
In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies Guidance for Industry

DRAFT GUIDANCE

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For questions regarding this draft document, contact (CDER) Office of Clinical Pharmacology, Guidance and Policy Team at CDER_OCP_GPT@fda.hhs.gov.

**U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)**

**October 2017
Clinical Pharmacology**

In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies Guidance for Industry

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1 **In Vitro Metabolism- and Transporter-Mediated Drug-Drug**
2 **Interaction Studies**
3 **Guidance for Industry¹**
4

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6 This draft guidance, when finalized, will represent the current thinking of the Food and Drug
7 Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not
8 binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the
9 applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible
10 for this guidance as listed on the title page.
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14
15 **I. INTRODUCTION**
16

17 This guidance is intended to help drug developers plan and evaluate studies to determine the
18 drug-drug interaction (DDI) potential of an investigational drug product.² It focuses on in vitro
19 experimental approaches to evaluate the interaction potential between investigational drugs that
20 involves metabolizing enzymes and/or transporters. This guidance also discusses how in vitro
21 results can inform future clinical DDI studies. The appendices of this guidance include
22 considerations when choosing in vitro experimental systems, key issues regarding in vitro
23 experimental conditions, and more detailed explanations regarding model-based DDI prediction
24 strategies. See section VIII for a list of terms used in this guidance and their definitions.
25

26 If an in vitro assessment suggests that the sponsor should conduct a clinical DDI study, the
27 sponsor should refer to a related guidance addressing the conduct and interpretation of clinical
28 drug interaction studies (draft guidance for industry entitled *Clinical Drug Interaction Studies —*
29 *Study Design, Data Analysis, and Clinical Implications*).³ Together, these two guidances
30 describe a systematic, risk-based approach to assessing the DDI potential of investigational drugs
31 and making recommendations to mitigate DDIs and will replace the 2012 draft guidance entitled

¹ This guidance has been prepared by the Office of Clinical Pharmacology, Office of Translational Sciences in the Center for Drug Evaluation and Research at the Food and Drug Administration.

² Only small molecule drugs are covered in this guidance. Interactions involving biologics (therapeutic proteins) are beyond the scope of this guidance.

³ When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA Drugs guidance Web page at <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.

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32 *Drug Interaction Studies – Study Design, Data analysis, Implications for Dosing, and Labeling*
33 *Recommendations.*

34
35 In general, FDA’s guidance documents do not establish legally enforceable responsibilities.
36 Instead, guidances describe the Agency’s current thinking on a topic and should be viewed only
37 as recommendations, unless specific regulatory or statutory requirements are cited. The use of
38 the word *should* in Agency guidances means that something is suggested or recommended, but
39 not required.

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41

42 **II. BACKGROUND**

43

44 Evaluating the DDI potential of an investigational new drug involves: (1) identifying the
45 principal routes of the drug’s elimination; (2) estimating the contribution of enzymes and
46 transporters to the drug’s disposition; and (3) characterizing the effect of the drug on enzymes
47 and transporters. This evaluation often starts with in vitro experiments to identify potential
48 factors influencing drug disposition to elucidate potential DDI mechanisms and to yield kinetic
49 parameters for use in further studies. Results of in vitro experiments, along with clinical
50 pharmacokinetic (PK) data, provide mechanistic information that can inform the need and proper
51 design of potential future clinical studies. Various modeling approaches can translate in vitro
52 observations into in vivo predictions of potential clinical DDIs. For example, when evaluating
53 the drug as a perpetrator (i.e., an inhibitor or inducer) of a metabolism-mediated DDI, basic
54 models (Einolf 2007; Einolf, Chen, et al. 2014; Vieira, Kirby, et al. 2014), static mechanistic
55 models (Einolf 2007; Fahmi, Hurst, et al. 2009; Einolf, Chen, et al. 2014), or dynamic
56 mechanistic models including physiologically-based pharmacokinetic (PBPK) models (Zhao,
57 Zhang, et al. 2011; Zhao, Rowland, et al. 2012; Jones, Chen, et al. 2015; Wagner, Zhao, et al.
58 2015; FDA draft guidance for industry *Physiologically Based Pharmacokinetic Analyses —*
59 *Format and Content*⁴) can guide decisions on when and how to conduct a clinical DDI study.
60 This guidance outlines a general framework for conducting in vitro experiments and interpreting
61 in vitro study results to determine the potential for clinical DDIs.

62

63 The recommendations in this guidance are based on current scientific understanding. The
64 recommendations outlined here may be periodically updated as the scientific field of DDIs
65 evolves and matures. Refer to the appendices for general considerations regarding in vitro
66 systems for drug development and regulatory purposes.

67

68

69 **III. EVALUATING METABOLISM-MEDIATED DRUG INTERACTIONS**

70

71 Many drugs undergo metabolism as a major mechanism of bioactivation (e.g., in the case of

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72 prodrugs) or clearance from the body. Drugs can be metabolized in several organs, including,
73 but not limited to, the liver, kidney, gut wall, and lung. Drug metabolism primarily occurs in the
74 liver and intestine. These organs express a wide variety of drug metabolizing enzymes and are
75 responsible for the biotransformation of many drugs. Hepatic metabolism occurs primarily
76 through the cytochrome P450 (CYP) family of enzymes located in the hepatic endoplasmic
77 reticulum but can also occur through non-CYP enzymes, including Phase II glucuronosyl- and
78 sulfo-transferases. Sponsors should examine the potential for interactions between these
79 metabolizing enzymes and investigational drugs. Although certain clinical PK information is
80 necessary to quantify the potential for interactions between metabolizing enzymes and
81 investigational drugs, sponsors should initiate in vitro metabolic studies before first-in-human
82 studies to inform the need and design of these clinical studies. We recommend that the sponsor
83 conducts the following in vitro studies to evaluate the potential for metabolism-mediated drug
84 interactions.

A. Determining if the Investigational Drug is a Substrate of Metabolizing Enzymes

1. Conducting In Vitro Studies

91 The sponsor should routinely evaluate CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19,
92 CYP2D6, and CYP3A4/5 using in vitro phenotyping experiments to determine which enzymes
93 metabolize the investigational drug. However, it is possible that the investigational drug
94 undergoes significant in vivo metabolism that is not mediated by these major CYP enzymes. In
95 this event, the investigational drug is probably a substrate for additional enzymes, and the
96 sponsor should determine what additional enzymes contribute to the metabolism of the
97 investigational drug. These additional enzymes include but are not limited to:

- 98 • CYP enzymes including CYP2A6, CYP2J2, CYP4F2, and CYP2E1
- 99 • Other Phase I enzymes including monoamine oxidase (MAO), flavin monooxygenase
100 (FMO), xanthine oxidase (XO), and alcohol/aldehyde dehydrogenase
- 101 • Phase II enzymes including UDP glucuronosyl transferases (UGTs)
- 102
- 103
- 104

2. Data Analysis and Interpretation

105
106
107
108 The contribution of a specific metabolizing enzyme to an investigational drug's clearance is
109 considered significant if the enzyme is responsible for $\geq 25\%$ of the drug's elimination based on
110 the in vitro phenotyping studies and human PK data. Under these circumstances, the sponsor
111 should conduct clinical DDI studies using strong index inhibitors and/or inducers of the enzyme
112 (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study*

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113 *Design, Data Analysis, and Clinical Implications*⁵). Refer to the appendix, section VII.A.1, for
114 additional considerations regarding data analysis.

115

116 **B. Determining if the Investigational Drug is an Inhibitor of Metabolizing** 117 **Enzymes**

118

119 1. *Conducting In Vitro Studies*

120

121 The sponsor should evaluate an investigational drug's potential to inhibit CYP1A2, CYP2B6,
122 CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 in both a reversible manner (i.e.,
123 reversible inhibition) and time-dependent manner (i.e., time-dependent inhibition (TDI)).

124

125 2. *Data Analysis and Interpretation*

126

127 For basic models of reversible inhibition, the sponsor should calculate the predicted ratio of the
128 area under the plasma concentration-time curve (AUC) for the victim (substrate) drug in the
129 presence and absence of an inhibitor. This ratio is referred to as R_1 (see the appendix, section
130 VII.C1). For CYP3A, $R_{1,gut}$ should also be calculated as shown in Figure 1.

131

132 **Figure 1: Equations to Calculate the Predicted Ratio of Victim Drug's AUC in the** 133 **Presence and Absence of an Inhibitor for Basic Models of Reversible Inhibition**

134

$$135 R_1 = 1 + (I_{max,u} / K_i)$$

136

$$137 R_{1,gut} = 1 + (I_{gut} / K_i)$$

138

139 R_1 or $R_{1,gut}$ is the predicted ratio of the victim drug's AUC in the presence and absence of an inhibitor for basic
140 models of reversible inhibition.

141 $I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug.*

142 I_{gut} is the intestinal luminal concentration of the interacting drug calculated as the dose/250 mL.

143 K_i is the unbound inhibition constant determined in vitro.

144

145 Note: I and K_i need to be expressed in the same unit (e.g., in a molar concentration unit).

146

147 *Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1%
148 (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

149

150

151 For basic models of TDI, the sponsor should calculate the predicted ratio of the victim drug AUC
152 in the presence and absence of an inhibitor (R_2) as described in Figure 2.

153

154

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Figure 2: Equations to Calculate the Predicted Ratio of the Victim Drug's AUC in the Presence and Absence of an Inhibitor for Basic Models of TDI (Yang, Liao, et al. 2008; Grimm, Einolf, et al. 2009; Vieira, Kirby, et al. 2014)

$$R_2 = (k_{obs} + k_{deg}) / k_{deg}$$

$$\text{Where } k_{obs} = (k_{inact} \times 50 \times I_{max,u}) / (K_I + 50 \times I_{max,u})$$

R_2 is the predicted ratio of the victim drug's AUC in the presence and absence of an inhibitor for basic models of enzyme TDI.

k_{obs} is the observed (apparent first order) inactivation rate constant of the affected enzyme.

k_{deg} is the apparent first-order degradation rate constant of the affected enzyme.

K_I is the inhibitor concentration causing half-maximal inactivation.

k_{inact} is the maximal inactivation rate constant.

