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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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d) Nonstandard abbreviations: AUCR = area under the curve ratio; CL_{bile} = biliary (canalicular) efflux clearance; CL_{int} = intrinsic clearance; CL_{met} = metabolic clearance; CL^{s}_{in} = sinusoidal influx clearance; CL^{s}_{ef} = sinusoidal efflux clearance; DDI = drug – drug interaction; ECM = extended clearance model; IVIVE = *in vitro* to *in vivo* extrapolation; HPGL = 10^6 hepatocytes per gram

liver; NDA = new drug application; NME = new molecular entity; OATP = organic anion transporting polypeptide; $PI_{met+bile}$ = percent inhibition of $CL_{met+bile}$ necessary for RDS_{uptake} to switch to RDS_{all}; PBPK = physiologically based pharmacokinetics; MPPGL = microsomal protein per gram liver; SCRH = sandwich cultured rat hepatocytes; SCHH = sandwich cultured human hepatocytes; RDS = rate-determining step

ABSTRACT

For dual transporter/enzyme substrate drugs, the extended clearance model (ECM) can be used to predict the rate-determining step(s) (RDS) of a drug and hence predict its drug-drug interaction (DDI) liabilities (i.e. transport, metabolism, or both). If the RDS of the hepatic clearance of the drug is sinusoidal uptake clearance (CL^s_{in}), even if the drug is mainly eliminated by hepatic metabolism, its DDI liability (as viewed from changes to systemic drug concentrations) is expected to be inhibition or induction of uptake transporters but not hepatic enzymes. However, this is true only if the condition required to maintain CL^s_{in} as the RDS is maintained. Here, we illustrate through theoretical simulations that the RDS condition may be violated in the presence of a DDI. That is, the RDS of a drug can switch from CL^s_{in} to all hepatobiliary clearances (i.e. metabolic/biliary clearance [$CL_{met+bile}$] and CL^s_{in}) leading to unexpected systemic DDI's, such as metabolic DDI's when only transporter DDI's are anticipated. As expected, these analyses revealed that the RDS switch depends on the ratio of $CL_{met+bile}$ to sinusoidal efflux clearance (CL^s_{ef}). Additional analyses revealed that for intravenously administered drugs, the RDS switch also depends on the magnitude of CL^s_{in}. We analyzed published *in vitro* quantified hepatobiliary clearances and observed that most drugs have $CL_{met+bile}/CL^s_{ef}$ ratio < 4, and hence in practice, the magnitude of CL^s_{in} must be considered when establishing the RDS. These analyses provide insights, previously not appreciated, and a theoretical framework to predict DDI liabilities for drugs that are dual transporter/enzyme substrates.

INTRODUCTION

Identifying liabilities with respect to drug – drug interactions (DDI) is important in drug development. In 2015, 25 out of the 33 new drug applications (NDA's) contained *in vitro* transporter data and out of 20 clinical trials using the new molecular entities (NME's) as victim drugs, only 9 resulted in a significant area under the curve (AUC) change (Yu et al., 2017). These data acknowledge that drug transporters are important in determining drug disposition (Giacomini et al., 2010; Hillgren et al., 2013; Patel et al., 2016).

As shown by the hepatic extended clearance model (ECM), when a drug is both transported into and metabolized/biliary excreted by the liver, the rate-determining step (RDS) in the systemic clearance of the drug can be its hepatic uptake clearance, metabolic clearance, biliary (canalicular efflux) clearance, or all hepatobiliary clearances (Miyauchi et al., 1987; Sirianni and Pang, 1997; Shitara et al., 2006; Kusuhara and Sugiyama, 2009; Li et al., 2014; Patilea-Vrana and Unadkat, 2016). The RDS of a drug can be identified using models such as the Extended Clearance Concept Classification System (ECCCS) and the Extended Clearance Classification System (ECCS) that use the drug's *in vitro* quantified hepatobiliary clearance values or the drug's physicochemical properties, respectively (Camenisch and Umehara, 2012; Varma et al., 2015). Using such models is advantageous since the RDS of a drug helps identify where the DDI liabilities lie. Of note, unless indicated otherwise, all subsequent reference to DDI should be interpreted as those DDI that can be observed from measurement of the systemic concentrations of the victim drug. For example, if the RDS of a drug is its hepatic uptake clearance (RDS_{uptake}), then the focus of the DDI studies should be transporter-based (e.g. hepatic organic anion-transporting polypeptide (OATP) – mediated uptake of atorvastatin, (Maeda et al., 2011)) or if the RDS is both hepatic uptake and metabolic/biliary clearance (RDS_{all}), the focus of DDI studies should be all hepatobiliary pathways (e.g. OATP and CYP-mediated clearance of cerivastatin, (Mück et al., 1999; Backman et al., 2002)).

Here, we asked if knowledge of the RDS of a drug is enough to predict DDI liabilities for drugs that are dual transporter/enzyme substrates? If it is not, the focus of DDI studies will be misdirected and will result either in a negative or unexpected DDI and therefore toxicity. Under the worst-case scenario, the latter will lead to discontinuation of drug development. The end result is that both outcomes will increase drug development cost (Paul et al., 2010). For these reasons, it is important to ask: can the RDS switch from hepatic uptake clearance to all hepatobiliary clearance pathways, thus resulting in unexpected systemic DDIs? Using the ECM theory and simulations, we aimed to: i) provide a theoretical framework of when the RDS_{uptake} switches to RDS_{all} in the presence of a DDI, and ii) apply the RDS framework to predict DDI liabilities through theoretical and practical examples. The resulting analyses and simulations provide novel insights, hitherto not appreciated, into factors that determine when a victim drug experiences the RDS_{uptake} switch to RDS_{all} and elucidate important considerations when predicting DDI liabilities for drugs that are substrates of both hepatic transporters and enzymes.

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MATERIALS AND METHODS

Theoretical background

The ECM describes complex hepatobiliary clearance in terms of transport at the sinusoidal membrane via sinusoidal influx (CL^s_{in}) and efflux (CL^s_{ef}), transport at the canalicular membrane via biliary efflux (CL_{bile}), metabolism (CL_{met}), hepatic blood flow (Q_h), and fraction unbound in blood (fu_b) (Eq. 1). CL^s_{in} and CL^s_{ef} terms incorporate both transport-mediated plus passive diffusion clearance while CL_{bile} describes active transport only. The interrelationships between the hepatobiliary clearances defined by the ECM create the RDS in the hepatic clearance of a drug. As described by us and others (Miyauchi et al., 1987; Sirianni and Pang, 1997; Shitara et al., 2006; Patilea-Vrana and Unadkat, 2016), these can be: i) $RDS_{met+bile}$ when the metabolic and biliary efflux clearances of the drug are much less than sinusoidal efflux clearance ($CL_{met+bile} << CL^s_{ef}$) and the drug is highly permeable (passive diffusion >> active transport, $CL^s_{in} \approx CL^s_{ef}$) and can thus rapidly distribute across the sinusoidal membrane, ii) RDS_{uptake} when the metabolic plus biliary efflux clearances are much greater than the sinusoidal efflux clearance ($CL_{met+bile} >> CL^s_{ef}$), or iii) RDS_{all} when a drug has both active transport and metabolism but the two extreme scenario from above do not apply ($CL^s_{in} \neq CL^s_{ef}$).

$$CL_h = \frac{Q_h fu_b CL^s_{in}(CL_{met} + CL_{bile})}{Q_h(CL^s_{ef} + CL_{met} + CL_{bile}) + fu_b CL^s_{in}(CL_{met} + CL_{bile})} \quad (1)$$

Identifying the RDS of a drug can be used to predict the liability of transporter versus metabolic DDI's (see Patilea-Vrana and Unadkat, 2016 for simulations of systemic and hepatic AUC when hepatobiliary clearances are inhibited). For example, while a victim drug has RDS_{uptake}, inhibition of $CL_{met+bile}$ will not result in a significant increase in the systemic AUC even though such DDI could result in significant drug accumulation in the liver and hence potentially enhanced hepatic efficacy or toxicity of the drug. That is, from the point of view of a systemic (e.g. victim plasma concentrations) measurements, inhibition of $CL_{met+bile}$ will be incorrectly

interpreted as negative because there will be no change in systemic concentrations of the drug. On the other hand, inhibition of CL^{s}_{in} will result in an increase in the drug's systemic AUC (and therefore potentially non-hepatic efficacy and toxicity of the drug) but will result in no changes in the hepatic AUC provided the liver is the primary eliminating organ (see Patilea-Vrana and Unadkat, 2016 for examples). However, less appreciated is the fact that in the presence of metabolic/biliary efflux DDI, the RDS of a drug can switch from RDS_{uptake} to RDS_{all} and hence switch the DDI liability from uptake transporters to both metabolic/biliary and uptake pathways. Consequently, the drug's systemic AUC will significantly change due to metabolic and biliary efflux DDI's even though uptake was the RDS of the drug in the absence of a DDI. This would lead to unexpected DDIs as viewed from the systemic concentrations of the victim drug. Therefore, through MATLAB simulations (R2016a; MathWorks, Natick, MA), we illustrated when the RDS_{uptake} to RDS_{all} switch occurs for a victim drug in the presence of a DDI. We then applied our proposed RDS framework to published *in vitro* hepatobiliary clearances to determine if *in vivo* observed DDI liabilities can be correctly predicted. While the insights illustrated can be derived from analytical solutions of the ECM equation (Eq. 1), for clarity, we chose to use simulations to illustrate the principles of these DDI liabilities within the RDS framework.

Simulation assumptions

The hepatic ECM was simulated using the governing differential equations as previously described (Endres et al., 2009; Patilea-Vrana and Unadkat, 2016) and for simplicity, the following assumptions about the victim drug were made: i) it was administered intravenously (IV); ii) fraction unbound (fu) in blood and tissue (liver) was set to 1; iii) liver was the only eliminating organ; iv) Q_h was set to 1 L/min. All references to systemic AUC are derived from drug concentrations in blood. Our conclusions regarding the RDS switch are generalizable to when victim drugs are administered orally but our conclusions of the RDS dependence on CL^{s}_{in} only apply for IV administered drugs (see text below). Furthermore, for oral drug administration,

our findings apply only to changes to the hepatic clearance/bioavailability of the victim drug and do not address the intestinal availability of the victim drug. If there is significant non-hepatic clearance, our conclusions will stand except that the magnitude of the change observed in the systemic and/or hepatic AUC of the drug will differ (Patilea-Vrana and Unadkat, 2016).

Identifying when the RDS_{uptake} switches to RDS_{all} and factors that influence this switch

First, we determined when the RDS of a drug switches from uptake clearance to all hepatobiliary clearance pathways. This requires violating the condition $CL_{met+bile} >> CL^{s}_{ef}$, the condition necessary for uptake clearance to be the RDS in the hepatic clearance of drug. To illustrate this effect, for three theoretical victim drugs where $CL_{met+bile} >> CL^{s}_{ef}$, ($CL_{met+bile}$ = 1, 10, 100 L/min, CL^{s}_{ef} = 0.1 L/min, and $CL^{s}_{in} = 1xQ_h$), the systemic AUC ratio (AUCR) of the victim drug in the absence and presence of 10-99% inhibition of $CL_{met+bile}$ was simulated. Following the FDA guidelines, an AUCR of 1.25 was considered to be significant.

