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1. **Overview**

This User Guide describes the features of PharmaPendium’s Drug–Drug Interaction Risk Calculator (DDIRC). This feature enables the prediction of drug–drug interactions between proprietary substances and marketed drugs based on published results from in vitro and in vivo experiments.

1.1 **Conventions**

The instructions in this guide use this formatting to call attention to particular details of this software.

**Note**: This is an example of text explaining a point of interest.

**Important**: This is an example of text explaining something required for successful DDIRC operation.

**Tip**: This is an example of text that contains a tip for faster or easier use of the DDIRC.

1.2 **Key features**

**Table 1. Key features of PharmaPendium’s DDIRC**

<table>
<thead>
<tr>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully compliant with FDA guidance on the evaluation of DDIs</td>
</tr>
<tr>
<td>Enzyme induction in the liver is included in the DDI prediction</td>
</tr>
<tr>
<td>Enzyme induction in the gut is included in the DDI prediction</td>
</tr>
<tr>
<td>Library of CYP inducers, including EC\textsubscript{50}, E\textsubscript{max} and nh parameters</td>
</tr>
<tr>
<td>Induction results using mRNA or enzymatic reaction included</td>
</tr>
<tr>
<td>( fu\text{{hep}} ) prediction (Austin or Houston Methods)</td>
</tr>
<tr>
<td>Competitive and mechanism-based inhibition is included</td>
</tr>
<tr>
<td>More than 600 marketed compounds are included in the Drug Library</td>
</tr>
<tr>
<td>Estimation of perpetrator concentration in the liver is optimized based on the perpetrator mechanism of action (competitive, mechanism-based inhibition and induction)</td>
</tr>
<tr>
<td>Filters for drug status include Approved, Withdrawn and Investigational</td>
</tr>
<tr>
<td>Export: Induction risk of DDIs is included</td>
</tr>
<tr>
<td>Queries can be saved</td>
</tr>
</tbody>
</table>
2. **DDIRC Approach**

The DDIRC is a unique application designed to predict potential metabolic drug–drug interactions between proprietary drugs in development and a panel of marketed drugs that are automatically selected within PharmaPendium’s DDIRC Knowledge Database (also called the Drug Library). The methodology implemented in the DDIRC is based on a general in vitro–in vivo extrapolation (IVIVE) method that uses mechanistic static modeling (MSM). Figure 1 illustrates the DDIRC workflow.

![DDIRC Diagram](image)

**Figure 1.** An illustration of the Drug–Drug Interaction Risk Calculator workflow
If the proprietary drug is a victim (substrate), the DDI calculation is made against perpetrators and related data: $K_i$, $IC_{50}$, $kinact$, $EC_{50}$, $E_{max}$, $C_{max}$, etc.) from the DDIRC database. If the proprietary compound is a perpetrator (inhibitor and/or inducer), the calculation is made with victims and the corresponding mandatory data: $fm(E)$, $Fg$. The result is a profile of drug drug–interactions between your proprietary drug and potential co-medication drugs on the market (Figure 2).

![Figure 2. An illustration of the DDIRC workflow for victim and perpetrator drugs](image)

### 2.1 Mechanistic static model used in the DDIRC

#### 2.1.1 Assumptions

The DDIRC applies to: orally administered victim drugs undergoing linear first pass and systemic hepatic metabolism; and IV-administered victim drugs with low clearance (i.e., low hepatic extraction ratio $E_H < 0.3$) according to the so-called well-stirred model.

It ignores transient plasma binding displacement during the absorption phase and time variant inhibitor concentration (leading to a different extent of inhibition of substrate metabolism during the “first” and subsequent passes through the liver).

#### 2.1.2 Methods

For the major CYPs involved in drug metabolism (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4), assessments of CYP metabolism and inhibition are determined as early as possible in the research and development stages. Determining the mechanisms of CYP inhibition—reversible or mechanism-based inhibition (MBI), which is also called time-dependent inhibition (TDI)—and CYP induction can potentially influence drug development strategies. Early prediction of the magnitude of clinical DDIs is imperative to avoid DDI-related adverse events.
Most approaches for the prediction of metabolic DDIs are based on in vitro–in vivo extrapolation (IVIVE). However, the effects of inhibition of drug metabolism on in vivo pharmacokinetics are highly variable and depend on the properties of the drug, the route of administration, and other factors (Rowland, et al., 1973; Tucker, 1992).

Equation 1 describes the average increase in the area under the plasma concentration–time curve (area under curve, AUC) of a victim drug following administration of one or more perpetrator drug(s), where $f_m(E_k)$ is the fraction of victim clearance mediated by the inhibited metabolic pathway $k$ and $Cl_{int_k}$ is the intrinsic metabolic clearance of substrate for the pathway $k$ in the liver.

$$\frac{AUC_i}{AUC} = \frac{1}{\sum_{k=1}^{n} \text{Fold reduction} \cdot Cl_{int_k}} + (1 - \sum_{k=1}^{n} f_m(E_k))$$

Equation 1

The ratio of AUC estimated by Equation 1 only takes into account the victim metabolism in the liver (i.e., inhibition or induction no longer occurred during the first pass metabolism of the victim drug in the gut wall). This equation could be applied: after oral administration of perpetrator drugs with no inhibition or induction occurring in the gut; or after IV administration assuming oral administration of the victim drug.

However, due to the high levels of inhibitors in the gut lumen, the contribution of the intestine to the magnitude of DDIs is significant when perpetrator drugs are administered orally. Intestinal inhibition was incorporated in the DDI prediction model as the ratio of intestinal wall availability in the presence and absence of the inhibitor ($F_g'$ and $F_g$, respectively) by Rostami-Hodjegan, et al (2004), Obach, et al (2006) and Wang, et al (2004). See Equation 2.

$$\frac{AUC_i}{AUC} = \frac{F_g'}{F_g} \times \frac{1}{\sum_{k=1}^{n} \text{Fold reduction} \cdot Cl_{int_k}} + (1 - \sum_{k=1}^{n} f_m(E_k))$$

Equation 2

The concept of the fold reduction clearance can also be applied for the determination of the ratio of the intestinal wall availability in the presence and absence of the perpetrator. See Equation 3.

$$\frac{F_g'}{F_g} = \frac{1}{F_g + (1-F_g) \cdot \frac{Cl_{int_g}}{Cl_{int_g}}}$$

Equation 3
2.1.2.1 Hepatic metabolism

2.1.2.1.1 Fold reduction in intrinsic hepatic clearance

The fold reduction in intrinsic hepatic clearance is defined according to the type of inhibition that occurs.

2.1.2.1.1.1 Competitive binding

In the presence of competitive inhibition, the fold reduction in intrinsic hepatic clearance is also determined by the unbound concentration of perpetrator(s) in the liver \([I_u]_H\) and its associated unbound equilibrium dissociation constant \((K_i,u)\) as in Equation 4.

\[
\text{Fold reduction } C_{\text{Cl,unb}} = 1 + \sum_{j=1}^{P} \left( \frac{[I_u]_H}{K_i,u_j} \right)
\]

Equation 4

where \([I_u]_H\) is the unbound concentration of perpetrator \(j\) at the enzyme site and \(K_i,u_j\) the unbound inhibition constant for perpetrator \(j\) obtained from in vitro studies after accounting for non-specific binding. The use of non-specific binding in DDI prediction is discussed in a separate section.

2.1.2.1.1.2 Inhibitory constant and substrate probe selection

In theory, if different substrates are used at the same \(K_m\) concentration, the same \(I_C_{50}\) value should be generated for inhibition of the same enzyme when assessed under the same conditions (protein concentration, solvent concentration, buffer, enzyme source). However, this is often not the case. There are differences in the inhibition activities of test compounds, dependent on the substrate chosen for some CYPs, in particular CYP3A4 and CYP2C9.

Due to the substrate-differential response observed for CYP3A4, the recommended approach for CYP3A4 DDI analysis is to use multiprobes (Tucker, et al., 2001; Bjornsson, et al., 2003), where the lowest inhibition constant \((K_i)\) indicates the worst-case scenario for a potential interaction. An analysis of twenty-six in vivo interaction studies between four different CYP3A4 substrates and 3 competitive inhibitors (ketoconazole, itraconazole and fluconazole) was done by Galetin, et al (2005). Based on the percentage of AUC ratio predictions that were within the two-fold standard, midazolam, testosterone, nifedipine and quinidine respectively predicted 77, 71, 69 and 81% of the inhibitory DDIs accurately when used as in vitro substrates for CYP3A4. Galetin, et al (2005) concluded that midazolam and quinidine provided the best assessment of drug–drug interactions involving CYP3A4.

This notwithstanding, the preferred substrates proposed by the FDA (FDA, 2012) for CYP3A4 were midazolam and testosterone. Quinidine was not proposed as preferred or acceptable substrate. See Table 2.
Table 2. Preferred and acceptable chemical substrates for in vitro experiments (FDA: 9/25/2006)

<table>
<thead>
<tr>
<th>CYP</th>
<th>Substrate preferred</th>
<th>Km (µM)</th>
<th>Substrate acceptable</th>
<th>Km (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>phenacetin-O-deethylation</td>
<td>1.7–152</td>
<td>7-ethoxyresorufin-O-deethylation</td>
<td>0.18–0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>theophylline-N-demethylation</td>
<td>280–1230</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>caffeine-3-N-demethylation</td>
<td>220–1565</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tacrine 1-hydroxylation</td>
<td>2.8, 16</td>
</tr>
<tr>
<td>2A6</td>
<td>coumarin-7-hydroxylation nicotine C-oxidation</td>
<td>0.30–2.3</td>
<td>propofol hydroxylation</td>
<td>3.7–94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13–162</td>
<td>S-mephenytoin-N-demethylation</td>
<td>1910</td>
</tr>
<tr>
<td>2B6</td>
<td>efavirenz hydroxylase bupropion-hydroxylation</td>
<td>17–23</td>
<td>flurbiprofen 4'-hydroxylation</td>
<td>6–42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67–168</td>
<td>phenytoin-4-hydroxylation</td>
<td>11.5–117</td>
</tr>
<tr>
<td>2C8</td>
<td>Taxol 6-hydroxylation</td>
<td>5.4–19</td>
<td>amodiaquine N-deethylation</td>
<td>2.4, 4.3–7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rosiglitazone para-hydroxylation</td>
<td></td>
</tr>
<tr>
<td>2C9</td>
<td>tolbutamide methyl-hydroxylation S-warfarin 7-hydroxylation</td>
<td>67–838</td>
<td>flurbiprofen 4'-hydroxylation</td>
<td>6–42</td>
</tr>
<tr>
<td></td>
<td>diclofenac 4'-hydroxylation</td>
<td>1.5–4.5</td>
<td>phenytoin-4-hydroxylation</td>
<td>11.5–117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4–52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C19</td>
<td>S-mephenytoin 4'-hydroxylation</td>
<td>13–35</td>
<td>omeprazole 5-hydroxylation</td>
<td>17–26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>fluoxetine O-dealkylation</td>
<td>3.7–104</td>
</tr>
<tr>
<td>2D6</td>
<td>(±)-bufuralol 1'-hydroxylation dextromethorphan O-demethylation</td>
<td>9–15</td>
<td>debrisoquine 4-hydroxylation</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.44–8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td>chlorzoxazone 6-hydroxylation</td>
<td>39–157</td>
<td>p-nitrophenol 3-hydroxylation</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lauric acid 11-hydroxylation</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>aniline 4-hydroxylation</td>
<td>6.3–24</td>
</tr>
<tr>
<td>3A4/5</td>
<td>midazolam 1-hydroxylation testosterone 6b-hydroxylation</td>
<td>1–14</td>
<td>erythromycin N-demethylation</td>
<td>33–88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52–94</td>
<td>dextromethorphan N-demethylation</td>
<td>133–710</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>triazolam 4-hydroxylation</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>terfenadine C-hydroxylation</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>nifedipine oxidation</td>
<td>5.1–47</td>
</tr>
</tbody>
</table>
Important: The DDIRC uses the (FDA, 2012) recommendation for the substrate probe selection. Therefore, when inhibitory constants exist for a perpetrator and are automatically selected in the Drug Library, certain priority orders are followed (Figure 3). For example, for an inhibitor of CYP3A4 with 3 IC\textsubscript{50} values on HLMs with midazolam as the substrate and 2 Ki values on recombinant CYPs with testosterone as the substrate, only the 2 Ks is involved in the predictions.

**Step 1: Parameter**

\[ Ki > IC_{50} \]

**Step 2: Substrate and system types**

<table>
<thead>
<tr>
<th>Preferred + acceptable substrate</th>
<th>Preferred + acceptable + other substrate</th>
<th>Other substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM</td>
<td>Recombinant</td>
<td>HLM</td>
</tr>
</tbody>
</table>

![Figure 3. inhibitory constants and substrate probes selection used in the DDIRC: priority order](image)

**Note:** An additional complicating issue in the assessment of CYP3A inhibition potential is the occurrence of homo- or heterotropic cooperativity in vitro. These atypical phenomena, attributed to the existence of multiple binding sites, have been associated with CYP3A. Recent publications indicate similar behavior for CYP2C9 (Egnell, et al., 2003; Hutzler, et al., 2003; Foti, et al., 2008) and UDP glucuronosyltransferase enzymes (Uchaipichat, et al., 2004). These cooperativity effects are not taken into account in the DDIRC.

2.1.2.1.2 **Mechanism-based or time-dependent inhibition**

The inactivation of CYPs by reactive products that form heme or protein adducts or a metabolic inhibitory complex (MIC) is referred to as mechanism-based inhibition (MBI) or time-dependent inhibition (TDI). MBI is characterized as an irreversible or quasi-irreversible inactivation of the CYP, requiring synthesis of new enzyme for recovery of activity. TDI is generally involved in more complex drug–drug interaction than reversible competitive inhibition as it can result in a more profound and prolonged effect than therapeutic dose or exposure might suggest. For CYP3A4, the number of TDI interactions accounted for around 60% of all the significant interactions (AUC ratio > 2). See Figure 4.
In the presence of inactivation, the fold reduction in intrinsic hepatic clearance is also determined by the pseudo-first-order apparent inactivation rate ($k_{\text{obs}}$). This apparent inactivation rate is dependent upon $[\text{Iu}]_H$, $K_{\text{Iu}}$ and the true first-order inactivation rate constant ($k_{\text{inact}}$). See Equation 5.

$$\text{Fold reduction } \text{Cl}_{\text{HINTK}} = 1 + \sum_{j=1}^{p} \left( \frac{k_{\text{inact}} \times [\text{Iu}]_H}{k_{\text{deg,H}} \times ([\text{Iu}]_H + K_{\text{Iu}})} \right)$$

Equation 5

where $k_{\text{deg,H}}$ is the natural degradation rate constant for the enzyme in the liver.

Such prediction clearly depends on reliable determinations of all the parameters involved in calculations.
2.1.2.1.2.1  Enzyme turnover

Mechanisms proposed to be involved in the degradation of native and inactivated CYPs involve lysosomal, ubiquitin-independent or -dependent, and 20S- or 26S-proteasomal systems (Correia, et al., 2007). It is commonly accepted that the kinetic change in enzyme level is a zero order rate for enzyme synthesis and a first order rate for enzyme degradation. In the steady state, enzyme synthesis \( (K_{\text{syn}}) \) equals the degradation rate \( (k_{\text{deg},h} \times E_{\text{ss}}; \text{Equation 6}) \), but when this balance is disturbed by external events, the enzyme level will change to reach a new steady state (Figure 5).

\[ K_{\text{syn}} = k_{\text{deg},h} \times E_{\text{ss}} \]

\text{Equation 6}

Figure 5. Schematic of in vivo enzyme turnover (A), mechanism-based inactivation (B), and competition with a probe substrate (C).

The lack of information on in vivo turnover rates \( (k_{\text{deg}}) \) of several human CYP isoforms is an important source of uncertainty in the IVIVE process. Determination of the rate constant of degradation in humans remains a challenge even if different types of experiments have been used. Nevertheless, a great variability was measured depending on the methods but also on the sources of hepatocytes, the enzymes for the in vitro experiment, and the patient population for the in vivo experiment.

For example, the estimate of the average CYP3A4 half-life ranges from 26 to 140 h based on 13 determinations using in vivo and in vitro methods. Clearly, all in vitro methods will have some experimental deficiencies in reflecting the in vivo reality (Yang, et al., 2008).
Because of the kinetic profile of enzyme turnover—enzyme synthesis was a zero order rate ($K_{syn}$) and enzyme degradation was a first order rate ($k_{deg}$)—the same increase in the final level produced either by the same (absolute value) fold increase in enzyme synthesis rate or by the same fold decrease in the enzyme degradation rate, the time profile up to the final enzyme level is varies considerably. A decrease of the enzyme degradation rate constant prolongs the turnover half-life but an increase of the rate of synthesis does not. When the DDI predictions involve mechanism-based inactivators, the in vivo impact should be carefully managed and analyzed and the use of extreme values should be avoided.