$I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug.*

Note: I and K_I need to be expressed in the same unit (e.g., in a molar concentration unit).

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

If $R_1 \geq 1.02$, $R_2 \geq 1.25$ (Vieira, Kirby et al. 2014) or the $R_{1, gut} \geq 11$ (Tachibana, Kato, et al. 2009; Vieira, Kirby, et al. 2014), the sponsor should further investigate the DDI potential by either using mechanistic models (see the appendix, section VII.C) or conducting a clinical DDI study with a sensitive index substrate. If the predicted AUC ratio (AUCR) of a sensitive index substrate in the presence and absence of an investigational drug is ≥ 1.25 based on static mechanistic models or dynamic mechanistic models (e.g., PBPK models) (see appendix, section VII.C), the sponsor should conduct a clinical DDI study using a sensitive index substrate.

When static mechanistic models or PBPK models (see appendix, section VII.C) are used for predicting DDIs caused by enzyme inhibition, the models should include the inhibition mechanism only (i.e., the model should not include concurrent induction predictions for an investigational drug that is hypothesized to be both an inducer and inhibitor) to definitively assess the potential of the investigational drug to inhibit metabolizing enzymes.

C. Determining if the Investigational Drug is an Inducer of Metabolizing Enzymes

1. Conducting In Vitro Studies

The sponsor should evaluate the potential of an investigational drug to induce CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5. Initially, sponsors can conduct experiments to evaluate CYP1A2, CYP2B6, and CYP3A4/5 only. If no induction of CYP3A4/5 enzymes is observed, evaluating the induction potential of CYP2C enzymes is not necessary

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because both CYP3A4/5 and CYP2C enzymes are induced via activation of the pregnane X receptor (PXR). If the investigational drug induces CYP3A4/5, however, the sponsor should evaluate the potential of the investigational drug to induce CYP2C.

2. Data Analysis and Interpretation

Several basic methods can assess the potential of an investigational drug to induce metabolizing enzymes (Fahmi, Kish, et al. 2010; Fahmi and Ripp 2010; Einolf, Chen, et al. 2014). Three of them are described in detail below:

- **Fold-change method:** The sponsor can examine the fold-change in CYP enzyme mRNA levels when incubated with the investigational drug by using a cutoff determined from known positive and negative controls to calibrate the system. For example, a ≥ 2 -fold increase in mRNA and a response $\geq 20\%$ of the response of the positive control in the presence of an investigational drug are interpreted as a positive finding.
- **Correlation methods:** The sponsor may use correlation methods with predicted positive criteria defined by known positive (e.g., known inducers of the same enzyme) and negative controls as described in Figure 3.

Figure 3: Two Correlation Methods to Assess the Potential of an Investigational Drug to Induce Metabolizing Enzymes (Fahmi and Ripp, 2010)

Correlation Method 1: Calculate a relative induction score (RIS) using $(E_{\max} \times I_{\max,u}) / (EC_{50} + I_{\max,u})$

OR

Correlation Method 2: Calculate $I_{\max,u} / EC_{50}$ values

Determine the magnitude of a clinical induction effect (e.g., AUC ratio of index substrate in the presence and absence of inducers) according to a calibration curve of RIS scores or $I_{\max,u} / EC_{50}$ for a set of known inducers of the same enzyme.

E_{\max} is the maximum induction effect determined in vitro.

EC_{50} is the concentration causing half-maximal effect determined in vitro.

$I_{\max,u}$ is the maximal unbound plasma concentration of the interacting drug.*

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

- **Basic kinetic model:** To use this method, the sponsor should calculate the ratio of victim drug AUC in the presence and absence of an inducer (R_3) as described in Figure 4.

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Figure 4: An Equation to Calculate the Predicted Ratio of the Victim Drug's AUC in the Presence and Absence of an Inducer for Basic Models of Induction

$$R_3 = 1 / [1 + (d \times E_{\max} \times 10 \times I_{\max,u}) / (EC_{50} + (10 \times I_{\max,u}))]$$

R_3 is the predicted ratio of the victim drug's AUC in the presence and absence of an inducer for basic models of enzyme induction.

d is the scaling factor and is assumed to be 1 unless supported by prior experience with the system used.

E_{\max} is the maximum induction effect determined in vitro.

$I_{\max,u}$ is the maximal unbound plasma concentration of the interacting drug.*

EC_{50} is the concentration causing half-maximal effect determined in vitro.

*Considering uncertainties in the protein binding measurements, the unbound fraction should be set to 1% if experimentally determined to be <1%.

If any of these methods indicates that the investigational drug has the potential to induce metabolizing enzymes (using specific cutoff values developed by individual laboratories for Methods 1 and 2 or if $R_3 \leq 0.8$), the sponsor should further investigate the enzyme induction potential of the investigational drug by using mechanistic models (see the appendix, section VII.C) or by conducting a clinical DDI study with a sensitive index substrate. If the predicted AUCR of a sensitive index substrate in the presence and absence of an investigational drug is ≤ 0.8 based on static mechanistic models or dynamic mechanistic models (e.g., PBPK models; see appendix, section VII.C), the sponsor should further investigate potential DDIs by conducting a clinical DDI study using a sensitive index substrate.

When static mechanistic models or dynamic mechanistic models (e.g., PBPK models; see appendix, section VII.C) are used for predicting DDIs caused by enzyme induction, the models should include the induction mechanism only (i.e., the model should not include concurrent inhibition predictions for an investigational drug that is hypothesized to be both an inducer and inhibitor) to definitively assess the potential of an investigational drug to induce metabolizing enzymes.

3. Additional Considerations

The AUCR cutoffs of > 0.8 (for induction) and < 1.25 (for inhibition) using mechanistic models are the suggested default values to indicate that the investigational drug has no effect on the levels of metabolizing enzymes.

When evaluating whether an investigational drug is an inhibitor of multiple CYP enzymes, the sponsor can prioritize in vivo DDI evaluations for various CYP enzymes with sensitive index substrates of respective pathways (see the FDA's draft guidance for industry *Clinical Drug*

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286 *Interaction Studies — Study Design, Data Analysis, and Clinical Implications*⁶) based on rank-
287 ordered R_1 , R_2 , or the predicted AUCR values, preferably using the in vitro inhibition parameters
288 obtained in the same study.⁷ That is, the sponsor may first carry out an in vivo study with a
289 sensitive index substrate of the CYP with the largest R or AUCR value. If this in vivo study
290 shows no interaction, in vivo evaluations of other CYPs with lower potencies (e.g., smaller R or
291 AUCR) are not needed. However, if this in vivo study shows a positive interaction between the
292 drug and the sensitive index CYP substrate, the sponsor should conduct additional in vivo studies
293 for other CYPs, starting with the CYP with the next largest R or AUCR value. Alternatively, the
294 sponsor can use a PBPK model to inform the need for the conduct of additional studies. The
295 sponsor should verify and update any PBPK models to demonstrate that the model can
296 adequately describe the observed findings from the first in vivo study with a sensitive index
297 substrate.

298
299

300 **IV. EVALUATING TRANSPORTER-MEDIATED DRUG INTERACTIONS**

301
302 Membrane transporters can have clinically relevant effects on the pharmacokinetics and
303 pharmacodynamics of a drug in various organs and tissues by controlling its absorption,
304 distribution, and elimination (Giacomini, Huang, et al. 2010; Giacomini and Huang 2013). In
305 contrast to drug metabolizing enzymes that are largely expressed in the liver and small intestines,
306 transporters are expressed in tissues throughout the human body and govern the access of
307 endogenous and exogenous substances to various sites in the body. Transporters, in concert with
308 metabolizing enzymes, can govern a drug's disposition and pharmacological action. Conversely,
309 a drug can also modulate transporter expression or activity, resulting in altered disposition of
310 endogenous (e.g., creatinine, glucose) or exogenous substances.

311
312 Several transporters interact with drugs in clinical use (Giacomini, Huang, et al. 2010; Giacomini
313 and Huang 2013), for example:

314

- 315 • P-glycoprotein (P-gp or Multi-drug Resistance 1 (MDR1) protein)
- 316 • Breast cancer resistance protein (BCRP)
- 317 • Organic anion transporting polypeptide 1B1/1B3 (OATP1B1/OATP1B3)
- 318 • Organic anion transporter 1/3 (OAT1/OAT3)
- 319 • Multidrug and toxin extrusion (MATE) proteins
- 320 • Organic cation transporter 2 (OCT2)

⁶ When final, this guidance will represent the FDA's current thinking on this topic.

⁷ An orally administered drug may inhibit intestinal metabolic enzymes (e.g., CYP3A) in addition to hepatic enzymes. Therefore, in vivo DDI for CYP3A inhibition needs to be considered if $R_{1,\text{gut}}$ is greater than or equal to 11, even if R_1 for CYP3A is not the largest value among the major CYPs evaluated.

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321
322 The consequence of a drug interaction mediated by transporters may not be apparent if a clinical
323 DDI study only measures systemic drug exposures. However, understanding whether the drug is
324 a substrate or perpetrator (i.e., inhibitor or inducer) of these key transporters can explain some
325 clinical consequences, such as increased toxicity or altered efficacy, that result from altered
326 tissue distribution of a drug that is a substrate of a transporter.

327
328 This section focuses on transporters that have clinical evidence suggesting their involvement in
329 drug interactions (Giacomini, Huang, et al. 2010; Brouwer, Keppler, et al. 2013; Giacomini and
330 Huang 2013; Tweedie, Polli, et al. 2013; Zamek-Gliszczynski, Lee, et al. 2013). The sponsor
331 should evaluate the interactions between investigational drugs acting as substrates and/or
332 perpetrators of these transporters as outlined below. The timing of the in vitro evaluation of each
333 transporter may vary depending on the therapeutic indications of the investigational drug. For
334 example, if the intended population is likely to use statins, the sponsor should examine the
335 potential of the investigational drug to interact with OATP1B1/1B3 before clinical studies in
336 patients. If in vitro experiments indicate a low potential for an interaction between the
337 transporter and investigational drug, subjects taking statins may be included in clinical studies to
338 better represent the intended patient population.

