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Regulatory recommendations for calculating the unbound maximum hepatic inlet concentration: A complicated story with a surprising and happy ending

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Running Title: The unbound maximum hepatic inlet concentration

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The European Medicines Agency (EMA), the US Food and Drug Administration (FDA), and the Japanese Pharmaceutical and Medical Devices Agency (PMDA) all recommend interpreting the results of *in vitro* studies of CYP inhibition, transporter inhibition, and CYP induction based on equations that incorporate a relevant *in vivo* concentration of test drug (also known as the investigational drug, new chemical or molecular entity, inhibitor, inducer, perpetrator or precipitant) (EMA, 2013; FDA, 2017; PMDA, 2018). For the so-called basic static models of drug interactions, the three main *in vivo* concentrations are I_{gut}, $I_{max,u}$, and $I_{in,max,u}$, each of which is used with a safety factor to minimize false negative predictions. For interactions with intestinal CYP3A and efflux transporters, the relevant *in vivo* concentration is I_{gut}, the intestinal concentration of an orally administered test drug, which all agencies recommend calculating from dose/250 mL. For interactions with hepatic CYP enzymes (both inhibition and induction), hepatic efflux transporters, and renal uptake and efflux transporters, the relevant *in vivo* concentration is $I_{max,u}$, the unbound maximum concentration of test drug in plasma following dosing to steady state with the highest intended clinical dose, which all agencies recommend calculating from plasma $C_{max,ss}$ x fu_P, where fu_P is the fraction of unbound drug in plasma with a lower limit of 0.01 (for the basic static models). For interactions with hepatic uptake transporters, such as OATP1B1, OATP1B3, and OCT1, the relevant *in vivo* concentration is $I_{in,max,u}$, the unbound maximum hepatic inlet concentration of an orally administered test drug. Here the agencies diverge. This letter points out some potential sources of confusion stemming from differences in how regulatory agencies recommend calculating $I_{in,max,u}$. It ends on a happy note.

To dispense with the easiest difference right away: the unbound maximum hepatic inlet concentration is called $I_{in,max,u}$ by the FDA, $I_{inlet,max,u}$ by the PMDA, and $[I]_{u,inlet,max}$ by the EMA. The first potential source of confusion is that, in the case of the FDA, the term $I_{in,max,u}$ refers to the unbound maximum hepatic inlet concentration of test drug in *plasma*. However, the EMA's term $[I]_{u,inlet,max}$ and the PMDA's term $I_{inlet,max,u}$ refer to the corresponding concentration in *blood.* How did these differences arise and, perhaps more importantly, what impact do they have on interpreting the results of *in vitro* drug interaction studies? Is it possible that a negative result according to one agency might be positive according to another agency?

All three agencies base their equation for estimating the hepatic inlet concentration of an orally administered drug on a method described by Ito *et al.* (1998). The original equation was

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developed for the concentration of test drug in *blood* (which is evident from Fig. 4 in Ito's paper). The equation developed by Ito *et al.* (1998) is described below because the corresponding equation described by all three agencies contains ambiguities (at best) or errors (at worst).

Based on Ito *et al*. (1998), the total (bound + unbound) maximum hepatic inlet concentration of a test drug in blood ($I_{in,max,B}$) can be estimated from the sum of two blood concentrations, namely the maximum concentration of drug in systemic blood ($I_{max,B}$, which is also known as $C_{max,B}$) and the maximum concentration of drug that was absorbed from the gut into hepatic portal blood, which is estimated from four parameters: (1) oral dose; (2) F_a, the fraction of parent drug absorbed from the gastrointestinal tract into hepatic portal blood; (3) the absorption rate constant, k_a (the fraction of dose absorbed per unit time), and (4) hepatic blood flow, Q_H (or Q_h).

$$I_{in,max,B} = I_{max,B} + \left(\frac{Dose \cdot F_a \cdot k_a}{Q_H}\right) \quad Equation\ 1$$

Equation 1 is an abbreviated version of Equation 22 in Ito *et al.* (1998). The subscript 'B' was added to clarify that the concentrations of test drug are those in blood, not plasma.