Table 3. Mean values of the half-lives of degradation in the liver used in the DDIRC (based on literature analysis)

<table>
<thead>
<tr>
<th>CYP</th>
<th>Sources</th>
<th>$k_{deg}$ (min$^{-1}$)</th>
<th>t1/2 (h)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>In vivo (smokers)</td>
<td>0.00030</td>
<td>38.6</td>
<td>Faber, et al., 2004</td>
</tr>
<tr>
<td>2A6</td>
<td>In vitro (slices)</td>
<td>0.00044</td>
<td>26</td>
<td>Renwick, et al., 2000</td>
</tr>
<tr>
<td>2B6</td>
<td>In vitro (slices)</td>
<td>0.00036</td>
<td>32</td>
<td>Renwick, et al., 2000</td>
</tr>
<tr>
<td>2C8</td>
<td>In vitro (slices)</td>
<td>0.00050</td>
<td>23</td>
<td>Renwick, et al., 2000</td>
</tr>
<tr>
<td>2C9</td>
<td>In vitro (slices)</td>
<td>0.00011</td>
<td>104</td>
<td>Renwick, et al., 2000</td>
</tr>
<tr>
<td>2C19</td>
<td>In vitro (slices)</td>
<td>0.00044</td>
<td>26</td>
<td>Renwick, et al., 2000</td>
</tr>
<tr>
<td>2D6</td>
<td>In vivo (paroxetine)</td>
<td>0.000226</td>
<td>51</td>
<td>Liston, et al., 2002; Kaye, et al., 1989; Venkatakrishnan, et al., 2005</td>
</tr>
<tr>
<td>2E1</td>
<td>In vivo (disulfiram)</td>
<td>0.00023</td>
<td>50</td>
<td>Emery, et al., 1999</td>
</tr>
<tr>
<td>3A4</td>
<td>In vivo</td>
<td>0.000321</td>
<td>36</td>
<td>Fromm, et al., 1996</td>
</tr>
<tr>
<td>3A5</td>
<td>In vitro (slices)</td>
<td>0.000321</td>
<td>36</td>
<td>Renwick, et al., 2000</td>
</tr>
</tbody>
</table>

For other enzymes involved in MBI, a default value of 0.00025 min$^{-1}$ was used for $k_{deg}$

2.1.2.1.2 $k_{inact}$ and $K_I$ determination

The common determination of MBI was proposed by (Silverman, 1988) and involved two different experimental protocols with a common part involving a preincubation of the enzyme and cofactors with different inhibitor concentrations for varying incubation times:

- Followed by a dilution of the reaction mixture and further incubation with a probe substrate to assess the capability of the inactivator to block the active enzyme
- Without dilution but after direct addition of a large amount of substrate probe to the preincubation mixture

The natural logarithm of remaining enzyme activity is plotted against the pre-incubation time and according to Equation 7, the slopes of the initial log-linear phases represent the observed inactivation rate constants ($k_{obs}$).
Two methods are generally used to obtain $k_{inact}$ and $K_I$ values: a linear regression method (Kitz, et al., 1962) and a non-linear regression method.

**Note:** A modification of the conventional protocol (CEP) was proposed recently by Yang, et al. (2007a) and named a mechanistically based experimental protocol (MEP). This arose because using CEP for characterizing MBI may introduce substantial bias in estimating parameter values (Yang, et al., 2005), which may influence the ability to predict the in vivo consequences of MBI from in vitro data.

The MEP relies on the simultaneous fitting of three differential equations to estimate the relevant CYP inhibition constants more precisely than with traditional methods. Inhibitory constants ($k_{inact}$, $K_I$ and $K_i$) are determined in the MEP and optimized using a genetic algorithm.

MEP is considered by (Yang, et al., 2007a) to be superior to the CEP with regard to accuracy, precision and efficiency. Its application may allow better prediction of the in vivo implications of MBI.

**Important:** The DDIRC use $K_I$ and $k_{inact}$ determined by CEP or MEP without any priority order based on the method. Nevertheless, when more than one $K_I$, $k_{inact}$ couple is found in the Drug Library, HLM results are preferred to recombinant experiment results without any refinement on substrate used.

### 2.1.2.1.3 Induction

When induction occurs, the fold change in intrinsic clearance is also determined using inducer concentrations at the enzyme site $[I]_{h,g}$ (in the liver or in the gut), the maximum fold induction, and the concentration of inducer associated with half-maximum induction ($EC_{50}$), but the way of combining these three elements can vary. The predictive model used in the DDIRC to calculate the fold reduction in intrinsic clearance is based on the Hill equation developed by Shou, et al. (2008). See Equation 8.

$$\text{Fold change } Clinit, u_{\text{h},k} = \frac{1}{1 + \sum_{j=1}^{p} \left( \frac{E_{\text{max},j} \times [I]_{h,j}}{[I]_{h,j} + EC_{50,j}} \right)}$$

*Equation 8*

In many cases, $E_{\text{max}}$ and $EC_{50}$ are not readily obtained from concentration–response curves measured using in vitro induction assays due limitations imposed by drug solubility, cell permeability or toxicity. In these instances, the slope of the induction response curve (equivalent to $E_{\text{max}}/EC_{50}$) at a more experimentally feasible low concentration range of the inducer can be used for the prediction. This method (Equation 9) is only applicable if in vivo concentrations of an inducer are low ($[I]_{h,j} << EC_{50,j}$).
An alternative approach was investigated by Fahmi et al. (2008), who proposed a $d$ parameter to scale induction from in vitro to in vivo.

### 2.1.2.2 Parallel pathway: fraction metabolized

We have considered the factors related to the perpetrator (inactivation, competitive inhibition) and to the enzyme being inactivated ($k_{dgi}$) as determinants of interaction magnitude. However, the magnitude of a drug–drug interaction is dependent not only on the characteristics of the perpetrator but also on the pharmacokinetic properties of the victim. These considerations are common to IVIVE of metabolic inhibitory drug interactions in general.

Consideration of the fraction of total clearance of the victim drug mediated via the enzyme being inactivated, $fm(E)$, is crucial when an assessment of risk for interaction between a specific victim and perpetrator drug pair is needed. The relationship between fold increase in AUC and the $fm(E)$ of the victim drug is illustrated in Equation 1.

Getting initial estimates of $fm(E)$ of the victim drug is not always straightforward. The fraction of the victim drugs that is metabolized is estimated using in vitro or in vivo approaches.

#### 2.1.2.2.1 $fm(E)$ in vitro determination

In vitro approaches mainly involve reaction phenotyping of major metabolic pathways using factors to scale to in vivo metabolic clearance from recombinant CYP experiments. The fraction metabolized for an enzyme, $fm(E)_{\text{vivo}}$, is estimated by dividing the estimated clearance for this enzyme (different pathways) by the total in vitro clearance (Equation 10).

$$ fm(E_{i})_{\text{vivo}} = \frac{\sum_{i=1}^{P} Clint_{i,k}(rE_{i}) \times SF_{i}}{\sum_{j=1}^{P} (\sum_{i=1}^{P} Clint_{i,k}(rE_{i}) \times SF_{i,j})} $$

**Equation 10**

where there are $j$ enzymes with corresponding $Clint_{i,k}(rE_{i})$ values calculated from enzyme kinetic parameters for different pathways $k$ in each recombinant system and where $SF_{i,j}$ was a scaling factor corresponding to the pathway $k$ and enzyme $j$. 

$$ \text{Fold reduction } Cl\text{u}_{\text{int}k} = \frac{1}{1 + \sum_{p=1}^{P} (\text{Slope}_{k,p} \times [I]_{hj})} $$

**Equation 9**
A relative activity factor (RAF) was proposed as scaling factor to allow for differences in the activity of the enzymes per unit of microsomal protein compared with that in human liver microsomes (HLMs; Crespi, 1995). Initially, the RAF was defined relatively to $V_{\text{max}}$ (Equation 9), but RAF based on intrinsic clearance was also used. It was demonstrated that prediction accuracy of metabolic clearance in HLMs increased when RAFClint (Equation 11) was used instead of RAF$V_{\text{max}}$ (Emoto, et al., 2007). Furthermore, recombinant CYP microsomes co-expressed with cytochrome b5 might be suitable for the prediction, at least in the case of recombinant microsomes from insect cells (Emoto, et al., 2006; Nakajima, et al., 2002). They suggested that the expression level of cytochrome b5 recombinant CYP microsomes and NADPH-CYP oxidoreductase used for the prediction was not crucial while cytochrome b5 and NADPH-CYP oxidoreductase are expressed in recombinant systems. Co-expression of cytochrome b5 might facilitate the scaling of data in recombinant CYP microsomes to the predicted value in HLMs.

$$\text{RAF}_{k} = \frac{\text{Clint}_{u_k}(\text{HLM})}{\text{Clint}_{u_k}(rE_j)}$$

Equation 11

RAF has units equating to pmol of enzymes/mg of protein. The contribution of the activity of each enzyme involved in the metabolism of a new chemical entity (NCE) could be scaled to human liver microsomes. An example of RAF approach application was for the determination of the relative contribution of metabolic pathways to net Mirtazapine biotransformation (Stormer, et al., 2000). This parameter was originally suggested for CYPs, but was also used for UGTs with a slight modification (Toide, et al., 2004).

The RAF approach does not take into account interindividual variability because it is very difficult to differentiate experimental variability from the true interindividual variability in liver samples.

Note: In the DDIRC, default values of RAF based on Clint,u were provided for CYPs (Table 3). These values were obtained through a literature analysis using the following conditions:

- Preferred substrate probes were used exclusively (see Table 1 for a full list).
- Recombinant systems considered were based on B-lymphoblastoid cells (L), baculovirus-transformed insect cells (B), *Escherichia coli* cells (E) and *Saccharomyces cerevisiae* cells (Y) for Clint determination.
- The median of Clint,u for the HLMs was used for each CYP, based on the preferred substrate probes (109 values).
Table 4. Median of RAF for each recombinant system and CYP enzyme (number of recombinant Clint used)

<table>
<thead>
<tr>
<th>rhCYP</th>
<th>Baculo (n)</th>
<th>E. coli (n)</th>
<th>Lympho (n)</th>
<th>Yeast (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>43.75 (4)</td>
<td>528.61 (1)</td>
<td>435.61 (4)</td>
<td>1221.19 (2)</td>
</tr>
<tr>
<td>2A6</td>
<td>X</td>
<td>71.15 (3)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2B6</td>
<td>37.94 (2)</td>
<td>X</td>
<td>62.22 (1)</td>
<td>X</td>
</tr>
<tr>
<td>2C8</td>
<td>14.55 (2)</td>
<td>26.45 (2)</td>
<td>X</td>
<td>45.12 (1)</td>
</tr>
<tr>
<td>2C9</td>
<td>60.13 (9)</td>
<td>101.21 (4)</td>
<td>47.67 (11)</td>
<td>59.13 (11)</td>
</tr>
<tr>
<td>2C19</td>
<td>3.93 (5)</td>
<td>3.66 (2)</td>
<td>13.2 (2)</td>
<td>17.52 (2)</td>
</tr>
<tr>
<td>2D6</td>
<td>7.77 (16)</td>
<td>17.62 (3)</td>
<td>16.82 (4)</td>
<td>81.2 (4)</td>
</tr>
<tr>
<td>2E1</td>
<td>496.68 (2)</td>
<td>110.43 (1)</td>
<td>551.39 (2)</td>
<td>1956.96 (1)</td>
</tr>
<tr>
<td>3A4</td>
<td>55.32 (11)</td>
<td>147.1 (8)</td>
<td>266.81 (7)</td>
<td>609.78 (2)</td>
</tr>
</tbody>
</table>

Alternatively, enzyme abundance could be used as scaling factor (Equation 12) to estimate the fraction metabolized in vitro through extrapolation of recombinant clearances to HLM clearances (Rodrigues, 1999).

\[
f_{\text{m(Ei)vitro}} = \frac{\sum_{i=1}^{p} \text{Clint}_{i}(rE_{i}) \times E_{i}\text{abundance (HLM)}}{\sum_{i=1}^{p} (\sum_{k=1}^{p} \text{Clint}_{i}(rE_{i}) \times E_{j}\text{abundance (HLM)})}
\]

where there are j CYPs with corresponding Clint\textsubscript{i}(rE\textsubscript{i}) values calculated from enzyme kinetic parameters for different pathways k in each recombinant system, and where enzyme abundance, E\textsubscript{i}, was the amount of j enzyme per mg microsomal protein (pmol/mg prot) in HLMs.

However, abundances are needed to correct for the relative contribution of each enzyme to the total metabolism and this is not always a straightforward factor to establish. Shimada, et al. (1994) remains the most cited source for abundance information, despite the study having been based on liver samples from only 30 Caucasian and 30 Japanese subjects. A meta-analysis of the literature was done by Rowland, et al. (2004) but it only included Caucasian subjects (over 200 liver samples).

**Note:** In the DDIRC, the abundance of enzymes in the liver (pmol/mg prot) was determined from a literature analysis of 18 separate studies based on 315 livers using a weighted mean. Only data from adult Caucasians (more than 16 years old) were included, and sources were verified to exclude duplication of individual data in the analysis.
Table 5. Abundances of enzymes in the liver determined by literature analysis of 18 separate studies based on 315 livers and used as default values in the DDIRC

<table>
<thead>
<tr>
<th>CYP</th>
<th>Abundance in HLMs, measured in pmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>48.8</td>
</tr>
<tr>
<td>2A6</td>
<td>21.5</td>
</tr>
<tr>
<td>2B6</td>
<td>14.6</td>
</tr>
<tr>
<td>2C8</td>
<td>15.5</td>
</tr>
<tr>
<td>2C9</td>
<td>69.6</td>
</tr>
<tr>
<td>2C19</td>
<td>15.4</td>
</tr>
<tr>
<td>2C18</td>
<td>2.5</td>
</tr>
<tr>
<td>2D6</td>
<td>9.5</td>
</tr>
<tr>
<td>2E1</td>
<td>74.3</td>
</tr>
<tr>
<td>3A4</td>
<td>173</td>
</tr>
<tr>
<td>3A5</td>
<td>59.4</td>
</tr>
</tbody>
</table>

Using enzyme abundances as scaling factor does not allow for any difference in variability activity per unit amount of CYP between the expression system and the liver. Proctor, et al. (2004) proposed incorporating a scaling factor (ISEF: intersystem extrapolation factor) in the abundance method to take into account different intrinsic activity in recombinant enzyme systems.

ISEF is a dimensionless number used as scaling factor to convert data obtained with a recombinant enzyme system to an HLM system. ISEF could be defined based on $V_{max}$ or Clint.u. Since it was demonstrated that the prediction accuracy of metabolic clearance in HLMs was increased when RAFClint (Equation 11) was used instead of RAF$V_{max}$ (Emoto, et al., 2007), we have decided to use ISEFClint (Equation 13) instead of ISEF$V_{max}$.

\[
ISEF_{kj} = \frac{Clint.u_{kj}(HLM)}{Clint.u(rE_{kj}) \times E_{kj} \text{abundance}(HLM)}
\]

Equation 13

where $E_{kj}$ abundance (HLM) refers to the abundance of the $j^{th}$ enzyme in the liver and Clint,u refers to the metabolism of a probe substrate by an individual enzyme. The units of Clint,u(HLM), Clint,u(rE) and abundance are µl/min/mg protein, µl/min/pmol enzyme and pmol enzyme/mg protein, respectively.
Using ISEFs, abundances and intrinsic clearance for each metabolic pathway allows the estimation of the fraction metabolized for each enzyme involved in the metabolism of the victim (Equation 14).

\[
fm(E_j)^{in vitro} = \frac{\sum_{k=1}^{p} Clint_u (rE_i) \times ISEF_k \times E_j \times abundance(\text{HLM})}{\sum_{i=1}^{n} \left( \sum_{k=1}^{p} Clint_u (rE_i) \times ISEF_k \times E_j \times abundance(\text{HLM}) \right)}
\]

Equation 14

where there are \( j \) CYPs with corresponding \( Clint_u (rE_i) \) values calculated from enzyme kinetic parameters for different pathways \( k \) in each recombinant system and where enzyme, abundance, \( E_j \), was the amount of \( j \) enzyme per mg microsomal protein (pmol/mg prot) in HLMs and ISEF\(_k j \) was an intersystem extrapolation factor corresponding to the pathway \( k \) and enzyme \( j \).

**Note:** In the DDRC, default values of ISEF based on Clint,\( u \) were provided for CYPs. These values were obtained through a literature analysis with the following conditions:

- Preferred substrate probes were used exclusively (see Table 1 for a full list).
- Recombinant systems considered were based on B-lymphoblastoid cells (L), baculovirus-transformed insect cells (B), *Escherichia coli* cells (E) and *Saccharomyces cerevisiae* cells (Y) for Clint,\( u \) determination.
- The median of Clint,\( u \) for the HLMs was used for each CYP based on the preferred substrate probes (109 values).