To illustrate that the $CL_{met+bile}/CL^{s}_{ef}$ ratio and not the absolute magnitude of $CL_{met+bile}$ and/or CL^{s}_{ef} determines when the RDS_{uptake} switches to RDS_{all} we conducted the following simulations: the systemic AUC of the drug was simulated for CL^{s}_{ef} values ranging from 0.1 to 10 L/min (representing 0.1x to 10xQ_h) with $CL_{met+bile}$ set to 1-20 fold the value of the corresponding CL^{s}_{ef} value. The simulated systemic AUC's when $CL_{met+bile}/CL^{s}_{ef}$ ratio was held constant were compared to the simulated systemic AUC's when $CL_{met+bile}/CL^{s}_{ef}$ ratio varied.

Next, we defined the tipping point as the $CL_{met+bile}/CL^{s}_{ef}$ ratio at which RDS_{uptake} switches to RDS_{all}. Following the same strategy as above, we simulated the AUCR for various $CL_{met+bile}/CL^{s}_{ef}$ ratios for victim drugs that originally had RDS_{uptake} to illustrate the $CL_{met+bile}/CL^{s}_{ef}$ ratio at which AUCR = 1.25, thus signifying that RDS_{uptake} switched to RDS_{all}. The systemic AUC where the RDS is uptake was simulated such as $CL_{met+bile}/CL^{s}_{ef}$ ratio = 1000 ($AUC_{ratio\ =\ control}$, $CL_{met+bile}$ = 100 L/min, CL^{s}_{ef} = 0.1 L/min). Then, systemic AUC was simulated for $CL_{met+bile}/CL^{s}_{ef}$

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"test" ratios ranging from 0.1 – 10 ($CL_{met+bile}$ = 0.01 - 1 L/min, CL^s_{ef} = 0.1 L/min) and the resulting AUC ($AUC_{ratio = test}$) was normalized to the control simulation (AUCR = $AUC_{ratio = test}/AUC_{ratio = control}$). The decrease in $CL_{met+bile}/CL^s_{ef}$ ratio is akin to inhibition of $CL_{met+bile}$ since CL^s_{ef} is held constant. The $CL_{met+bile}/CL^s_{ef}$ ratio which resulted in a significant change to the systemic AUC (AUCR = 1.25) compared to control was identified as the tipping point.

To illustrate that the magnitude of CL^s_{in} contributes to the tipping point, we simulated the tipping point for CL^s_{in} values ranging from $0.01xQ_h$ – $4xQ_h$ (henceforth, for simplicity, CL^s_{in} notation will be used instead of $fu_bCL^s_{in}$ since fu_b = 1). The tipping point can be explicitly derived from the ECM (Eq. 1) by defining the RDS switch for any chosen AUCR as AUCR = RDS_{uptake}/RDS_{all} and solving for the $CL_{met+bile}/CL^s_{ef}$ ratio (Eq. 2). This relationship (Eq. 2 with AUCR = 1.25) was used later to identify DDI liabilities when considering CL^s_{in} magnitude and $CL_{met+bile}/CL^s_{ef}$ ratio of a drug.

$$\text{Tipping point} = \frac{1}{(AUCR - 1)\left(1 + {CL^s_{in}}/{Q_h}\right)} \quad (2)$$

Quantifying when a drug with RDS_{uptake} will switch to RDS_{all} due to metabolic/biliary efflux DDI's

We defined $PI_{met+bile}$ as the percent inhibition of $CL_{met+bile}$ required for RDS_{uptake} to switch to RDS_{all}. This quantifies when a significant DDI (AUCR ≥ 1.25) will occur due to inhibition of $CL_{met+bile}$ even when uptake was the RDS in the absence of DDI. For $CL_{met+bile}/CL^s_{ef}$ ratios ranging from 1-100, $CL_{met+bile}$ was inhibited 10-99%. Simulations were conducted for CL^s_{in} values = 0.25x, 1x, $4xQ_h$. CL^s_{in} values were chosen to represent ER = 0.2, 0.5, and 0.8 (low, mid, and high extraction ratio (ER), respectively) and were back calculated from Eq. 3-4. The percent inhibition of $CL_{met+bile}$ at which the $CL_{met+bile}/CL^s_{ef}$ ratio reaches the tipping point (i.e. $PI_{met+bile}$) and thus causes the RDS_{uptake} to switch to RDS_{all} was calculated as shown in Eq. 5.

$$\mathrm{CL_h} = \mathrm{Q_h} * \mathrm{ER}\ (3)$$

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$$CL_h = \frac{Q_h fu_b CL_{in}^s}{Q_h + fu_b CL_{in}^s} \quad (4)$$

$$PI_{met+bile}\ (\%) = \frac{CL_{met+bile}/CL_{ef}^s - \text{tipping point}}{CL_{met+bile}/CL_{ef}^s} \text{x } 100 \quad (5)$$

Applying the RDS framework to *in vitro* and *in vivo* examples

Published data sets where all hepatobiliary clearance pathways (CL^s_{in}, CL^s_{ef}, CL_{bile}, CL_{met}) were quantified *in vitro* were collected. The *in vivo* hepatobiliary clearances must be used to identify the RDS of a drug. As such, the provided *in vitro* to *in vivo* extrapolated (IVIVE) clearances were utilized; otherwise, *in vitro* hepatobiliary clearance values were scaled to *in vivo* using IVIVE scaling factors (i.e. MPPGL, HPGL, liver weight) as provided by the authors. For all drugs, $fu_bCL^s_{in}/Q_h$ was used to calculate the tipping point using Eq. 2 (see Results section below). RDS was labeled as RDS_{uptake} and RDS_{all} if the $CL_{met+bile}/CL^s_{ef}$ ratio was above and below the tipping point, respectively. For drugs with RDS_{uptake}, the $PI_{met+bile}$ was calculated using Eq. 5. Finally, for selected drugs, the predicted DDI liabilities using the RDS and $PI_{met+bile}$ were compared to the observed *in vivo* data. To ensure that only the systemic clearance, and not bioavailability of the victim drug was affected, clinical DDI studies were included if the victim was a dual transporter/enzyme substrate and co-administered with a selective enzyme inhibitor administered IV. It should be noted that the availability of such studies was limited.

RESULTS

Identifying the tipping point (i.e. when RDS_{uptake} switches to RDS_{all}) and factors that influence this switch

As described under theoretical background, RDS_{uptake} occurs when $CL_{met+bile} >> CL^{s}_{ef}$, and as such, inhibition of $CL_{met+bile}$ will not manifest in the systemic AUC of a victim drug. However, when the above condition is violated due to extensive inhibition of $CL_{met+bile}$, there will be a significant increase in the systemic AUC of the victim drug when $CL_{met+bile}$ is inhibited further. In other words, when $CL_{met+bile}$ is no longer >> CL^{s}_{ef}, then RDS_{uptake} switches to RDS_{all}. In Fig.1A, 84%, 98%, 99.8% inhibition of $CL_{met+bile}$ led to a clinically significant increase in the systemic AUC of the three theoretical victim drugs shown (AUCR ≥ 1.25). Even though the victim drugs had different pre-inhibition $CL_{met+bile}$ values (1, 10, 100 L/min), the post-inhibition $CL_{met+bile}$ values were all the same (0.2 L/min). Since CL^{s}_{ef} was kept constant (0.1 L/min), an AUCR of 1.25 was observed when $CL_{met+bile}/CL^{s}_{ef} = 2$ for all three victim drugs. This simulation illustrates that the RDS_{uptake} switch to RDS_{all} depends on the $CL_{met+bile}/CL^{s}_{ef}$ ratio and not the extent of $CL_{met+bile}$ inhibition.

To further emphasize the dependence on the $CL_{met+bile}/CL^{s}_{ef}$ ratio, we simulated the systemic AUC of the victim drug (in the absence of DDI) for different $CL_{met+bile}$ and CL^{s}_{ef} values while holding CL^{s}_{in} constant. The systemic AUC remained unchanged when the $CL_{met+bile}/CL^{s}_{ef}$ ratio remained fixed even though the $CL_{met+bile}$ and CL^{s}_{ef} values varied, demonstrating that the RDS in the hepatic clearance of a drug is dependent on the $CL_{met+bile}/CL^{s}_{ef}$ ratio and not on the absolute value of these clearances (Fig. 1B). This was true for both when $CL_{met+bile}$ was higher and lower than CL^{s}_{ef} (also see Supplementary Fig. 1). Since the systemic AUC decreased as the $CL_{met+bile}/CL^{s}_{ef}$ ratio increased, only the $CL_{met+bile}/CL^{s}_{ef}$ ratio needs to be considered when determining when the RDS_{uptake} switches to RDS_{all} for a victim drug.

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Next, we identified the tipping point, defined here as the $CL_{met+bile}/CL^{s}_{ef}$ ratio when RDS_{uptake} switches to RDS_{all}. The RDS_{uptake} switch to RDS_{all} signifies when DDI's due to inhibition of $CL_{met+bile}$ start to become significant for a victim drug that has RDS_{uptake}. As demonstrated above, the RDS_{uptake} switch to RDS_{all} depends on the $CL_{met+bile}/CL^{s}_{ef}$ ratio. As such, we identified the tipping point as the $CL_{met+bile}/CL^{s}_{ef}$ ratio at which the systemic AUC increases significantly (AUCR = 1.25) due to decrease in the $CL_{met+bile}/CL^{s}_{ef}$ ratio for a victim drug that has RDS_{uptake} (Fig. 1C). As demonstrated in Fig. 1C, the tipping point for a low, mid, and high ER drug was 3.2, 2, and 0.8, respectively.

Since the tipping point varied for a low, mid, and high ER, the magnitude of CL^{s}_{in} is also an important factor in determining when the RDS_{uptake} switches to RDS_{all} (Fig. 1C). Extending the simulations to identify the tipping point across a range of CL^{s}_{in} values, we established a theoretical (Eq. 2) and practical (Fig 2.) relationship between CL^{s}_{in}/Q_h and the tipping point. The tipping point decreases as CL^{s}_{in} increases. In other words, as a drug's CL^{s}_{in} (and therefore its ER) increases, the drug is more likely to have RDS_{uptake} and a larger $PI_{met+bile}$, therefore making the drug more resistant to switching its RDS. In addition, as the influx across the sinusoidal membrane becomes large, hepatic clearance becomes limited by blood flow and therefore less likely to result in a change in AUCR when either CL^{s}_{in} (or for that matter $CL_{met+bile}$) is inhibited. On the other hand, when CL^{s}_{in} (or ER) is small and the hepatic clearance becomes proportional to CL^{s}_{in}, the victim drug becomes more susceptible to a change in RDS. This demonstrates that low ER drugs are more susceptible to RDS_{uptake} switching to RDS_{all} whereas high ER drugs are more resistant to the RDS switch.

It should be noted that the relationship between CL^{s}_{in}/Q_h and the tipping point (Eq. 2 and Fig. 2) depends on the chosen AUCR cutoff. Here, an AUCR of 1.25 was chosen based off FDA guidelines of what constitutes a positive DDI. If a higher AUCR cutoff were to be selected (Supplementary Fig. 2), this would lead to estimation of lower tipping points, thus making it more

likely that drugs are labeled with RDS_{uptake}. Labeling a drug with RDS_{uptake} when in fact it has RDS_{all} can lead to underpredictions of DDI liabilities due to metabolic enzymes and biliary transporters.

