	6.8	0.52	3.09	0.61	0.98	–	all	2	2	Camenisch and Umehara, 2012
	577	276	111	6.9	0.52	3.07	0.43	0.98	–	all			Riede et al,. 2017
Quinidine	339	93	28	5.1	0.68	4.36	0.36	0.75	–	all	2	2	Camenisch and Umehara, 2012
	339	109	28	5.1	0.68	4.42	0.31	0.74	–	all			Riede et al,. 2017
Repaglinide	166	64	128	0.3	0.62	0.19	2.01	3.35	–	all	1B	n.d.	Varma et al,. 2014
	299	223	125	0.0	0.25	0.22	0.56	3.27	–	all			Jones et al., 2012
	1983[a]	64	128	0.3	0.97	2.32	2.01	1.21	>40%	uptake			Varma et al,. 2014
	1151[b]	64	128	0.3	0.94	1.35	2.01	1.71	>15%	uptake			Varma et al,. 2014
	3671[c]	352	125	0.0	0.90	2.73	0.35	1.07	–	all			Jones et al., 2012
Rosuvastatin	30	3.5	0.0	8.1	0.88	0.25	2.33	3.20	–	all	3B	4	Varma et al,. 2014
	52	25	1.5	5.7	0.52	0.43	0.29	2.80	–	all			Kunze et al,. 2015

	28	4.3	n.d.	3.8	0.84	0.22	0.89	3.27	–	all			Jones et al., 2012
	246[a]	3.5	0.0	8.1	0.99	2.06	2.33	1.31	>44%	uptake			Varma et al,. 2014
	282[b]	3.5	0.0	8.1	0.99	2.37	2.33	1.19	>49%	uptake			Varma et al,. 2014
	284[c]	0.4	n.d.	0	1.00	2.30	0.71	1.21	–	all			Jones et al., 2012
Simvastatin Acid	414	298	769	1.7	0.28	2.20	2.59	1.25	>52%	uptake	n.d.	1	Kunze et al,. 2015
	10	2.9	0.0	2.6	0.71	0.01	0.90	3.96	–	all			Varma et al,. 2014
	35	111	4.1	22	0.46	0.15	0.23	3.48	–	all			Camenisch and Umehara, 2012
	35	19	4.1	22	0.46	0.15	1.38	3.48	–	all			Riede et al,. 2017
Valsartan	6.8	1.5	n.d.	242	0.77	0.00	159	4.00	>97%	uptake	3B	4	Jones et al., 2012
	74 [a]	2.9	0.0	2.6	0.96	0.07	0.90	3.75	–	all			Varma et al,. 2014
	80[b]	2.9	0.0	2.6	0.96	0.07	0.90	3.75	–	all			Varma et al,. 2014
	592[c]	5.5	n.d.	6.0	0.99	0.05	1.09	3.80	–	all			Jones et al., 2012
Verapamil	258	8.7	128	8.1	0.00	1.62	15.6	1.53	>90%	uptake	2	2	Camenisch and Umehara, 2012
	258	258	128	8.1	0.00	1.62	0.53	1.53	–	all			Riede et al,. 2017

[a] Authors used individual empirical scaling factor (ranging from 1 to 101.8) for active sinusoidal uptake to match observed *in vivo* IV clearance assuming RDS_{uptake}

[b] Authors used geometric mean empirical scaling factor (10.6) for active sinusoidal uptake