339
340 **A. Determining if the Investigational Drug is a Substrate of the Transporters P-**
341 **gp and BCRP**

342
343 P-gp and BCRP are expressed in various tissues including the gastrointestinal tract, liver, kidney,
344 and brain. Thus, both transporters have the potential to impact the oral bioavailability, the tissue
345 distribution, and the hepatic and renal elimination of substrates.

346
347 *1. Conducting In Vitro Studies*

348
349 Sponsors should evaluate most investigational drugs in vitro to determine whether they are
350 substrates of P-gp and BCRP using the experimental systems described in the appendix, section
351 VII.B. P-gp and BCRP are not expected to impact the oral bioavailability of highly permeable
352 and highly soluble drugs. In vitro assessment of these drugs as P-gp or BCRP substrates is not
353 suggested unless there are potential safety concerns with the drug distributing into tissues (e.g.,
354 the kidney and brain). See the FDA's guidance for industry entitled *Waiver of In Vivo*
355 *Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms*
356 *Based on a Biopharmaceutics Classification System*⁸ to determine if the investigational drug can
357 be classified as highly permeable and/or highly soluble (e.g., biopharmaceutics classification
358 system class 1 drugs).

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⁸When final, this guidance will represent the FDA's current thinking on this topic.

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2. *Data Analysis and Interpretation*

The following results suggest that an investigational drug is an in vitro P-gp substrate:

- A net flux ratio (or efflux ratio (ER)) of ≥ 2 for an investigational drug in cells that express P-gp (e.g., Caco-2 cells or other cells overexpressing P-gp)⁹
- A flux that is inhibited by at least one known P-gp inhibitor at a concentration at least 10 times its K_i (e.g., the ER decreases to $< 50\%$ of the ER in the absence of inhibitor or the flux reduced to unity).

When using Caco-2 cells that express multiple efflux transporters, the sponsor should use more than one P-gp inhibitor to determine the specificity of the efflux. The sponsor may use a net flux ratio cutoff other than 2 or a specific relative ratio to positive controls if prior experience with the cell system justifies these alternative methods.

If in vitro studies indicate that a drug is a P-gp substrate, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known P-gp inhibitors in the indicated patient population (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*¹⁰).

The sponsor may also use the above procedures to determine whether the drug is a BCRP substrate by using known BCRP inhibitors. If in vitro studies indicate that a drug is a BCRP substrate, the sponsor should consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic index, and likely concomitant medications that are known BCRP inhibitors in the indicated patient population (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications*¹¹).

B. Determining if the Investigational Drug is a Substrate of the Hepatic Transporters OATP1B1 and OATP1B3

OATP1B1 and OATP1B3 are key uptake transporters expressed on the sinusoidal membrane of hepatocytes and play an important role in the hepatic uptake of various drugs.

⁹ The ER can be calculated as the ratio of the basal to apical (B-A) transport rate to the apical to basal (A-B) transport rate. The net flux ratio can be calculated as the ratio of the ER between cells expressing the transporter of interest to cells not expressing the transporter.

¹⁰ When final, this guidance will represent the FDA's current thinking on this topic.

¹¹ When final, this guidance will represent the FDA's current thinking on this topic.

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395 ***1. Conducting In Vitro Studies***

396

397 If in vitro studies or human/animal absorption, distribution, metabolism, and/or excretion
398 (ADME) data suggest that an investigational drug's hepatic uptake or elimination is significant
399 (i.e., the drug's clearance through hepatic metabolism or biliary secretion is $\geq 25\%$ of the total
400 drug's clearance), or the drug's uptake into the liver is clinically important (e.g., for
401 biotransformation or to exert a pharmacological effect), the sponsor should evaluate the
402 investigational drug in vitro to determine whether it is a substrate for the hepatic uptake
403 transporters OATP1B1 and OATP1B3 (see the appendix, section VII.B).

404

405 ***2. Data Analysis and Interpretation***

406

407 An investigational drug is considered an in vitro substrate for OATP1B1 or OATP1B3 if: (1) the
408 uptake of the drug in OATP1B1- or OATP1B3-transfected cells is ≥ 2 -fold of the drug's uptake
409 in empty vector-transfected cells; and (2) a known inhibitor (e.g., rifampin) can decrease the
410 drug's uptake to $\leq 50\%$ at a concentration at least 10 times that of the K_i or IC_{50} . The sponsor
411 may justify alternative cutoff ratios based on its prior experience with the cell system.

412

413 If in vitro studies indicate that a drug is an OATP1B1 or OATP1B3 substrate, the sponsor should
414 consider whether to conduct an in vivo study based on the drug's safety margin, therapeutic
415 index, and likely co-medications that are known OATP1B1 or OATP1B3 inhibitors in the
416 indicated patient populations (see the FDA's draft guidance for industry entitled *Clinical Drug
417 Interaction Studies — Study Design, Data Analysis, and Clinical Implications*¹²).

418

419 **C. Determining if the Investigational Drug is a Substrate of the Renal 420 Transporters OAT, OCT, and MATE**

421

422 OAT1, OAT3, and OCT2 are renal transporters expressed on the basolateral membrane of the
423 renal proximal tubule. MATE1 and MATE2-K are expressed on the brush border membrane.
424 All of the aforementioned renal transporters can play a role in the active renal secretion of
425 investigational drugs.

426

427 ***1. Conducting In Vitro Studies***

428

429 If the investigational drug's ADME data suggest that active renal secretion is significant for a
430 drug (i.e., active secretion of the parent drug by the kidney is $\geq 25\%$ of the total clearance), the
431 sponsor should evaluate the drug in vitro to determine whether it is a substrate of OAT1/3, OCT2

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432 and MATE1 and MATE2-K (see appendix, section VII.B). See Figure 5 for the equation to
433 calculate active secretion.

434

435 **Figure 5: An Equation to Calculate Active Secretion***

436

$$437 \text{Active secretion} = CL_r - (f_{u,p} \times \text{GFR})$$

438

439 CL_r is the renal clearance.

440 $f_{u,p}$ is the unbound fraction in plasma.

441 GFR is the glomerular filtration rate.

442

443 *This equation is valid assuming that there is no re-absorption. The GFR is set as 125 mL/min for subjects with

444 normal renal function if the GFR is not measured.

445

446

447 2. *Data Analysis and Interpretation*

448

449 The investigational drug is an in vitro substrate for the above renal transporters if: (1) the ratio
450 of the investigational drug's uptake in the cells expressing the transporter versus the drug's
451 uptake in control cells (or cells containing an empty vector) is ≥ 2 ; and (2) a known inhibitor of
452 the transporter decreases the drug's uptake to $\leq 50\%$ at a concentration at least 10 times its K_i or
453 IC_{50} . The sponsor may justify alternative cutoff ratios based on its prior experience with the cell
454 system.

455

456 If in vitro studies indicate that a drug is a substrate of one or more of these renal transporters, the
457 sponsor should consider whether to conduct an in vivo study based on the drug's safety margin,
458 therapeutic index, and likely concomitant medications that are known inhibitors of these renal
459 transporters in the indicated patient populations (see the FDA's draft guidance for industry
460 entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical*
461 *Implications*¹³).

462

463 **D. Determining if the Investigational Drug is an Inhibitor of a Transporter**

464

465 1. *Conducting In Vitro Studies*

466

467 The sponsor should conduct in vitro studies to evaluate whether an investigational drug is an
468 inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OCT2, MATEs (MATE-1, MATE-2K), OAT1,
469 and OAT3 (see appendix, section VII.B for considerations regarding in vitro systems).

470

471 2. *Data Analysis and Interpretation*

472

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473 **P-gp and BCRP:** The sponsor should conduct studies to determine if an investigational drug
474 inhibits the net flux of a known P-gp or BCRP substrate in Caco-2, P-gp- or BCRP-
475 overexpressed cells and determine the drug's inhibition potency (i.e., IC_{50} or K_i). The
476 investigational drug has the potential to inhibit P-gp or BCRP in vivo if the investigational drug
477 is administered orally, and the $I_{gut}/IC_{50} \geq 10$ where I_{gut} = dose of inhibitor/250 mL. To determine
478 the IC_{50} , a unidirectional assay based on the probe substrate can also be considered. This cutoff
479 value is based on a limited dataset (Zhang, Zhang, et al. 2008; Tachibana, Kato, et al. 2009;
480 Agarwal, Arya, et al. 2013; Ellens, Deng, et al. 2013). The sponsor may calibrate its internal in
481 vitro systems with known inhibitors and non-inhibitors and propose a different cutoff value with
482 proper justification (see appendix, section VII.B for detailed recommendations).

483
484 If in vitro studies indicate that a drug is a P-gp or BCRP inhibitor, the sponsor should consider
485 whether to conduct an in vivo study based on likely concomitant medications that are known P-
486 gp or BCRP substrates in the indicated patient populations (see the FDA's draft guidance for
487 industry entitled *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical*
488 *Implications*¹⁴).

489
490 **OATP1B1 and OATP1B3:** The sponsor should conduct studies to determine the inhibition
491 potency (i.e., IC_{50} or K_i) of the investigational drug on the uptake of a known OATP1B1 or
492 OATP1B3 substrate in cells overexpressing the relevant transporter. Because some known
493 OATP1B1/3 inhibitors demonstrate time-dependent inhibition, the sponsor should determine
494 IC_{50} values following pre-incubation with the investigational drug for a minimum of 30 minutes
495 (Amundsen, Christensen, et al. 2010; Gertz, Cartwright, et al. 2013; Izumi, Nozaki, et al. 2015).
496 The investigational drug has the potential to inhibit OATP1B1/3 in vivo if the R value (as
497 described in Figure 6 below) is ≥ 1.1 .

498
499 **Figure 6: Equation to Calculate the Predicted Ratio of the Victim Drug AUC in the**
500 **Presence and Absence of the Investigational Drug to Determine the Potential to Inhibit**
501 **OATP1B1/3***

$$R = 1 + ((f_{u,p} \times I_{in,max}) / IC_{50}) \geq 1.1$$

505 **R** is the predicted ratio of the victim drug's AUC in the presence and absence of the investigational drug as the
506 inhibitor.