By understanding the relationship between CL^{s}_{in} and the tipping point, the RDS can be identified for any combination of a drug's hepatobiliary clearance values (Fig. 2). For example, a high ER drug with a $CL_{met+bile}/CL^{s}_{ef}$ ratio of 3 will have RDS_{uptake} but a low ER drug with the same $CL_{met+bile}/CL^{s}_{ef}$ ratio will have RDS_{all}. Furthermore, a drug will always have RDS_{uptake} if the $CL_{met+bile}/CL^{s}_{ef}$ ratio is greater than 4, irrespective of the magnitude of CL^{s}_{in}. It should be noted that for orally administered drugs, the tipping point will no longer depend on the magnitude of CL^{s}_{in}, and therefore will always be 4, because blood flow limitations from systemic clearance are cancelled out by blood flow limitations of hepatic bioavailability.

Quantifying the $PI_{met+bile}$ for drugs with RDS_{uptake}

Identifying the RDS of a drug as well as when the RDS_{uptake} to RDS_{all} switch will happen identifies the drug's DDI liabilities. We quantified the $PI_{met+bile}$, defined here as the percent inhibition of $CL_{met+bile}$ needed to cause the RDS_{uptake} switch to RDS_{all}, to understand when inhibition of $CL_{met+bile}$ starts to become a DDI liability for victim drugs that have RDS_{uptake}. As the $CL_{met+bile}/CL^{s}_{ef}$ ratio of the victim drug (prior to inhibition) increases, the $PI_{met+bile}$ increases (Fig. 3A). This is because as $CL_{met+bile}$ becomes >> than CL^{s}_{ef}, the victim drug become resistant to the RDS_{uptake} switch to RDS_{all}. High ER drugs have a higher $PI_{met+bile}$ than low ER drugs, demonstrating again that high ER drugs are resistant to the RDS switch while low ER drugs are sensitive (Fig. 3A). Fig. 3B illustrates that while a low, mid, and high ER victim drug with $CL_{met+bile}/CL^{s}_{ef}$ ratio of 6 have RDS_{uptake} (prior to inhibition), inhibition of $CL_{met+bile}$ greater than 46%, 66%, and 87%, respectively, will cause the RDS_{uptake} to switch to RDS_{all}. This translates to observing a positive DDI due to inhibition of $CL_{met+bile}$ for a victim drug that has been identified to

have RDS_{uptake} (prior to inhibition). Without knowledge of the $PI_{met+bile}$, such a DDI may not be expected.

The purpose and conclusions of the simulations that have been used to established the RDS framework up to this point are summarized in Fig.4. As discussed, identifying the drug's RDS is not enough to correctly predict the drug's DDI liabilities. The tipping point concept is an important consideration when identifying DDI's for victim drugs are dual substrates of enzymes and transporters.

The flowchart in Fig. 5 can be used as a guide to identify the DDI liabilities for dual transporter/enzyme substrates. All drugs with $CL_{met+bile}/CL^{s}_{ef}$ ratio > 4 will have RDS_{uptake} whereas drugs with $CL_{met+bile}/CL^{s}_{ef}$ ratio < 4 will have RDS_{uptake} as long as this ratio is greater than the tipping point. Drugs with $CL_{met+bile}/CL^{s}_{ef}$ ratio less than the tipping point will have RDS_{all}. If the drug has RDS_{uptake}, then uptake transporters will become a DDI liability, whereas if the drug has RDS_{all}, then transporters and enzymes will be a DDI liability. However, even for drugs that have RDS_{uptake}, $CL_{met+bile}$ can become a DDI liability if inhibition of $CL_{met+bile}$ is greater than the predicted $PI_{met+bile}$ and thus causes the RDS_{uptake} switch to RDS_{all}. The flowchart identifies the $CL_{met+bile}/CL^{s}_{ef}$ ratios at which 25%, 50%, 75%, and 95% expected inhibition of $CL_{met+bile}$ is going to result in the RDS switch. This information can be used to assess when $CL_{met+bile}$ starts to become a DDI liability for drugs with RDS_{uptake}. It also helps answer the question of how much larger does $CL_{met+bile}$ needs to be compared to CL^{s}_{ef} in order for sinusoidal uptake clearance to become and maintain as the RDS in the hepatic clearance of any drug. Such information may be used during drug development to select drug candidates if a certain RDS is desired.

Applying the RDS framework to *in vitro* and *in vivo* examples

To provide context to the theoretical framework presented, examples from literature, where available, were utilized. For drugs with *in vitro* quantified hepatobiliary clearances that

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were extrapolated to *in vivo* via IVIVE, the tipping point and $PI_{met+bile}$ were calculated using Eq. 2 and 5, and a subset of the analyzed data set, which includes primarily statin drugs, is shown in Fig. 6 (also see Supplementary Table 1) (Camenisch and Umehara, 2012; Jones et al., 2012; Varma et al., 2014; Kunze et al., 2015; Riede et al., 2017). If no empirical scaling factors (such as for active uptake clearance to match observed *in vivo* clearance) are included in the IVIVE process, then almost all drugs have RDS_{all}, except for valsartan and pravastatin (Fig. 6A). This is because most $CL_{met+bile}/CL^{s}_{ef}$ ratios < 4 and since the IVIVE CL^{s}_{in} magnitudes were small, most $CL_{met+bile}/CL^{s}_{ef}$ ratios were less than the tipping point. Because many statins have been identified to have RDS_{uptake}, the trend in Fig. 6A suggests that CL^{s}_{in} was underestimated *in vitro*. When hepatobiliary clearances were adjusted by empirical scaling factors (Varma et al., 2014) or parameters were fitted from *in vivo* IV concentration-time profiles using a PBPK model (Jones et al., 2012), the distribution of drugs is altered such as low drugs (ER < 0.2) tended to have RDS_{all} whereas mid and high ER drugs (ER > 0.2) were more likely to have RDS_{uptake} (Fig. 6B). This analysis of the published *in vitro* hepatobiliary clearances provides insight that drugs with RDS_{uptake} exist within the moderate RDS framework space, meaning that in general their $CL_{met+bile}/CL^{s}_{ef}$ ratio is < 4 and are quite susceptible to the RDS switch (Supplementary Table 1). It further elucidates that current *in vitro* quantification techniques may underestimate CL^{s}_{in} which can lead to erroneous labeling of the RDS and thus incorrect DDI liability predictions (Fig 7, Supplementary Fig. 3).

To further illustrate the applicability of the RDS framework, predicted DDI liabilities using the RDS framework were compared to *in vivo* DDI examples. As indicated in Table 1, when empirical scaling factors are utilized during the IVIVE process or hepatobiliary clearances were estimated from *in vivo* via PBPK, atorvastatin and repaglinide have RDS_{uptake} and $PI_{met+bile}$ of 10-51% and 15-40%, respectively, while bosentan has RDS_{all}. For atorvastatin and repaglinide, the *in vitro* data predicted that uptake transporters (OATPs) are the primary DDI liability with the

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drugs' major metabolic enzymes (CYP3A and CYP2C8, respectively) becoming a potential liability only if the *in vivo* hepatic metabolic inhibition is greater than the $PI_{met+bile}$. For bosentan, the *in vitro* data predicted that both OATPs and CYP3A4 are a potential DDI liability. Clinically, for atorvastatin, co-administration of rifampin (an OATP inhibitor) lead to AUCR of 12 whereas 33% inhibition of CYP3A4 due to IV itraconazole (as measured using CYP3A4 probe midazolam) did not change atorvastatin systemic AUC even though inhibition of atorvastatin metabolism was observed via a decrease in the 2-hydroxyatorvastatin concentrations (Maeda et al., 2011). In a similarly conducted experiment, co-administration of rifampin resulted in AUCR of 3.2 and 1.9 for bosentan or repaglinide, respectively, whereas 73% inhibition of CYP3A4 due to IV itraconazole did not significantly change the systemic AUC of these drugs (Yoshikado et al., 2017). Furthermore, repaglinide co-administered with PO rifampin and trimethoprim (CYP2C8 selective inhibitor) resulted in AUCR was 2.6 and 1.8, respectively (Kim et al., 2016). The *in vivo* DDI liability for OATPs was well predicted for all three victim drugs. The *in vivo* DDI liability for CYP3A4 was well predicted for atorvastatin. Since a probe was not used to assess the degree of CYP2C8 inhibition, it is difficult to interpret if the significant DDI when repaglinide was co-administered with trimethoprim was because RDS_{uptake} switched to RDS_{all}, or because repaglinide truly has RDS_{all}. The *in vitro* metrics as well as a whole-body PBPK DDI model suggests that repaglinide has RDS_{uptake} (Varma et al., 2013) and thus the repaglinide-trimethoprim DDI is likely due to the RDS switch. Lastly, since bosentan was predicted to have RDS_{all}, a DDI was expected due to CYP3A4 inhibition but none was observed. It should be noted that the metabolic DDI liability prediction is assuming one main drug metabolizing enzyme and no significant biliary efflux (e.g. $CL_{met+bile} = CL_{CYP3A4}$ for atorvastatin and bosentan). This assumption predicts the highest DDI risk due to inhibition of $CL_{met+bile}$ and has a higher chance of predicting false positive DDI results.

In the published *in vitro* datasets, discrepancies in the *in vitro* quantified values, particularly for CL^{s}_{in}, can be observed (Table 1 and Supplementary Table 1). For example, in one report the authors used empirical scaling factors for active sinusoidal uptake clearance in order to match hepatic clearance with clinical observed data that ranged from 1.1 – 101.8 with geometric mean of 10.6 (Varma et al., 2014). However, the scaling factor used severely impacted the labeling of the RDS (e.g. fluvastatin, glyburide, and pravastatin) or impacted the predicted $PI_{met+bile}$ of drugs (e.g. atorvastatin, rosuvastatin, fluvastatin, and repaglinide) (Supplementary Table 1). Assumptions regarding CL^{s}_{ef} also caused discrepancies. In all reports, CL^{s}_{ef} was assumed to be equal to passive diffusion across the sinusoidal membrane, except in one report where CL^{s}_{ef} was back-calculated from total SCHH CL_{int} (Camenisch and Umehara, 2012). The assumptions surrounding CL^{s}_{ef} impacted the $CL_{met+bile}/CL^{s}_{ef}$ ratio which either changed how the RDS was labeled or the magnitude of the $PI_{met+bile}$ (e.g. aliskerin, ciprofloxacin, and digoxin) (Supplementary Table 1). All in all, mispredictions of any of the hepatobiliary clearances impact the RDS labeling, magnitude of the $PI_{met+bile}$, and DDI liability predictions.

Errors from *in vitro* quantification of hepatobiliary clearances can propagate when establishing the RDS and the predicted DDI liabilities. Underprediction of both CL^{s}_{in} and $CL_{met+bile}$ may erroneously label a drug with RDS_{all} when it is truly RDS_{uptake} (Fig. 7). $CL_{met+bile}$ is the more sensitive parameter when determining the RDS because underpredictions of CL^{s}_{in} may mislabel the RDS only for drugs with $CL_{met+bile}/CL^{s}_{ef}$ ratio < 4 (Supplementary Fig. 4). For such drugs, even moderate (e.g. 2-5 fold) underpredictions of either clearance pathway will lead to RDS mislabeling (Supplementary Fig. 4). Furthermore, underpredictions of both CL^{s}_{in} and $CL_{met+bile}$ leads to underprediction of $PI_{met+bile}$, resulting in predicting a larger DDI liability due to $CL_{met+bile}$ inhibition for a drug with RDS_{uptake} (Fig. 7; Supplementary Fig. 4). While underpredictions of hepatobiliary clearances will result in conservative DDI decisions, they also increase the chances of negative DDI studies.