**Table 6. Median of ISEF for each recombinant system and CYP enzyme (number of recombinant Clint used)**

<table>
<thead>
<tr>
<th>rhCYP</th>
<th>Baculo (n)</th>
<th><em>E. coli</em> (n)</th>
<th>Lympho (n)</th>
<th>Yeast (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>0.9 (4)</td>
<td>10.83 (1)</td>
<td>8.93 (4)</td>
<td>25.02 (2)</td>
</tr>
<tr>
<td>2A6</td>
<td>X</td>
<td>3.31 (3)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2B6</td>
<td>2.6 (2)</td>
<td>X</td>
<td>4.26 (1)</td>
<td>X</td>
</tr>
<tr>
<td>2C8</td>
<td>0.94 (2)</td>
<td>1.71 (2)</td>
<td>X</td>
<td>2.91 (1)</td>
</tr>
<tr>
<td>2C9</td>
<td>0.86 (9)</td>
<td>1.47 (4)</td>
<td>0.68 (1)</td>
<td>0.85 (11)</td>
</tr>
<tr>
<td>2C19</td>
<td>0.25 (5)</td>
<td>0.24 (2)</td>
<td>0.86 (2)</td>
<td>1.14 (2)</td>
</tr>
<tr>
<td>2D6</td>
<td>0.82 (16)</td>
<td>1.86 (3)</td>
<td>1.77 (4)</td>
<td>8.55 (4)</td>
</tr>
<tr>
<td>2E1</td>
<td>6.68 (2)</td>
<td>1.49 (1)</td>
<td>7.42 (2)</td>
<td>26.34 (1)</td>
</tr>
<tr>
<td>3A4</td>
<td>0.32 (11)</td>
<td>0.85 (8)</td>
<td>1.54 (7)</td>
<td>3.62 (2)</td>
</tr>
</tbody>
</table>
2.1.2.2.2 fm(E) in vivo determination based on fm(E)\text{vitro}

When fm(E)\text{vitro} is obtained and if an estimation of the total clearance and the non-hepatic clearance of the victim drug is available the hepatic fraction fh is evaluated (\text{Equation 15}) and consequently a fm(\text{vivo}) can be estimated (\text{Equation 16}).

\[
fh = 1 - \frac{Cl_{\text{non hepatic}}}{Cl_T}
\]
\text{Equation 15}

\[
fm(E_i) = fm(E_i)\text{vitro} \times fh
\]
\text{Equation 16}

Total clearance was estimated according to \text{Equation 17}.

\[
Cl = Cl_H + Cl_{\text{non hepatic}}
\]
\text{Equation 17}

\text{Note}: In the DDIRC, when the hepatic clearance (Cl_H) is not available for the victim, the user will have the opportunity to estimate it using the well-stirred model (\text{Equation 18}) and according to the scaling factor method chosen previously for the prediction of the fm(E)\text{vitro} (RAF: \text{Equation 19}, ISEF: \text{Equation 20}, Abundance: \text{Equation 21}, HLM: \text{Equation 22}; Proctor, et al., 2004).

\[
Cl_H = \frac{Q_h \times fu \times Cl_{H,\text{int}}}{Q_h + fu \times Cl_{H,\text{int}}}
\]
\text{Equation 18}
RAF

\[ Cl_{H,\text{int}} = \sum_{j=1}^{n} \left( \sum_{k=1}^{P} \frac{Clint_k(rE_j)}{fu_{(mic)}} \times RAF_k \right) \times MPPGL \times \text{Liver weight} \]

Equation 19

ISEF

\[ Cl_{H,\text{int}} = \sum_{j=1}^{n} \left( \sum_{k=1}^{P} \frac{Clint_k(rE_j)}{fu_{(mic)}} \times ISEF_k \times E_j \text{abundance(HLM)} \right) \times MPPGL \times \text{Liver weight} \]

Equation 20

Abundance

\[ Cl_{H,\text{int}} = \sum_{j=1}^{n} \left( \sum_{k=1}^{P} \frac{Clint_k(rE_j)}{fu_{(mic)}} \times E_j \text{abundance(HLM)} \right) \times MPPGL \times \text{Liver weight} \]

Equation 21

HLM

\[ Cl_{H,\text{int}} = \sum_{j=1}^{n} \left( \sum_{k=1}^{P} \frac{Clint_k(\text{MLEj})}{fu_{(mic)}} \times E_j \text{abundance(HLM)} \right) \times MPPGL \times \text{Liver weight} \]

Equation 22

MPPGL stands for milligrams of microsomal proteins per gram of liver, taken to be 32 mg/g (Barter, et al., 2008) for a liver weight of 1611 g.

2.1.2.2.3 \( fm(E) \) in vivo determination

In vivo approaches can also be applied to determine \( fm(E) \), such as phenotyping, chemical in vivo inhibition, non-linear regression or renal excretion.

The simplest case is that of polymorphic expression of the enzyme being inhibited with the existence of clearly definable extensive (EM) and poor metabolizer (PM) populations for example CYPs 2D6, 2C9 or 2C19. The fraction metabolized by the polymorphic enzyme could be expressed by the relationship shown in Equation 23, involving EM and PM clearance or AUC (Gibbs, et al., 2006).
where EF (enzyme function) is the ratio of polymorphic enzyme function in a PM relative to an EM subject. As described above, EF values greater than 0 and less than 1 could represent the degree of impairment in enzyme function resulting from the polymorphism.

For the specific case where CYP2D6 is the polymorphic enzyme of interest and EF = 0, meaning that the polymorphic enzyme pathway is absent in the PMs and that only one enzyme is subject to inhibition, Equation 23 can be simplified to Equation 24.

\[
\text{fm}(E) = 1 - \frac{\text{Cl}(PM)}{\text{Cl}(EM)} = 1 - \frac{\text{AUC}(EM)}{\text{AUC}(PM)}
\]

Equation 24

In such cases, knowledge of the pharmacokinetics in EM versus PM subjects will help to define fm(E). For example, this approach was extensively and successfully used to estimate fm(CYP2D6) by Ito, et al. (2005) for a set of 9 CYP2D6 victims.

Alternatively, initial estimates of fm(E) may be obtained from the results of clinical drug–drug interaction studies with selective potent inhibitors of enzymes that produce essentially complete inhibition of the enzyme of interest (in vivo chemical inhibition method). A total inhibition of \([I]/K_i<<1\) (for competitive inhibition) or

\[
\frac{k_{\text{inact}} \times [Iu]_Hj}{k_\text{deg} \times ([Iu]_Hj + K_iu)} >> 1
\]

(for MBI) produces the maximum possible AUC ratio that is solely dependent on fm(E). See Equation 25.

\[
\text{fm}(E) = 1 - \frac{1}{\text{AUC}_{\text{ratio}}} = 1 - \frac{1}{\text{Cl}_{\text{ratio}}}
\]

Equation 25

Total inhibition is assumed when, for example, ketoconazole is administered at 400 mg/day for CYP3A or when paroxetine is administered at 30 mg/day for CYP2D6. This method was used by Ohno, et al. (2007; 2008) to estimate the fraction of CYP3A4 victims metabolized using ketoconazole or itraconazole as selective competitive inhibitors and diltiazem as a mechanism-based inactivator. A similar study on CYP 3A4 was published by Shou, et al. (2008) but with an extended list of inhibitors (troleandomycin, ritonavir, mibefradil, fluconazole, itraconazole, clarithromycin, saquinavir, erythromycin and grapefruit juice).
An estimate of 0.93 for the $fm(CYP3A)$ value for the CYP3A probe substrate midazolam has been derived based on an observed mean 16-fold increase in total exposure following administration of 400 mg/day ketoconazole (Obach, et al., 2006)

When a significant amount of clinical interaction data exists for each victim (multiple inhibitors), a more sophisticated method, involving non-linear least-squares regression, was proposed. Clinical interactions were fitted to Equation 2 for competitive inhibitors (Brown, 2005 and Ito, 2005) and the total hepatic input concentration, $[I]_{in}$, was used as the inhibitor concentration for all the predictions. An estimate of 0.94 for $fm(CYP3A4)$ has been derived based on the AUC ratio observed in vivo and $[I]_{in}/K_i$ ratio for 10 drug–drug interactions involving midazolam as the victim drug.

A number of other approaches were employed to obtain $fm(E)$. Initial values were obtained from estimates of total metabolism calculated indirectly from urinary recovery of unchanged drug (renal excretion method) mainly for CYP3A4 (Brown, et al., 2005; Ito, et al., 2005). A good alternative for polymorphic enzymes is phenocopying, i.e., from the difference between the urinary recovery of metabolites in both the presence and absence of a selective inhibitor (Back, et al., 1989). This was employed for the determination of $fm(CYP2C9)$ for the substrate tolbutamide using sulphaphenazole as the inhibitor.

2.1.2.3 Non-specific binding

2.1.2.3.1 Microsomal binding

Microsomal binding of drug substrates and/or perpetrators is increasingly recognized as a potential source of artifacts arising in the course of in vitro studies of drug metabolism. Non-specific binding of the substrate to microsomal protein is an important aspect of the extrapolation of in vivo drug clearance from data obtained with liver or recombinantly expressed microsomal systems. These may in turn produce inaccurate predictions when in vitro data are used to estimate in vivo pharmacokinetics (Obach, 1996; 1997; Obach, et al., 1997; Venkatakrishnan, et al., 2000; 2001; Kalvass, et al., 2001). Inhibitor binding to microsomal systems may likewise influence estimation of potency of metabolic inhibitors (Gibbs, et al., 1999), resulting in an overestimation of $K_i$ (Margolis, et al., 2003; Brown, et al., 2006; Tran, et al., 2002). Consequently, in vivo hepatic clearance and the extent of inhibitory drug interactions were often underpredicted.

Investigators have tried to use relative low microsomal protein concentration to avoid non-specific binding (Jones, et al., 2004). However, relative high concentrations (1 to 2 mg/ml) were still needed when studying phase II metabolic reactions (Soars, et al., 2002) and in vitro assessment of the time-dependent inhibition potential (Ghanbari, et al., 2006). As such, it is essential to correct the metabolic kinetic parameters (Clint and $K_i$) using the fraction unbound to microsomes, $fu_{(mic)}$, to ensure accurate pharmacokinetic estimation of potential drug candidates.

The intrinsic clearance and the inhibitory constants ($K_i$, $K_I$, or $IC_{50}$) were corrected or not based on user choices.
Note: In the DDIRC, for proprietary drugs, corresponding \( f_{\text{mic}} \) values are estimated using the method of Austin, et al. (2002; Equation 26) or that of Halifax, et al. (2006; Equation 27).

\[
\begin{align*}
\text{Equation 26} \\
&= \frac{1}{1 + C \times 10^{0.56 \times \log P/D - 1.41}} \\
\text{Equation 27} \\
&= \frac{1}{1 + C \times 10^{0.072 \times \log P/D + 0.06 \times \log P/D - 1.126}}
\end{align*}
\]

where \( C \) is the microsomal protein concentration (g/L), and LogP/D refers to LogP for basic compounds (pKa > 7.4) and Log D7.4 for neutral or acidic compounds (pKa ≤ 7.4).

For \( f_{\text{mic}} \) values not provided by users, the DDIRC uses values based on literature results contained in the Drug Library (when available) with the priority order: human > other species (mouse, rat, rabbit, dog, monkey) as previously outlined (Obach, 1997).

When protein concentrations in the \( f_{\text{mic}} \) experiment and intrinsic clearance or inhibitory constant experiments are not the same, the \( f_{\text{mic}} \) is estimated at the protein concentration of the metabolic parameters using Equation 28 (Austin, et al., 2002).

\[
\text{Equation 28} \\
f_{\text{mic}}^2 = \frac{1}{C_2 \times \left(\frac{1 - f_{\text{mic}}}{f_{\text{mic}}^1}\right) + 1}
\]

where \( C_1 \) is the microsomal protein concentration (g/L) used in the \( f_{\text{mic}}^1 \) experiment, \( C_2 \) is the microsomal protein concentration (g/L) used in the inhibition experiment, \( f_{\text{mic}}^1 \) refers to the free fraction coming from database, and \( f_{\text{mic}}^2 \) refers to the estimated free fraction at \( C_2 \).

When the microsomal protein concentration used in the inhibition experiment is not available, a default protein concentration (C2) is assumed with the following rules:

- Competitive inhibition (Ki or IC50)
  - HLM: \( C_{\text{prot}} = 0.4 \) g/l
  - Recombinant: \( C_{\text{prot}} = 0.2 \) g/l
- Mechanism-based inhibition (K1)
  - HLM or recombinant \( C_{\text{prot}} = 1 \) g/l
Domain of applicability

An analysis done by Gertz et al (2008) highlighted the importance of drug lipophilicity as a highly sensitive parameter for the prediction of the \( f_{u(\text{mic})} \). Both Austin and Houston’s equations showed very good agreement in the \( f_{u(\text{mic})} \) estimates at low microsomal protein concentration (0.1 mg/ml), in particular for drugs with low lipophilicity (logP/D \( \leq 2.5 \)).

A significant difference in the \( f_{u(\text{mic})} \) estimates was seen in the area of intermediate lipophilicity due to the nature of the prediction equations and their sensitivity on the variability in the logP estimates. On average, the Hallifax equation provided more accurate \( f_{u(\text{mic})} \) predictions, in particular for lipophilic drugs (logP/D = 2.5–5) and at higher microsomal protein concentrations (1 mg/ml). The extent of non-specific binding for highly lipophilic drugs was poorly predicted by both equations, suggesting that the unbound fraction should be determined experimentally for drugs with logP/D > 5. This cutoff should be even lower (logP/D = 3) if microsomal protein concentrations above 0.1 mg/ml are used.

2.1.2.3.2 Hepatocyte binding

Hepatocyte binding of drug substrates and/or perpetrators is increasingly recognized as a potential source of artifact arising in the course of in vitro studies of drug metabolism. Non-specific binding of substrate to hepatocyte is an important aspect of the extrapolation of in vivo drug clearance from data obtained with hepatocyte systems. The EC\(_{50}\) estimated in induction experiments was corrected or not based on user choices using the relationship EC\(_{50,u}\) = \( f_{u(\text{hep})} \times \text{EC}_{50}\).

Note: For \( f_{u(\text{hep})} \) values not provided by users, the DDIRC uses values based on literature results contained in the Drug Library when available with the priority order: human > other species (mouse, rat, rabbit, dog, monkey).

If \( f_{u(\text{hep})} \) values were not found in the database, an estimation was performed using the Austin et al. method (Equation 29; Austin, et al., 2002) or the Kilford et al. method (Equation 30; Kilford, et al., 2008) assuming a fixed concentration of \( 10^6 \) cells/ml.

\[
\begin{align*}
\text{Equation 29} \\
\hat{f}_{u(\text{hep})} &= \frac{1}{1 + 10^{0.420 \times \log P/D - 1.38}}
\end{align*}
\]

\[
\begin{align*}
\text{Equation 30} \\
\hat{f}_{u(\text{hep})} &= \frac{1}{1 + 0.625 \times 10^{0.673 \times \log P/D^2 + 0.045 \log P/D - 1.136}}
\end{align*}
\]

where LogP/D refers to LogP for basic compounds (pKa > 7.4) and Log D7.4 for neutral or acidic compounds (pKa ≤ 7.4).
2.1.2.4 Estimated perpetrator concentration in the liver after oral administration route

[I] has been defined as the concentration of inhibitor in the liver, which in practice is unmeasureable. One of the key challenges is the estimation of most appropriate value of [I], which should be based on in vivo concentrations of a given perpetrator that change over time, raising the question of whether the systemic plasma concentration or the hepatic inlet concentration is the most relevant concentration (Table 7).

The impact of plasma–protein binding remains controversial even though the use of total plasma systemic concentration has been advocated in the past as a means of predicting reversible inhibition DDIs. However, this is contrary to the well-established ‘free-drug hypothesis’ of drug action in DDI predictions. The question is whether to take into account the total plasma concentration or the unbound plasma concentration for the estimation of [I].

In most of the publications dealing with the impact of the inhibitor concentration on the accuracy of DDI prediction (Table 7) correction to account for the free drug fraction was shown to be important and determined as the concentrations leading to the most accurate predictions not only for mechanism-based inhibitors but also for reversible inhibitor mechanisms. Nevertheless, it is essential to note that total concentration was also determined by other authors (Brown, et al., 2006; Houston, et al., 2005) as the best concentration leading to more accurate DDI predictions.

Consideration of the systemic plasma concentration as opposed to the hepatic inlet concentration is also crucial. Most of the publications predicting DDIs involving reversible competitive inhibitors have determined that the hepatic inlet concentration ([I]in,avg,u or [I]in,max,u; Table 7) provided the most accurate DDI predictions. Whereas, prediction of DDIs involving MBI or induction mechanisms are more accurate when systemic plasma concentration (Cmax,u) was introduced in the calculations. This result was also supported by our unpublished results, where it was found that using unbound systemic plasma concentrations (Cmax,u or Cavg,u) for the prediction of DDIs between a midazolam victim and eight inactivators of CYP3A4 significantly reduced overprediction due to the use of the hepatic inlet concentrations ([I]in,max,u or [I]in,avg,u) and increased the accuracy (Figure 6).
Figure 6. The relationship between the observed AUC ratio in vivo and the AUC ratio predicted for eight CYP3A4 inactivators (1: clarithromycin, 2: saquinavir, 3: erythromycin, 4: verapamil, 5: diltiazem, 6: fluoxetine, 7: azithromycin, 8: ethynylestradiol). The plot represents predictions using the average or maximum systemic unbound drug plasma concentration (red or blue dots respectively) or the average or maximal inlet hepatic (orange or yellow dots respectively). The solid line represents the line of unity, whereas dashed lines represent the twofold limit in prediction accuracy.

It was found that the reversible inhibition portion performed best when the unbound portal vein concentration was used for concentration in the liver, whereas for irreversible inactivation and induction, the unbound systemic concentration was best.

Even if these results appeared inconsistent from a physiological point of view, they can be rationalized. For reversible inhibition, much of the interaction occurs during absorption and the hepatic first pass after the concentrations of perpetrator decrease below values required to exhibit reversible interaction. Consequently, the use of the hepatic inlet concentration for predicting DDIs makes sense when reversible inhibitors are involved.

Conversely for inactivation and induction, the use of systemic concentration makes sense in that the DDIs continue to occur after first-pass exposure of the intestine and liver is over. An optimized choice of the concentration based on the above explanation was proposed in the DDIRC as a default result (Table 8).
Even if most of the results can be rationalized, some are contradictory. For example, total systemic plasma concentration ($C_{avg}$) was determined as the concentration leading to the most accurate prediction for reversible competitive inhibitors (Brown, et al., 2006; Houston, et al., 2005) These considerations may underline some limitations of the mathematical model.

Systemic or hepatic inlet concentrations can be entered into the mathematical model as total or unbound exposure. It is common practice with such approaches to empirically select surrogate measures of exposure in the liver and intestine that provide the best correlation of predicted and observed DDIs reported in the literature.

Although practically useful, such empirical oversimplifications surely limit predictive accuracy within the data set and it is reckless to apply the method outside the data set.

Furthermore, it would be inappropriate to conclude from publication of Houston et al. (2005) that CYP3A4 interactions are fundamentally driven by total systemic perpetrator concentration and could lead to an overinterpretation of the mathematical model.