507 **$f_{u,p}$** is the unbound fraction in plasma.

508 **IC_{50}** is the half-maximal inhibitory concentration.

509 **$I_{in,max}$** is the estimated maximum plasma inhibitor concentration at the inlet to the liver. It is calculated as:

$$I_{in,max} = (I_{max} + (F_a F_g \times k_a \times Dose)) / Q_h / R_B$$

Continued

¹⁴ When final, this guidance will represent the FDA's current thinking on this topic.

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513 **Figure 6 continued. Equation to Calculate the Predicted Ratio of the Victim Drug AUC in**
514 **the Presence and Absence of the Investigational Drug to Determine the Potential to Inhibit**
515 **OATP1B1/3***

516
517 **F_a** is the fraction absorbed.

518 **F_g** is the intestinal availability.

519 **k_a** is the absorption rate constant.

520 **Q_h** is the hepatic blood flow rate.

521 **R_B** is the blood-to-plasma concentration ratio.

522

523 *If unknown, $F_a F_g = 1$ and $k_a = 0.1/\text{min}$ can be used as a worst-case estimate.

524 Considering uncertainties in the protein binding measurements, the unbound fraction ($f_{u,p}$) should be set to 1% if
525 experimentally determined to be less than 1%.

526

527

528 The cutoff value described in Figure 6 is based on limited published data (Yoshida, Maeda, et al.
529 2012; Tweedie, Polli, et al. 2013; Vaidyanathan, Yoshida, et al. 2016). Sponsors may calibrate
530 their internal in vitro systems with known inhibitors and non-inhibitors of these transporter
531 systems and propose a specific cutoff value with proper justification.

532

533 If in vitro studies indicate that a drug is an OATP1B1 or OATP1B3 inhibitor, the sponsor should
534 consider whether to conduct an in vivo study based on whether the likely concomitant
535 medications used in the indicated patient populations are known OATP1B1 or OATP1B3
536 substrates (see the FDA's draft guidance for industry entitled *Clinical Drug Interaction Studies*
537 — *Study Design, Data Analysis, and Clinical Implications*¹⁵).

538

539 **OAT, OCT, and MATE**: Sponsors should conduct studies to determine the inhibition potency
540 (i.e., IC_{50} or K_i) of the investigational drug on the uptake of a known substrate for renal
541 transporters (i.e., OAT1, OAT3, OCT2, MATE1, and MATE2K) in cells overexpressing these
542 transporters. The investigational drug has the potential to inhibit these transporters in vivo if the
543 $I_{\max,u}/IC_{50}$ value is ≥ 0.1 for OAT1/OAT3/OCT2 or the $I_{\max,u}/IC_{50}$ value is ≥ 0.02 for MATEs.¹⁶
544 These cutoff values are based on limited data (Dong, Yang, et al. 2016a; Dong, Yang, et al.
545 2016b). Sponsors may calibrate their unique in vitro systems with known inhibitors and non-
546 inhibitors of these transporter systems and propose a different cutoff value with proper
547 justification. Creatinine is also a substrate for OCT2, MATEs, and OAT2 (Lepist, Zhang, et al.
548 2014). Elevated serum creatinine levels in observed in clinical studies could be due to inhibition
549 of these transporters by the investigational drug. Confirmation of the mechanism of an increase
550 in serum creatinine with the investigational drug requires additional evidence such as clinical

¹⁵ When final, this guidance will represent the FDA's current thinking on this topic.

¹⁶ Considering uncertainties in the protein binding measurements, the unbound fraction should be set to 1% if experimentally determined to be less than 1%.

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551 mechanistic studies.

552
553 If in vitro studies indicate that a drug is an inhibitor of these renal transporters, the sponsor
554 should consider whether to conduct an in vivo study based on whether the likely concomitant
555 medications used in the indicated patient populations are known substrates of these renal
556 transporters (see the FDA’s draft guidance for industry entitled *Clinical Drug Interaction Studies*
557 — *Study Design, Data Analysis, and Clinical Implications*¹⁷).

558 **E. Determining if the Investigational Drug is an Inducer of a Transporter**

560
561 Certain transporters such as P-gp are induced through mechanisms similar to those for CYP
562 enzymes (e.g., by activation of specific nuclear receptors). Because of these similarities,
563 information from CYP3A induction studies can inform P-gp induction studies (see the FDA’s
564 draft guidance for industry entitled *Clinical Drug Interaction Studies — Study Design, Data*
565 *Analysis, and Clinical Implications*¹⁸). However, in vitro methods to evaluate the induction of P-
566 gp and other transporters are not well established at this time. Therefore, the FDA does not
567 currently recommend in vitro evaluation of investigational drugs as transporter inducers.

568 569 570 **V. EVALUATION OF THE DDI POTENTIAL OF METABOLITES**

571
572 Sponsors should evaluate the DDI potential of an investigational drug’s metabolites for their
573 impact on the drug’s safety and efficacy using a risk-based assessment that considers safety
574 margins, likely concomitant medications, and therapeutic indications.

575
576 A metabolite with significant plasma exposure or pharmacological activities may need to be
577 evaluated for its DDI potential as a substrate or as an inhibitor of metabolizing enzymes (see
578 sections V.A and V.B below). In vitro studies normally use a synthesized or purified metabolite
579 standard or radiolabeled drug. Alternative methods are acceptable if the sponsor can justify that
580 the DDI potential of the metabolites can be adequately assessed (Callegari, Kalgutkar, et al.
581 2013; Yu and Tweedie 2013; Yu, Balani, et al. 2015).

582
583 Published data have shown that some Phase II metabolites can be better substrates (more polar
584 than the parent) or inhibitors of various transporters leading to a higher chance of DDIs than the
585 parent drug (Zamek-Gliszczyński et al, 2014). Therefore the DDI potential of a metabolite as a
586 substrate or a perpetrator of major drug transporters should be assessed on a case-by-case basis.
587 The same principles and strategies mentioned above for parent drug should be applied where

¹⁷ When final, this guidance will represent the FDA’s current thinking on this topic.

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588 applicable.

589

590 **A. Metabolite as a Substrate**

591

592 *1. Conducting In Vitro Studies*

593

594 The sponsor should evaluate the potential for a metabolite to cause DDIs by acting as a substrate
595 of metabolizing enzymes if the metabolite: (1) is active (i.e., has the potential to affect safety or
596 efficacy based on in vitro pharmacology and toxicology assessments); and (2) contributes to \geq
597 50% of the overall activity, considering both in vitro receptor potency and in vivo exposure.

598

599 *2. Data Analysis and Interpretation*

600

601 The sponsor should consider in vivo DDI studies of the metabolite based on in vitro assessments
602 using the same strategies as those for the parent drugs (see section III.A).

603

604 **B. Metabolite as an Inhibitor**

605

606 *1. Conducting In Vitro Studies*

607

608 If in vitro assessments suggest that the parent drug inhibits major CYP enzymes and transporters
609 and in vivo DDI studies are warranted, in vitro assessments of metabolites as enzyme or
610 transporter inhibitors may not be needed because the in vivo inhibition potential of metabolites
611 would be evaluated in vivo along with the parent drug, unless clinically relevant exposures of the
612 metabolite cannot be adequately represented in the in vivo DDI study (i.e., the study duration
613 does not allow the metabolite to accumulate). However, if in vitro assessments suggest that the
614 parent drug alone will not inhibit major CYP enzymes or transporters, in vivo DDIs caused by
615 metabolites may still be possible. In this situation, the sponsor should evaluate the in vitro
616 inhibition potential of a metabolite on CYP enzymes taking into account the following factors:
617 (1) the systemic exposure of the metabolite relative to the parent drug; and (2) any structural
618 alerts, such as Quantitative Structure-Activity Relationship (QSAR) for potential time-dependent
619 inhibition (Yu and Tweedie 2013; Yu, Balani, et al. 2015). Additional considerations are
620 discussed in detail below:

621

622 • The sponsor should conduct an in vitro inhibition study of the metabolite if a metabolite
623 is less polar than the parent drug and the $AUC_{\text{metabolite}} \geq 25\% \times \text{the } AUC_{\text{parent}}$ (i.e.,
624 $AUC_{\text{metabolite}} \geq 0.25 \times AUC_{\text{parent}}$).

625

626 • The sponsor should conduct an in vitro inhibition study of the metabolite if a metabolite
627 is more polar than the parent drug, and the $AUC_{\text{metabolite}} \geq 100\% \times AUC_{\text{parent}}$ (i.e.,
628 $AUC_{\text{metabolite}} \geq AUC_{\text{parent}}$).

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- The sponsor should conduct an in vitro inhibition study of the metabolite on CYP enzymes if a metabolite with a structural alert for TDI has an $AUC_{\text{metabolite}} \geq 25\% \times$ the AUC_{parent} and an $AUC_{\text{metabolite}} \geq 10\% \times$ the AUC of the total drugs (determined with radioactivity). If there are no radioactivity data but the $AUC_{\text{metabolite}} \geq 25\% \times$ the AUC_{parent} , then the sponsor should conduct an in vitro DDI assessment with the metabolite.

2. Data Analysis and Interpretation

Based on the results of in vitro DDI assessments of the metabolite, the sponsor should consider an in vivo DDI study of the metabolite using the same strategies as those for the parent drug (see section III.B).

VI. LABELING RECOMMENDATIONS

Prescription information must include a summary of drug interaction information that is essential for the safe and effective use of the drug product by the health care provider and must be based on data derived from human experience whenever possible.¹⁹ In the absence of clinical information, the sponsor should include in vitro information regarding the characterization of metabolic and transporter pathways as well as PK interactions between the drug and other prescription drugs, over-the-counter drugs, classes of drugs, dietary supplements, and foods or juices (including inhibition, induction, and genetic characteristics) in prescription drug labeling, if clinically significant. In addition, the results of pertinent in vitro studies that establish the absence of an effect must be included.²⁰ In vitro information that has been superseded by clinical information should not be included in the prescription drug labeling unless it is essential to understanding the clinical results.