DISCUSSION

We built a theoretical RDS framework and identified important considerations when predicting DDI liabilities for dual transporter/enzymes substrate drugs. First, inhibition of $CL_{met+bile}$ can cause the RDS of a victim drug to switch from RDS_{uptake} to RDS_{all} and hence result in an unexpected systemic DDI. Two metrics have been developed to identify when the RDS switch occurs: the tipping point, defined as the $CL_{met+bile}/CL^{s}_{ef}$ ratio at which RDS_{uptake} will switch to RDS_{all} and the $PI_{met+bile}$, defined as the percent inhibition of $CL_{met+bile}$ at which a significant AUC change (AUCR > 1.25) for a drug with RDS_{uptake} will start to be observed. The tipping point depends on the drug's $CL_{met+bile}/CL^{s}_{ef}$ ratio and on the magnitude of CL^{s}_{in}. The former but not latter condition is relevant when victim drugs are administered orally. Second, we showed that the $CL_{met+bile}/CL^{s}_{ef}$ ratio must be > 4 in order for any drug to have RDS_{uptake}. Third, we applied the RDS framework to *in vitro* quantified hepatobiliary clearances and observed that most drugs have $CL_{met+bile}/CL^{s}_{ef}$ ratio < 4, and hence in practice, the magnitude of CL^{s}_{in} must be considered when establishing the RDS.

Our theoretical analysis demonstrates that the $CL_{met+bile}/CL^{s}_{ef}$ ratio and not the absolute magnitudes of the clearances determines the RDS in the hepatic clearance of a drug. Previous publications allude to this relationship. The authors of the ECCCS observed through experimental data that when $CL_{met+bile}$ is $2xCL^{s}_{ef,}$ drugs that have RDS_{uptake} can be separated from those that do not (Riede et al., 2016). Furthermore, β value ($\beta = CL_{met+bile}/(CL_{met+bile} + CL^{s}_{ef})$) introduced by Yoshikado et al., can be used to differentiate the RDS, such as when β approaches unity (i.e. $CL_{met+bile} >> CL^{s}_{ef}$), a drug has RDS_{uptake} (Yoshikado et al., 2016). Our analyses corroborate and expand upon these results to provide a quantitative definition of the demarcation point between RDS_{uptake} and RDS_{all}, i.e. the tipping point, and illustrate that the magnitude of CL^{s}_{in} in addition to the $CL_{met+bile}/CL^{s}_{ef}$ ratio is an important factor in determining the

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RDS of a drug. That is, as a drug's CL^{s}_{in} value increases, the drug is more likely to have RDS_{uptake} and to become resistant to the RDS_{uptake} switch to RDS_{all}.

We found good agreement for atorvastatin *in vivo* predicted DDI liabilities (Table 1). For bosentan, the overprediction of expected DDI due to inhibition of $CL_{met+bile}$ may be due to errors in the quantification of the hepatobiliary clearances. Indeed, a study in cynomolgus monkey where bosentan plasma and liver drug concentrations were quantified found that the *in vitro* scaled CL^{s}_{in} and CL_{met} were 28 and 13-fold underpredicted while CL^{s}_{ef} (assumed equal to passive diffusion) was overpredicted by 2-fold when compared to the *in vivo* fitted values (Morse et al., 2017). Combining the *in vitro* metrics that identify RDS_{uptake} for repaglinide with *in vivo* repaglinide DDI's, it appears CYP2C8 but not CYP3A4 inhibition may lead to RDS_{uptake} switch to RDS_{all}. Indeed, inhibition of repaglinide with gemfibrozil (CYP2C8 and OATP1B1 inhibitor) led to an 8-fold increase in systemic AUC while co-administration of itraconazole or cyclosporine (OATP1B1 and CYP3A4 inhibitor) led to much more modest 1.4 and 2.4-fold increase in systemic AUC (Niemi et al., 2003; Kajosaari et al., 2005).

The DDI liabilities discussed so far are relevant for systemic drug exposure but not necessarily for hepatic drug exposure and thus efficacy/toxicity if the site of action is in the liver. For example, the LDL cholesterol lowering effect mediated by atorvastatin does not change for subjects with OATP1B1 polymorphism c.521T>C even though there is a significant increase in atorvastatin systemic AUC (Maeda, 2015). This is because if the liver is the main eliminating organ, changes to sinusoidal uptake alters the hepatic concentration-time profile but not the hepatic AUC. However, systemic increase of atorvastatin may lead to off-target toxicity, such as muscle myopathy. We refer the readers to our previous publication (Patilea-Vrana and Unadkat, 2016), where simulations demonstrate the impact of inhibition of uptake or metabolism on both systemic and hepatic AUC when the liver is and is not the main eliminating organ.

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The contrast between *in vitro* quantified CL^{s}_{in} with and without empirical scaling factors in Fig. 6 demonstrates that IVIVE of accurate transporter mediated clearance remains challenging (Chu et al., 2013; Feng et al., 2014). The system used for *in vitro* quantification may be crucial, since CL^{s}_{in} for statins quantified in SCHH appeared to be lower in magnitude then when quantified in suspended hepatocytes (Table 1 and Supplementary Table 1). This may be mediated by significant intracellular localization of plasma membrane transporters (Kumar et al., 2017), or high interindividual variability when using individual donors (Vildhede et al., 2014). These reasons may also cause underpredictions of CL^{s}_{in} or $CL_{bile.}$ For transporter IVIVE, we have previously recommended using a bottom-up proteomic approach and adjusting for *in vitro* activity via *in vitro* to *in vivo* transporter expression based scaling factors (Prasad and Unadkat, 2014). We have recently demonstrated the successful prediction of hepatobiliary clearance of rosuvastatin in rat using the aforementioned approach (Ishida et al., 2017).

Special emphasis needs to be put on quantifying CL^{s}_{ef} along with $CL_{met+bile}$ since the $CL_{met+bile}/CL^{s}_{ef}$ ratio is one of the anchor points when establishing the RDS. Because CL^{s}_{ef} is a difficult parameter to quantify *in vitro*, it is typically assumed to be equal to passive diffusion across the sinusoidal membrane. However, there are examples of active sinusoidal efflux transport, such as MRP3 efflux of rosuvastatin (Pfeifer et al., 2013). Active sinusoidal efflux would increase the magnitude of CL^{s}_{ef} and decrease the $CL_{met+bile}/CL^{s}_{ef}$ ratio, making a drug more likely to have RDS_{all}. One approach to measuring CL^{s}_{ef} is to use an integrative temporal modeling approach in SCRH (Pfeifer et al., 2013; Ishida et al., 2017).

Errors in the quantification of CL^{s}_{in} and/or the $CL_{met+bile}/CL^{s}_{ef}$ ratio can impact DDI liability predictions. For example, patients with OATP1B1 polymorphism c.521T>C have a ~2-fold higher atorvastatin AUC compared to the wild type allele (Maeda, 2015). Because of the lower CL^{s}_{in} and therefore higher susceptibility to the RDS_{uptake} to RDS_{all} switch, patients with OATP1B1 polymorphism may experience a DDI due to inhibition of CYP3A whereas patients with the wild-

type allele may not. The same trend would be true for patients with polymorphic enzymes that result in lower $CL_{met+bile}$ and thus lower $CL_{met+bile}/CL^{s}_{ef}$ ratios. Polypharmacy use can also impact DDI liability predictions. For example, highly active antiretroviral therapy (HAART) typically includes potent CYP3A4 and moderate OATP inhibitor ritonavir among other drugs, which can impact the $CL_{met+bile}/CL^{s}_{ef}$ ratio more severely than if only one drug is administered. Indeed, the systemic AUC of atorvastatin increased 3.9 and 9.4-fold when co-administered with saquinavir/ritonavir and tipranavir/ritonavir, respectively (Fichtenbaum et al., 2002; Pham et al., 2009). Lastly, saturation of enzymes, leading to a lower CL_{met} with increased dose, may lower the $CL_{met+bile}/CL^{s}_{ef}$ ratio and cause DDI's due to the RDS_{uptake} switch to RDS_{all}.

If a victim drug has RDS_{all} but it has been mislabeled as RDS_{uptake}, then the DDI liability due to inhibition of both transporter and metabolic activity could be underestimated. Considering potential DDI risks, it would be most conservative to assume a drug has RDS_{all}; however, making such an assumption would lead to an increase in negative DDI studies, particularly when conducting metabolic/biliary efflux DDI studies if the drug has RDS_{uptake}. An analysis of the DDI's performed for a cohort of NME's in 2013 showed a modest return on investment because 57% (n=141) of all *in vivo* DDI's were negative (Lesko and Lagishetty, 2016). Given the high prevalence of negative DDI's, it may be more appropriate to make mechanistic-based rather than conservative decisions with regard to DDI liabilities.

The RDS framework presented here should be used as a guide when identifying the DDI liabilities whereas PBPK models should be used to predict the direction and magnitude of complex transporter-enzyme DDI's. Several examples of such models (e.g. repaglinide, simvastatin, rosuvastatin) exist that predict complex interactions due to chemical inhibition or genetic polymorphism (Varma et al., 2013; Rose et al., 2014; Tsamandouras et al., 2015). Even with PBPK models, there are limitations. For example, when a drug has RDS_{uptake}, the $CL_{met+bile}$ is unidentifiable from plasma concentrations data since only CL^{s}_{in} plays a significant role in

determining hepatic clearance. Focusing on capturing the correct $CL_{met+bile}$ magnitude and not the $CL_{met+bile}/CL^{s}_{ef}$ ratio can be misleading and will impact PBPK predictions. For instance, in an atorvastatin PBPK model, when cyclosporine CYP3A4 K_i was modulated 100-fold, a maximum 1.6–fold AUCR was achieved (Duan et al., 2017). While the tendency is to run sensitivity analysis on the active components (transport and metabolism), a sensitivity analysis on CL^{s}_{ef} value (in the model it was assumed to be equal to passive diffusion) should also be run as, for the specific example provided, it would likely have revealed a larger impact of cyclosporine on atorvastatin systemic AUC. Such an analysis may be helpful in consolidating *in vitro* K_i data with observed *in vivo* DDI data.