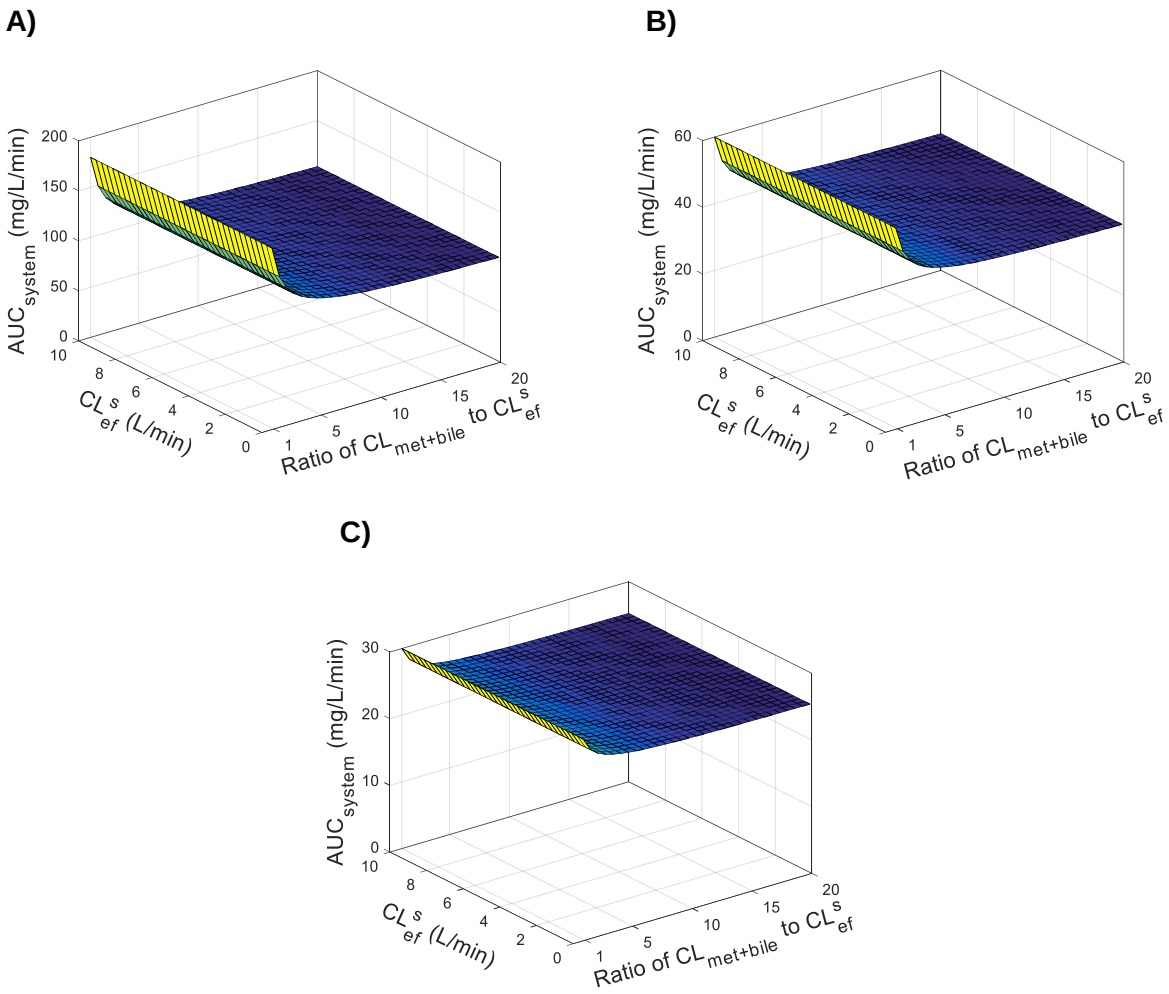
[c] Parameters estimated using a PBPK model and IV data where all parameters were fixed except for active uptake clearance, passive diffusion, and $CL_{met+bile}$

[d] Composite of $CL_{met+bile}$

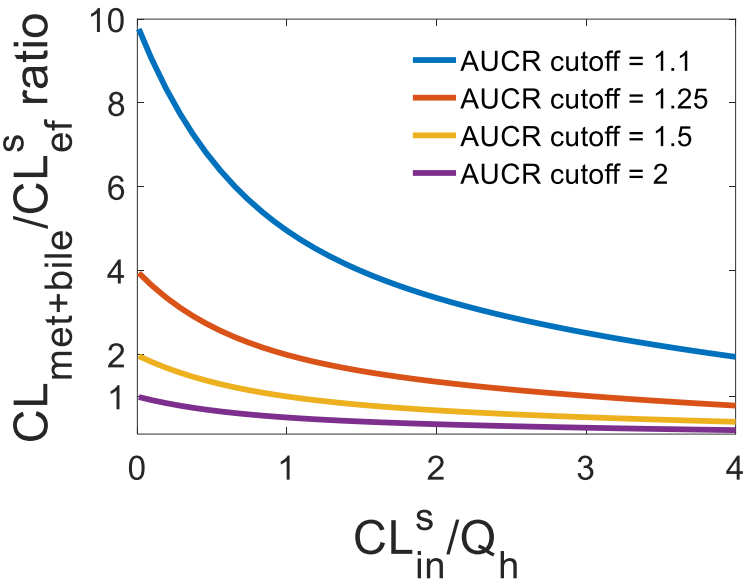
[e] ECCS classes: 1A – metabolism, 1B – uptake, 2 – metabolism, 3A – renal, 3B – uptake or renal, 4 – renal

[f] ECCCS classes: 1 – passive diffusion, 2 – metabolism + biliary efflux, 3 – uptake, 4 – all hepatobiliary pathways