This in vitro information should generally be placed under the *12.3 Pharmacokinetics* subsection of the CLINICAL PHARMACOLOGY section. In rare cases, the clinical significance of the in vitro information may require placement in other sections of the prescription drug labeling (e.g., BOXED WARNING, CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS, and/or DRUG INTERACTIONS sections).

See the following FDA guidances for industry for labeling recommendations relevant to drug metabolism and transporter pathways as well as clinical DDIs:

¹⁹ 21 CFR 201.56(a)(3).

²⁰ 21 CFR 201.57(c)(13)(c)(i)(C).

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- 667 • *Clinical Pharmacology Labeling for Human Prescription Drug and Biological Products*
668 *— Considerations, Content, and Format*²¹
669
- 670 • *Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical*
671 *Implications*²²
672

²¹ This guidance is available on the FDA Drugs guidance Web page at <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>

²² When final, this guidance will represent the FDA’s current thinking on this topic.

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673 **VII. APPENDICES**

674

675 **A. Evaluating Metabolism-Based Drug Interactions In Vitro**

676

677 Various hepatic in vitro systems can be used to evaluate the drug interaction potential of an
678 investigational drug, including:

679

680 (1) Subcellular human liver tissue fractions such as reconstituted microsomal systems,
681 supernatants after 9000 g centrifugation of liver homogenate (S9), and cytosol (adding
682 appropriate co-factors as necessary)

683

684 (2) Recombinant CYP enzymes in various expression systems that can identify the
685 production of individual drug metabolites and the involvement of certain classes of enzymes

686

687 (3) Human liver tissues, including freshly prepared hepatocytes and cryopreserved
688 hepatocytes that preserve enzyme architecture and contain the full complement of Phase I
689 and Phase II drug metabolizing enzymes

690

691 Although the main focus of this guidance is on CYP and hepatic metabolism, sponsors should
692 consider non-CYP, enzyme-based metabolism (e.g., Phase II enzymes) and metabolism
693 occurring in extra-hepatic tissues when relevant for their investigational drugs.

694

695 *1. Determining if the Investigational Drug is an Enzyme Substrate*

696

697 Drug metabolizing enzyme identification studies, often referred to as reaction phenotyping
698 studies, are a set of in vitro experiments that identify the specific enzymes responsible for the
699 metabolism of a drug. Along with other information (e.g., in vivo pharmacokinetics, enzyme
700 polymorphism or DDI data), in vitro phenotyping data are often used to quantify disposition
701 pathways of an investigational drug.

702

703 *a. Conducting metabolic pathway identification experiments*

704

705 Metabolic pathway identification experiments identify the number and structures of metabolites
706 produced by a drug and whether the metabolic pathways are parallel or sequential. These
707 experiments use intact human liver systems (e.g., human hepatocytes), human liver microsomes,
708 or recombinant enzyme systems. Data obtained from metabolic pathway identification
709 experiments help to determine whether and how to conduct a reaction phenotyping study.

710

711 *b. Identifying the enzymes that metabolize an investigational drug*

712

713 The sponsor should conduct in vitro experiments to identify specific metabolizing enzymes that
714 are involved in the metabolism of an investigational drug, preferably before first-in-human
715 studies. There are two widely used methods for identifying the individual CYP enzymes

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716 responsible for a drug's metabolism: (1) the first method uses chemicals, drugs, or antibodies as
717 specific enzyme inhibitors in pooled (e.g., a pool of more than 10 donors) human liver
718 microsomes and (2) the second method uses individual human recombinant CYP enzymes. The
719 sponsor should consider the following recommendations when performing reaction phenotyping
720 experiments:

- 721
- 722 • The sponsor should use both methods to identify the specific enzymes responsible for a
723 drug's metabolism.
- 724
- 725 • When using individual recombinant CYP enzymes, the sponsor should consider the
726 difference in the amount of CYP contents between recombinant CYP enzyme systems
727 and the human liver.
- 728
- 729 • The in vitro system for these studies should: (1) be robust and reproducible; and (2)
730 include the necessary selective in vitro probe substrate as a positive control to prove the
731 activity of each enzyme. A list of probe substrates can be found on the FDA's Web site
732 on Drug Development and Drug Interactions.²³
- 733
- 734 • Whenever possible, the sponsor should conduct all experiments with drug concentrations
735 deemed appropriate by kinetic experiments and under initial rate conditions (linearity of
736 metabolite production rates with respect to time and enzyme concentrations). The
737 sponsor should conduct an adequate number of replicates (e.g., three or more replicates
738 per drug concentration) in a single study.
- 739
- 740 • When conducting an in vitro study to examine the contribution of individual CYP
741 enzymes to the overall metabolism of an investigational drug, the measurement of parent
742 drug depletion is preferred over the measurement of metabolite formation, unless all of
743 the major metabolites have been identified and quantified in metabolite formation
744 experiments.
- 745
- 746 • When conducting in vitro studies to examine the contribution of individual CYP enzymes
747 to the formation of a specific metabolite, the sponsor should measure the formation rate
748 of the metabolite.
- 749
- 750 • The sponsor should develop validated and reproducible analytical methods to measure
751 levels of the parent drug and each metabolite.
- 752

²³ A list of probe substrates:

<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1>.

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- 771
- The use of a radiolabeled drug substrate is advantageous because samples can be analyzed using liquid chromatography coupled with a radioactivity detector or a mass spectrometer to identify and quantify drug-related species.
 - The sponsor should separately evaluate individual isomers of racemic drugs when it is important to understand the different disposition characteristics of each isomer (e.g., when two isomers have different pharmacological activities).
 - Most chemical inhibitors are not specific for an individual CYP enzyme. The sponsor should verify the selectivity and potency of inhibitors in the same experimental conditions using probe substrates for each CYP enzyme. Commonly used in vitro CYP enzyme inhibitors can be found on the FDA’s Web site on Drug Development and Drug Interactions.²⁴
 - The sponsor should test the inhibitory effect of an antibody to a CYP enzyme at sufficiently low and high concentrations to establish a titration curve and ensure the maximal inhibition of a particular pathway (ideally resulting in greater than 80 percent inhibition). The sponsor should verify the effect of an antibody using probe substrates of each CYP isoform and with the same experimental conditions.

772

773

774

2. *Determining if the Investigational Drug is an Enzyme Inhibitor or Inducer*

775

776

a. *Conducting in vitro enzyme inhibition studies*

777

778

779

780

781

782

783

The potential of an investigational drug to inhibit CYP enzymes is usually investigated in human liver tissue systems using probe substrates to determine the inhibition mechanisms (e.g., reversible or time-dependent inhibition) and inhibition potencies (e.g., K_i for reversible inhibition, and K_I and k_{inact} for time-dependent inhibition). The in vitro systems used for these studies include human liver microsomes, microsomes obtained from recombinant CYP-expression systems, or hepatocytes (Bjornsson, Callaghan, et al. 2003).

784

785

786

787

788

Kinetic data from in vitro inhibition studies of an investigational drug can be used in quantitative models to predict the investigational drug’s effects on the pharmacokinetics of other drugs in humans. These analyses inform the decision on whether to conduct an in vivo DDI study using sensitive enzyme index substrates (see section III.B.2).

789

The sponsor should consider the following recommendations when designing an in vitro CYP

²⁴ Examples of in vitro selective inhibitors for P450-mediated metabolism:
<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1-2>

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790 inhibition study:

- 791
- 792 • A probe substrate should be selective (e.g., predominantly metabolized by a single
793 enzyme in pooled human liver microsomes or recombinant CYPs) and have simple
794 metabolic schemes (ideally, the drug does not undergo sequential metabolism).
795 Commonly used in vitro probe substrates and their marker reactions can be found on the
796 FDA Web site on Drug Development and Drug Interactions.²⁵
797
 - 798 • The sponsor should use a validated and reproducible analytical assay to measure the
799 formation of a probe substrate's metabolite.
800
 - 801 • The in vitro system of choice for enzyme inhibition should be robust and reproducible
802 and include the appropriate strong probe inhibitors as positive controls (see the FDA's
803 Web site on Drug Development and Drug Interactions).²⁶ Kinetic constants (K_i , IC_{50} , K_I ,
804 and/or k_{inact}) of the probe inhibitors should be comparable to literature-reported values.
805 In vitro systems may be pooled human liver microsomes (e.g., pooled from more than 10
806 donors), pooled cryopreserved hepatocytes (e.g., pooled from more than 10 donors), or
807 individual microsomes expressing recombinant CYP enzymes. To obtain inhibition
808 parameters, the sponsor may consider primary hepatocytes enriched with human plasma
809 as an in vitro system that represents physiological conditions (Lu, Miwa, et al. 2007; Mao,
810 Mohutsky, et al. 2012).
811
 - 812 • When used as an inhibitor, the investigational drug concentrations should generally be as
813 high as possible to maximize the inhibition effect. However, the drug concentration
814 should not exceed the drug's solubility limits or cause deleterious effects (e.g.,
815 cytotoxicity) in the cell models.
816
 - 817 • The sponsor should test four to six different concentrations of the investigational drug
818 with the probe substrate. The sponsor should first conduct experiments with a high
819 concentration of test drug to study its inhibition potential on a particular enzyme (e.g., 50
820 times the unbound C_{max} , or 0.1 times the dose/250 mL). If the initial high concentration

²⁵ Examples of in vitro marker reactions for P450-mediated metabolism and in vitro selective inhibitors for P450-mediated metabolism:
<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1>

²⁶ Examples of in vitro selective inhibitors for P450-mediated metabolism:
<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm#table1-2>

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821 of the test drug is able to inhibit a particular enzyme, the sponsor should test lower drug
822 concentrations to calculate the drug's IC_{50} or K_i value.