In Equation 1, the term on the left, namely $I_{max,B}$, represents drug entering the liver from the systemic circulation whereas the term on the right, namely $(Dose \cdot F_a \cdot k_a)/Q_H$, represents drug entering the liver from the gut via the hepatic portal vein. It should be noted that Ito *et al.* (1998) defined F_a as the fraction of oral dose that reaches the hepatic portal vein. For the basic static model, all three regulatory agencies replaced Ito's term F_a with a new term, namely $F_a \cdot F_g$. The agencies redefined F_a to mean the fraction of drug that is absorbed from the lumen of the gut, and they defined F_g as the fraction of drug that passes through the gut wall into the hepatic portal vein. Accordingly, Ito's term F_a is identical to the term $F_a \cdot F_g$ used by regulatory agencies. The advantage of the term $F_a \cdot F_g$ is that it captures additional mechanistic information. For example, if the value of $F_a \cdot F_g$ is 0.5, it could reflect the situation where 100% of the drug is absorbed from the gut ($F_a = 1$) but only 50% reaches the hepatic portal blood ($F_g = 0.5$), perhaps due to intestinal metabolism, or it could reflect the situation where only half the drug is absorbed from the gut ($F_a = 0.5$), perhaps due to poor aqueous solubility or efflux by intestinal transporters, but all of the drug that is absorbed reaches the hepatic portal blood ($F_g = 1.0$). Accordingly, to reflect the new terms used by regulatory agencies, Ito's equation can be modified as follows:

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$$I_{in,max,B} = I_{max,B} + \left(\frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H}\right) \quad Equation\ 2$$

where k_a is the rate of intestinal absorption (time^{-1}), F_a is the fraction of dose absorbed from the gut lumen, F_g is the fraction of drug that passes through the intestinal wall into blood, and Q_H is hepatic blood flow. Unless it is determined experimentally, k_a is assumed to have a value of 0.1 min^{-1}, meaning that 0.1 of the dose (*i.e.*, 10%) is absorbed every minute. Q_H, hepatic blood flow, is 97 L/h for a 70-kg person (the value used by all 3 regulatory agencies), but in Equation 2 this is expressed on a per-minute basis to match the units of k_a; hence, Q_H = 1.62 L/min. If blood concentrations are expressed in µM (to match values of K_i or IC_{50}), then *Dose* is expressed in µmol provided Q_H is expressed in *liters* per unit time (*i.e.*, 1.62 L/min). The term $F_a \cdot F_g$ represents the fraction of drug that is absorbed from the intestine into hepatic portal blood. Unless it is determined experimentally, $F_a \cdot F_g$ is assumed to have a value of 1, which assumes that all of the orally administered parent drug is absorbed from intestine (F_a = 1) and all of it crosses the intestinal lining and enters hepatic portal blood (F_g = 1).

R_b is the ratio of drug concentration in blood (C_B) to drug concentration in plasma (C_P); hence $R_b = C_B/C_P$. Accordingly, $C_P = C_B/R_b$, and $C_B = C_P \cdot R_b$. In other words, R_b can be used to convert blood concentrations to plasma concentrations, and *vice versa*. Dividing all sides of Equation 2 by R_b gives Equation 3:

$$\frac{I_{in,max,B}}{R_b} = \frac{I_{max,B}}{R_b} + \frac{\left(\frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H}\right)}{R_b} \quad Equation\ 3$$

$I_{in,max,B}/R_b$ is the maximum hepatic inlet concentration of total drug in *plasma*, which is written by the FDA as $I_{in,max}$ (without a subscripted 'P' to clarify this is a *plasma* concentration). Likewise, $I_{max,B}/R_b$ is the maximum systemic concentration of total drug in *plasma*, which is invariably written as I_{max}; it corresponds to plasma C_{max}. Accordingly, Equation 3 can be simplified as follows:

$$Plasma\ I_{in,max} = Plasma\ I_{max} + \frac{\left(\frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H}\right)}{R_b} \quad Equation\ 4$$

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Equation 4 describes how to calculate the *total* (bound + unbound) maximum hepatic inlet concentration of drug in plasma. The *unbound* maximum hepatic inlet concentration in plasma, namely $I_{in,max,u}$, is calculated according to Equation 5:

$$Plasma\ I_{in,max,u} = fu_P \left(Plasma\ I_{max} + \frac{\left(\frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H}\right)}{R_b} \right) \qquad Equation\ 5$$

where fu_p is the fraction of unbound drug in plasma (with a lower limit of 0.01 for the basic static model according to all three regulatory agencies).

What equations do regulatory agencies recommend for estimating the maximum hepatic inlet concentration of an orally administered drug?