Table 7. Literature analysis of perpetrator concentrations used for predicting drug–drug interactions using the MSM or [I]/Ki approach

<table>
<thead>
<tr>
<th>Model</th>
<th>Perpetrators</th>
<th>CYPs involved</th>
<th>Best [I]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>3A4</td>
<td>[I]in,avg,u (competitive)</td>
<td>Fahmi, et al., 2009</td>
</tr>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>3A4, 2D6, 2C9, 2C19</td>
<td>[I]in,max,u (MBI and Inducer)</td>
<td>Einolf, 2007</td>
</tr>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>3A4, 2D6, 2C9, 1A2</td>
<td>[I]in,max,u</td>
<td>McGinnity, et al., 2008</td>
</tr>
<tr>
<td>MSM</td>
<td>MBI</td>
<td>3A4, 1A2, 2D6, 2C9, 2C19</td>
<td>[I]max,u</td>
<td>Obach, et al., 2007</td>
</tr>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>2C9, 2D6, 3A4</td>
<td>[I]max</td>
<td>Brown, et al., 2006</td>
</tr>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>3A4, 2A2,2D6, 2C9, 2C19</td>
<td>[I]max,u</td>
<td>Obach, et al., 2006</td>
</tr>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>3A4</td>
<td>[I]max</td>
<td>Houston, et al., 2005</td>
</tr>
<tr>
<td>[I]/Ki</td>
<td>Competitive</td>
<td>2C9, 2D6, 3A4</td>
<td>[I]avg</td>
<td>Ito, et al., 2004</td>
</tr>
<tr>
<td>[I]/Ki</td>
<td>Competitive</td>
<td>3A4, 2A2, 2D6, 2C9, 2C19</td>
<td>[I]max,u</td>
<td>Blanchard, et al., 2004</td>
</tr>
<tr>
<td>[I]/Ki</td>
<td>Competitive</td>
<td>3A4, 2A2, 2D6, 2C9, 2C19</td>
<td>[I]max,u</td>
<td>Ito, et al., 2002</td>
</tr>
<tr>
<td>MSM</td>
<td>Competitive</td>
<td>2C9</td>
<td>[I]in,max,u</td>
<td>Kanamitsu, et al., 2000</td>
</tr>
</tbody>
</table>
Note: In the DDIRC, hepatic inlet or systemic concentrations are estimated in the total blood for repeated or single administration using the blood-to-plasma ratio (Rb) and the hematocrit concentration (H, default value: 0.45; Table 8).

Table 8. Methods used in the DDIRC for computing values for [I]

<table>
<thead>
<tr>
<th>Choice of [I]</th>
<th>Estimation of [I]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum steady-state blood concentration</td>
<td>[l]lmax=Rb×Cmax</td>
</tr>
<tr>
<td>Unbound concentration</td>
<td>[l]lmax,u=((1-H)\times fu)×[l]lmax</td>
</tr>
<tr>
<td>Average steady-state blood concentration</td>
<td>[l]avg=Rb×Cavg</td>
</tr>
<tr>
<td>Unbound concentration</td>
<td>[l]avg,u=((1-H)\times fu)×[l]avg</td>
</tr>
<tr>
<td>Maximum hepatic inlet blood concentration</td>
<td>[l]lin,max=Rb×Cmax+(\frac{Fabs\times kabs\times D}{Qh})</td>
</tr>
<tr>
<td>Unbound concentration</td>
<td>[l]lin,max,u=((1-H)\times fu)×[l]lin,max</td>
</tr>
<tr>
<td>Average hepatic inlet blood concentration</td>
<td>[l]lin,avg=Rb×Cavg+(\frac{Fabs\times kabs\times D}{Qh})</td>
</tr>
<tr>
<td>Unbound concentration</td>
<td>[l]lin,avg,u=((1-H)\times fu)×[l]lin,avg</td>
</tr>
<tr>
<td>Optimized blood concentration</td>
<td>For competitive inhibition, [l]lin,max or [l]lin,max,u</td>
</tr>
<tr>
<td>Unbound concentration</td>
<td>For MBI, [l]max or [l]max,u</td>
</tr>
<tr>
<td></td>
<td>For induction, [l]max or [l]max,u</td>
</tr>
</tbody>
</table>

2.1.2.4.1 Rate constant of absorption

Rate constant of absorption (kabs) values, when available from the Drug Library, are used for [l]in vivo determination and, if necessary, estimated assuming a first order absorption rate according to Equation 31.

\[
\text{Equation 31}
\]

\[
\text{Tmax} = \frac{\ln\left(\frac{kabs}{kel}\right)}{kabs-kel}
\]

where Tmax (h) is the time to reach the maximum concentration and kel (h\(^{-1}\)) is the elimination rate constant coming from the same experiment. When kabs values are not available; a default value of 0.1 min\(^{-1}\) is assumed corresponding to gastric emptying.
2.1.2.4.2 Non-equilibrium conditions

When perpetrators are in non-equilibrium conditions (concentration increase or decrease in the liver compare to plasma or blood concentration), this phenomenon is taken into account to estimate the total or free blood concentration. The liver-to-blood concentration ratio (Kb), liver-to-plasma concentration ratio (Kp) or cell-to-medium concentration ratio were used to estimate concentration of perpetrators in non-equilibrium condition (Table 9).

Table 9. Parameters used in the DDIRC for computing non-equilibrium values for [I]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kp</td>
<td>Kp values, when available from the Drug Library, are used depending on blood plasma species with the priority order human &gt; mouse, rat, rabbit, dog, monkey. When Kp values are not available; a default value of 1 is assumed. Kp and Rb values are used to calculate the liver-to-blood concentration ratio (Kb) using the formula Kb = Kp/Rb.</td>
</tr>
<tr>
<td>Kb</td>
<td>Kb values, when available from the Drug Library, are used depending on whole blood species with the priority order human &gt; mouse, rat, rabbit, dog, monkey, and if necessary estimated from liver-to-plasma concentration ratio (Kp) and Rb, using the formula Kb = Kp/Rb.</td>
</tr>
<tr>
<td>C/M</td>
<td>C/M is an in vitro estimation of in vivo hepatic uptake (Kb unbound) and is used to preduct perpetrator concentration in the liver (hepatic uptake) using the formula ( I_{\text{nonequilibrium}} = I_{\text{liver}} = \frac{C}{M} \times [I]<em>{\text{b}} \times fb ), where ([I]</em>{\text{b}}) is the perpetrator concentration in the whole blood and fb is the unbound fraction in the whole blood (Yamano, et al., 1999).</td>
</tr>
</tbody>
</table>

2.1.2.5 Estimated perpetrator concentration in the liver after IV administration route

Estimation of [I], even after IV administration of perpetrator, remains a challenge. Furthermore, bolus and infusion administration types have to be taken into account to estimate the more relevant perpetrator concentration in the liver, [I], raising again the question of whether the total systemic plasma concentration or the unbound systemic plasma concentration is the most relevant concentration.

After bolus administration of the perpetrator drug, the systemic plasma concentration decreases over time, so the highest measureable concentration must be used in the DDIRC to estimate [I]. Calculation of the AUC ratio performed with the highest perpetrator concentration corresponds to the worst-case scenario (i.e., an overprediction of AUC ratio).

After slow infusion administration of the perpetrator drug the systemic plasma concentration at steady state (Css) must be used be in the DDIRC to estimate [I]. Perpetrator concentration is estimated after a fast infusion using the maximal concentration reached most of the time assimilated to \( C_{\text{max}} \).
Note: In the DDIRC, systemic concentrations are estimated in the total blood for bolus or infusion using the blood-to-plasma ratio (Rb) and the hematocrit concentration (H, default value: 0.45) when concentration is estimated in the free total blood (Table 10).

Table 10. Methods used in the DDIRC to compute values for [I] after bolus or infusion of the perpetrator

<table>
<thead>
<tr>
<th>Administration</th>
<th>Choice of [I]</th>
<th>Estimation of [I]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolus</td>
<td>Maximum steady-state blood concentration</td>
<td>$[I]_{\text{max}} = R_b \times C_0$</td>
</tr>
<tr>
<td></td>
<td>Unbound concentration</td>
<td>$[I]<em>{\text{max}, u} = \frac{(1-H) \times [I]</em>{\text{max}}}{R_b}$</td>
</tr>
<tr>
<td>Fast infusion</td>
<td>Maximum steady-state blood concentration</td>
<td>$[I]<em>{\text{max}} = R_b \times C</em>{\text{ss}}$</td>
</tr>
<tr>
<td></td>
<td>Unbound concentration</td>
<td>$[I]<em>{\text{max}, u} = \frac{(1-H) \times [I]</em>{\text{max}}}{R_b}$</td>
</tr>
<tr>
<td>Slow infusion</td>
<td>Maximum steady-state blood concentration</td>
<td>$[I]<em>{\text{max}} = R_b \times C</em>{\text{max}}$</td>
</tr>
<tr>
<td></td>
<td>Unbound concentration</td>
<td>$[I]<em>{\text{max}, u} = \frac{(1-H) \times [I]</em>{\text{max}}}{R_b}$</td>
</tr>
</tbody>
</table>

2.1.2.5.1 Non-equilibrium conditions

Non equilibrium conditions described for oral administration of perpetrator drug are applicable to IV administration. See 2.1.2.4.2 for a full description.

2.1.2.6 Gut metabolism

Many enzymes are expressed in the human small intestine: cytochromes and P450 enzymes (CYP3A4, CYP3A5, CYP1A1, CYP2C9, CYP2C19, CYP2D6 and CYP2J2) as well as transporters (Pgp, MRP1 and MRP2). CYP3A4 was determined as the most expressed enzyme in the small intestine, accounting for 80% of the total P450 amount, followed by CYP2C9 (14%), CYP2C19 (2%) and CYP2J2 (1.4%; Lin, et al., 1999; Paine, et al., 2006). The total amount of CYP3A expressed in the human small intestine (65.7–70.5 nmol) represents approximately 1% of the hepatic estimate (Paine, et al., 1997).

The contribution of intestinal interaction is incorporated into the prediction equation based on a hepatic enzyme interaction as the ratio of intestinal wall availability in the presence or absence of the inhibitor ($F'g$ and $F_g$, respectively). This approach is applicable for reversible and irreversible inhibition interactions. The concept of fold reduction clearance can be applied for the determination of the ratio of the intestinal wall availability in the presence and absence of the perpetrator (Equation 3).

$$\frac{F'g}{F_g} = \frac{1}{F_g + (1-F_g) \times \frac{C_{\text{int}, g}}{C_{\text{int}, g}}}$$

Equation 3
Using the DDIRC, the $F'g/Fg$ ratio can be estimated in two different ways as outlined below. In all cases, the $Fg$ control values can be provided by the user or estimated based on the well stirred model using in vitro data.

Maximal: $F'g/Fg$

Model predicted: $F'g/Fg$

2.1.2.6.1 Maximal $F'g/Fg$

Assuming the worst-case scenario, i.e., maximal inhibition of intestinal CYPs resulting in $F'g = 1$, and therefore the maximal ratio as $1/Fg$. Maximal inhibition can be used as a pragmatic estimation of the $F'g/Fg$ (Galetin, et al., 2007), mostly when perpetrators are potent competitive inhibitors or inactivators.

2.1.2.6.2 Model-predicted $F'g/Fg$ ratio

The model-predicted $F'g/Fg$ ratio is obtained from the decrease in the intestinal intrinsic clearance in the presence of an inhibitor using the in vitro obtained inhibitory constant (competitive or mechanism-based) and the estimated inhibitor concentration in the intestinal wall during the absorption phase ([I,u]g; Obach, et al., 2006; Rostami-Hodjegan, et al., 2004).

Prediction of the $F'g/Fg$ ratio using the model-predicted approach is more accurate than the worst-case scenario when perpetrators are weak competitive inhibitors or inactivators (unpublished results).

When more than one cytochrome (CYP3A4 and CYP3A5 are taken into account in the DDIRC) are involved in the intestinal metabolism, the fold reduction in intestinal intrinsic clearance can be estimated using Equation 32.

\[
\frac{C_{\text{int},g}'}{C_{\text{int},g}} = \sum_{k=1}^{n} \frac{f_{m}(E)_{k,g}}{C_{gk} \times M_{gk} \times T_{gk}} + 1 \sum_{k=1}^{n} f_{m}(E)_{k,g}
\]

Equation 32

where $f_{m_{k,g}}$ is the fraction metabolized by the enzyme $k$ in the gut wall, and $C_{gk}$ (Equation 33), $M_{gk}$ (Equation 34) and $T_{gk}$ (Equation 35) are the competitive, mechanism-based inhibition and induction components, respectively.

\[
M_{gk} = 1 + \sum_{i=1}^{p} \frac{[I_{u}]_{g} \times k_{\text{inact,ki}}}{K_{\text{deg}_g} \times ([I_{u}]_{g} + K_{I,u})}
\]

Equation 33

\[
C_{gk} = 1 + \sum_{j=1}^{m} \frac{[I_{u}]_{g}}{K_{I,u_{ij}}}
\]

Equation 34

\[
T_{gk} = \frac{1}{1 + \sum_{i=1}^{p} \frac{[I_{u}]_{g}^{n}}{K_{I,u_{ij}} + E_{C_{S_{2}},u_{k,i}}}}
\]

Equation 35
Concerning the concentration of the perpetrator in the gut, \([I]_g\), FDA guidance recommends estimating it using the equation described by Rostami-Hodjegan, et al. (2004; Equation 36)

\[
[I]_g = \frac{F_{abs} \times k_{abs} \times D}{Q_g}
\]

**Equation 36**

### 2.1.2.6.2.1 Fraction metabolized in the gut (fm(E),g) and intestinal intrinsic clearance

All the intestinal enzymes involved in the metabolism of the compound contribute to the net intrinsic metabolic clearance in the gut (Clint_{int,g}). Assuming that intrinsic metabolic clearance in the gut and in the liver are the same when expressed per pmol of enzyme (Yang, et al., 2004), the contribution of a particular enzyme to the net intrinsic metabolic clearance in the gut may be estimated from the product of the intrinsic unbound clearance per unit of enzyme multiplied by a scaling factor (Equation 37). This scaling factor could be the same as used in the liver (ISEF, RAF or abundance).

\[
fm(E)_g = \frac{\sum_{i=1}^{n} \text{Clint}_{u,g}(rE_i) \times SF_{E_i}}{\sum_{i=1}^{n} (\sum_{k=1}^{p} \text{Clint}_{u,k}(rE_k) \times SF_{E_k})}
\]

**Equation 37**

The DDIRC uses only abundance in the gut as a scaling factor for CYP3A4 and CYP3A5. Absolute intestinal abundances of CYP3A4 and CYP3A5 used were determined by Yang, et al. (2007b) as 62 and 23 nmol, respectively, in populations that express active intestinal CYP3A5.

### 2.1.2.6.2.2 Qg

Qg is the gut blood flow. Authors have used either total intestinal blood flow (37.2 l/h) or mucosal blood flow (29.8 l/h) to represent Qg.

**Note:** In the DDIRC, Qent (enterocytic blood flow: 18 l/h) was chosen as the relevant intestinal blood flow because cytochrome enzymes are located at the villous tips.

### 2.1.2.6.3 Fg prediction

Predictions of intestinal availability (Fg) are based on the well-stirred gut model adapted from the well-stirred hepatic model and use in vitro data on gut intrinsic clearance, the abundance of enzyme in gut (determined by publication analysis) and the gut blood flow (fixed value; Yang et al.).

\[
F_g = \frac{Q_g}{Q_g + f_{u,g} \times Cl_{int,g}}
\]

**Equation 38**
Where \( Q_g \) is gut blood flow, \( f_{ug} \) is the fraction of drug unbound in the enterocyte, and \( C_{\text{int},g} \) is the net intrinsic metabolic clearance in the gut based on unbound drug concentration.

**Note:** In the DDIRC, \( Q_{\text{ent}} \) (enterocytic blood flow: 18 l/h) was chosen as the relevant intestinal blood flow because cytochrome enzymes are located at the villous tips, \( f_{ug} \) was set as 1, and the net intrinsic metabolic clearance in the gut was estimated using intrinsic unbound clearance per unit of enzyme multiplied by the abundance of enzymes in the gut (Equation 39).

\[
C_{\text{int},g} = \sum_{k=1}^{P} C_{\text{int},u}(E_k) \times E_k \text{abundance (gut)}
\]

**Equation 39**

Absolute intestinal abundances of CYP3A4 and CYP3A5 used were determined by (Yang, et al., 2007b) at 62 and 23 nmol, respectively, in populations that express active intestinal CYP3A5.