- 823
- 824 • Typical experiments to determine the IC_{50} value of a drug involve incubating the
825 substrate at a concentration at or below its K_m to more closely relate the inhibitor's IC_{50}
826 to its K_i . For K_i determinations, the sponsor should vary both the substrate and inhibitor
827 concentrations to cover ranges above and below the substrate's K_m and the inhibitor's K_i .
828
 - 829 • Microsomal protein concentrations are usually less than 1 mg/mL. The sponsor should
830 correct for nonspecific binding during the incubation if this binding is expected to
831 influence the analysis of kinetic data. Nonspecific binding can be measured
832 experimentally (e.g., using equilibrium dialysis or ultrafiltration) (Hallifax and Houston
833 2006) or predicted using in silico methods (Gertz, Kilford, et al. 2008).
834
 - 835 • Because buffer strength, type, and pH can all significantly affect the determination of
836 V_{max} and K_m , the sponsor should use standardized assay conditions.
837
 - 838 • In general, the sponsor should avoid any significant depletion of the substrate or inhibitor.
839 However, when substrates have a low K_m , it may be difficult to avoid substrate depletion
840 at low substrate concentrations. In these circumstances, the sponsor should consider
841 substrate depletion when determining inhibition kinetics.
842
 - 843 • The sponsor should choose an incubation time and an enzyme amount that result in linear
844 formation of the metabolite (at an initial rate of the metabolite formation).
845
 - 846 • The sponsor should use any organic solvents at low concentrations (<1%
847 (volume/volume) and preferably < 0.5%) because some solvents can inhibit or induce
848 enzymes. The experiment should include a no-solvent control and a solvent (vehicle)
849 control.
850
 - 851 • The sponsor should determine inhibition kinetics according to appropriate mechanisms
852 (e.g., competitive, noncompetitive, or TDI).
853
 - 854 • The sponsor should routinely study TDI in standard in vitro screening protocols by pre-
855 incubating the investigational drug (e.g., for at least 30 min) before adding any substrate.
856 Any significant time-dependent and co-factor-dependent (e.g., NADPH for CYPs) loss of
857 initial product formation may indicate TDI. In these circumstances, the sponsor should
858 conduct definitive in vitro studies to obtain TDI parameters (i.e., k_{inact} and K_I) (Grimm,
859 Einolf, et al. 2009).

860 b. Evaluating enzyme induction in vitro

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863 The sponsor can investigate the potential of an investigational drug to act as an inducer of CYP
864 enzymes in plateable, cryopreserved or freshly isolated, human hepatocytes. Other, alternative in
865 vitro systems such as immortalized hepatic cell lines may be used and are acceptable methods to
866 determine the CYP induction potential of investigational drugs. Cell receptor assays may be
867 used, but the results from these studies are considered supportive, rather than definitive, in nature.
868 The sponsor should justify both why any alternative in vitro system is appropriate for the
869 purpose of the study as well as the method to interpret the data. Acceptable study endpoints
870 include mRNA levels and/or enzyme activity levels using a probe substrate (Fahmi and Ripp
871 2010; Einolf, Chen, et al. 2014). A major challenge of measuring only the enzyme's activity is
872 that the induction may be masked in the presence of concomitant inhibition. Transcriptional
873 analysis through the measurement of mRNA levels may address this challenge. Regardless of
874 which in vitro system and endpoint are chosen, the sponsor should validate the system to show
875 that all major CYP enzymes are functional and inducible with positive controls.

876

877 When using in vitro systems to study enzyme induction, the sponsor should consider the
878 following recommendations:

879

- 880 • The sponsor should evaluate the ability of an investigational drug to induce the major
881 CYPs, including CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4/5.
882
- 883 • The sponsor should individually evaluate CYP1A2, CYP2B6, and CYP3A4/5 first
884 because they are induced via different nuclear receptors.
885
- 886 • Activation of a nuclear receptor, PXR, may lead to co-induction of CYP3A4/5 and
887 CYP2C enzymes. Thus, a negative in vitro result for CYP3A4/5 induction eliminates the
888 need for additional in vitro or in vivo induction studies for CYP3A4/5 and CYP2C
889 enzymes. If in vitro CYP3A4/5 induction results are positive, the sponsor should
890 evaluate the ability of the investigational drug to induce CYP2C8, CYP2C9, and
891 CYP2C19 either in vitro or in vivo.
892
- 893 • The in vitro system of choice to evaluate enzyme induction should be robust and
894 reproducible and include appropriate clinical inducers and/or non-inducers as positive
895 and negative controls (see the FDA's Web site on Drug Development and Drug
896 Interactions).²⁷ When applicable, the sponsor should conduct pilot experiments to
897 establish a test system (e.g., a particular lot of cryopreserved human hepatocytes) for

²⁷ For more information, see:

<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm>.

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898 routine studies of CYP induction (Fahmi, Kish et al. 2010; Fahmi and Ripp 2010; Einolf,
899 Chen et al. 2014).

- 900
- 901 • The sponsor should investigate drug concentrations that reflect the expected or observed
902 human plasma drug concentrations or intestinal drug concentrations (for CYP3A4/5).
903 Drug concentrations should span the range of therapeutic exposures. If the drug
904 solubility permits, this range of drug concentrations should include at least one drug
905 concentration that is an order of magnitude greater than the maximum unbound steady-
906 state plasma drug concentration in vivo. The sponsor should conduct three replicate
907 experiments per drug concentration. The sponsor should measure the concentration of
908 unbound test drug to help predict the magnitude of clinical DDIs.
909
 - 910 • The sponsor should use hepatocyte preparations from at least three donors. If the result
911 from at least one donor's hepatocytes exceeds the predefined threshold, the sponsor
912 should consider the drug an inducer in vitro and conduct a follow-up evaluation.
913
 - 914 • The sponsor should demonstrate that the experimental approach is capable of identifying
915 the absence and presence of the investigational drug's induction potential and avoids
916 false negative predictions with the selected system and endpoints.
917
 - 918 • Incubation of an investigational drug usually lasts for 48-72 hours to allow complete
919 induction to occur. Incubations include a daily addition of the investigational drug, and
920 the medium containing the drug is changed regularly. The optimal time course for
921 incubation should allow the sponsor to detect enzyme induction without causing cell
922 toxicity. The sponsor should justify the rationale for shorter incubation durations.
923
 - 924 • Data on the actual concentration of drug in the system are important for extrapolating in
925 vitro results to in vivo scenarios. The sponsor should measure concentrations of the
926 parent drug in the medium at several time points during the last day of the incubation,
927 unless loss of the parent drug due to in vitro drug metabolism, degradation, or lysosomal
928 trapping is negligible, or if loss of the parent drug was quantified in the system before the
929 induction assay and compensated for through the amount of drug added or the intervals
930 between medium changes.

931 **B. Evaluating Transporter-Mediated Drug Interactions In Vitro**

932 In vitro transporter assays can determine whether an investigational drug is a substrate or
933 inhibitor of a particular transporter. Coupled with appropriate in vitro-to-in vivo extrapolation
934 methods (see section IV), these assays can determine if the sponsor should conduct an in vivo
935 drug interaction study. Currently, in vitro methods to evaluate transporter induction are not well
936 understood.
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940 1. *General Considerations When Using In Vitro Experimental Systems to Evaluate*
941 *Transporter-Mediated Drug Interactions*

942
943 a. Selecting an in vitro test system

944
945 The sponsor should choose an in vitro test system that is suitable for a specific transporter, such
946 as a membrane vesicle system, a polarized cell-based bidirectional assay for efflux transporters,
947 or a cell-based assay for uptake transporters. Selecting the in vitro model may depend on the
948 purpose of the study and the questions to be addressed. Table 1 summarizes examples of in vitro
949 systems to investigate potential transporter-mediated drug interactions with an investigational
950 drug as either a substrate or an inhibitor of a specific transporter.

951

952 **Table 1. Examples of In Vitro Systems to Investigate Transporter-Mediated Drug**
953 **Interactions**

Transporter	In Vitro Systems
<i>ABC Transporters</i>	
BCRP, P-gp	Caco-2 cells, commercial or in-house membrane vesicles, knock-out/down cells, transfected cells (MDCK, LLC-PK ₁ , etc.)
<i>Solute Carrier (SLC) Transporters</i>	
OATPs	Hepatocytes, transfected cells (CHO, HEK293, MDCK, etc.)
OATs, OCTs	Transfected cells (CHO, HEK293, MDCK, etc.)
MATEs*	Commercial or in-house membrane vesicles, transfected cells (CHO, HEK293, MDCK)

954 CHO: Chinese hamster ovary cell

955 HEK293: human embryonic kidney 293 cell

956 LLC-PK₁: Lewis-lung cancer porcine kidney 1 cell

957 MDCK: Madin-Darby canine kidney cell

958

959 *The function of MATEs depends on the driving force from oppositely directed proton gradient; therefore, the
960 appropriate pH of MATE assay system should be employed.

961

962 Details regarding each in vitro test system to investigate transporter-mediated drug interactions
963 are described below:

964

965 • Membrane vesicles:

966

967 - In vitro systems using inside-out membrane vesicles evaluate whether an
968 investigational drug is a substrate or inhibitor of P-gp or BCRP but may fail to
969 identify highly permeable drugs as substrates.

970

971 - Assays using membrane vesicles should directly measure the adenosine
972 triphosphate (ATP)-dependent, transporter-mediated uptake of drugs.

973

974 • Bi-directional transport assays with cell-based systems:

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- Bidirectional assays evaluate whether an investigational drug is a substrate or inhibitor of efflux transporters such as P-gp or BCRP.
 - Cell monolayers grow on semi-porous filters in a device with apical (AP) and basolateral (BL) chambers.
 - The sponsor should add the test drug to either the AP or BL side of the cell monolayer and measure the amount of the drug permeating through the cell monolayers in the receiver chamber over time.
 - The sponsor should calculate the apparent permeability (P_{app}) of the drug in both the AP→BL (absorption) and BL→AP (efflux) directions and calculate an efflux ratio from the ratio of BL→AP to AP→BL P_{app} values for the substrate.
 - When using transfected cell lines, the sponsor should compare the efflux ratios of the transfected cell line to the parental or empty vector-transfected cell line.
- Uptake assays with cell-based systems:
 - Uptake assays evaluate whether an investigational drug is a substrate or inhibitor of SLC transporters such as OCT, OAT, OATP and MATE.
 - When transfected cell lines are used, the sponsor should compare the drug uptake in the transfected cell line to the parental or empty vector-transfected cell line.
 - The sponsor may use human hepatocytes or hepatic cell lines in suspension, plated, or sandwich-cultured assays.

b. Determining in vitro test conditions

The sponsor should validate the model system and experimental conditions, including culture and transport assay conditions, within the same laboratory. The sponsor should include appropriate positive and negative controls in the test study to ensure the validity of the study's results. The sponsor should consider the following recommendations during assay development and validation:

- The sponsor should develop and optimize transport assays to ensure consistent transporter expression (e.g., mRNA expression, protein expression) and transporter function (e.g., uptake, efflux).