FDA – Total hepatic inlet concentration

The FDA calculates the maximum hepatic inlet concentration of total (bound + unbound) drug in plasma based on the following equation (as written in Figure 6 of the 2017 FDA Guidance for Industry), where $I_{in,max}$ is the total maximum hepatic inlet concentration in *plasma*:

$$I_{in,max} = (I_{max} + (F_a F_g\ x\ k_a\ x\ Dose))/Q_H/R_b \qquad Equation\ 6$$

For clarity an expanded form of this equation is shown below.

$$I_{in,max} = \frac{\frac{I_{max} + \left(k_a \cdot Dose \cdot F_a \cdot F_g\right)}{Q_H}}{R_b} \quad Equation\ 7$$

The expanded form of the equation makes it clear that systemic plasma concentration (I_{max}) is being divided by Q_H and R_b, which is applicable only to the estimate of the concentration of drug absorbed from the intestine into hepatic portal blood. The linear form of the FDA equation should be written as follows:

$$I_{in,max} = I_{max} + \left(\left(\left(F_a F_g\ x\ k_a\ x\ Dose\right)/Q_H\right)/R_b\right) \quad Equation\ 8$$

This single line equation is identical to the expanded version shown in Equation 4.

PMDA – Total hepatic inlet concentration

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Between issuing its draft guidelines in September, 2017 and its final guidelines in February, 2018, the PMDA changed its equation for calculating $I_{inlet,max}$, the agency's term for the maximum hepatic inlet concentration of total (bound + unbound) drug. The equations appear in the footnote to Figure 2-5 in both documents. The draft version is shown in Equation 9A; the final version in Equation 9B

$$I_{inlet,max} = C_{max} + \left(k_a \cdot dose \cdot F_a \cdot F_g / Q_H\right) \qquad Equation\ 9A$$

$$I_{inlet,max} = C_{max} + \left(k_a \cdot dose \cdot F_a \cdot F_g / Q_H\right) \qquad Equation\ 9B$$

The fact the equations are identical conceals an important difference, namely that, in Equation 9A, C_{max} is defined as the maximum concentration of total drug in *plasma*, whereas in Equation 9B it is defined as the maximum concentration of total drug in *blood*. Accordingly, in the draft version, $I_{inlet,max}$ was calculated as the sum of two concentrations, one in plasma (C_{max}) and one in portal blood, such that $I_{inlet,max}$ was neither plasma concentration nor blood concentration but a combination of both. By changing the definition of C_{max} from a plasma concentration to a blood concentration, the final version of the PMDA guidelines corrected this error and made $I_{inlet,max}$ the maximum hepatic concentration of total drug in blood. For clarity, the PMDA's final equation can be written as follows:

$$Blood\ I_{inlet,max} = C_{max,B} + \left(\frac{k_a \cdot dose \cdot F_a \cdot F_g}{Q_H}\right) \qquad Equation\ 9C$$

EMA – Total hepatic inlet concentration

The EMA does not describe an equation for calculating the *total* maximum hepatic inlet concentration but it does describe an equation for calculating the *unbound* maximum hepatic inlet concentration in blood, which is described later. Had the EMA described a separate equation for calculating the total maximum hepatic inlet concentration in blood, it would have been the same as Equation 9C (the PMDA's equation).

FDA – The unbound maximum hepatic inlet concentration in plasma

The FDA equation shown above (Equation 4) is intended to calculate the total (bound + unbound) maximum hepatic inlet drug concentration in *plasma* (called $I_{in,max}$ by the FDA). The corresponding unbound concentration is calculated as follows: $I_{in,max,u} = fu_P \cdot I_{in,max}$, as shown in Equation 5.

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EMA and PMDA – The unbound maximum hepatic inlet concentration in blood

The PMDA does not show an expanded equation for calculating the unbound maximum hepatic concentration in blood, but the flowchart in Figure 2-5 shows that this is calculated as fu_B x $I_{inlet,max}$, where fu_B is the unbound concentration of drug in blood, and $I_{inlet,max}$ is the total maximum hepatic inlet concentration in blood, which is calculated as shown in Equation 9C.

The EMA describes an equation for estimating the unbound hepatic inlet concentration of drug in *blood*, termed $[I]_{u,inlet,max}$, as follows:

$$[I]_{u,inlet,max} = fu_B \cdot \left([I]_{max,B} + F_a \cdot F_g \cdot k_a \cdot Dose/Q_H\right) \quad Equation\ 10$$

This equation is correct provided the term $F_a \cdot F_g \cdot k_a \cdot Dose$ is divided by Q_H before it is added to $[I]_{max,B}$. Adding brackets around the term $F_a \cdot F_g \cdot k_a \cdot Dose$ /Q_H would remove any ambiguity. Accordingly, the EMA and PMDA both describe an equation (Equation 10) for calculating the unbound maximum hepatic inlet concentration of drug in *blood*, whereas the FDA describes an equation (Equation 5) for calculating the corresponding concentration in *plasma.*