In summary, we introduced a theoretical RDS framework to better predict DDI liabilities for drugs that are dual transporter/enzyme substrates. We provide useful insights, such as: i) the RDS_{uptake} switch to RDS_{all} depends on the ratio of $CL_{met+bile}/CL^{s}_{ef}$ and the magnitude of CL^{s}_{in}, ii) $CL_{met+bile}/CL^{s}_{ef}$ ratio > 4 ensures RDS_{uptake} independent of CL^{s}_{in} magnitude or administration route iii) existing drugs exist within a moderate space within the RDS framework and are susceptible to the RDS_{uptake} switch to RDS_{all}. While the above insights were obtained from the hepatic ECM, they can be equally applied to other organs such as the kidneys where vectorial (basal to apical) transport of drugs is possible.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Patilea-Vrana and Unadkat

Conducted experiments: Patilea-Vrana

Contributed new reagents or analytical tool: Patilea-Vrana

Performed data analysis: Patilea-Vrana

Wrote or contributed to the writing of the manuscript: Patilea-Vrana and Unadkat

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Identifying when RDS_{uptake} switches to RDS_{all}, i.e. the tipping point. A) Extensive inhibition of $CL_{met+bile}$ can lead to a significant increase in the systemic AUC for three theoretical victim drugs that have RDS_{uptake} (i.e. $CL_{met+bile} >> CL^{s}_{ef}$) in the absence of DDI. When inhibition of $CL_{met+bile}$ eventually violates the condition required for RDS_{uptake}, the RDS_{uptake} switches to RDS_{all}. An AUCR ≥ 1.25 was observed when $CL_{met+bile}$ was inhibited ≥84%, ≥98%, and ≥99.8% for $CL_{met+bile}$ = 1, 10, 100 L/hr, respectively. However, for all three victim drugs, the $CL_{met+bile}$ value after such inhibition was similar (0.2 L/min) as was the $CL_{met+bile}/CL^{s}_{ef}$ ratio (= 2). Simulations were performed as follows: $CL^{s}_{in} = 1xQ_h$, $CL_{met+bile}$ = 1, 10, 100 L/hr, CL^{s}_{ef} = 0.1 L/min. **B)** The systemic AUC (in the absence of any DDI) of a theoretical drug remains unchanged when the $CL_{met+bile}/CL^{s}_{ef}$ ratio remains fixed (blue bars) but not when the $CL_{met+bile}/CL^{s}_{ef}$ ratio is varied (yellow bars) even though the absolute value of $CL_{met+bile}$ and CL^{s}_{ef} is varied in both scenarios. This trend was observed irrespective of the value of CL^{s}_{in} (Supplementary Fig. 1). Furthermore, this trend is true for when $CL_{met+bile} > CL^{s}_{ef}$ or $CL_{met+bile} < CL^{s}_{ef}$ (also refer to Supplementary Fig. 1). Thus, the $CL_{met+bile}/CL^{s}_{ef}$ ratio, irrespective of the magnitude of the absolute values of these clearances, is important for establishing the RDS and henceforth when the RDS switches from uptake to all hepatobiliary clearances. Simulations were performed as follows: $CL^{s}_{in} = 0.25xQ_h$ and the other input clearance values for scenarios A-E are shown in the table provided. **C)** Since the RDS depends on the $CL_{met+bile}/CL^{s}_{ef}$ ratio, we define the tipping point as the $CL_{met+bile}/CL^{s}_{ef}$ ratio at which RDS_{uptake} switches to RDS_{all}. Similar to panel A, the RDS_{uptake} switch to RDS_{all} is represented by an AUCR of 1.25 and the decrease in $CL_{met+bile}/CL^{s}_{ef}$ ratio is akin to inhibition of $CL_{met+bile}$ when CL^{s}_{ef} is kept constant. As shown by the gray arrows, the tipping point for a low, mid, and high ER drug is 3.2, 2, and 0.8, respectively. For example, if the $CL_{met+bile}/CL^{s}_{ef}$ ratio for the low ER drug is above the tipping point (i.e. $CL_{met+bile}/CL^{s}_{ef} > 3.2$), then the drug will have RDS_{uptake} and therefore DDI's due to

CL^{s}_{in} but not $CL_{met+bile}$ should be expected. However, inhibition of $CL_{met+bile}$ that makes the $CL_{met+bile}/CL^{s}_{ef}$ ratio lower than the tipping point (i.e. $CL_{met+bile}/CL^{s}_{ef} < 3.2$) will lead to a significant increase in the systemic AUC. Crossing the tipping point is indicative of the RDS_{uptake} switch to RDS_{all}. Simulations were performed as follows: systemic AUC were simulated for CL^{s}_{in} = 0.25x, 1x, 4xQ_h (representing low, mid, and high ER, respectively) and $CL_{met+bile}/CL^{s}_{ef}$ ratios from 1-10, then normalized to a control simulation where the $CL_{met+bile}/CL^{s}_{ef}$ ratio was set to 1000 (i.e. RDS_{uptake}).

Figure 2. The RDS framework helps identify DDI liabilities. The $CL_{met+bile}/CL^{s}_{ef}$ ratio and CL^{s}_{in} magnitude of a drug determines the RDS of the drug and when RDS_{uptake} switches to RDS_{all}. Combinations of hepatobiliary clearances found in the shaded area have RDS_{all} while those in the non-shaded area have RDS_{uptake}. Any alterations in hepatobiliary clearances that causes a drug to switch from the non-shaded to the shaded area will cross the tipping point (dashed line – Eq. 2) and therefore switch the RDS from uptake to all hepatobiliary clearances. The consequence of this switch is that DDI's due to inhibition of $CL_{met+bile}$ will now manifest in the systemic AUC of a victim drug that originally had RDS_{uptake}. Consistent with Fig. 1C, the tipping point decreases as the magnitude of CL^{s}_{in} (and therefore the drug's ER) increase. This suggests that the greater the ER of the drug, the more likely it will have RDS_{uptake} and it will be more resistant to switch to RDS_{all}. Furthermore, when $CL_{met+bile}/CL^{s}_{ef} > 4$, the RDS will always be uptake clearance irrespective of the value of CL^{s}_{in}/Q_h. However, when $CL_{met+bile}/CL^{s}_{ef} < 4$, the RDS can be either uptake or all hepatobiliary pathways depending on the magnitude of CL^{s}_{in}. It should be noted that if a drug is administered orally, the tipping point will always be 4 because the blood flow limitations are no longer relevant. Simulations were performed as follows: the tipping point was simulated for CL^{s}_{in} values (0.01xQ_h – 4xQ_h) using Eq. 2.

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Figure 3. Identifying when drugs with RDS_{uptake} will start to experience a DDI due to inhibition of $CL_{met+bile}$. A) The $PI_{met+bile}$, defined as the % inhibition of $CL_{met+bile}$ required for the RDS_{uptake} switch to RDS_{all}, depends on the $CL_{met+bile}/CL^{s}_{ef}$ ratio (prior to inhibition) and the magnitude of CL^{s}_{in} (represented as low, mid, and high ER drugs). The $PI_{met+bile}$ identifies when a positive DDI due to inhibition of $CL_{met+bile}$ for a drug with RDS_{uptake} would be expected. Lower $CL_{met+bile}/CL^{s}_{ef}$ ratios as well as low ER drugs are the most susceptible for the RDS_{uptake} switch to RDS_{all} due to $CL_{met+bile}$ inhibition. **B)** In order for RDS_{uptake} to switch to RDS_{all} for a theoretical victim drug with $CL_{met+bile}/CL^{s}_{ef}$ ratio of 6, $CL_{met+bile}$ must be inhibited by >46%, >66%, or >87% if the drug is low, mid, and high ER, respectively. Visually, the RDS_{uptake} switch to RDS_{all} happens when the theoretical victim drug crosses the dashed line (the tipping point) from the unshaded area (RDS_{uptake}) to the shaded area (RDS_{all}). Additional examples of $PI_{met+bile}$ are given in the table provided. Simulations were performed as follows: $PI_{met+bile}$ was calculated using Eq. 5 for $CL_{met+bile}/CL^{s}_{ef}$ ratios ranging 1-40 and for CL^{s}_{in} = 0.25x, 1x, $4xQ_h$ (representing low, mid, and high ER, respectively).

Figure 4. Summary of the purpose and conclusions for the simulations used to establish the RDS framework.

Figure 5. Applying the RDS framework to identify DDI liabilities for dual transporter/enzyme substrate drugs. If $CL_{met+bile}/CL^{s}_{ef} > 4$, then the drug will have RDS_{uptake}, irrespective of the magnitude of CL^{s}_{in}. For drugs with $RDS_{uptake,}$ DDI's due to inhibition of $CL_{met+bile}$ can become significant depending on the drug's $CL_{met+bile}/CL^{s}_{ef}$ ratio and the expected inhibition of $CL_{met+bile}$. For example, 50% inhibition of $CL_{met+bile}$ may result in a significant DDI for a drug with RDS_{uptake} and $CL_{met+bile}/CL^{s}_{ef}$ ratio < 8 but no DDI will be observed if the drug has $CL_{met+bile}/CL^{s}_{ef}$ ratio > 8. The DDI liability due to inhibition of $CL_{met+bile}$ increases as the $CL_{met+bile}/CL^{s}_{ef}$ ratio decrease and the expected $CL_{met+bile}$ inhibition increases.

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Figure 6. The distribution of drugs within the RDS framework using hepatobiliary clearance quantified *in vitro* and extrapolated to *in vivo*. Published *in vitro* hepatobiliary clearance values, when extrapolated to *in vivo* via IVIVE, can identify the RDS based on $fu_bCL^s_{in}/Q_h$ and $CL_{met+bile}/CL^s_{ef}$ ratio (Eq. 2). **A)** When no empirical scaling factors, such as to scale up active transport, are applied during the IVIVE process, all drugs except for valsartan and pravastatin have RDS_{all}. Most drugs had $CL_{met+bile}/CL^s_{ef}$ ratio < 4, indicating drugs primarily exist within the moderate RDS framework space. Furthermore, most drugs have $fu_bCL^s_{in}/Qh$ < 0.4, indicating severe underprediction of CL^s_{in}. **B)** When empirical scaling factors are used or hepatobiliary clearances are estimates from *in vivo* data using PBPK modeling, the RDS of the drugs is altered severely. Now, RDS_{uptake} occurs more often for mid and high ER drugs with RDS_{all} primarily for low ER drugs (ER was calculated from *in vivo* hepatic clearance and blood flow). Furthermore, since all drugs have $CL_{met+bile}/CL^s_{ef}$ ratio < 4, information about both the magnitude of $fu_bCL^s_{in}$ and the $CL_{met+bile}/CL^s_{ef}$ ratio is necessary to correctly predict DDI liabilities. The dashed line represents the tipping point (Eq. 2). The data shown are from Jones et al., 2012 and Varma et al., 2014, and represent a subset of the complete data set presented in Supplementary Table 1.

Figure 7. The impact of underpredictions of hepatobiliary clearance on DDI liability predictions. A representative 3-fold underprediction of either **A)** CL^s_{in} or **B)** $CL_{met+bile}$ can lead to erroneous labeling of the RDS for low, mid and high ER drugs (shown by the filled circles crossing from the non-shaded to shaded area, i.e. RDS_{uptake} switches to RDS_{all}). Mislabeling the RDS impacts the expected DDI risk due to transporters versus enzymes. Furthermore, 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. Please refer to Supplementary Fig. 4 for more detailed simulations.

Table 1. Comparison of predicted DDI liabilities from *in vitro* data to *in vivo* clinical studies

Hepatobiliary clearances, following IVIVE, can be used to identify the RDS of a drug, such as if the $CL_{met+bile}$:CL^s_{ef} ratio is > or < than the tipping point (Eq. 2), then the drug will have RDS_{uptake} or RDS_{all}, respectively. If a drug has RDS_{uptake}, then the $PI_{met+bile}$ can be quantified (Eq. 5) in order to predict when a significant DDI should be expected due to inhibition of metabolic/biliary efflux clearance. An expanded analysis is shown in Supplementary Table 1.