### 2.1.2.7 Integrated general models

#### 2.1.2.7.1 Oral administration of victim drug

#### 2.1.2.7.1.1 Oral administration of perpetrator drug

This combined model is based on calculating the net effect of competitive inhibition, inactivation and induction in both the intestine and liver, assuming an oral administration of the victim and perpetrator drug.

\[
\frac{AUC_i}{AUC} = \frac{1}{F_g + (1-F_g) \times \left( \sum_{k=1}^{n} \frac{f_{m(E)}E_k}{C_{g,k} \times M_{g,k} \times T_{g,k}} + 1 \right) \times \left( \sum_{k=1}^{n} \frac{f_{m(E)}E_k}{C_{h,k} \times M_{h,k} \times T_{h,k}} + 1 \right)}
\]

**Equation 40**

where \( C_{h,k} \) and \( C_{g,k} \) are the terms for reversible inhibition in the liver and in the gut, respectively, associated to enzyme \( k \) and assuming inhibition by multiple perpetrators.

\[
C_{h,k} = 1 + \sum_{i=1}^{p} \frac{I_{i,h,k}}{K_{i,h,k}}
\]

\[
C_{g,k} = 1 + \sum_{i=1}^{p} \frac{I_{i,g,k}}{K_{i,g,k}}
\]

**Equation 4**  **Equation 34**
\( M_{h,k} \) and \( M_{g,k} \) are the terms for time-dependent inhibition in the liver and in the gut, respectively, associated to enzyme \( k \) and assuming inhibition by multiple perpetrators.

\[
M_{h,k} = 1 + \sum_{j=1}^{p} \frac{k_{m,ij} \times [I_{h,k}]_j}{k_{d,ij} \times ([I_{h,k}]_j + K_{I_{h,k}})}
\]

\[
M_{g,k} = 1 + \sum_{j=1}^{p} \frac{k_{m,ij} \times [I_{g,k}]_j}{k_{d,ij} \times ([I_{g,k}]_j + K_{I_{g,k}})}
\]

Equation 5

Equation 33

\( T_{h,k} \) and \( T_{g,k} \) are the terms for induction in the liver and in the gut, respectively, associated to enzyme \( k \) and assuming induction by multiple perpetrators.

\[
T_{h,k} = \frac{1}{1 + \sum_{j=1}^{p} \frac{E_{m,ij} \times [I_{h,k}]_j}{[I_{h,k}]_j \times EC_{50,h,k}^{i,j}}}
\]

\[
T_{g,k} = \frac{1}{1 + \sum_{j=1}^{p} \frac{E_{m,ij} \times [I_{g,k}]_j}{[I_{g,k}]_j \times EC_{50,g,k}^{i,j}}}
\]

Equation 8

Equation 35

The combined model (Equation 40) can be simplified into Equation 41 when no inhibition or induction of victim metabolism by perpetrators occurred in the gut.

\[
\frac{AUC_i}{AUC} = \frac{1}{\sum_{k=1}^{n} \frac{fm(E)_{h,k}}{C_{h,k} \times M_{h,k} \times T_{h,k}} + 1 - \sum_{k=1}^{n} \frac{fm(E)_{h,k}}{C_{h,k} \times M_{h,k} \times T_{h,k}}}
\]

Equation 41

2.1.2.7.1.2 IV administration of perpetrator drug

The AUC ratio after IV administration of a perpetrator drug can be estimated using Equation 41. Indeed, after IV administration of the perpetrator drug, no inhibition or induction occurred in the gut, so this equation could be applicable after IV administration of perpetrator drugs assuming oral administration of the victim drug.

2.1.2.7.2 IV administration of victim drug

Calculation of the AUC ratio after IV administration of a victim drug is partially treated in the DDIRC, but only for low-clearance victim drugs.

MSM has been widely used for oral administration, basically because it is the most common route. Even so, MSM can also be used for IV dosing.
The change in the AUC of the victim after intravenous bolus administration (AUC\textsubscript{iv}) and during intravenous infusion (C\textsubscript{ss}) can be expressed by Equation 42, if the dose or infusion rate is constant (Ito, et al., 1998).

\[ \frac{\text{AUC}_{\text{iv},i}}{\text{AUC}_{\text{iv}}} = \frac{C_{\text{ss},i}}{C_{\text{ss}}} = \frac{1}{f_h \times \frac{Cl_{\text{h},i}}{Cl_h} + 1 - f_h} \]

Equation 42

where \( f_h \) represents the fraction of the hepatic clearance in the total clearance and \( Cl_h \) and \( Cl_{h,i} \) the hepatic clearance without and with the perpetrator, respectively.

Prediction of DDIs after intravenous administration of the victim can be described by Equation 42 but this remains poorly reported in the literature. Predictions involving high and low clearance drugs have been addressed by Ito, et al. (1998).

For low-clearance victim drugs (\( E_H < 0.3 \)) where the first-pass hepatic availability is close to unity, Equation 42 can be rearranged to Equation 41.

For high-clearance drugs, the basal clearance value (\( E_H \) without inhibitor) has to be taken into account for DDI prediction. Consequently, a mechanistic static model has been developed (Equation 43) to predict the change in AUC (not included in the DDIRC) after intravenous administration (Kirby, et al., 2010; Lopez, 2010). It is based on the following parameters: the fraction of hepatic blood clearance to the total blood clearance of the victim drug, \( f_h \), the fraction of affected drug cleared by each enzyme, \( fm(E_k) \), the intrinsic clearance values for each enzyme affected or not affected by the perpetrator, and the hepatic extraction ratio of the victim drug, \( E_H \).

\[ \frac{\text{AUC}_{\text{iv},i}}{\text{AUC}_{\text{iv}}} = \frac{1}{\frac{f_h}{1 - E_H} + 1 - f_h} + \sum_{k=1}^{n} \frac{f_m(E_k)h}{C_{hk} \times M_{hk} \times T_{hk}} + \sum_{k=1}^{n} f_m(E_k)h \]

Equation 43

A sensitivity/error analysis of the model was performed by Kirby, et al. (2010) to determine the boundaries (degree of inhibition or induction) at which ignoring the \( E_H \) of commonly used victim drugs results in a > 30% error in the predicted AUC ratio.

Eighteen in vivo clinical studies retrieved from publications were used by Lopez, et al. (2010) to compare actual and predicted DDIs with (Equation 43) and without (Equation 41) the basal clearance value of the victim drug (\( E_H \) ranging from 0.01 to 0.63). Predicted values derived from Equation 41, without considering the \( E_H \) of the victim drug, tend to overpredict DDIs whereas values predicted with \( E_H \) are more accurate.
2.1.2.8 The DDIRC and FDA guidance

The DDIRC uses a mechanistic static model identical to the one described in the 2012 FDA guidance (Table 11). A slight difference between the two models appears for the calculation of contribution of induction.

The 2012 FDA guidance used the equation developed by Fahmi, et al. (2009), whereas the DDIRC used the one proposed by Shou, et al. (2008).

The two models remain exactly the same when the $E_{\text{max}}$ value used in the DDIRC takes into account the in vitro to in vivo scaling factor $d$ and the hill coefficient $n$ value is equal to one.

Concerning equations and default values of hepatic ($Q_h = 1.61 \, \text{l/min}$) and gut blood flow ($Q_g = 18 \, \text{l/h}$) used to calculate perpetrator concentration in the liver and gut, there is an exact match between the two systems.

Table 11. Comparison of mechanistic models from the 2012 FDA guidance and the DDIRC

<table>
<thead>
<tr>
<th>MSM</th>
<th>FDA Draft Guidance 2012</th>
<th>DDIRC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reversible inhibition</strong></td>
<td></td>
<td>$A_{h/g}$</td>
</tr>
<tr>
<td>$A_{h/g}$</td>
<td>$AUC_i = \frac{1}{\text{AUC}} \times \frac{1}{F_g + ((1-F_g) \times (A_g \times B_g \times C_p) \times (A_h \times B_h \times C_h) \times \text{fm(E)}_h + 1 - \text{fm(E)}_h}$}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\frac{1}{1 + \frac{[I]_{h/g}}{K_i}}$</td>
</tr>
<tr>
<td><strong>Time-dependent inhibition</strong></td>
<td></td>
<td>$B_{h/g}$</td>
</tr>
<tr>
<td>$B_{h/g}$</td>
<td>$AUC_i = \frac{1}{\text{AUC}} \times \frac{1}{F_g + ((1-F_g) \times (A_g \times B_g \times C_p) \times (A_h \times B_h \times C_h) \times \text{fm(E)}_h + 1 - \text{fm(E)}_h}$}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1 + \frac{k_{\text{int}} \times [I]<em>{h/g}}{k</em>{\text{deg},h} \times ([I]<em>{h/g} + E</em>{\text{max}})}$</td>
</tr>
<tr>
<td><strong>Induction</strong></td>
<td></td>
<td>$C_{h/g}$</td>
</tr>
<tr>
<td>$C_{h/g}$</td>
<td>$AUC_i = \frac{1}{\text{AUC}} \times \frac{1}{F_g + ((1-F_g) \times (A_g \times B_g \times C_p) \times (A_h \times B_h \times C_h) \times \text{fm(E)}_h + 1 - \text{fm(E)}_h}$}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1 + \frac{d \times E_{\text{max}} \times [I]<em>{h/g}}{[I]</em>{h/g} + E_{50}}$</td>
</tr>
<tr>
<td><strong>Perpetrator concentration, liver $[I]_h$</strong></td>
<td></td>
<td>$[I]<em>h = \text{fib} \times \left( \frac{C</em>{\text{max,b}} + \frac{F_g \times \text{kabs} \times D}{Q_h}}{Q_h} \right)$</td>
</tr>
<tr>
<td><strong>Perpetrator concentration, gut $[I]_g$</strong></td>
<td></td>
<td>$[I]_g = \frac{F_g \times \text{kabs} \times D}{Q_g}$</td>
</tr>
</tbody>
</table>
2.2 Drug Library used in the DDIRC

2.2.1 Input data

The DDIRC requires prior information, including the concentration of the inhibitor at the enzyme site, the mechanism of inhibition, inhibition constants for each route, and the proportional clearance of the substrate via different routes (including renal excretion).

Input requirements for proprietary victim (Table 12) and perpetrator (Table 13) drugs are detailed below and classified as Minimum, Optimal and Not required.

**Note:** Accuracy of predictions will increase in conjunction with the amount of data provided by user.

### Table 12. Input data requirements for a proprietary victim drug

<table>
<thead>
<tr>
<th>Input data</th>
<th>DDI Risk Calculator</th>
</tr>
</thead>
<tbody>
<tr>
<td>fm</td>
<td>if fm Predicted</td>
</tr>
<tr>
<td>HLM Clint or rhCYP Clint</td>
<td>if fm Predicted</td>
</tr>
<tr>
<td>RAF, ISEF, Abundance</td>
<td>Predicted</td>
</tr>
<tr>
<td>fu(mic)</td>
<td>Predicted</td>
</tr>
<tr>
<td>Fg</td>
<td>Default</td>
</tr>
<tr>
<td>Qg</td>
<td>Predicted</td>
</tr>
<tr>
<td>CIH</td>
<td>Optional</td>
</tr>
<tr>
<td>CIR</td>
<td>Optional</td>
</tr>
<tr>
<td>ka</td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td></td>
</tr>
<tr>
<td>Ff</td>
<td></td>
</tr>
<tr>
<td>Vss</td>
<td></td>
</tr>
</tbody>
</table>

### Table 13. Input data requirements for a proprietary perpetrator drug

<table>
<thead>
<tr>
<th>Input data</th>
<th>DDI Risk Calculator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax or Cavg</td>
<td>predicted</td>
</tr>
<tr>
<td>Ki or IC50</td>
<td>Optional</td>
</tr>
<tr>
<td>Ki and kinact (for MBI)</td>
<td>Predicted</td>
</tr>
<tr>
<td>EC50 and Emax</td>
<td>Optional</td>
</tr>
<tr>
<td>fu(mic)</td>
<td></td>
</tr>
<tr>
<td>fub</td>
<td></td>
</tr>
<tr>
<td>ka</td>
<td></td>
</tr>
<tr>
<td>FaFg</td>
<td></td>
</tr>
<tr>
<td>Dose</td>
<td></td>
</tr>
<tr>
<td>Qh</td>
<td></td>
</tr>
<tr>
<td>Qg</td>
<td></td>
</tr>
<tr>
<td>CIR</td>
<td></td>
</tr>
<tr>
<td>HLM Clint or rhCYP Clint</td>
<td></td>
</tr>
<tr>
<td>Vss</td>
<td></td>
</tr>
</tbody>
</table>
2.2.2 Drug Library

The DDIRC Drug Library includes mainly drugs that are on the market, but also includes a small number of drugs that are withdrawn. More than 400 perpetrators and 200 victims along with their own in vitro and in vivo data are included in the DDIRC. This Drug Library covers the most popular cytochrome P450s (CYP3A4, 2D6, 2C9, 2C19, 2C8, 2B6, 1A2 and 2E1) and to a lesser extent, UGTs involved in the metabolism of drugs on the market (Figure 7). The Drug Library covers more than 100 therapeutic classes, including the most commonly prescribed ones.

![Perpetrators and Victims within DDIRC Library](image)

*Figure 7. The count of victims and perpetrators available in the DDIRC Drug Library for the most popular cytochromes. Only victims with fm values and perpetrators with inhibitory or induction constants supplemented with $C_{max}$ values in repeated or single doses were added to the Drug Library.*

In the last 2012 FDA guidance, lists of in vivo perpetrators and victims were provided to conduct in vivo DDIs trials. Perpetrators were classified into strong, moderate and weak inhibitors or inducers based on the fold increase or decrease in the AUC ratio. The DDIRC allows users to predict DDIs with most of the perpetrators and victims recommend in the 2012 FDA guidance.
2.2.2.1 FDA Victims

Sensitive and narrow therapeutic range substrates of CYPs are recommended in the 2012 FDA draft guidance. The DDIRC allows users to predict DDIs rapidly and accurately with 87% of all the exemplified FDA in vivo substrates (Figure 8). Fm values for each substrate are provided by the Drug Library when available and used by the DDIRC. Fm values (maximum) for CYPs involved in the metabolism of sensitive and narrow therapeutic range substrates are displayed in Figures 9 and 10.

Figure 8. Sensitive and narrow therapeutic ranges for in vivo substrates proposed by the FDA and available in the DDIRC for CYPs.
Figure 9. Fm values for the sensitive and narrow therapeutic range for in vivo substrates proposed by the FDA and used in the DDIRC for CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6. Only maximum values of Fm are displayed. Other values are also used to predict DDIs when available.

Figure 10. Fm values for the sensitive and narrow therapeutic range for in vivo substrates proposed by the FDA and used in the DDIRC for CYP3A4. Only maximum values of Fm are displayed. Other values are also used to predict DDIs when available.
Perpetrators in the FDA guidance are classified into strong, moderate and weak inhibitors or inducers based on the fold increase or decrease in the AUC ratio (Table 14). 73% (120/164) of the perpetrators recommended by FDA are included in the DDIRC.

Table 14. Percentage of in vivo perpetrators from the 2012 FDA guidance and included in the DDIRC with their in vitro and in vivo data needed to predict DDIs

<table>
<thead>
<tr>
<th>CYP</th>
<th>Inhibitor</th>
<th>Inducer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
<td>Moderate</td>
<td>Weak</td>
</tr>
<tr>
<td>1A2</td>
<td>100%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>2B6</td>
<td>100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>2C8</td>
<td>100%</td>
<td>67%</td>
<td></td>
</tr>
<tr>
<td>2C9</td>
<td>70%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>2C19</td>
<td>100%</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>2D6</td>
<td>100%</td>
<td>100%</td>
<td>75%</td>
</tr>
<tr>
<td>3A4</td>
<td>100%</td>
<td>73%</td>
<td>93%</td>
</tr>
<tr>
<td>Total</td>
<td>100%</td>
<td>73%</td>
<td>77%</td>
</tr>
</tbody>
</table>
3 Getting Started With the DDIRC

3.1 Overview

The following sections are intended to help users quickly get started with the DDIRC.

Home page
General overview of the interface
Predict DDI: Proprietary Victim Drug
Predict DDI: Proprietary Perpetrator Drug
Results
Export
Glossary

3.2 Home page

Choose whether you want to predict the DDI risk of a proprietary victim drug or proprietary perpetrator drug using the two entry options. Simply click **Start** under the relevant option (1).
3. 3  General overview of the interface

1. Fill in the **Victim** tab using in vitro data generated from the drug candidate of interest. Use the **Perpetrators** tab to refine which drugs are assessed for drug–drug interaction risks.
2. Enter data for victim(s) or perpetrator(s) and define parameters for data retrieval in these fields.
3. Click **Predict Interactions** to calculate the AUC ratio based on your input.
3.4 Predict DDI: Proprietary Victim Drug

3.4.1 Performing your first Prediction: fm(E) user defined

3.4.1.1 No metabolism in the gut

1. Enter the compound name.
2. Check User Defined to enable user fm(E) definition; for default values, see the next section.
3. Select the enzyme(s) involved in your proprietary drug metabolism.
   Note: You can delete any row by clicking on the x at the end of the line.
   Important: Each enzyme must only be selected once, even if it is involved in multiple pathways. In such cases, enter the sum of the clearance for each pathway.
4. For mechanism-based inhibition, when selecting a target, a default value of kdeg is proposed taken from peer-reviewed publications, but this can be edited by the user (see section 2.1.2.1.2.1 Enzyme turnover). Click i for more information on the kdeg values.
5. Enter the fm(E) value (the total clearance fraction mediated by the selected enzyme for your victim drug).
6. Click Predict Interactions.
   Note: Perpetrators are automatically selected from the DDIRC Drug Library.
### 3.4.1.2 Metabolism in the gut

1. Select CYP3A and/or CYP3A5 to open the **Intestinal Metabolism** form.
2. Bioavailability in the gut (Fg) can be defined by the user or predicted as described in section 3.4.1.3 **Fg prediction**. The default value is 1.
3. Microsomal non-specific binding, fu(mic), can be defined by the user or predicted by clicking **Calculate fu(mic)**. The methods of Austin and Houston are available for fu(mic) prediction, as described in section 2.1.2.3 **Non-specific binding**.
   **Tip**: If correction of clearance by fu(mic) is not wanted or fu(mic) is not known, the user can enter the same protein concentration for each enzyme as the one used for fu(mic) and use a value of 1 for fu(mic).
4. The predicted intestine inhibition model is the default selection. It has a default value of Qg = 18 l/h.
5. Enter the metabolic clearance expressed per pmol of enzymes (recombinant or HLM). If the clearance is not available, Km and Vmax (per pmol of enzymes) are required and clearance is automatically calculated.
   **Note**: The protein concentration used for each clearance or Km and Vmax experiment is required to estimate fu(mic) at the right concentration.
   **Tip**: Rate constant of degradation and abundance are defined for CYP3A4 and CYP3A5 in the gut.
   **Tip**: Metabolic clearance, rate constant of degradation (kdeg) and abundance are required only when the model **Predicted** is selected.
3.4.1.3 Fg prediction