The concentration of a circulating test drug is rarely measured in blood; it is nearly always measured in plasma. $[I]_{max,B}$ can be estimated by multiplying plasma C_{max} by R_b. Likewise, protein binding of a test drug is usually measured in plasma, not blood. The unbound fraction of drug in blood (fu_B) can be estimated by dividing the unbound fraction in plasma (fu_P) by R_b. These relationships are summarized in Equation 11.

$$C_B(blood\ concentration) \cdot fu_B = C_P(plasma\ concentration) \cdot fu_P \qquad Equation\ 11$$

Accordingly, the EMA and PMDA equations can be written as follows (where the subscripts B and P represent blood and plasma, respectively):

$$[I]_{u,inlet,max,B} = \left(\frac{fu_P}{R_b}\right) \cdot \left(\left([I]_{max,P} \cdot R_b\right) + \left(\frac{F_a \cdot F_g \cdot k_a \cdot Dose}{Q_H}\right)\right) \quad Equation\ 12$$

Blood *versus* plasma

How do plasma levels of *total* maximum hepatic inlet concentration compare with blood levels, and how do plasma values of *unbound* maximum hepatic inlet concentration compare with blood values? Simulated comparisons were made for drugs with an R_b of 0.5, 1.0, and 2.0. The oral dose of each drug was 325 µmol. Each drug had a systemic plasma C_{max} of 4.0 µM, and each

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was 80% bound to plasma protein (fu_P = 0.2). In each case, $F_a \cdot F_g$ was 1, and k_a was 0.1 min^{-1}. Hepatic blood flow (Q_H) was 1.62 mL/min. Table 1 shows calculations of *total* (bound + unbound) and *unbound* maximum hepatic inlet concentration in *plasma*, as recommended by the FDA, and calculations of the corresponding concentrations in *blood*, as recommended by the EMA and PMDA. The *total* maximum hepatic inlet concentrations in plasma (the FDA's approach) differ from those in blood (the EMA and PMDA's approach) depending on the value of R_b. However, the values of *unbound* maximum hepatic inlet concentration are *identical* in plasma and blood for a given value of R_b. This is because to convert plasma concentration to blood concentration you *multiply* by R_b ($C_B = C_P \times R_b$) but to convert the unbound fraction of drug in plasma to unbound concentration in blood you *divide* by R_b ($fu_B = fu_P/R_b$), based on the relationship shown in Equation 11. The two cancel each other out.

A formal proof that the EMA/PMDA and FDA's equations for calculating the unbound maximum hepatic inlet concentration in blood and plasma, respectively, are identical is as follows:

Equation 12 - the EMA's (and PMDA's) equation - is as follows:

$$Blood\ [I]_{u,inlet,max} = \left(\frac{fu_P}{R_b}\right) \cdot \left(\left([I]_{max,P} \cdot R_b\right) + \left(\frac{F_a \cdot F_g \cdot k_a \cdot Dose}{Q_H}\right)\right) \quad Equation\ 12$$

Multiplying each of the terms for systemic blood concentration and portal blood concentration by fu_p/R_b gives the expanded version Equation 13:

$$Blood\ [I]_{u,inlet,max} = \left(fu_p \cdot \left([I]_{max,P}\right) + \frac{fu_p}{R_b} \cdot \left(\frac{F_a \cdot F_g \cdot k_a \cdot Dose}{Q_H}\right)\right) \quad Equation\ 13$$

Factoring out fu_p gives the contracted equation:

$$Blood\ [I]_{u,inlet,max} = fu_p \cdot \left(\left([I]_{max,P}\right) + \frac{1}{R_b} \cdot \left(\frac{F_a \cdot F_g \cdot k_a \cdot Dose}{Q_H}\right)\right) \quad Equation\ 14$$

Equation 14, a rearrangement of the EMA's equation for calculating the unbound maximum hepatic inlet concentration in *blood*, is identical to Equation 5, the FDA's equation for calculating the unbound maximum hepatic inlet concentration in *plasma*:

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$$Plasma\ I_{in,max,u} = fu_P \left(Plasma\ I_{max} + \frac{\left(\frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H}\right)}{R_b} \right) \qquad Equation\ 5$$

One point needs to be emphasized. I stated previously that the values of *unbound* maximum hepatic inlet concentration are *identical* in blood and plasma for a given value of R_b. The qualification "for a given value of R_b" is important. As shown in Table 1, values of R_b do have an impact on the unbound maximum hepatic inlet concentration. But for a given drug with a given R_b value, the unbound maximum hepatic inlet concentration in blood is the same as that in plasma, so it makes no difference whether you calculate the FDA's value of *plasma* $I_{in,max,u}$ according to Equation 5 or calculate the EMA/PMDA's value of *blood* $[I]_{u,inlet,max}$ according to Equation 12.