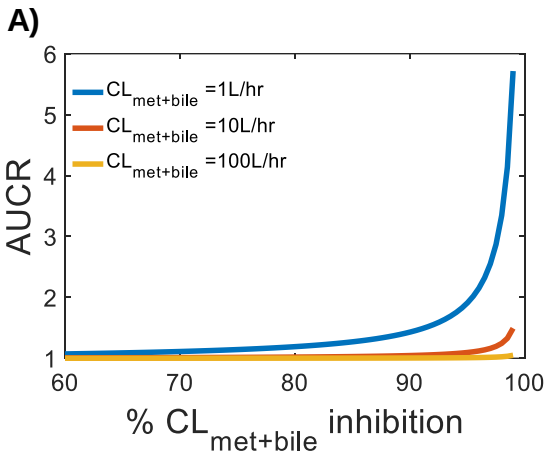
Drug	Hepatobiliary clearance (ml/min/kg)				$fu_bCL^s_{in}$ /Qh[f]	$CL_{met+bile}$ /CL^s_{ef}	Tipping point	$PI_{met+bile}$	RDS *in vitro*	RDS *in vivo*	REF
	CL^s_{in}	CL^s_{ef}	CL_{met}	CL_{bile}							
Atorvastatin	61[a]	24.9	58.3	4.3	0.12	2.52	3.59	--	all	uptake[g]	Varma et al., 2014 Tamenisch and Umehara, 2012 Kunze et al., 2015 Maeda et al., 2011
	1194[b]	24.9	58.3	4.3	2.27	2.52	1.22	>51%	uptake		
	405[c]	24.9	58.3	4.3	0.77	2.52	2.26	>10%	uptake		
	198[a]	359	64.6	11.8	0.80	0.21	2.22	--	all		
	198[a]	57.7	64.6	11.8	0.80	1.32	2.22	--	all		
Bosentan	132[a]	28.9	19.5	5.8	0.36	0.87	2.95	--	all	uptake[g]	Varma et al., 2014 Jones et al., 2012 Yoshikado et al., 2017
	142[b]	28.9	19.5	5.8	0.38	0.87	2.90	--	all		
	1117[c]	28.9	19.5	5.8	3.02	0.87	0.99	--	all		
	35[a]	12.1	--	39[e]	0.02	3.24	3.93	--	all		
	2035[d]	14	--	5.0[e]	1.09	0.36	1.91	--	all		
Repaglinide	166[a]	63.6	128	0.3	0.19	2.01	3.35	--	all	uptake[g] all[h]	Varma et al., 2014 Jones et al., 2012 Yoshikado et al., 2017
	1983[b]	63.6	128	0.3	2.32	2.01	1.21	>40%	uptake		
	1151[c]	63.6	128	0.3	1.35	2.01	1.71	>15%	uptake		
	299[a]	223	125	0.0	0.22	0.56	3.27	--	all		
	3671[d]	352	125	0.0	2.73	0.35	1.07	--	all		

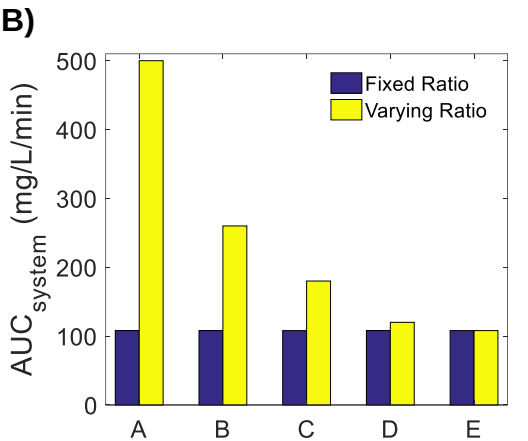
[a] – *in vitro* quantified + IVIVE
[b] – *in vitro* quantified + IVIVE + empirical scaling factor for active uptake transport (individual scaling factor)
[c] – *in vitro* quantified + IVIVE + empirical scaling factor for active uptake transport (geometric mean scaling factor)
[d] – fitted parameters from *in vivo* using PBPK model
[e] – composite $CL_{met+bile}$
[f] – fu_b and Q_h values as noted in each reference were used for analysis – note that fu_b may vary for the same drug across different references
[g] – RDS_{uptake} was established *in vivo* for atorvastatin and bosentan, since there was no significant AUC change to victim drugs when co-administered with IV itraconazole (CYP3A inhibitor) which resulted in 33% and 73% CYP3A inhibition, respectively (Maeda et al., 2011; Yoshikado et al., 2017). Midazolam, a CYP3A

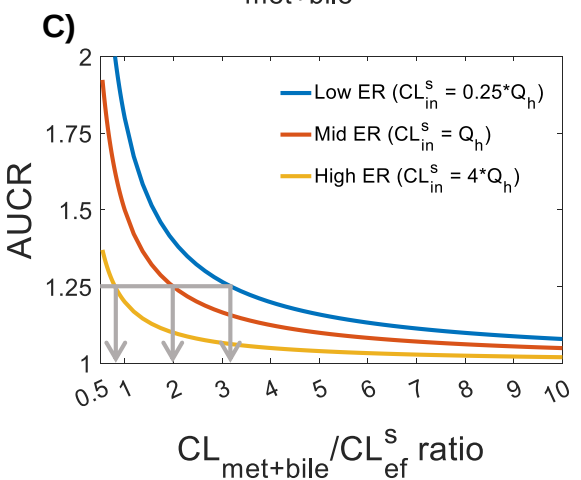
probe, was used to asses magnitude of CYP3A inhibition. RDS_{uptake} was established for repaglinide via whole-body PBPK modeling of complex transporter- and enzyme- mediated DDI's (Varma et al., 2013).

[h] – RDS_{all} was established *in vivo* for repaglinide since even though there was no change to systemic AUC by IV itraconazole, because CYP2C8 is the major hepatic drug metabolizing enzyme (Yoshikado et al., 2017). In a different study, PO trimethoprim, a selective CYP2C8 inhibitor, increased repaglinide AUC by 1.8-fold (Kim et al., 2016).

Figure 1







		A	B	C	D	E
Fixed Ratio	$CL_{met+bile}$ (L/min)	1	4	10	40	100
	CL^s_{ef} (L/min)	0.1	0.4	1	4	10
	$CL_{met+bile}/CL^s_{ef}$	10	10	10	10	10
Varying Ratio	$CL_{met+bile}$ (L/min)	0.2	0.4	1	4	10
	CL^s_{ef} (L/min)	1	1	1	1	1
	$CL_{met+bile}/CL^s_{ef}$	0.2	0.4	1	4	10

Figure 2

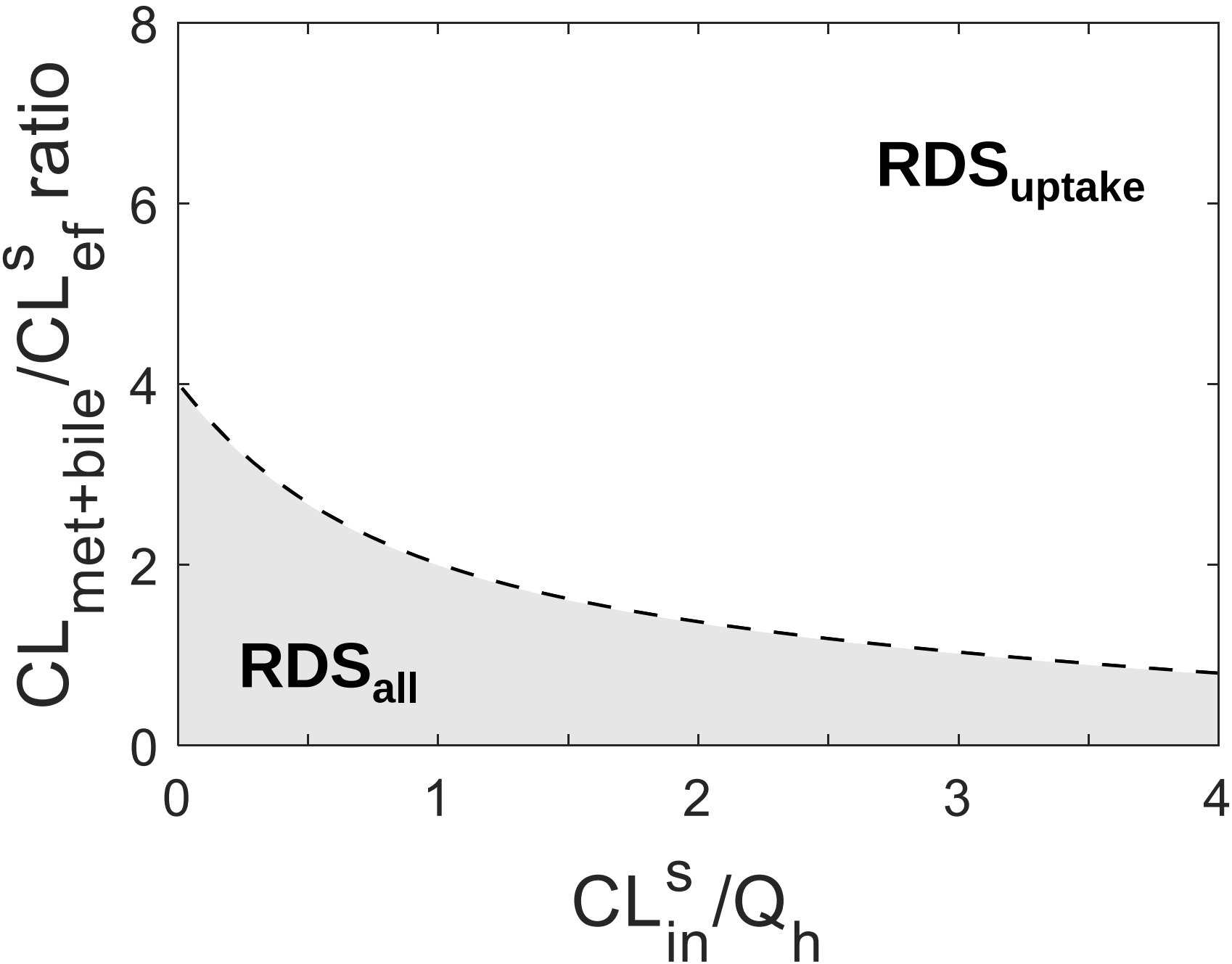
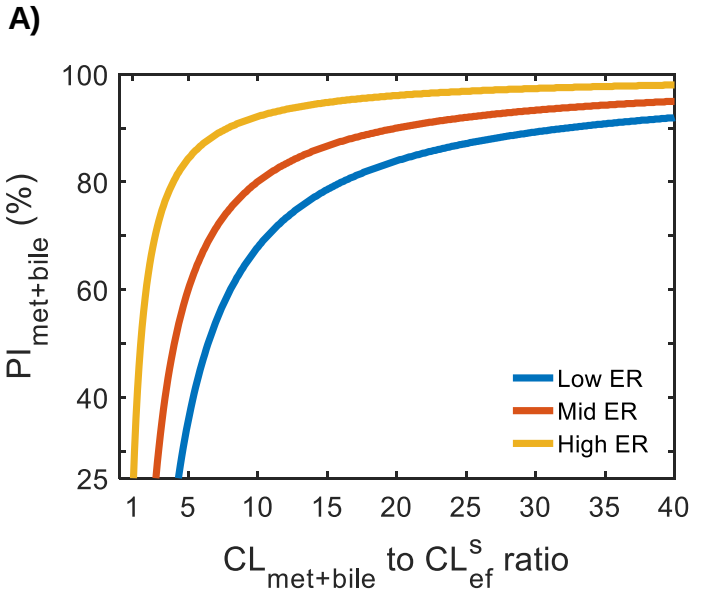
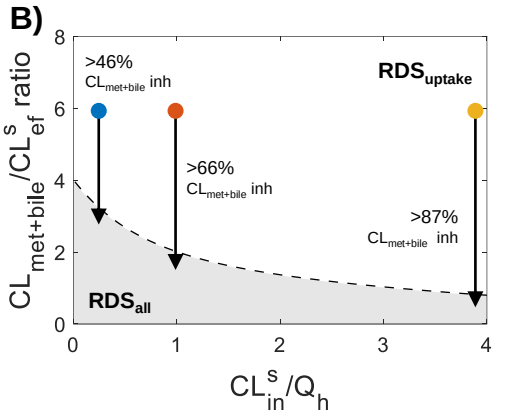

8
6
4
2
0
0
1
2
3
4
$CL^s_{met+bile}/CL^s_{ef}$ ratio
CL^s_{in}/Q_h
RDS$_{uptake}$
RDS$_{all}$