1. When Predicted is selected, the Fg value becomes unavailable for user entry.
2. Clearance or Km, \( V_{\max} \) and Protein concentration can be entered when Predicted is selected.
3. The abundances of CYP3A4 and/or CYP3A5 are given by default, but they can be adjusted to commonly used values.
4. When all data are entered, click Predict to calculate Fg. The calculated value will appear in orange in 1 and clearance values will disappear, replaced with the dropdown selection header Fg and fm(E)g Prediction >. Clicking on the blue arrow reveals the clearance table, enabling modifications and new predictions, if needed.
3.4.1.4 \( \text{Fu} \text{(mic)} \) Calculation

1. Click \( \text{Calculate} \ \text{fu} \text{(mic)} \) to predict \( \text{fu} \text{(mic)} \).
2. The \( \text{fu} \text{(mic)} \) Calculation box allows the selection of the Halifax/Houston method (selected as default) or the Austin method for the calculation. See section 2.1.2.3 Non-specific binding. Depending on the pKa value, LogD or LogP will appear. The protein concentration must be entered. Click \( \text{Calculate} \) to get the \( \text{fu} \text{(mic)} \) estimate, which will appear in orange (3).
3.4.2 Advanced prediction features: fm(E) prediction from victim kinetic experiments (IVIVE)

3.4.2.1 No metabolism in the gut

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Choose <strong>predicted</strong> to estimate fm(E) based on clearances.</td>
</tr>
<tr>
<td>2.</td>
<td>Choose the type of hepatic metabolism experiment performed: <strong>HLM</strong> or <strong>hRecombinant</strong> (which is the default selection).</td>
</tr>
</tbody>
</table>
| 3.   | When **hRecombinant** is selected, the scaling factors **ISEF**, **RAF** and **Abundance** can be selected.  
**Tip**: ISEF and RAF default values depend on the recombinant system chosen. A complete list of the default values used is available by clicking on the **i** near the recombinant system. |
| 4.   | When **ISEF** or **RAF** are selected, the recombinant system can be chosen. |
| 5.   | Select the enzyme(s) involved in the metabolism of your proprietary drug. Enter metabolic clearance expressed per pmol of enzymes (recombinant or HLM). If the clearance is not available, Km and \( V_{\text{max}} \) (per pmol of enzymes) are required and clearance is automatically calculated.  
**Note**: You can delete any row by clicking on the **x** at the end of the line.  
**Important**: Each enzyme must only be selected once, even if it is involved in multiple pathways. In such cases, enter the sum of the clearance for each pathway. |
| 6.   | Hepatic fraction (fh) is defined by the user. The default value of 1 means that the total clearance is exclusively hepatic. fh can be predicted based on hepatic clearance and non-hepatic clearance. |
| 7.   | Click on Predict fm(E) for fm(E) estimation. The table with intrinsic clearance disappears when fm(E) is predicted (see the next screenshot). |
3.4.2.1 No metabolism in the gut (continued)

**PharmaPendium**

**Proprietary Victim Drug**

**Victim Perpetrators**

Please enter proprietary data for the victim drug:

*Compound name: PharmaPendium-1*

**Hepatic Metabolism**

<table>
<thead>
<tr>
<th>Enzyme(s)</th>
<th>kdeg (min⁻¹)</th>
<th>fmE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**In Vitro enzyme contribution: fm(E)\text{vitro}**

Click on the blue arrow (1) beside In vitro enzyme contribution: fm(E)\text{vitro} to see the clearance table. Modify values for a new estimation of fm(E) and click again on Predict Fme.
3.4.2.2 fh and hepatic clearance prediction

1. Choose **Predicted** to estimate the hepatic fraction (fh) based on total and non-hepatic clearance.
2. In the fh prediction field, choose **User Defined** (which is the default selection) for total clearance (Cl) if you have total and non-hepatic clearance results from in vivo experiential results. If you do not, see the tip on seeing and modifying predicted hepatic clearance (next page). Click **Predict fh** to obtain the fh value based on in vivo clearance.

---

1. If you choose **Predicted** in the fh prediction field, more fields will become available to allow you to estimate total and hepatic clearance based on intrinsic clearance and scaling factors using the well-stirred model.
2. Enter the non-hepatic clearance value (Cl non hepatic) in l/h.
3. Click **Predict fh** to obtain the fh value based on predicted total and hepatic clearance.
3.4.2.2 fh and hepatic clearance prediction (continued)

**Tip:** How to see and modify the predicted hepatic clearance?

Click on the blue arrow (1) beside fh **prediction** to see the total, hepatic and non-hepatic clearance. Modify values for a new estimation of fh and click **Predict fh**.

![Image of hepatic clearance prediction interface](image)

Note: In addition to "Cl non hepatic" and "fup" values, please make sure that the values for Clint, [Cl] prot, and the microsomal binding values (fubmic) and Prot. Conc.) are entered. Only then you will be able to complete the prediction of fh.
3.4.2.3 Metabolism in the gut

When metabolism in the gut is taken into account (CYP3A4 and/or CYP3A5) for DDI predictions, intrinsic clearances from recombinant or HLM experiments provided for estimation of fm(E) are automatically used to estimate metabolism in the gut, i.e., gut clearance and, when necessary, fm(E) in the gut.

1. Select CYP3A and/or CYP3A5 to open the Intestinal Metabolism form.
2. Bioavailability in the gut (Fg) can be defined by the user or predicted as described in section 3.4.1.3 Fg prediction. The default value is 1.
3. Metabolic clearance or Km and Vmax and protein concentration are automatically filled with the values defined in the hepatic metabolism. Clearance is automatically calculated per pmol of enzyme.
   Note: The protein concentration used for each clearance or Km and Vmax experiment is required to estimate fu(max) at the right concentration.
   Tip: Rate constant of degradation and abundance are defined for CYP3A4 and CYP3A5 in the gut.
   Tip: Metabolic clearance, rate constant of degradation (kdeg) and abundance are required only when the model Predicted is selected.
3.4.2.3 Metabolism in the gut (continued)

4. The default intestinal inhibition has a $Q_g$ value of 18 l/h.
5. Microsomal non-specific binding, $f_{u_{(mic)}}$, can be defined by the user or predicted by clicking Calculate $f_{u_{(mic)}}$. The methods of Austin and Houston are available for $f_{u_{(mic)}}$ prediction, as described in section 2.1.2.3 Non-specific binding. 
   **Tip:** If correction of clearance by $f_{u_{(mic)}}$ is not wanted or $f_{u_{(mic)}}$ is not known, the user can enter the same protein concentration for each enzyme as the one used for $f_{u_{(mic)}}$ and use a value of 1 for $f_{u_{(mic)}}$. 
3.4.3 Advanced prediction features: Perpetrator tuning

3.4.3.1 No metabolism in the gut

By default, perpetrators are administered in repeat doses and the hepatic concentration is estimated using the Optimized [1] value (not corrected for blood or plasma binding).

This screenshot shows the perpetrator tab with the default settings under Dosing regimen and Estimated liver concentration.

**Proprietary Victim Drug**

<table>
<thead>
<tr>
<th>Victim</th>
<th>Perpetrator</th>
<th>Parameter</th>
<th>Value</th>
<th>IC50 (µM)</th>
<th>Perpetrator name</th>
<th>Victim C (µM)</th>
<th>Victim Km (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mechanism Based (MBM)</th>
<th>Enzyme(s)</th>
<th>Parameter</th>
<th>Value</th>
<th>IC50 (µM)</th>
<th>Perpetrator name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>Enzyme(s)</th>
<th>Parameter</th>
<th>Value</th>
<th>Emax</th>
<th>nh</th>
<th>Induction type</th>
<th>Perpetrator name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Predict Interactions**
3.4.3.2 Dosing regimen and hepatic perpetrator concentration

Users can choose between single or repeated dosing regimens. The choice opens different possibilities for further estimations.

Selection of **Repeated** dosing regimen

Selection of **Single** dosing regimen

Perpetrators are retrieved from the Drug Library. Hepatic perpetrator concentrations, $[\text{j}]_{\text{in}}$, are selected from the options available. See section 2.1.2.4 Estimated perpetrator concentration in the liver for more details. The hepatic blood flow value corresponding to that of a healthy Caucasian male is given by default. This can be modified by the user.

**Important:** hepatic perpetrator concentrations are estimated in the total blood using the blood-to-plasma ratio ($R_b$).
3.4.3.3 Blood or plasma binding, microsomal binding and hepatocyte binding

By default the fu (blood or plasma binding) is not taken into account. If you want to use an unbound concentration of perpetrators in the prediction, tick the box (1) and the value is retrieve automatically from the Drug Library in the priority order human > rat, mouse, rabbit or monkey. If no fu values are available in Drug Library, a default value of 1 is used as detailed in section 2.1.2.4 Estimated perpetrator concentration in the liver.

By default the fu(mic) (fraction unbound in the microsome) is not taken into account. If you want to use a different value of fu(mic) including unbound Ki, IC50 or KI in the calculation of DDI predictions, tick the box (2). The value is retrieved from the Drug Library if it is available. If no fu(mic) values are available in the Drug Library, they are estimated using the Hallifax/Houston (default value) or Austin methods as detailed in section 2.1.2.3.1 Microsomal binding.

By default the fu(hep) (fraction unbound in the hepatocyte) is not taken into account. If you want to use a different value of fu(hep) including unbound EC50 in the calculation of DDI predictions for inducers, tick the box (3). The value is retrieved from the Drug Library if it is available. If no fu(hep) values are available in the Drug Library, they are estimated using the Killford et al. (default value) or Austin methods as detailed in section 2.1.2.3.2 Hepatocyte binding.

Important: fu for the blood, fu(mic) and fu(hep) can be managed separately, but for accurate prediction these items should be used together.
### 3.4.3.4 Perpetrator inhibitory constant

The use of perpetrator inhibitory constants is optional. When perpetrator inhibitory constants are provided by the user, they are used by the DDIRC instead of those found in the Drug Library.

**Important**: If the perpetrator selected is not available (i.e., necessary parameters are missing), the AUC ratio will not be calculated.

<table>
<thead>
<tr>
<th>Competitive inhibition</th>
<th>Parameter (µM)</th>
<th>Value</th>
<th>Perpetrator name</th>
<th>Victim C (µM)</th>
<th>Victim Km (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>Ki</td>
<td></td>
<td>select molecule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>IC50</td>
<td>0.4</td>
<td>select molecule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mechanism Based (MBI)</th>
<th>Parameter (µM)</th>
<th>Value</th>
<th>[C]prot (g/l)</th>
<th>Perpetrator name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>kdeg (min⁻¹)</td>
<td>0.0032</td>
<td>1</td>
<td>erythromycin</td>
</tr>
<tr>
<td>Select</td>
<td></td>
<td></td>
<td></td>
<td>select molecule</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Induction</th>
<th>Parameter (µM)</th>
<th>Value</th>
<th>Emax</th>
<th>nh</th>
<th>Induction type</th>
<th>Perpetrator name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>IC50</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>mP450A</td>
<td>rifampicin</td>
</tr>
<tr>
<td>Select</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>select molecule</td>
<td></td>
</tr>
</tbody>
</table>

1. Instead of using Ki or IC50 from the Drug Library for competitive inhibitors, you can enter your own values measured for your drug candidate and perpetrator(s) by selecting the enzyme inhibited, the parameter, and the perpetrator used during the inhibition experiment. Your values then replace those from the Drug Library.

**Note**: Enter the values of Ki, IC50 or KI in µM and k\text{inact} value in min⁻¹.

When IC50 values are entered, the Km of the victim and concentration used during the inhibition experiment can be entered to estimate a Ki based on the equation:

$$\text{Ki} = \frac{\text{IC50}}{2} \times \text{Km}$$

If Km and concentration are not provided, the victim concentration is assume equal to its Km value, therefore: Ki = IC50/2.

2. Enter the protein concentration used in your competitive- and/or mechanism-based inhibition experiments.

**Tip**: By default, a protein concentration of 0.4 g/l for competitive and 1 g/l for mechanism-based inhibition is proposed. If you have selected fu\text{(mic)} then the protein concentration entered is used to estimate the fu\text{(mic)} value.

3. Select the perpetrator name in the list.

**Tip**: Competitive- and mechanism-based inhibition parameters can be entered for the same perpetrator. Several values may also be entered for the same parameter. For example: 2 Ki values and to Ki/K\text{inact} could be entered for erythromycin on CYP3A4.
3.5  Predict DDI: proprietary perpetrator drug

3.5.1  Performing your first prediction

If your proprietary drug is a perpetrator, the victims are automatically selected from the Drug Library based on the enzymes inhibited. Up to 2 perpetrators can be entered. Perpetrator 2 can be used when you have information on the metabolite of your proprietary drug.

By default, perpetrators are administered in repeated doses and the hepatic concentration is estimated using the [][in,max] value not corrected for blood or plasma binding.
3.5.1.1 No metabolism in the gut of victims

1. Enter perpetrator name, molecular weight and dose.
2. Select the dosing regimen of your perpetrator drug: Repeated (selected by default) or Single.
3. Enter absorption properties for your drug candidate: Fraction Absorbed (Fa) and rate constant of absorption (kabs).
   **Tip:** kabs can be estimated assuming a first order absorption model by clicking on the calculator.
4. Select the hepatic perpetrator concentration you wish to use in the calculation and fill in the required parameters to calculate it.
   **Note:** For more details about hepatic perpetrator concentration, click the information point i.
   **Important:** Hepatic perpetrator concentrations are estimated in the total blood using the blood-to-plasma ratio (Rb).
5. Under non-equilibrium conditions, the perpetrator accumulates in the liver due to binding or hepatic uptake. In this case, the liver-to-blood (Kb), liver-to-plasma (Kp) or cell-to-medium (C/M) portion ratio can be taken into account by user.

6. Enter the values for the fraction unbound in plasma (fup) and blood-to-plasma ratio (Rb) of your perpetrator. By default a fup of 1 and an Rb of 0.55 are used.

7. Enter the value for the fraction unbound in the microsome, fu\textsubscript{mic}, and the corresponding protein concentration. If no value is entered, a default fu\textsubscript{mic} value of 1 is used.
   **Tip:** fu\textsubscript{mic} can be estimated by clicking on the calculator.

8. Enter the value for the fraction unbound in the hepatocyte, fu\textsubscript{hep}. If no value is entered, a default fu\textsubscript{hep} value of 1 is used.
   **Tip:** fu\textsubscript{hep} can be estimated by clicking on the calculator.

9. Enter Ki, IC\textsubscript{50} or KI and k\textsubscript{inact} values for perpetrator by selecting parameters and adding the values. Competitive-and mechanism-based inhibition parameters can be simultaneously used for your perpetrator.

When IC\textsubscript{50} values are entered, the Km of the victim and concentration used during the inhibition experiment can be entered to estimate a Ki based on the equation:

\[
Ki = \frac{IC_{50}}{1 + \frac{C}{Km}}
\]

If Km and concentration are not provided, the victim concentration is assume equal to its Km value, therefore: Ki = IC\textsubscript{50}/2.

Select the target subject to inhibition by perpetrator (one target at a time). For a mechanism-based inhibitor, when selecting a target, a default value of k\textsubscript{deg} is proposed but can be modified by the user. The default value used for each enzyme has been taken from peer reviewed publications. Click the information point i for more details about these default values.
3.5.1.1 kabs calculation

kabs can be estimated assuming a first-order absorption model by clicking on Calculate kabs. The kabs calculation box will appear showing the half-life of elimination in hours and $T_{\text{max}}$ in hours. Fill these in and click Calculate. The kabs estimate in min$^{-1}$ is shown. It can be re-calculated at any time. The maximum value is 0.1 min$^{-1}$ corresponding to the gastric emptying rate.
3.5.1.2 Metabolism of victims in the gut

**Use Fg** is selected by default in the intestine inhibition model used to estimate the F'g/Fg ratio and the Qg value is set to 18 l/h.

The Fg is retrieved from the Drug Library. If no value is found, a default value of 1 is taken.

When CYP3A4 and/or CYP3A5 are inhibited by one of the perpetrators, the user can decide to avoid metabolism in the gut for the victims retrieved from the DDIRC knowledgebase.

**Tip:** If **Use Fg** is not ticked, a default value of 1 is used for the calculation, assuming no metabolism in the gut for the victim.

---

**PharmaPendium®**

**Proprietary Perpetrator Drug**

**Perpetrator 1 Perpetrator 2 Victim**

Please select the database retrieval rules for the victim drugs:

- First Pass Metabolism (gut wall)

  1. **Use Fg**
  2. Intestine inhibition: Estimation of F'g/Fg ratio
  3. Maximal Inhibition (Fg/Fg=1/Fg)
  4. Model Predicted
  5. Qg: 18 l/h

[Predict interactions]
3.5.1.3 Dosing regimen and hepatic perpetrator concentration

3.5.1.3.1 Single dose

1. Select the single dosing regimen for the perpetrator.
2. Select the hepatic perpetrator concentration, \([I]_{\text{in}}\), to be used in the calculation. For more details about hepatic perpetrator concentration click i on the interface or go to section 2.1.2.4 Estimated perpetrator concentration in the liver.
   Tip: Cabs should be used for competitive inhibitors and \(C_{\text{max}}\) for inactivators.
3. Enter corresponding values depending on your choice of \([I]_{\text{in}}\), always in ng/ml.
3.5.1.3.2 Repeated dose

Interface with repeated dose and:

1. Select the repeated dosing regimen for the perpetrator.
2. Select the Hepatic Perpetrator concentration, [I]in, to be used in the calculation. For more details about hepatic perpetrator concentration click i on the interface or go to section 2.1.2.4 Estimated perpetrator concentration in the liver. **Tip:** [I]in,avg and [I]in,max should be used for competitive inhibitors, and C_{max} and C_{avg} for inactivators.
3. Enter corresponding values depending on your choice of [I]in, always in ng/ml.
3.5.1.3.3 Perpetrator inhibitory constant

1. Enter your own measured values between your drug candidate and victims(s) by selecting the enzyme inhibited or induced, the parameter, the victim used during the inhibition experiment.