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- 1016 • The sponsor should verify the functionality of the assay by conducting studies with
1017 known positive and negative controls (see the FDA’s Web site on Drug Development and
1018 Drug Interactions²⁸).
1019
- 1020 • The sponsor should characterize the following conditions whenever applicable: the
1021 source of the membrane vesicles or cells, the cell culture conditions (e.g., cell passage
1022 number, seeding density, monolayer age), the probe substrate/inhibitor concentrations,
1023 the incubation time, the buffer/pH conditions, the sampling interval, and the methods for
1024 calculating parameters such as the IC₅₀, K_i, and K_m.
1025
- 1026 • The sponsor should use any organic solvents at low concentrations (< 1% volume/volume
1027 and preferably < 0.5%) because some solvents can affect cell integrity or transporter
1028 function. The experiment should include a no-solvent control and a solvent (vehicle)
1029 control.
1030
- 1031 • For both substrate and inhibitor studies, the sponsor should attempt to assess the impact
1032 of the following factors:
1033
- 1034 - The stability of the test drug for the duration of study
 - 1035 - The effect of nonspecific binding of the test drug to cells/apparatus
 - 1036 - The test drug’s solubility limits
 - 1037 - The effect of prefiltration
 - 1038 - The effect of adding serum protein to the media
 - 1039 - The effect of other experimental steps involved in transport studies
- 1040
- 1041 • The sponsor should conduct transport studies under linear transport rate conditions.
1042
- 1043 • The sponsor should establish laboratory acceptance criteria for study results (e.g.,
1044 monolayer integrity, passive permeability, efflux or uptake of probe substrates, K_m for a
1045 probe substrate, IC₅₀ for probe inhibitor). The K_m value of a probe substrate or the IC₅₀
1046 value of a probe substrate or inhibitor should be comparable to literature-reported values.
1047
- 1048 • The substrate (which may be the test drug) should be readily measured with no
1049 interference from the assay matrix.

2. *Determining if the Investigational Drug is a Transporter Substrate*

²⁸ For more information, see:

<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm>.

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1053 When using in vitro systems to study whether an investigational drug is a substrate of
1054 transporters, the sponsor should consider the following factors:

- 1055
- 1056 • The sponsor should evaluate multiple concentrations of the test drug to cover the range of
1057 clinically relevant concentrations.
- 1058
- 1059 • Several factors may limit test drug concentrations in the in vitro assays, including
1060 aqueous solubility, nonspecific binding to the culture vessel, and cytotoxicity.
- 1061
- 1062 • The sponsor should evaluate the recovery (mass balance), stability, and/or nonspecific
1063 binding of the test drug.
- 1064
- 1065 • If the in vitro system expresses multiple transporters, the sponsor should conduct
1066 additional experiments to confirm the findings with two or more known potent inhibitors.
- 1067

3. *Determining if the Investigational Drug is a Transporter Inhibitor*

1068

1069

1070 When using in vitro systems to study whether an investigational drug is an inhibitor of
1071 transporters, the following should be considered:

- 1072
- 1073 • Test-drug concentrations should generally be as high as possible to maximize the
1074 inhibition effect. However, the drug concentration should not exceed the drug's
1075 solubility limits or cause deleterious effects (e.g., cytotoxicity) in the cells.
- 1076
- 1077 • The sponsor should evaluate approximately four to six concentrations of the test drug
1078 with the probe substrate. The sponsor should start with a high concentration of the test
1079 drug, at least an order of magnitude higher than the drug's clinically relevant
1080 concentration. Because transporters are expressed in different locations in tissues, the
1081 sponsor should consider different clinically relevant concentrations (e.g., the unbound
1082 C_{\max} for renal uptake transporters, unbound maximum hepatic inlet concentration for
1083 hepatic uptake transporters (see Figure 6), or dose/250 mL for apical intestinal
1084 transporters). If the test drug demonstrates inhibitory activity, the sponsor should test
1085 additional concentrations to calculate IC_{50} or K_i values. The sponsor can then compare
1086 these values to clinical plasma or intestinal concentrations to predict the potential for
1087 DDIs.
- 1088
- 1089 • Experiments should include a probe substrate concentration range that results in linear
1090 transport of the substrate. The probe substrate concentration should be at or below its K_m
1091 for the transporter.
- 1092
- 1093 • The sponsor should consider a pre-incubation step with the test drug (for a minimum of
1094 30 minutes) for OATP1B1 and OATP1B3 inhibition to evaluate whether TDI could result

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1095 in a lower IC₅₀ of the test drug. For example, recent data show that cyclosporine and its
1096 metabolite AM1 are time-dependent OATP1B inhibitors (Amundsen, Christensen et al.
1097 2010; Gertz, Cartwright et al. 2013; Izumi, Nozaki et al. 2015).
1098

- 1099 • Inhibition can be substrate dependent; therefore, the sponsor should determine the
1100 inhibition constant of the test drug with a probe substrate that may also be used in later
1101 clinical studies. Alternatively, the sponsor may use a probe substrate that usually
1102 generates a lower IC₅₀ for known inhibitors to avoid underestimating the interaction
1103 potential of the investigational drug.
1104
- 1105 • The sponsor could use positive and negative controls to calibrate their internal in vitro
1106 systems to generate cutoff values to inform potential future clinical DDI studies.
1107

C. Using Model-Based Predictions to Determine a Drug’s Potential to Cause DDIs

1111 Mathematical models can evaluate the results of in vitro and in vivo DDI studies to determine
1112 whether, when, and how to conduct further clinical DDI studies in drug development. In many
1113 cases, negative findings from early in vitro and clinical studies, in conjunction with model-based
1114 predictions, can eliminate the need for additional clinical investigations of an investigational
1115 drug’s DDI potential.
1116

1117 Mathematical models that integrate in vitro findings and are verified with clinical PK data can
1118 play an important role in predicting the DDI potential of an investigational drug under various
1119 scenarios. There are several models to consider when evaluating the drug as a perpetrator of a
1120 metabolism-based DDI. *Basic models* generally serve simple purposes, such as the identification
1121 of low levels of inhibition or induction of metabolizing enzymes by an investigational drug.
1122 *Static mechanistic models* can account for the disposition characteristics of both the perpetrator
1123 and the index substrate drugs (Fahmi, Hurst, et al. 2009). *Dynamic mechanistic models*,
1124 including PBPK models that integrate system-dependent parameters (e.g., based on human
1125 physiology) and drug-dependent parameters (Zhao, Zhang, et al. 2011) and their time course of
1126 changes, can support decisions on when and how to conduct a clinical DDI study. Furthermore,
1127 these models can quantitatively predict the magnitude of DDI in various clinical situations, such
1128 as in patients with renal impairment or patients with genetic deficiencies in certain metabolizing
1129 enzymes.
1130

1. General Considerations When Using Predictive Models to Evaluate Enzyme-Based DDIs

- 1134 a. Basic models to predict the effect of a drug as an enzyme modulator
1135

1136 Evaluating a drug as a potential enzyme inhibitor or inducer begins with the use of a basic
1137 model, which includes the following components:

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1139 • The R value is the ratio of intrinsic clearance values of an index substrate for an
1140 enzymatic pathway in the absence and presence of a potential modulator (perpetrator).
1141 Assuming changes in intrinsic clearance are proportional to those in total clearance, the R
1142 value can be used to represent the AUC ratio of a victim drug in the presence and absence
1143 of a potential modulator (perpetrator). This guidance uses R_1 (including $R_{1,g}$), R_2 , and R_3
1144 for reversible inhibition, time-dependent inhibition, and induction, respectively. The R
1145 value is calculated using the concentration of the interacting drug available at the enzyme
1146 site (defined as [I]) and the appropriate kinetic parameters for each basic model (see
1147 section III)).

1148

1149 • [I] represents the concentration of the interacting drug (potential inhibitor or inducer)
1150 available at the enzyme site. The basic models described in this guidance use the
1151 maximal unbound plasma concentration of the interacting drug for [I]. For CYP3A at the
1152 gut, [I] is I_{gut} , which is calculated as dose/250 mL for the basic model.

1153

1154 • Kinetic parameters are estimates for each basic model (reversible inhibition, time-
1155 dependent inhibition, and induction).

1156

1157 The sponsor should compare the calculated R values to the predefined cutoff criteria to
1158 determine whether it is possible to rule out the potential for a DDI. If the basic model does not
1159 rule out the potential for a DDI, the sponsor should further evaluate the DDI potential of the
1160 investigational drug by conducting additional modeling analyses, using static mechanistic
1161 models or PBPK models (see below) or by conducting an in vivo DDI study.

1162

1163 b. Using static mechanistic models to predict the effect of a drug as an
1164 enzyme modulator

1165

1166 Static mechanistic models incorporate more detailed drug disposition and drug interaction
1167 mechanisms for both interacting and substrate drugs (Fahmi, Hurst, et al. 2009). The following
1168 equation can be used to calculate the overall effect (inhibition or induction) of the investigational
1169 drug on substrate drugs (represented as the AUCR) (see Figure 7).

1170

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1172 **Figure 7: Equation to Calculate AUCR of the Substrate Drugs (AUC_{plus investigational drug}/AUC**
 1173 **minus investigational drug)**

1174

$$AUCR = \left(\frac{1}{[A_g \times B_g \times C_g] \times (1 - F_g) + F_g} \right) \times \left(\frac{1}{[A_h \times B_h \times C_h] \times f_m + (1 - f_m)} \right)$$

1175

1176

1177 **A** is the effect of reversible inhibitions.

1178 **B** is the effect of TDI.

1179 **C** is the effect of induction.

1180 **F_g** is the fraction available after intestinal metabolism.