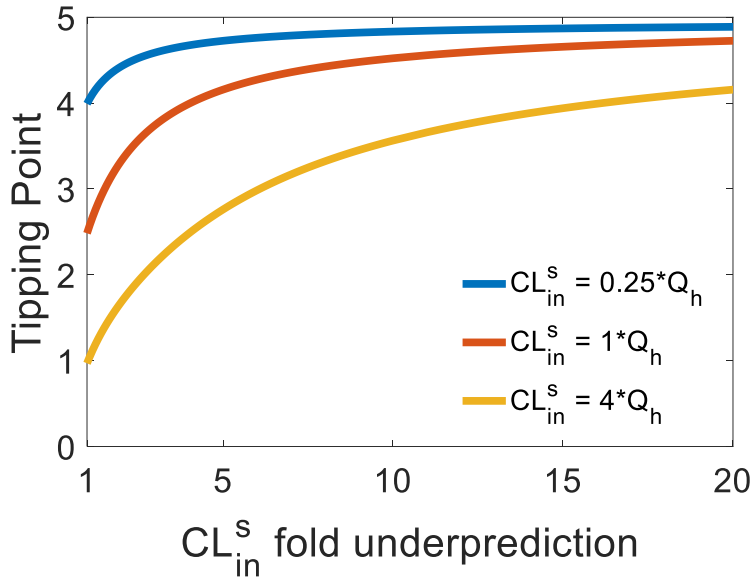
n.d. - not determined



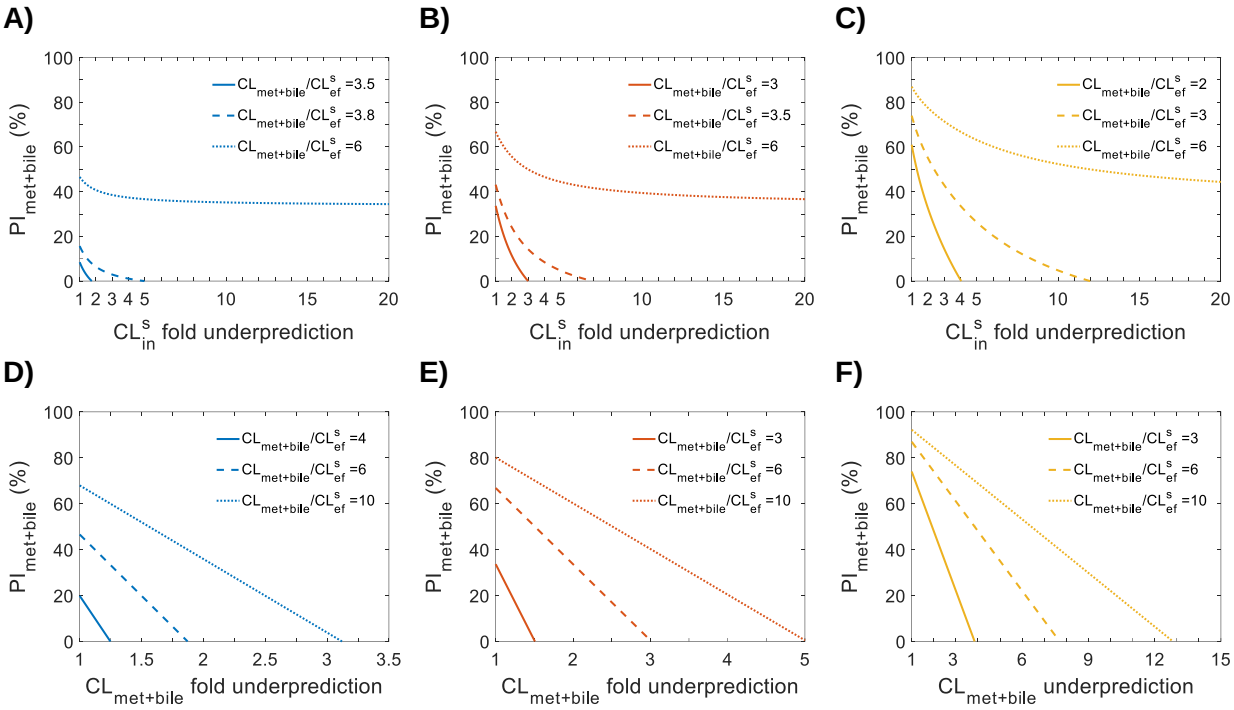
Supplementary Figure 1. Irrespective of the CL^s_{in} value, the systemic AUC of a drug is determined by the $CL_{met+bile}/CL^s_{ef}$ ratio and not the magnitude of the $CL_{met+bile}$ and CL^s_{ef} clearance. The systemic AUC decreases as the $CL_{met+bile}/CL^s_{ef}$ ratio (x-axis) increases but there is no change when the $CL_{met+bile}/CL^s_{ef}$ ratio remains the same even though CL^s_{ef} (y-axis) and $CL_{met+bile}$ magnitudes are different. Note that the x-axis is $CL_{met+bile}/CL^s_{ef}$ and therefore represents varying magnitude of $CL_{met+bile}$ and CL^s_{ef}. This trend persists irrespective of different CL^s_{in} values as in **A)** $CL^s_{in} = 0.25xQ_h$, **B)** $CL^s_{in} = 1xQ_h$, and **C)** $CL^s_{in} = 4xQ_h$. The simulated systemic AUC is i) lower for higher CL^s_{in} values because hepatic clearance approaches blood flow limitations, ii) higher for lower $CL_{met+bile}/CL^s_{ef}$ ratios irrespective of the nominal CL^s_{ef} value, iii) unchanged for different CL^s_{ef} values as long as the $CL_{met+bile}/CL^s_{ef}$ ratio remains constant.