All of this suggests that, when evaluating the inhibition of hepatic uptake transporters based on the unbound maximum hepatic inlet concentration, a negative result obtained with the FDA's approach would also be negative according to the EMA and PMDA's approach. That would be true if the three agencies used the same equations and cutoff criteria, but they don't. The FDA and PMDA's equation and cutoff for inhibition of hepatic uptake transporters (OATP1B1 and OAPT1B3) is shown in Equation 15.

$$1 + \frac{I_{in,max,u}}{K_i\ or\ IC_{50}} \geq 1.1 \qquad Equation\ 15$$

The EMA's equation and cutoff for inhibition of hepatic uptake transporters (OATP1B1, OAPT1B3, and OCT1) is shown in Equation 16.

$$\frac{25 \cdot [I]_{u,inlet,max}}{K_i\ or\ IC_{50}} \geq 1 \qquad Equation\ 16$$

The EMA's equation is equivalent to an FDA/PMDA-style equation (1 + ratio) with a cutoff value of 1.04 (=1 + $^1/_{25}$), compared with 1.1 for the FDA and PMDA. In other words, the EMA is more conservative than the other two agencies.

Another important difference is the FDA, which updated its Guidance in 2017, and the PMDA, which updated its guidelines in 2018, now recommend evaluating test drugs as inhibitors of OATPs with and without a 30-min preincubation of the test drug with the test system to assess the potential for time-dependent inhibition of OATPs (FDA, 2017; PMDA 2018).

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In the case of the basic static models, the unbound maximum hepatic inlet concentration is used only to evaluate the potential for a test drug to cause clinically relevant inhibition of hepatic uptake transporters (OATP1B1, OATP1B3 and, for the EMA, OCT1). It is also used when drug interactions are assessed by the static mechanistic model (FDA, 2017), the mechanistic static model (EMA, 2013), or the mechanism-based static pharmacokinetic (MSPK) model (PMDA, 2018), which are all one-and-the-same. This model provides an assessment of the net effect of reversible inhibition, irreversible inhibition, and induction of CYP enzymes in the liver and intestine. Despite describing this model, the EMA and FDA both recommend that an assessment of CYP induction potential should not be combined with assessments of reversible and irreversible CYP inhibition potential, but the static mechanistic model can be used to assess the combined impact of reversible and irreversible CYP inhibition. When the static mechanistic model is used to assess the impact of a test drug on hepatic enzymes, all three agencies recommend basing the assessment on the unbound maximum hepatic inlet concentration, which all three agencies call $[I]_h$. The EMA makes it clear that $[I]_h$ corresponds to $[I]_{u,inlet,max}$ (its term for the unbound maximum hepatic inlet concentration) and both are calculated from the same equation (Table 1 in EMA, 2013). In the body of its updated guidelines, the PMDA does not specify that $[I]_h$ corresponds to $I_{inlet,max,u}$ (its term for the unbound maximum hepatic inlet concentration) nor does it provide an equation for calculating $[I]_h$. It simply states that $[I]_h$ is the "concentration of the investigational drug in the liver hepatocytes." However, the 2018 PDMA guidelines end with a Question & Answer section. In response to Question 15, the PMDA clarifies that [I]h corresponds to $I_{inlet,max,u}$, which the agency recommends calculating from Equation 10, which is the EMA's equation for calculating $[I]_{u,inlet,max}$. The FDA does not specify that $[I]_h$ corresponds to $I_{in,max,u}$ (its term for the unbound maximum hepatic inlet concentration) but it does provide an equation for calculating $[I]_h$ (Figure 7 in FDA, 2017). However, the equation for calculating $[I]_h$ is not identical to the equation for calculating $I_{in,max,u}$. For reasons outlined earlier, to calculate $I_{in,max,u}$ for the basic static model, the FDA replaced Ito's original term F_a with the combined term $F_a \cdot F_g$ (see Equation 5). However, to calculate $[I]_h$ for the static mechanistic model, the FDA retained Ito's original term and definition of F_a (Figure 7 in FDA, 2017); hence, F_g appears in the equation to calculate $I_{in,max,u}$ but not in the equation to calculate $[I]_h$, even though they are the same thing, namely the unbound maximum hepatic inlet concentration. Equation 5 should be used to calculate plasma $I_{in,max,u}$ and $[I]_h$.

I will touch on 3 related topics before I close with a summary.