Figure 3





$PI_{met+bile}$ (%)			
$CL_{met+bile}/CL^{s}_{ef}$	Low ER	Mid ER	High ER
4 – fold	>19%	>50%	>80%
8 – fold	>60%	>75%	>87%
16 – fold	>80%	>90%	>95%

Figure 4

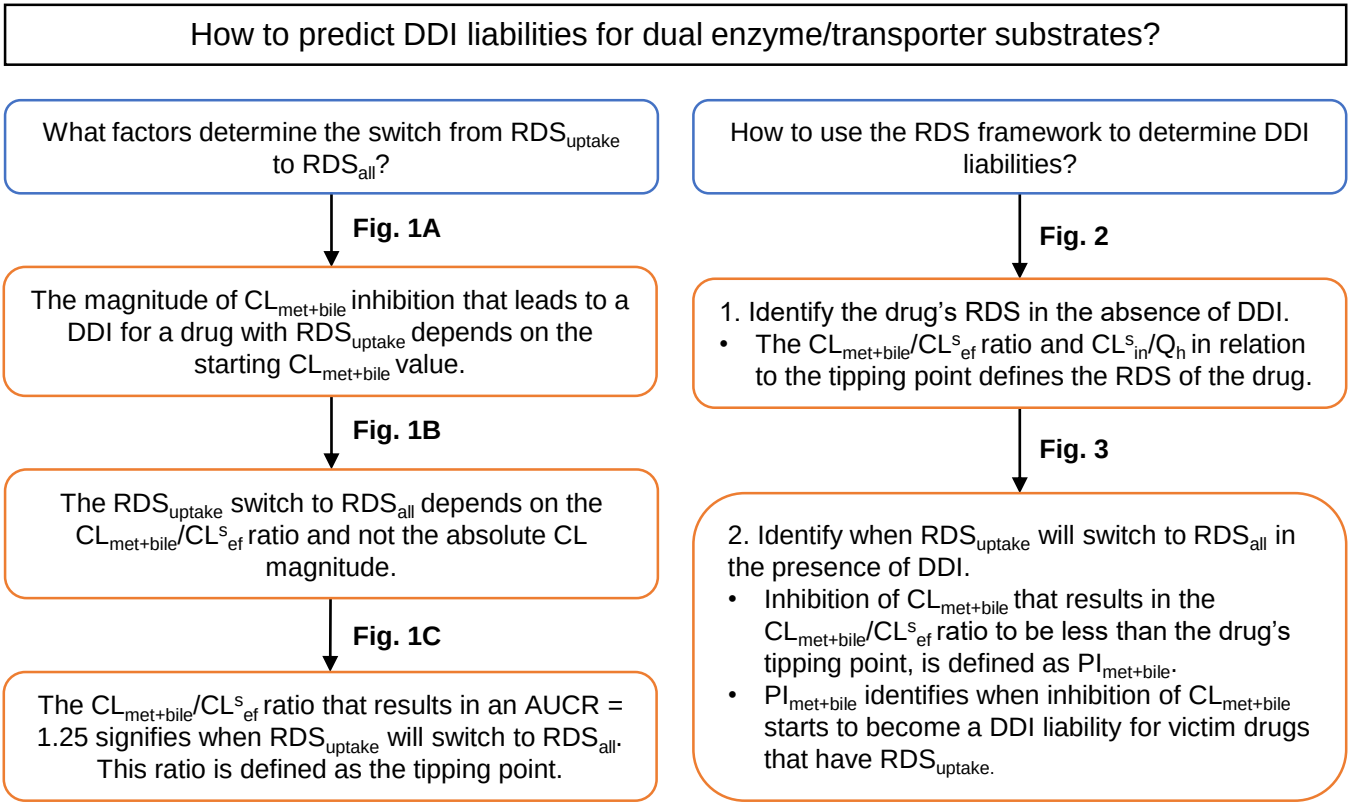

How to predict DDI liabilities for dual enzyme/transporter substrates?
What factors determine the switch from RDS$_{uptake}$ to RDS$_{all}$?
Fig. 1A
The magnitude of CL$_{met+bile}$ inhibition that leads to a DDI for a drug with RDS$_{uptake}$ depends on the starting CL$_{met+bile}$ value.
Fig. 1B
The RDS$_{uptake}$ switch to RDS$_{all}$ depends on the CL$_{met+bile}$/CL$^{s}_{ef}$ ratio and not the absolute CL magnitude.
Fig. 1C
The CL$_{met+bile}$/CL$^{s}_{ef}$ ratio that results in an AUCR = 1.25 signifies when RDS$_{uptake}$ will switch to RDS$_{all}$. This ratio is defined as the tipping point.
How to use the RDS framework to determine DDI liabilities?
Fig. 2
1. Identify the drug's RDS in the absence of DDI.
• The CL$_{met+bile}$/CL$^{s}_{ef}$ ratio and CL$^{s}_{in}$/Q$_{h}$ in relation to the tipping point defines the RDS of the drug.
Fig. 3
2. Identify when RDS$_{uptake}$ will switch to RDS$_{all}$ in the presence of DDI.
• Inhibition of CL$_{met+bile}$ that results in the CL$_{met+bile}$/CL$^{s}_{ef}$ ratio to be less than the drug's tipping point, is defined as PI$_{met+bile}$.
• PI$_{met+bile}$ identifies when inhibition of CL$_{met+bile}$ starts to become a DDI liability for victim drugs that have RDS$_{uptake}$.

Figure 5

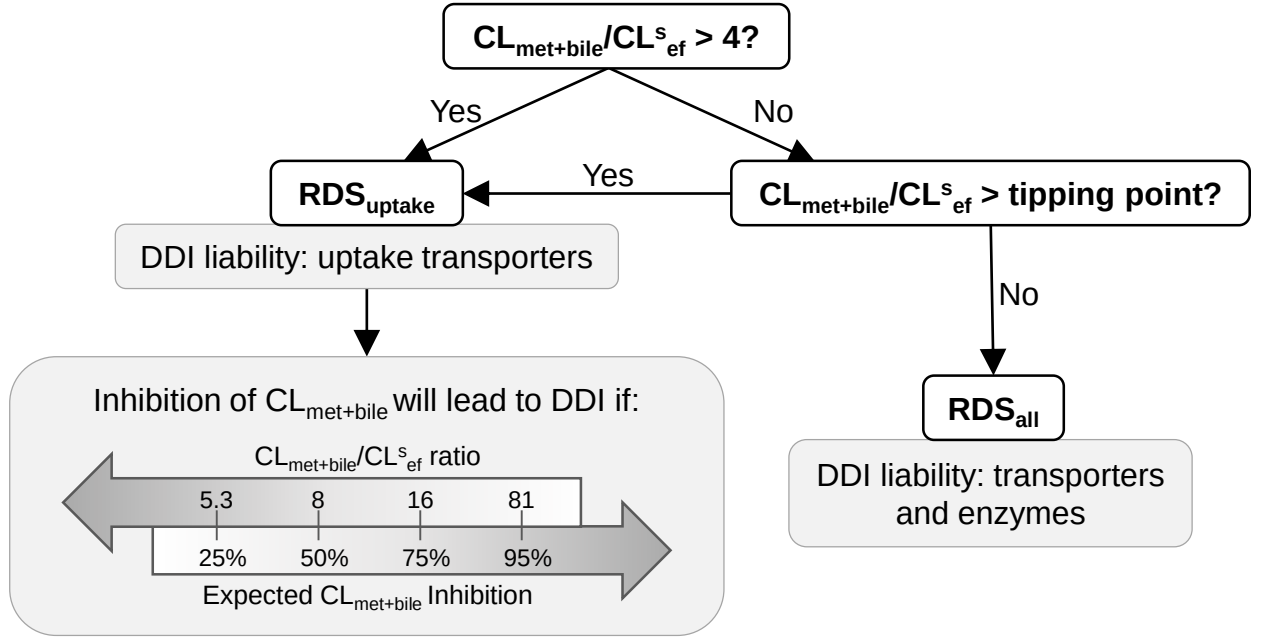

CLmet+bile/CLsef > 4?
Yes
No
RDSuptake
Yes
CLmet+bile/CLsef > tipping point?
DDI liability: uptake transporters
No
RDSall
DDI liability: transporters and enzymes
Inhibition of CLmet+bile will lead to DDI if:
CLmet+bile/CLsef ratio
5.3
8
16
81
25%
50%
75%
95%
Expected CLmet+bile Inhibition

Figure 6

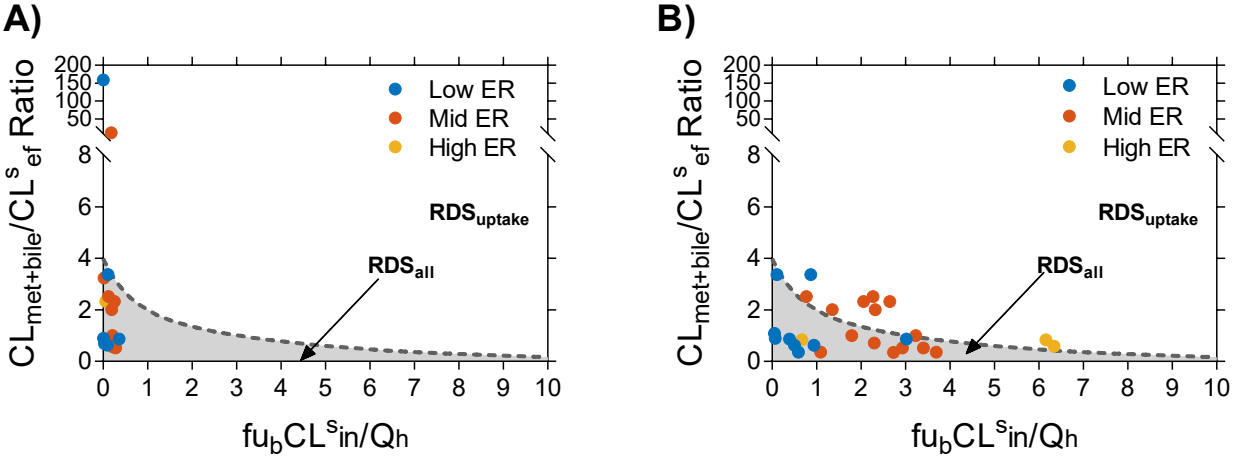
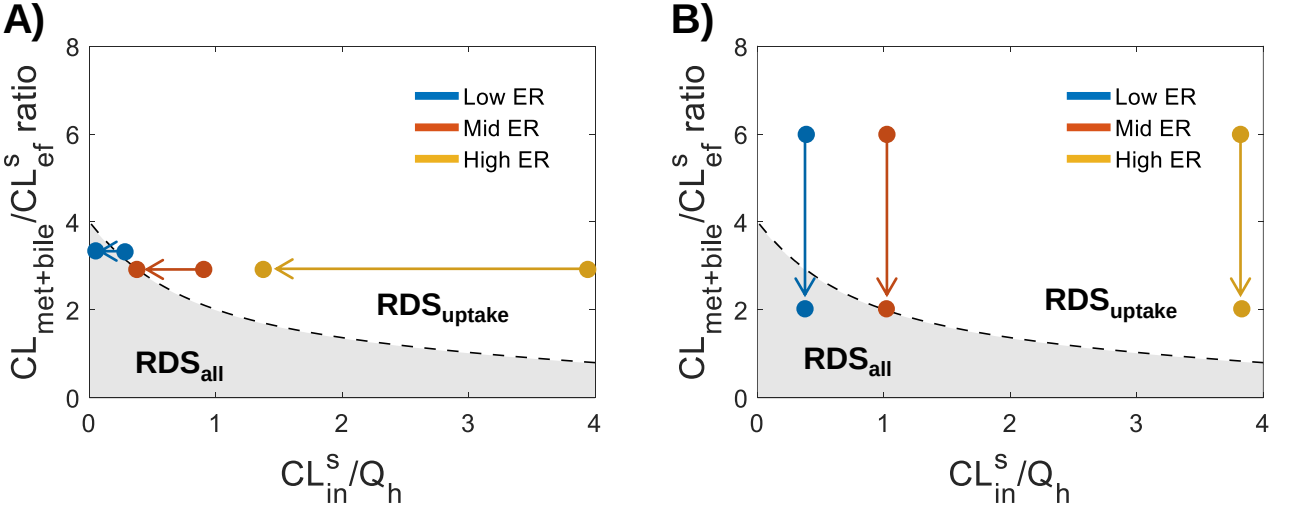