2. Enter the values of $K_i$, $IC_{50}$, $K_I$ or $EC_{50}$ in µM, $k_{inact}$ value in min$^{-1}$, and $E_{max}$ and IndSlope in their units.
   
   **Note:** When $EC_{50}$ is entered, maximal effect ($E_{max}$) and hill slope (nh) are required. When IndSlope ($EC_{50}/E_{max}$) is selected, only the hill coefficient is required.
   
   When $IC_{50}$ values are entered, the Km of the victim and concentration used during the inhibition experiment can be entered to estimate a $K_i$ based on the equation:

   $$K_i = \frac{IC_{50}}{K_m}$$

   If Km and concentration are not provided, the victim concentration is assume equal to its Km value, therefore: $K_i = IC_{50}/2$.
   
   When $K_i/k_{inact}$ is selected, a default value of kdeg from the literature is proposed depending on the enzyme. If there are no values from the literature, a default value of 0.00025 min$^{-1}$ is used (see section 2.1.2.1.2.1 Enzyme turnover).

3. Enter the protein concentration used in your competitive and mechanism-based inhibition experiments.
   
   **Tip:** By default, a protein concentration of 0.4 g/l for competitive and 1 g/l for mechanism-based inhibition is proposed. If you have selected $f_u(mic)$ (microsomal binding), then the protein concentration entered is used to estimate the $f_u(mic)$.

4. Select victim name from the list.
   
   **Tip:** Competitive and mechanism-based inhibition parameters can be entered for the same enzyme with different victims. For example, 2 $K_i$s can be entered for CYP3A4 with midazolam and testosterone as victims (see section 2.1.2.1.1.2 Inhibitory constant and substrate probe selection).

5. When $IC_{50}$ are entered, the Km of the victim and concentration used during the inhibition experiment can be entered to estimate a $K_i$. 

3.5.2 Two perpetrators

DDIRC allows the prediction of interactions involving two perpetrator drugs and one victim; or one perpetrator, its metabolite and one victim, acting on the same or different enzymes. The mechanisms of inhibition for each perpetrator can be different.

3.5.2.1 Setup for two perpetrators

For the two perpetrators, select the dosing regimen, hepatic concentration estimation and mechanism of inhibition. These can be the same or different.

3.5.2.2 One perpetrator and its metabolite

In this case, the dosing regimen must be the same for both the perpetrator and its metabolite. The first pass effect does not exist for the metabolite, so the concentration at the enzyme site is estimated using the systemic hepatic concentration $C_{\text{avg}}$ or $C_{\text{max}}$ if the perpetrator is administered in repeated doses and using only $C_{\text{max}}$ if it is a single dose.

**Important**: If you use Perpetrator 2 for the metabolite, molecular weight, dose, $k_{\text{abs}}$ and $F_{\text{abs}}$ are mandatory fields but will not be used in the calculation.
3.6 Results

3.6.1 Global results

The bar displayed in the DDI table is a color-coded graphical overview of the risk assessment. Color codes represent AUCi/AUC ratio ranges corresponding to the FDA classification (2012 FDA guidance) of CYP inhibitor and inducer potency. The size of each colored segment in the bar represents the percentage of the total number of calculated AUC ratios (for a given victim/perpetrator couple) that falls into one of the following categories:

<table>
<thead>
<tr>
<th>Category</th>
<th>AUC ratio range</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk (induction)</td>
<td>AUC ratio &lt; 0.8</td>
<td></td>
</tr>
<tr>
<td>No risk</td>
<td>0.8 ≤ AUC ratio &lt; 1.25</td>
<td></td>
</tr>
<tr>
<td>Low risk</td>
<td>1.25 ≤ AUC ratio &lt; 2</td>
<td></td>
</tr>
<tr>
<td>Medium risk</td>
<td>2 ≤ AUC ratio &lt; 5</td>
<td></td>
</tr>
<tr>
<td>High risk</td>
<td>5 ≤ AUC ratio</td>
<td></td>
</tr>
</tbody>
</table>

1. Filters for molecules and therapeutic classes
2. Results with links to detailed calculations
3. Help on charts describes the color-coding for risk assessment
4. Export button allows export of calculation results for all drugs
3.6.1.1 Global results: Predict DDI for proprietary victim drug

1. The number of victim–perpetrator couples for which prediction was assessed
2. The Drug Library perpetrator name, therapeutic class and a link to drug information
3. Dose (in g or g/kg) for which predictions were done. When the term Multiple is displayed instead of the dose, bar charts represent an overview of the DDI for all doses. Details by dose can be retrieved by clicking on Multiple (see screenshot below).
4. Color-coded overview of the risk assessment
5. Count of AUC ratios calculated for a victim/perpetrator couple. This also a link to calculation details (parameters, values, conditions).
6. Min., Max., Mean, SD, Med. and 5–95th percentile of AUC ratios. All these statistical items are calculated for a range of doses when the term Multiple is displayed in the dose column or is shown for each dose after clicking on the Multiple link.

Note: Clicking on the column headers (Perpetrator, Dose, Count, etc.) allows you to order the results.

Results by dose display for a victim with multiple doses used for prediction

<table>
<thead>
<tr>
<th>Dose</th>
<th>AUC Ratio</th>
<th>Count</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>Med</th>
<th>5–95th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.018 g</td>
<td></td>
<td></td>
<td>11</td>
<td>1.01</td>
<td>1.20</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01-1.02</td>
</tr>
<tr>
<td>0.026 g</td>
<td></td>
<td></td>
<td>11</td>
<td>1.01</td>
<td>1.23</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01-1.02</td>
</tr>
<tr>
<td>0.034 g</td>
<td></td>
<td></td>
<td>11</td>
<td>1.01</td>
<td>1.20</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01-1.02</td>
</tr>
<tr>
<td>0.012 g</td>
<td></td>
<td></td>
<td>11</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01-1.02</td>
</tr>
</tbody>
</table>
3.6.1.2 Global results: Predict DDI for proprietary perpetrator drug

The results tab is the same than the one described in the previous section. When the drug candidate is a perpetrator, a new tab is provided named **Default results**. This tab contains victims that do not have fm(E) values in the Drug Library but are metabolized by at least one of the inhibited enzymes (victims having clearance or \(V_{\text{max}}\) results on the corresponding enzymes). For the drugs falling into this category, an fm(E) value of 1 is used as default for the calculation. The predictions presented in this tab are qualitative results and should be used as alerts for potential risk of drug–drug interaction (values of AUC ratio are given for information only).
### 3.6.1.3 Detailed chemical information

Click on the ID number just below the name to retrieve detailed chemical information including: name, synonyms, therapeutic class, calculated physiochemical properties, and Lipinski and Veber rules.

<table>
<thead>
<tr>
<th></th>
<th>Drug Type: Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td><strong>Amitriptyline</strong></td>
</tr>
<tr>
<td></td>
<td>4815</td>
</tr>
<tr>
<td></td>
<td>Analgesic: non narcotic</td>
</tr>
<tr>
<td></td>
<td>Antidepressant</td>
</tr>
<tr>
<td></td>
<td>Drug Type: Approved</td>
</tr>
<tr>
<td>7</td>
<td><strong>Atomoxetine</strong></td>
</tr>
<tr>
<td></td>
<td>9737</td>
</tr>
<tr>
<td></td>
<td>Antidepressant</td>
</tr>
<tr>
<td></td>
<td>Psychostimulant</td>
</tr>
<tr>
<td></td>
<td>Devc:</td>
</tr>
</tbody>
</table>
Click on **Show filters** to access three types of filters: Therapeutic class, Molecule and Drug type. Click on **Hide filters** to hide these filter options.

**Therapeutic Classes**: Note that these therapeutics classes may differ in some regards to therapeutic classes which are presented in PharmaPendium due to the fact that the DDIRC was not co-developed with PharmaPendium.

**Molecules**: This presents a list of all of the drugs listed in the Perpetrator column.

**Drug Type**: This shows various drug classifications associated with drugs. Note that these classifications are in the process of being updated, so use with caution. They may not align with PharmaPendium drug statuses. Currently, **Approved** refers to approval for use on the U.S. drug market; **Experimental/Investigation** refers to drugs in developmental phases I, II or III. **Unspecified** means no status is available; and **Withdrawn** means withdrawn from the U.S. drug market.
3.6.2 Accessing detailed results

1. Click on the Count for the AUC Ratio of the victim–perpetrator couple.

2. This provides access to all the parameter values used in the calculation of each AUC ratio.

3. Each link provides additional information available with a click or by mousing over the link.
   - Clicking on parameters gives the number of values used in the calculation and their source(s).
   - Clicking on values opens protocol details.
   - Clicking on plus signs gives details pertaining to the values if they were estimated based on other values from the knowledge database.
   - Clicking on the drug name reveals detailed chemical information.

Example 1: Information available when Cmax (µM) header is clicked

[Image showing the DDI Results details table and a message box indicating 45 value(s) found]

Using value(s) found in database
Example 2. Information available when Cmax (µM) value is clicked

Example 3. Information available when the + beside kabs (min-1) is clicked

Example 4. Information available when Drug Name is clicked
3.7 Exporting

3.7.1 Overview of Excel file

1. Click on Export to open the option to export the data as an Excel document (.xls).

Note: If the drug is a perpetrator, the option to include default results will be given. Tick the box to include them.

The downloaded Excel file contains all of the information with color coding using the same key as the DDIRC interface. The tabs are explained below.

1. **Overview**: Color-coded results overview
2. **Input Parameters**: A summary of the input parameters used for predicting DDIs
3. **Therap. Classes (All Doses)**: A graph displaying the potential co-medications between the NCE and drugs on the market based on therapeutic classes.
4. **Therap. Classes (Max Dose)**: A graph displaying the potential co-medications between the NCE and drugs on the market based on therapeutic classes and maximum dose.
5. **Drugs**: Graphs showing AUC ratio of drugs for each therapeutic class involved in the predicted DDIs.
3.7.2 Input Parameters tab

3.7.3 Therapeutic Classes (All Doses) tab – victim drug

These graphs provide an overview of the DDI risk by therapeutic class. Counts are related to the total amount of predicted AUCi/AUC ratio falling into the defined categories. AUC ratios are predicted for a perpetrator at dose(s) retrieved from the database.

**Risk per Therapeutic Classes (Inhibitors)**

Therapeutic Classes

- Risk (Induction)
- No Risk
- Low Risk
- Medium Risk
- High Risk

Graph for a victim drug
3.7.4 Therapeutic Classes (Max Dose) tab – victim drug

Risk per Therapeutic Classes (Inhibitors Max Dose)

[Graph showing risk distribution across different therapeutic classes]

Therapeutic Classes
- Risk (Induction)
- No Risk
- Low Risk
- Medium Risk
- High Risk

Graph for a victim drug
3.7.5 Therapeutic Classes (All Doses) tab – perpetrator drug

Risk per Therapeutic Classes (Inhibitors)

<table>
<thead>
<tr>
<th>Therapeutic Classes</th>
<th>Risk (Induction)</th>
<th>No Risk</th>
<th>Low Risk</th>
<th>Medium Risk</th>
<th>High Risk</th>
</tr>
</thead>
</table>

Graph for a perpetrator drug

3.7.6 Drugs tab

The Drugs tab contains graphs showing the AUC ratios of drugs for each therapeutic class involved in the predicted DDIs.

When the proprietary drug is a victim, the graph contains perpetrators and doses (g) included in each therapeutic class. When the proprietary drug is a perpetrator, the graph contains victims included in each therapeutic class.

Graph for a victim

Graph for a perpetrator
3.8 Saved and open queries

At the moment, we do not support saved searches and alerts in PharmaPendium. However, should a user perform a search and within the same browser session want to access a search that they recently conducted then they may do so. The saved search functionality will be developed in the near future.

Click on **Save Query**. A pop-up will appear enabling users to save their query by name and include any notes about the query.

**Note:** Once the browser session is closed, the saved queries are no longer available. These saved queries are only available as long as the browser remains open.
4 Glossary

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Details</th>
</tr>
</thead>
</table>
| fm(E) | fm(E): fraction of total clearance mediated by enzyme of interest (E)  
If necessary, fm(E) values are selected when available from the Drug library. |
| fm(E)_vitro | fm(E)_vitro: fraction of hepatic metabolic clearance mediated by enzyme of interest (E)  
fm(E)_vitro values are selected when available from the Drug library.  
When fm(E)_vitro values are not available, a default value of 1 is used assuming complete metabolism by the enzyme of interest.  
fm(E)_vitro values could be estimated using recombinant metabolism experiment based on the following equation using abundance:  
\[
fm(E)_{vitro} = \frac{\sum_{k=1}^{j} Clint_{u_k}(E) \times SF_k}{\sum_{k=1}^{j} Clint_{u_k}(E) \times SF_k}
\]  
where there are j CYPs with corresponding Clint(k(E)) values calculated from enzyme kinetic parameters for different pathways k in each recombinant system and where SF_k was a scaling factor corresponding to the pathway k and enzyme j. The scaling factor could be abundance, RAF or ISEF. |
| RAF | RAF: relative activity factor  
RAF is used as a direct scaler to convert data obtained with a recombinant enzyme system to an HLM one.  
\[
RAF = \frac{Clint_{u_l}(HLM)}{Clint_{u_l}(rE)}
\]  
where Clint_{u_l} (HLM) refer to metabolism of a probe substrate by the l^th enzyme in the liver and Clint_{u_l} (rE) refers to metabolism of the same probe substrate by recombinant enzyme. The units of Clint_{u_l}(HLM) and Clint_{u_l}(rE) are µl/min/mg protein and µl/min/pmol enzyme, respectively. Thus, RAF units are pmol enzyme/mg protein. For a publication on RAF, see Crespi C. et al. (1995). |

**RAF Clint Calculation**

The median of RAF in relation to recombinant expression system and enzymes was determined from a literature analysis. Only data on preferred substrate (defined by the 2012 FDA guidance) were included, and sources were verified to exclude duplication of individual data in the analysis.

Data sets for enzyme kinetic data (Km and V_{max}) were compiled for rhCYP systems (111 sets) and HLM (109 sets). The recombinant systems considered were based on B-lymphoblastoid cells, baculovirus-transformed insect cells, Escherichia coli cells and Saccharomyces cerevisiae cells.

<table>
<thead>
<tr>
<th>rhCYP</th>
<th>Baculo (n)</th>
<th>E. coli (n)</th>
<th>Lympho (n)</th>
<th>Yeast (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>43.75 (4)</td>
<td>528.61 (1)</td>
<td>435.61 (4)</td>
<td>1221.19 (2)</td>
</tr>
<tr>
<td>2A6</td>
<td>&lt;</td>
<td>71.15 (3)</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td>2B6</td>
<td>37.94 (2)</td>
<td>&lt;</td>
<td>62.22 (1)</td>
<td>X</td>
</tr>
<tr>
<td>2C8</td>
<td>14.55 (2)</td>
<td>26.45 (2)</td>
<td>&lt;</td>
<td>45.12 (1)</td>
</tr>
<tr>
<td>2C9</td>
<td>60.13 (9)</td>
<td>101.21 (4)</td>
<td>47.67 (11)</td>
<td>59.13 (11)</td>
</tr>
<tr>
<td>2C19</td>
<td>3.93 (5)</td>
<td>3.66 (2)</td>
<td>13.2 (2)</td>
<td>17.52 (2)</td>
</tr>
<tr>
<td>2D6</td>
<td>7.77 (16)</td>
<td>17.62 (3)</td>
<td>16.82 (4)</td>
<td>81.2 (4)</td>
</tr>
<tr>
<td>2E1</td>
<td>496.68 (2)</td>
<td>110.43 (1)</td>
<td>551.39 (2)</td>
<td>1956.96 (1)</td>
</tr>
<tr>
<td>3A4</td>
<td>55.32 (11)</td>
<td>147.1 (8)</td>
<td>266.81 (7)</td>
<td>609.78 (2)</td>
</tr>
</tbody>
</table>
ISEF: inter-system extrapolation factor

ISEF is a dimensionless number used as a direct scaler to convert data obtained with a recombinant enzyme system to an HLM one.

\[
\text{ISEF} = \frac{\text{Clint,u}(\text{HLM})}{\text{Clint,u}(\text{E}) \times \text{E abundance}(\text{HLM})}
\]

where \( E_j \) abundance (HLM) refers to the abundance of the \( j \)th enzyme in the liver and Clint,u refers to metabolism of a probe substrate by an individual enzyme. The units of Clint,u(HLM), Clint,u(E) and abundance are \( \mu l/min/mg \) protein, \( \mu l/min/pmol \) enzyme and pmol enzyme/mg protein, respectively. Thus ISEF is a dimensionless number.

**ISEF Clint Calculation**

The median of ISEF in relation to recombinant expression system and enzymes was determined from a literature analysis. Only data on preferred substrate (defined by the 2012 FDA guidance) were included, and sources were verified to exclude duplication of individual data in the analysis.

Data sets for enzyme kinetic data (Km and \( V_{\text{max}} \)) were compiled for rhCYP systems (111 sets) and HLM (109 sets). The recombinant systems considered were based on B-lymphoblastoid cells, baculovirus-transformed insect cells, *Escherichia coli* cells and *Saccharomyces cerevisiae* cells.