First, the concentration of test drug in hepatic portal blood is estimated from dose, k_a, $F_a \cdot F_g$, and Q_H. I don't know why blood flow is based on total hepatic blood flow (97 L/h) and not on hepatic portal blood flow, which is roughly 80% of total hepatic blood flow (Carlisle *et al*., 1992).

Second, as a general rule, non-ionized and basic drugs tend to distribute evenly between plasma and erythrocytes with R_b values close to 1, whereas zwitterionic and acidic drugs tend to be excluded from erythrocytes with R_b values of roughly 0.55 (which is 1–hematocrit) (Hallifax *et al.*, 2010). However, some drugs have a high blood-to-plasma concentration ratio. For example, R_b values for tacrolimus average about 15 but the values are concentration-dependent and vary from 4 to 114 (Wallemacq and Verbeeck, 2001). The partitioning of tacrolimus into erythrocytes does not restrict its hepatic clearance, which is due mainly to metabolism by CYP3A. Accordingly, whereas the hepatic clearance of intravenously administered tacrolimus from blood ranges from 2.1 to 6.3 L/h (for a 70 kg person), which is well below hepatic blood flow (97 L/h), hepatic clearance from plasma ranges from 42 to 378 L/h, which greatly exceeds hepatic blood flow (97 L/h) and hepatic plasma flow (~53 L/h) (Wallemacq and Verbeeck, 2001). The finding that the plasma clearance of tacrolimus (CL_P) is equal to blood clearance (CL_B) x R_b suggests that the hepatic clearance of tacrolimus is not restricted by its extensive partitioning into erythrocytes (due to its binding to an intracellular immunophilin known as FK-binding protein-12 or FKBP12). However, not all drugs with a high R_b value behave like tacrolimus. Some diuretic drugs, such as chlorthalidone, dorzolamide, and methazolamide, bind with high affinity to carbonic anhydrase, an abundant enzyme in erythrocytes (Hinderling, 1997). These drugs have R_b values ranging from 30 to 240 but they do not move freely between erythrocytes and plasma; hence, for these drugs – drugs whose movement out of erythrocytes is restricted – it is their concentration in *plasma* that is relevant to their potential to cause drug interactions (Hinderling, 1997). When a drug has a high value of R_b, it would be prudent to establish that partitioning into erythrocytes is readily reversible before using R_b to interconvert values of drug concentration in blood and plasma, protein binding in blood and plasma, and/or clearance from blood and plasma. Such interconversions are not appropriate when high values of R_b reflect restrictive binding of drugs to erythrocytes.

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Third, hoping to find some clarification on issues surrounding equations for calculating the unbound maximum hepatic inlet concentration, I turned to a publication authored by representatives from the FDA, EMA, pharma and academia (Vieira *et al.*, 2014). Their equation for calculating the unbound maximum hepatic inlet concentration in blood (called $[I]_{u,inlet,max}$ after the EMA) is shown in Figure 1. I include it here for two reasons. First, on the positive side, the equation reinforces the importance of using blood concentration in equations that include one or more terms for organ blood flow. This same point was made by Yang *et al.* (2007) for estimating hepatic clearance according the well-stirred model, an equation that includes a term for hepatic blood flow. Both publications point to the importance of taking R_b into account. On the negative side, the published equation for calculating the unbound maximum hepatic inlet concentration is missing a plus sign, as shown in Figure 1, which supports my contention that this is a rather tricky area to navigate. It is also worth noting that, for the fraction of drug absorbed from the intestine into hepatic portal blood, the equation shown in Figure 1 uses F_a, the term originally used by Ito *et al.*, (1998), rather than $F_a \cdot F_g$, the term now used by all three regulatory agencies, as discussed earlier.

In summary, the maximum hepatic inlet concentration of a test drug is the sum of two concentrations: systemic C_{max} and hepatic portal C_{max}. As summarized in Table 2, the FDA bases the maximum hepatic inlet concentration on the sum of these two concentrations in *plasma* whereas the EMA and PMDA base it on the sum of these two concentrations in *blood*. Estimates of the total (bound + unbound) maximum hepatic inlet concentration in plasma differ from those in blood unless R_b, the blood-to-plasma concentration ratio, is 1, as shown in Table 1. However, assessing the potential for a test drug to cause clinically relevant inhibition of hepatic uptake transporters, such as OATP1B1, OATP1B3, and OCT1 (according to the basic static models), or inhibition of hepatic CYP enzymes (according to the static mechanistic models) is based on the *unbound* maximum hepatic inlet concentration. Surprisingly, for a given value of R_b, the *unbound* maximum hepatic inlet concentration in plasma is *identical* to the *unbound* maximum hepatic inlet concentration in blood, as shown in Table 1. For FDA submissions, plasma $I_{in,max,u}$ can be calculated from Equation 5. For EMA and PMDA submissions, blood $[I]_{u,inlet,max}$ (EMA) and blood $I_{inlet,max,u}$ (PMDA) can be calculated from Equation 12. But, I'm happy to say, all these values of unbound maximum hepatic inlet concentration are the same.