A)
B)
Low ER
Mid ER
High ER
RDS_uptake
RDS_all
CL_met+bile/CL^s_ef Ratio
fu_bCL^sin/Qh
0
1
2
3
4
5
6
7
8
9
10
50
100
150
200

Figure 7


A)
B)
Low ER
Mid ER
High ER
RDS_uptake
RDS_all
CL^s_met+bile/CL^s_ef ratio
CL^s_in/Q_h
0
1
2
3
4
6
8

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

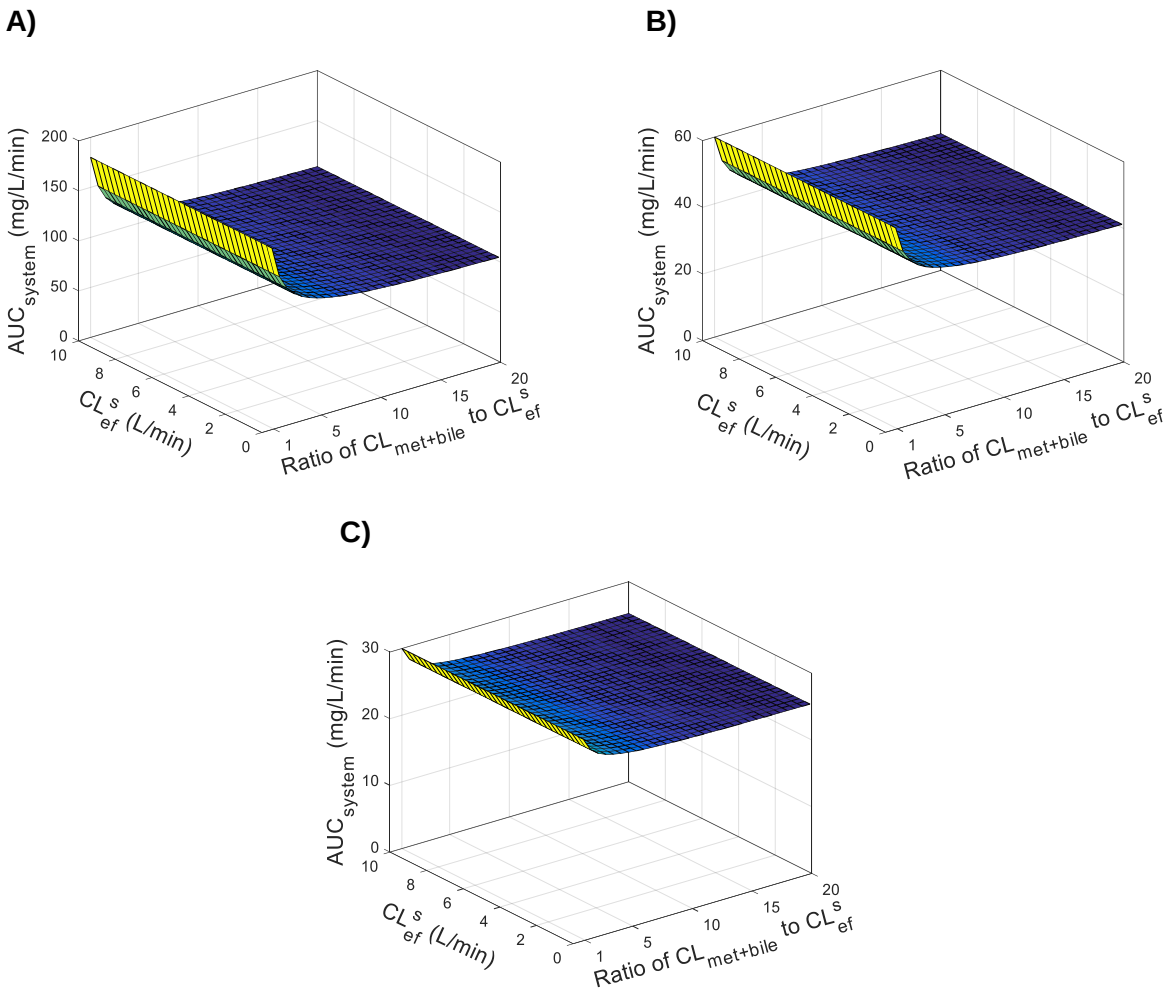
[c] Parameters estimated using a PBPK model and IV data where all parameters were fixed except for active uptake clearance, passive diffusion, and $CL_{met+bile}$

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

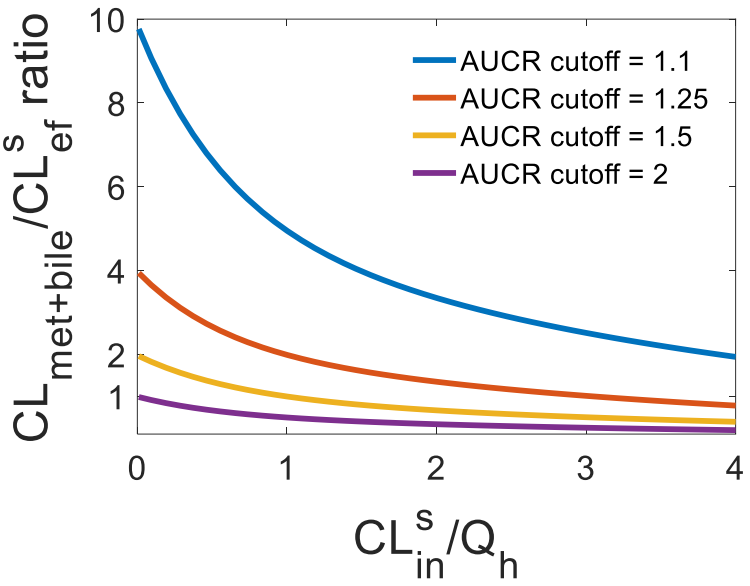
[e] ECCS classes: 1A – metabolism, 1B – uptake, 2 – metabolism, 3A – renal, 3B – uptake or renal, 4 – renal

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

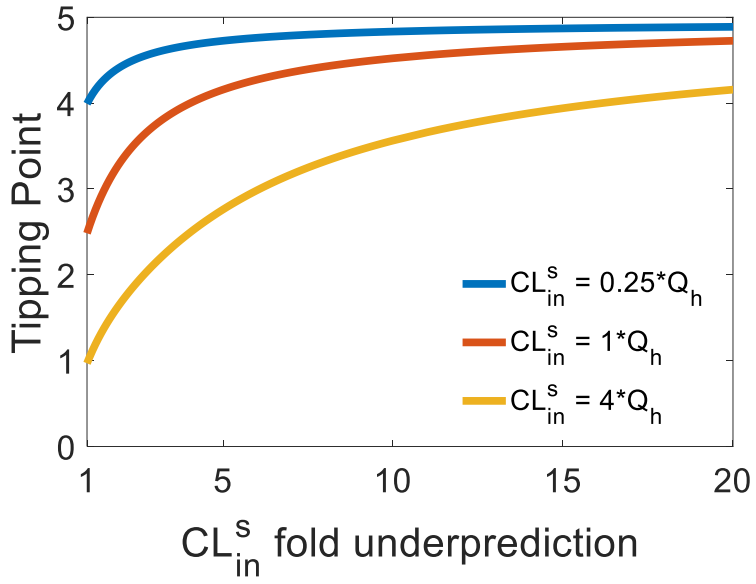
n.d. - not determined



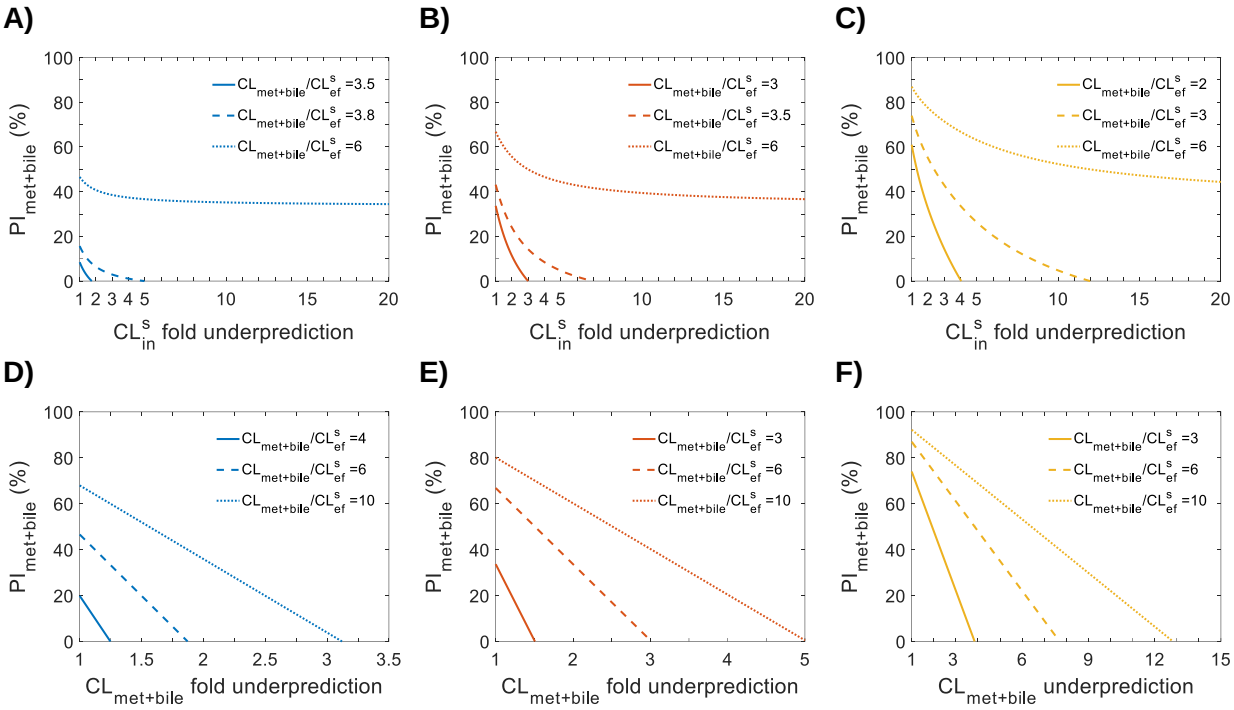
Supplementary Figure 1. Irrespective of the CL^s_{in} value, the systemic AUC of a drug is determined by the $CL_{met+bile}/CL^s_{ef}$ ratio and not the magnitude of the $CL_{met+bile}$ and CL^s_{ef} clearance. The systemic AUC decreases as the $CL_{met+bile}/CL^s_{ef}$ ratio (x-axis) increases but there 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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