Supplementary Figure 2. The tipping point depends on the AUCR cutoff chosen to represent a significant DDI. The larger the AUCR cutoff, the lower the $CL_{met+bile}/CL^{s}_{ef}$ ratio at which RDS_{uptake} switches to RDS_{all} (tipping point). In other words, if a larger AUCR cutoff is chosen, drugs are more likely to be labeled with RDS_{uptake} rather than RDS_{all}. Consequently, a larger $PI_{met+bile}$ will be predicted. The tipping point is sensitive even for small differences in the AUCR cutoff (e.g. AUCR cutoff of 1.1 versus 1.25). The lines were simulated using Eq. 2 for the different AUCR. As shown in Fig. 2, combinations of hepatobiliary clearances in the area above and below the tipping point line will have RDS_{uptake} and RDS_{all}, respectively.



Supplementary Figure 3. Impact of CL^{s}_{in} underprediction on the tipping point. The tipping point will be overpredicted when CL^{s}_{in} is underpredicted. A high ER drug will have the largest error in the tipping point predictions. Since the tipping point has been overpredicted, the $PI_{met+bile}$ will be underpredicted. Ultimately, this leads to an overestimation of the metabolic/biliary efflux DDI liability for drugs with $RDS_{uptake.}$ Simulations were performed as follows: for CL^{s}_{in} = 0.25x, 1x, 4x$Q_{h,}$ the tipping point following 1-20 fold underprediction of CL^{s}_{in} was calculated from Eq. 2.



Supplementary Figure 4. Underprediction of hepatobiliary clearances impacts DDI liability predictions. *In vitro* quantification often results in under-prediction of hepatobiliary clearances which can impact how the RDS is labeled and consequently how DDI liabilities are predicted. The impact on $PI_{met+bile}$ due to CL^s_{in} (**A-C**) or $CL_{met+bile}$ (**D-F**) underpredictions for a low (**A,D**), mid (**B,E**) and high (**C,F**) ER drug at various $CL_{met+bile}/CL^s_{ef}$ ratios is illustrated. Underpredictions of both CL^s_{in} and $CL_{met+bile}$ will underestimate the $PI_{met+bile}$. For example, for a mid ER drug with $CL_{met+bile}/CL^s_{ef}$ ratio = 6, a 3-fold underprediction of CL^s_{in} estimates $PI_{met+bile}$ of ~50% when the true value is 67% (top dashed line, panel **A**), whereas a 3-fold underprediction of $CL_{met+bile}$ for the same drug estimates $PI_{met+bile}$ of ~0% when the true value is 67% (middle dashed line, panel **D**). When $PI_{met+bile}$ = 0% is estimated, the tipping point has been crossed, (see Fig. 7), and the RDS is labeled as RDS_{all} rather than RDS_{uptake}. For the example given above, a >3-fold underprediction of $CL_{met+bile}$ would mislabel the RDS of the drug as RDS_{all} when it is truly RDS_{uptake} (middle dashed line, panel **D**). If the $CL_{met+bile}/CL^s_{ef}$ ratio is > 4, CL^s_{in}

underpredictions cannot wrongfully identify the RDS (top dashed lines, panels **A-C**). Mislabeling the RDS impacts the expected DDI risk due to transporters versus enzymes. Low ER drugs are most susceptible to having the RDS erroneously labeled. Furthermore, mislabeling of the RDS is more susceptible to underpredictions of $CL_{met+bile}$ than CL^{s}_{in}. Pooling together these trends, underpredictions of either CL^{s}_{in} or $CL_{met+bile}$ leads to identifying both transporters and enzymes as DDI liabilities when truly only uptake transporters are the true DDI liability. Simulations were performed as follows: 1-20 fold underprediction of CL^{s}_{in} or $CL_{met+bile}$ was simulated for drugs with starting values of CL^{s}_{in} = 0.25x, 1x, $4xQ_h$ (representing low, mid, and high ER, respectively) and $CL_{met+bile}/CL^{s}_{ef}$ ratios as shown in the legends. Underprediction of CL^{s}_{in} necessitated identifying a new tipping point using Eq. 2 and the new $PI_{met+bile}$ was established using Eq. 5.

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