References

Carlisle KM, Halliwell M, Read AE and Wells PN. Estimation of total hepatic blood flow by duplex ultrasound. *Gut* **33**: 92-97, 1992.

EMA (2013). European Medicines Agency: Guideline on the investigation of drug interactions. Available at:
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf

FDA (2017). US Food and Drug Administration: Guidance for Industry. In Vitro Metabolism and Transporter-Mediated Drug-Drug Interaction Studies. Draft issued October, 2017. Available at:
http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/UCM237160.pdf

Hallifax D, Foster JA, Houston JB. Predication of human metabolic clearance from in vitro systems: retrospective analysis and prospective view. *Pharm Res* **27**: 2150-2161, 2010.

Hinderling PH. Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. *Pharmacol Rev* **49**: 279-295, 1997.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H and Sugiyama Y. Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* **50**: 387-412, 1998.

PMDA (2017). Pharmaceuticals and Medical Devices Agency (Japan): Drug Interaction Guideline for Drug Development and Labelling Recommendations. Draft version issued September, 2017.
Available in English at:
https://www.xenotech.com/scientific-resources/regulatory-documents/2017/2017-japanese-pmda-guidance-english.aspx#.W-sJyDbruP

PMDA (2018). Pharmaceuticals and Medical Devices Agency (Japan): Drug Interaction Guideline for Drug Development and Labelling Recommendations. Final version issued February, 2018.
Available in English at: https://www.pmda.go.jp/files/000228122.pdf

Vieira ML, Kirby B, Ragueneau-Majlessi I, Galetin A, Chien JY, Einolf HJ, Fahmi OA, Fischer V, Fretland A, Grime K, Hall SD, Higgs R, Plowchalk D, Riley R, Seibert E,

Skordos K, Snoeys J, Venkatakrishnan K, Waterhouse T, Obach RS, Berglund EG, Zhang L, Zhao P, Reynolds KS, Huang SM. Evaluation of various static in vitro-in vivo extrapolation models for risk assessment of the CYP3A inhibition potential of an investigational drug. *Clin Pharmacol Ther* **95**: 189-198, 2014

Wallemacq PE and Verbeeck RK. Comparative clinical pharmacokinetics of tacrolimus in paediatric and adult patients. *Clin Pharmacokinet* **40**: 283-295, 2001.

Yang J, Jamei M, Yeo KR, Rostami-Hodjegan A and Tucker GT. Misuse of the well-stirred model of hepatic drug clearance. *Drug Metab Dispos* **35**: 501-502, 2007.

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Legend for Figure

Fig. 1. A published equation for calculating the unbound maximum hepatic inlet concentration in blood that contains an error, namely a missing plus sign.

This equation is equation 3 from Vieira *et al.* (2104). $[I]_{u.inlet,max}$ is the unbound maximum hepatic inlet concentration of drug in blood, $f_{u,b}$ is the unbound fraction of drug in blood, $[I]_{max,b}$ is the maximum concentration of total drug in systemic blood, and (Dose x F_a x k_a)/Q_h is the maximum concentration of drug in hepatic portal blood. Q_h is the sum of the blood flow in hepatic artery and portal vein (1.617 L/h), k_a is the rate of absorption of drug from the intestine, and F_a is the fraction of drug absorbed from the intestine into portal blood, as originally described by Ito *et al.* (1998), which all three regulatory agencies have replaced with $F_a \cdot F_g$, as described in the text.

TABLE 1

Total (bound + unbound) and unbound maximum hepatic inlet concentration in plasma (the FDA approach) and blood (the EMA and PMDA approach) for drugs with different R_b values

Simulated comparisons were made for Drugs A, B, and C with an R_b of 0.5, 1.0, and 2.0, respectively. The oral dose of each drug was 325 µmol. Each drug had a total (bound + unbound) systemic plasma C_{max} of 4.0 µM, and each was 80% bound to plasma protein (fu_P = 0.2). In each case, $F_a \cdot F_g$ was 1, and k_a was 0.1 min^{-1}. Hepatic blood flow (Q_H) was 1.62 L/min.