<table>
<thead>
<tr>
<th>rhCYP</th>
<th>Baculo (n)</th>
<th>E. coli (n)</th>
<th>Lympho (n)</th>
<th>Yeast (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>0.9 (4)</td>
<td>10.83 (1)</td>
<td>8.93 (4)</td>
<td>25.02 (2)</td>
</tr>
<tr>
<td>2A6</td>
<td>X</td>
<td>3.31 (3)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2B6</td>
<td>2.6 (2)</td>
<td>X</td>
<td>4.26 (1)</td>
<td>X</td>
</tr>
<tr>
<td>2C8</td>
<td>0.94 (2)</td>
<td>1.71 (2)</td>
<td>X</td>
<td>2.91 (1)</td>
</tr>
<tr>
<td>2C9</td>
<td>0.86 (9)</td>
<td>1.47 (4)</td>
<td>0.68 (1)</td>
<td>0.85 (11)</td>
</tr>
<tr>
<td>2C19</td>
<td>0.25 (5)</td>
<td>0.24 (2)</td>
<td>0.86 (2)</td>
<td>1.14 (2)</td>
</tr>
<tr>
<td>2D6</td>
<td>0.82 (16)</td>
<td>1.86 (3)</td>
<td>1.77 (4)</td>
<td>8.55 (4)</td>
</tr>
<tr>
<td>2E1</td>
<td>6.68 (2)</td>
<td>1.49 (1)</td>
<td>7.42 (2)</td>
<td>26.34 (1)</td>
</tr>
<tr>
<td>3A4</td>
<td>0.32 (11)</td>
<td>0.85 (8)</td>
<td>1.54 (7)</td>
<td>3.62 (2)</td>
</tr>
</tbody>
</table>

Abundance

Abundances of enzymes in the liver (pmol/mg prot) were determined from a literature analysis of 18 separate studies based on 315 live rs using weighted means. Only data from Caucasian adults (over 16 years old) were included, and sources were verified to exclude duplication of individual data in the analysis.

**Publications used:**

- Stresser (1999)

fh: fraction of hepatic metabolic clearance (Cl_{H}) to total clearance (Cl)

When Cl_{H} or Cl_{R} and Cl are available from the Drug Library, fh is estimated as: \( fh = \frac{Cl_{H}}{Cl} = \frac{Cl_{R}}{Cl} \)

When fh values are not available, a default value of 1 is used, assuming total clearance by metabolism in the liver as detailed in Ito, et al. (1998)
Cl and Cl<sub>H</sub> Total clearance was estimated according to:

\[ Cl = Cl_H + Cl_{non\ hepatic} \]

When the hepatic clearance (Cl<sub>H</sub>) is not available for the victim, the user has the opportunity to estimate it using the well-stirred model or according to the scaling factor method chosen previously for the prediction of the fm(E) <sub>vitr</sub> (RAF, ISEF, Abundance, HLM; Proctor, et al., 2004).

**Well-stirred model**

RAF

\[ Cl_H = \frac{Q_h \times \text{fu} \times Cl_{H, int}}{Q_h + \text{fu} \times Cl_{H, int}} \]

ISEF

\[ Cl_{H, int} = \sum_{i=1}^{n} \left( \sum_{k=1}^{p} \frac{Clint_k(rE_k)}{\text{fu(mic)}} \times RAF_k \right) \times MPPGL \times \text{Liver weight} \]

Abundance

\[ Cl_{H, int} = \sum_{i=1}^{n} \left( \sum_{k=1}^{p} \frac{Clint_k(rE_k)}{\text{fu(mic)}} \times E_i \text{abundance(HLM)} \right) \times MPPGL \times \text{Liver weight} \]

HLM

\[ Cl_{H, int} = \sum_{i=1}^{n} \left( \sum_{k=1}^{p} \frac{Clint_k(HLE_k)}{\text{fu(mic)}} \times E_i \text{abundance(HLM)} \right) \times MPPGL \times \text{Liver weight} \]

MPPGL stands for milligrams of microsomal proteins per gram of liver, taken to be 32 mg/g (Barter, et al., 2008) for a liver weight of 1611 g.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Qg</td>
<td>Qg represents the gut blood flow. For DDIRC, Qent (enterocytic blood flow) with a value of 18 l/h was chosen as the relevant intestinal blood flow because cytochromes tend to locate at the villous tips.</td>
</tr>
<tr>
<td>Fg</td>
<td>Fg: fraction of victim dose escaping metabolism within the walls of the gastrointestinal tract (intestinal wall availability)</td>
</tr>
<tr>
<td></td>
<td>Fg prediction uses the well-stirred gut model adapted from the well-stirred hepatic model.</td>
</tr>
<tr>
<td></td>
<td>[ F_g = \frac{Q_g}{Q_g + \text{fu,g} \times Cl_{int,g}} ]</td>
</tr>
<tr>
<td></td>
<td>where Qg is gut blood flow, fu,g is the fraction of drug unbound in the enterocyte, and Clint,g is the net intrinsic metabolic clearance in the gut based on unbound drug concentration. If gut metabolism is mainly due to CYP3A4 and/or 3A5, intrinsic metabolic clearance in the gut will be:</td>
</tr>
<tr>
<td></td>
<td>[ Cl_{int,g} = Cl_{int,g}(3A4) + Cl_{int,g}(3A5) ]</td>
</tr>
<tr>
<td></td>
<td>Assuming that intrinsic metabolic clearance in the gut and in the liver are the same when expressed per pmol of enzyme, the net intrinsic metabolic clearance in the gut can be estimated thus:</td>
</tr>
<tr>
<td></td>
<td>[ Cl_{int,g} = \left( Cl_{int(CYP3A4)} \times \text{Abund.}(3A4),g \right) + Cl_{int(CYP3A5)} \times \text{Abund.}(3A5),g ]</td>
</tr>
</tbody>
</table>
### Fg/Fg

The model-predicted Fg ratio was obtained from the decrease in the intestinal intrinsic clearance in the presence of an inhibitor ($C_{\text{int},g}$) using the in vitro inhibitory constant (competitive or mechanism-based) and the estimated inhibitor concentration in the intestinal wall during absorption ($\{I\}_u,g$).

$$
\frac{F_g}{F_g} = \frac{1}{f_r^g + \frac{C_{\text{int},g}}{C_{\text{int},g}}}
$$

where $f_m,k$ is the fraction metabolized by the enzyme k in the gut wall, and $C_{\text{int},k}$ and $M_{\text{int},k}$ are the competitive and mechanism-based inhibition components, respectively.

Assuming that the perpetrator and victim were given at the same time, the concentration in the intestinal wall during the absorption phase is:

$$
[I]_{u,g} = \frac{F_{\text{abs}} \times \mathrm{kabs} \times \mathrm{D}}{Q_g}
$$

### kdeg (gut)

$k_{\text{deg}}$ for the gut was determined using grapefruit juice in a single dose as described in Greenblatt, et al. (2003). For CYP3A4 it is 62 nmol and for 3A5 it is 23 nmol.

### fu

$fu$: fraction unbound in the plasma or serum

When available from the Drug Library, $fu$ values are used with the priority order: human > other species (mouse, rat, rabbit, dog, monkey). When they are not available, a default value of 1 is assumed. They are used with $Rb$ values to calculate the fraction unbound in the whole blood: $fb = [(1-H) \times fu]/Rb$ (Howgate et al., 2006).

They are also used to determine unbound perpetrator concentration in the whole blood: $[I, u] = fb \times [I]$.

### Rb

$Rb$: blood-to-plasma ratio

When available from the Drug Library, $Rb$ values are used with the priority order: human > other species (mouse, rat, rabbit, dog, monkey). When they are not available, a default value of 0.55 is assumed. They are used with $fu$ values to calculate the fraction unbound in the whole blood: $fb = [(1-H) \times fu]/Rb$ (Howgate et al., 2006).

### fu(mic)

$fu_{\text{mic}}$: fraction unbound in the microsomes, which is used to convert $K_i$, $K_I$ and $IC_{50}$ values into corresponding unbound parameters

When available from the Drug Library, $fu_{\text{mic}}$ values are used with the priority order: human > other species (mouse, rat, rabbit, dog, monkey) and if necessary, estimated at the microsomal protein concentration used in inhibition experiments according to:

$$
fu_{\text{mic}(i)} = \frac{1}{C_2 \times \left(\frac{fu_{\text{mic}(i)}}{fu_{\text{mic}(1)}}\right) + 1}
$$

where $C_1$ is the microsomal protein concentration (g/L) used in the $fu_{\text{mic}(1)}$ experiment, $C_2$ is the microsomal protein concentration (g/L) used in the inhibition experiment, $fu_{\text{mic}(1)}$ refers to the free fraction coming from database, and $fu_{\text{mic}(2)}$ refers to the estimated free fraction at $C_2$.

When the microsomal protein concentration used in the inhibition experiment is not available, a default protein concentration ($C_2$) is assumed with the following rules:

1. Competitive inhibition ($K_i$ et $IC_{50}$)
   - HLM: $C_{\text{prot}} = 0.4$ g/l
   - Recombinant: $C_{\text{prot}} = 0.2$ g/l
2. Mechanism-based inhibition ($K_I$)
   - HLM or recombinant $C_{\text{prot}} = 1$ g/l

Further reading on the impact of in vitro binding on DDI prediction: Austin, et al. (2006); Grime & Riley (2006); and Obach (1997).

### fu(hep)


### fu(hyp)

The unbound fraction in hepatocytes is used to convert $EC_{50}$ values from induction assays into corresponding unbound parameters.

Optimized [D] automatically selects the concentration of perpetrator to use in the calculation of DDIs based on the perpetrator mechanism of action based on the rules:

<table>
<thead>
<tr>
<th>Concentration used (parameter)</th>
<th>Reversible inhibition</th>
<th>MBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic inlet ([l]in,max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic (C\text{max})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rationale

DDIs occur during the first pass exposure

DDIs continue after first pass exposure

\( C_{\text{max}} \): The maximal systemic concentration in the plasma

\( C_{\text{max}} \) and \( R_b \) values are used to calculate maximal systemic concentration in the blood \([l]_{\text{max}}\) using:

\[
[l]_{\text{max}} = R_b \times C_{\text{max}}
\]

\( C_{\text{avg}} \): The average systemic concentration in the plasma

\( C_{\text{avg}} \) and \( R_b \) values are used to calculate average systemic concentration in the blood \([l]_{\text{avg}}\) using:

\[
[l]_{\text{avg}} = R_b \times C_{\text{avg}}
\]

\( [l]_{\text{in,max}} \): The maximal systemic concentration in the blood entering into the liver

\[
[l]_{\text{in,max}} = \frac{F_{\text{abs}} \times k_{abs} \times D}{Q_h}
\]

When \( k_{abs} \) values are available from the Drug Library, they are used for \([l]_{\text{in,vivo}}\) determination and if necessary, estimated, assuming a first-order absorption rate, using the equation:

\[
T_{\text{max}}(h) = \frac{\ln\left(\frac{k_{abs}}{k_{el}}\right)}{k_{el}}
\]

where \( T_{\text{max}}(h) \) is the time to reach the maximum concentration and \( k_{el}(h^{-1}) \) is the elimination rate constant coming from the same experiment. When \( k_{abs} \) values are not available, a default value of 0.1 min\(^{-1}\) is assumed, corresponding to gastric emptying (Ito, et al., 1998).

\( [l]_{\text{in,avg}} \): The average systemic concentration in the blood entering into the liver

When \( k_{abs} \) values are available from the Drug Library, they are used for \([l]_{\text{in,vivo}}\) determination and if necessary, estimated, assuming a first-order absorption rate, using the equation:

\[
T_{\text{max}}(h) = \frac{\ln\left(\frac{k_{abs}}{k_{el}}\right)}{k_{el}}
\]

where \( T_{\text{max}}(h) \) is the time to reach the maximum concentration and \( k_{el}(h^{-1}) \) is the elimination rate constant coming from the same experiment. When \( k_{abs} \) values are not available, a default value of 0.1 min\(^{-1}\) is assumed, corresponding to gastric emptying (Ito, et al., 1998).

\( C_{\text{abs}} \): concentration in the blood coming only from the contribution of absorption (single dose)

\[
C_{\text{abs}} = \frac{k_{abs} \times F_{\text{abs}} \times F_s \times D}{Q_h}
\]

When \( k_{abs} \) values are available from the Drug Library, they are used for \([l]_{\text{in,vivo}}\) determination and if necessary, estimated, assuming a first-order absorption rate, using the equation:

\[
T_{\text{max}}(h) = \frac{\ln\left(\frac{k_{abs}}{k_{el}}\right)}{k_{el}}
\]

where \( T_{\text{max}}(h) \) is the time to reach the maximum concentration and \( k_{el}(h^{-1}) \) is the elimination rate constant coming from the same experiment. When \( k_{abs} \) values are not available, a default value of 0.1 min\(^{-1}\) is assumed, corresponding to gastric emptying (Ito, et al., 1998).

Estimated liver concentration

Different estimations of perpetrator concentration in the liver \([l]_{\text{in,vivo}}\) can be used to predict DDIs under equilibrium conditions (no hepatic uptake) and non-equilibrium conditions (hepatic uptake).

Under equilibrium:
- Average systemic concentration in the blood: \([l]_{\text{equilibrium}} = [l]_{\text{avg}} = R_b \times C_{\text{avg}}\)
- Maximal systemic concentration in the blood: \([l]_{\text{equilibrium}} = [l]_{\text{max}} = R_b \times C_{\text{max}}\)
- Maximal systemic concentration in the blood entering the liver: \([l]_{\text{equilibrium}} = [l]_{\text{in,max}}\)
- Average systemic concentration in the blood entering the liver: \([l]_{\text{equilibrium}} = [l]_{\text{in,avg}}\)
- Concentration in blood only coming from contribution of absorption (single dose): \([l]_{\text{equilibrium}} = C_{\text{abs}}\)

Under non-equilibrium conditions, perpetrator concentration in the liver is estimated using equilibrium conditions and liver-to-blood ratio \( (K_b) \), liver-to-plasma ratio \( (K_p) \) or cell-to-medium ratio \( (C/M) \).
**k_{abs}**

**k_{abs}**: rate constant of absorption (h⁻¹) in humans

When kabs values are available from the Drug Library, they are used for [I]b\_in\_vivo determination and if necessary, estimated, assuming a first-order absorption rate, using the equation:

\[
\text{Tmax} = \frac{\ln \left( \frac{k_{abs}}{k_{el}} \right)}{k_{abs} - k_{el}} 
\]

where Tmax (h) is the time to reach the maximum concentration and kel (h⁻¹) is the elimination rate constant coming from the same experiment. When kabs values are not available, a default value of 0.1 min⁻¹ is assumed, corresponding to gastric emptying (Ito, et al., 1998).

**Fa, Fg**

**Fa, Fg**: fraction of perpetrator dose entering into the portal vein after absorption and metabolism within the walls of the gastrointestinal tract in humans

When Fa, Fg values are available from Drug library, they are used for Cabs determination and if necessary estimated, using Fa and Fg values or according to equation:

\[
\text{Fa,Fg} = \frac{F}{F_{h}}
\]

where F is the total bioavailability and Fh hepatic availability.

When Fh values are not available, a calculation is made using the equation:

\[
\text{Fa,Fg} = \frac{F - \text{CL}_{H}}{1 - \text{CL}_{H}}
\]

and assuming no clearance other than CL_{H} and CL_{L}.

When Fa, Fg values are not available, a default value of 1 is assumed corresponding to entire absorption and no metabolism in gut wall for perpetrator drugs.

**Fh**

**Fh**: fraction of perpetrator dose escaping metabolism within the liver (hepatic availability) in humans

When Fh values are available from the Drug Library, they are used for Fa, Fg estimation and if necessary, estimated with CL_{H} or CL_{L} and ClH:

\[
\text{Fa,Fg} = \frac{F - \text{CL}_{H}}{1 - \text{CL}_{H}}
\]

and assuming no clearance other than CL_{H} and CL_{L}.

When Fh values are not available, a default value of 1 is assumed.

**Kp**

**Kp**: liver-to-plasma concentration ratio

When available from the Drug Library, Kp values are used with the priority order: human > other species (mouse, rat, rabbit, dog, monkey). When they are not available, a default value of 1 is assumed. They are used with Rb values to calculate liver-to-blood concentration ratio: Kb = Kp/Rb

**Kb**

**Kb**: liver-to-blood concentration ratio

When available from the Drug Library, Kb values are used with the priority order: human > other species (mouse, rat, rabbit, dog, monkey). When they are not available, a default value of 1 is assumed. They are used with Rb values to calculate liver-to-blood concentration ratio: Kb = Kp/Rb

**C/M**

**C/M**: cell-to-medium concentration ratio

C/M is an in vitro estimation of in vivo hepatic uptake (Kb unbound) and is used to predict perpetrator concentration in the liver (hepatic uptake).

\[
[I]_{\text{equilibrium}} = \frac{[I]_{\text{b}}}{C/M} \times [I]_{b} \times fb
\]

where [I]_{b} is the perpetrator concentration in the whole blood and fb is the fraction unbound in whole blood (Yamano, et al., 1999).

**k_{deg}**

**k_{deg}** (liver)

The values for k(deg) for these enzymes in the liver were established with sources listed. For all other enzymes involved in MBI, a default value of 0.00025 min⁻¹ is used.

- CYP1A2: in vivo, smokers (Faber & Fuhr, 2004)
- CYP2A6, 2B6, 2C8, 2C9, 2C19: in vitro, slices (Renwick, et al., 2000)
- CYP2E1: in vivo, disulfiram (Emery, et al., 1999)
- CYP3A4: in vivo, Rifampin (Fromm, et al., 2000)
- CYP3A5 in vitro, slices (Renwick, et al., 2000)
5 References


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