Drug	R_b	Total systemic C_{max}		Total portal C_{max}		Total maximum hepatic inlet concentration ($I_{in,max}$)		Fraction unbound		Unbound maximum hepatic inlet concentration ($I_{in,max,u}$)	
		Plasma[1]	Blood[2]	Plasma[3]	Blood[4]	Plasma[5]	Blood[6]	Plasma (fu_P)[7]	Blood (fu_B)[8]	Plasma[9]	Blood[10]
Drug A	0.5	4 µM	2 µM	40 µM	20 µM	44 µM	22 µM	0.2	0.4	8.8 µM	8.8 µM
Drug B	1.0	4 µM	4 µM	20 µM	20 µM	24 µM	24 µM	0.2	0.2	4.8 µM	4.8 µM
Drug C	2.0	4 µM	8 µM	10 µM	20 µM	14 µM	28 µM	0.2	0.1	2.8 µM	2.8 µM
FDA (based on drug concentrations in plasma)											
Drug A	0.5	4 µM		40 µM		44 µM		0.2		8.8 µM	
Drug B	1.0	4 µM		20 µM		24 µM		0.2		4.8 µM	
Drug C	2.0	4 µM		10 µM		14 µM		0.2		2.8 µM	
EMA and PMDA (based on drug concentrations in blood)											
Drug A	0.5		2 µM		20 µM		22 µM		0.4		8.8 µM
Drug B	1.0		4 µM		20 µM		24 µM		0.2		4.8 µM
Drug C	2.0		8 µM		20 µM		28 µM		0.1		2.8 µM

[1] The total drug concentration in systemic plasma is determined experimentally. It was set to 4 µM for Drugs A-C.

[2] Calculated as follows: $Total\ systemic\ C_{max}\ in\ blood = Total\ systemic\ C_{max} in\ plasma \cdot R_b$

[3] Calculated as follows: $Total\ portal\ C_{max}\ in\ plasma = \frac{\left(\frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H}\right)}{R_b} = \frac{\left(\frac{325\ \mu mol \cdot 1.0 \cdot 0.1\ min^{-1}}{1.62\ L/min}\right)}{R_b}$

[4] Calculated as follows: $Total\ portal\ C_{max}\ in\ blood = \frac{Dose \cdot F_a \cdot F_g \cdot k_a}{Q_H} = \frac{325\ \mu mol \cdot 1.0 \cdot 0.1\ min^{-1}}{1.62\ L/min}$

[5] Calculated as follows: $Total\ I_{in,max} in\ plasma = Total\ systemic\ C_{max}\ in\ plasma + Total\ portal\ C_{max}\ in\ plasma.$

[6] Calculated as follows: $Total\ I_{in,max} in\ blood = Total\ systemic\ C_{max}\ in\ blood + Total\ portal\ C_{max}\ in\ blood,$

[7] The unbound concentration of drug in plasma (fu_P) is determined experimentally. It was set to 0.2 for Drugs A-C.

[8] Calculated as follows: $fu_B = \frac{fu_P}{R_b}$

[9] Calculated as follows: $Plasma\ I_{in,max,u} = Plasma\ I_{in,max} \cdot fu_P.$

[10] Calculated as follows: $Blood\ I_{in,max,u} = Blood\ [I]_{in,max} \cdot fu_B,$

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TABLE 2

Summary of nomenclature and methods used by regulatory agencies to calculate the unbound maximum hepatic inlet concentration of an orally administered drug

Agency	Abbreviated terms for total and unbound hepatic inlet concentration	Matrix	Basic equation for calculating total and unbound hepatic inlet concentration[1,2]	Equation in text
FDA	$I_{in,max}$ $I_{in,max,u}$	Plasma	Total plasma $I_{in,max}$ = systemic C_{max} in plasma + portal C_{max} in plasma Plasma $I_{in,max,u}$ = plasma $I_{in,max}$ x fu_P	Equation 5
EMA	$[I]_{inlet,max}$ $[I]_{u,inlet,max}$	Blood	Total blood $[I]_{inlet,max}$ = systemic C_{max} in blood + portal C_{max} in blood Blood $[I]_{u,inlet,max}$ = blood $[I]_{inlet,max}$ x fu_B	Equation 12
PMDA	$I_{inlet,max}$ $I_{inlet,max,u}$		Total blood $I_{inlet,max}$ = systemic C_{max} in blood + portal C_{max} in blood Blood $I_{inlet,max,u}$ = blood $I_{inlet,max}$ x fu_B	

[1] *fup* and fu_B are the fraction of unbound drug in plasma and blood, respectively.

[2] The only difference between the EMA and PMDA equations is the abbreviated terms used for the total/unbound maximum hepatic inlet concentration.

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Figure 1