1181 **f_m** is the fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to
 1182 inhibition/induction.

1183 **Subscripts ‘h’** denote liver.

1184 **Subscripts ‘g’** denote gut.

1185

1186

Each value can be estimated with the following equations:

	Gut	Liver
Reversible inhibition	$A_g = \frac{1}{1 + \frac{[I]_g}{K_i}}$	$A_h = \frac{1}{1 + \frac{[I]_h}{K_i}}$
Time-dependent inhibition	$B_g = \frac{k_{deg,g}}{k_{deg,g} + \frac{[I]_g \times k_{inact}}{[I]_g + K_I}}$	$B_h = \frac{k_{deg,h}}{k_{deg,h} + \frac{[I]_h \times k_{inact}}{[I]_h + K_I}}$
Induction	$C_g = 1 + \frac{d \cdot E_{max} \cdot [I]_g}{[I]_g + EC_{50}}$	$C_h = 1 + \frac{d \cdot E_{max} \cdot [I]_h}{[I]_h + EC_{50}}$

1187

1188 $[I]_h = f_{u,p} \times (C_{max} + F_a \times k_a \times Dose / Q_h / R_B)$ (Ito, Iwatsubo, et al. 1998)

1189 $[I]_g = F_a \times k_a \times Dose / Q_{en}$ (Rostami-Hodjegan and Tucker 2004)

1190 **f_{u,p}** is the unbound fraction in plasma. When it is difficult to measure accurately due to high protein binding (i.e., **f_{u,p}**
 1191 <0.01) in plasma, a value of 0.01 should be used for **f_{u,p}**.

1192 **C_{max}** is the maximal total (free and bound) inhibitor concentration in the plasma at steady state.

1193 **F_a** is the fraction absorbed after oral administration; a value of 1 should be used when the data are not available.

1194 **k_a** is the first order absorption rate constant in vivo; a value of 0.1 min⁻¹ (Ito, Iwatsubo, et al. 1998) can be used
 1195 when the data are not available.

1196 **Q_{en}** is the blood flow through enterocytes (e.g., 18 L/hr/70 kg (Yang, Jamei, et al. 2007a)).

1197 **Q_h** is the hepatic blood flow (e.g., 97 L/hr/70 kg (Yang, Jamei, et al. 2007b)).

1198 **R_B** is the blood-to-plasma concentration ratio.

1199

1200

1201 One should separately use inhibition mechanisms (A and B only) to predict a drug’s enzyme
 1202 inhibition potential (i.e., assuming C is equal to 1), and use induction mechanisms (C only) to
 1203 predict a drug’s enzyme induction potential (i.e., assuming A and B are equal to 1).

1204

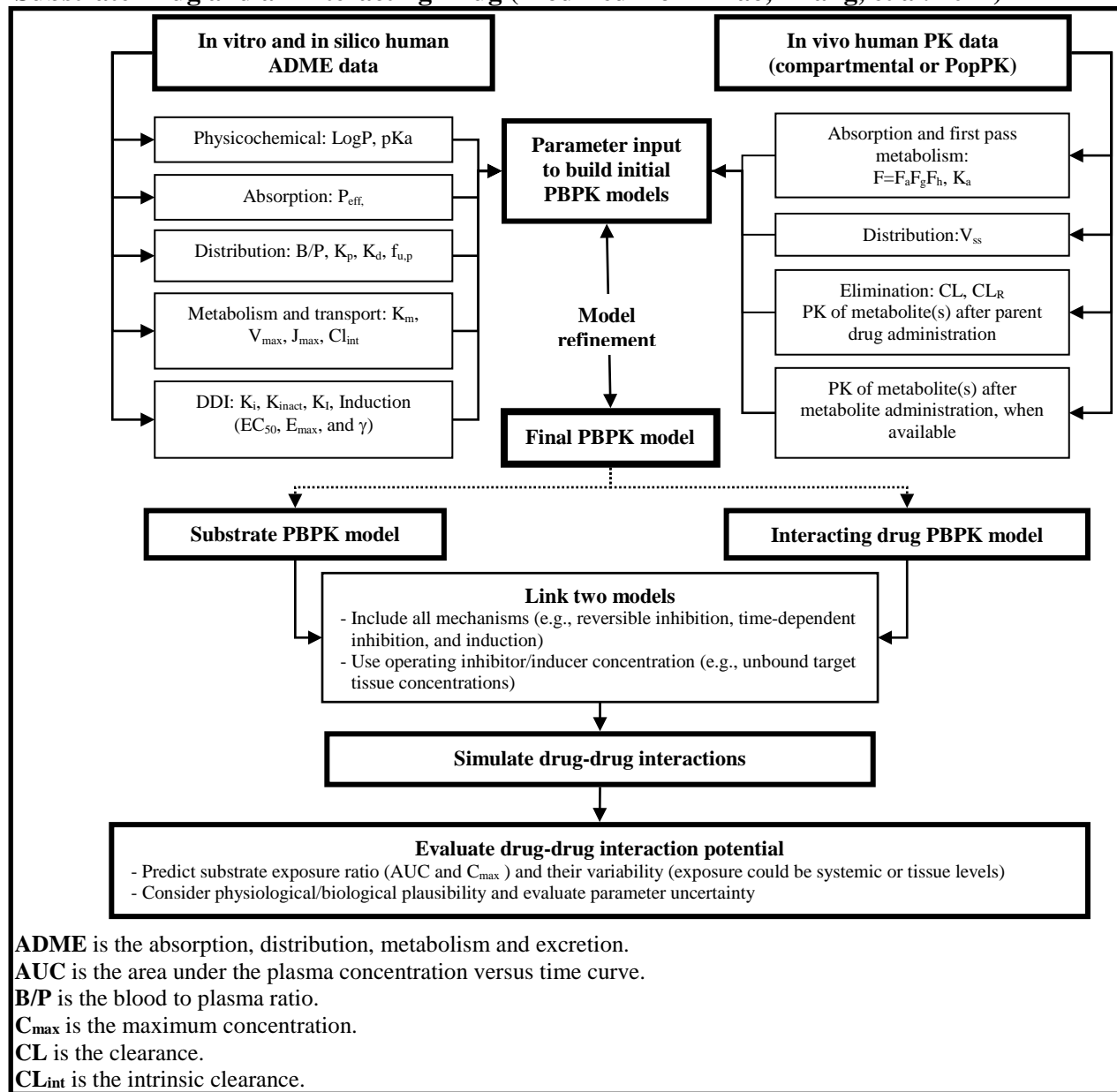
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c. Using PBPK models to predict enzyme-based DDIs

PBPK models can predict the DDI potential of an investigational drug as an enzyme substrate or an enzyme perpetrator. Figure 8 shows a general PBPK model-based framework to predict the DDI potential for the purposes of DDI study planning in clinical development.

Figure 8. A PBPK Model-Based Framework to Explore the DDI Potential Between a Substrate Drug and an Interacting Drug (Modified from Zhao, Zhang, et al. 2011)*



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ADME is the absorption, distribution, metabolism and excretion.
AUC is the area under the plasma concentration versus time curve.
B/P is the blood to plasma ratio.
 C_{max} is the maximum concentration.
CL is the clearance.
 CL_{int} is the intrinsic clearance.

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1221 **Figure 8 continued. A PBPK Model-Based Framework to Explore the DDI Potential**
1222 **Between a Substrate Drug and an Interacting Drug (Modified from Zhao, Zhang, et al.**
1223 **2011)***

1224
1225 **CL_R** is the renal clearance.
1226 **DDI** is a drug-drug interaction.
1227 **EC₅₀** is the concentration causing half maximal effect.
1228 **E_{max}** is the maximum effect.
1229 **F** is the bioavailability.
1230 **F_a** is the fraction absorbed.
1231 **F_g** is the bioavailability in the gut.
1232 **F_h** is the bioavailability in the liver.
1233 **f_{u,p}** is the unbound fraction in plasma.
1234 **γ** is the Hill coefficient.
1235 **IC₅₀** the concentration causing half maximal inhibition.
1236 **I_{max}** is the maximum effect or inhibition.
1237 **J_{max}** is the maximum rate of transporter-mediated efflux/uptake.
1238 **K_a** is the first-order absorption rate constant.
1239 **K_d** is the dissociation constant of a drug-protein complex.
1240 **K_i** is the reversible inhibition constant, concentration causing half maximal inhibition
1241 **K_I** is the apparent inactivation constant, concentration causing half maximum inactivation
1242 **k_{inact}** is the apparent maximum inactivation rate constant.
1243 **K_m** is the Michaelis-Menten constant, substrate concentration causing half maximal reaction or transport
1244 **K_p** is the tissue to plasma partition coefficient.
1245 **LogP** is the logarithm of the octanol-water partition coefficient.
1246 **MOA** is the mechanism of action.
1247 **PD** is the pharmacodynamics of a drug
1248 **P_{eff}** is the jejunum permeability.
1249 **PK** is pharmacokinetics of a drug.
1250 **PopPK** is population pharmacokinetics.
1251 **V** is the volume of distribution.
1252 **V_{max}** is the maximum rate of metabolite formation.

1253
1254 *Note: PBPK models for both substrate and interacting drug (inhibitor or inducer) should be constructed separately
1255 using in vitro and in vivo disposition parameters and be verified before they are linked through appropriate
1256 mechanisms to predict the degree of DDI.

- 1257
1258
- 1259 • When using PBPK modeling, the sponsor should provide comprehensive justifications on
1260 any model assumptions, the physiological and biochemical plausibility of the model,
1261 variability, and uncertainty measures. Submissions using advanced models like PBPK
1262 models should include a description of the structural model, the sources and justifications
1263 for both system- and drug-dependent parameters, the types of error models, all model
1264 outputs, the data analysis, and an adequate sensitivity analysis (see the FDA's guidance
1265 for industry *Physiologically Based Pharmacokinetic Analyses — Format and Content*²⁹).

²⁹ When final, this guidance will represent the FDA's current thinking on this topic.

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1266 When using predefined models (structural and error) from commercially available
1267 software, the sponsor should specify the software version and list any deviations from the
1268 predefined models (Zhao, Rowland, et al. 2012).
1269

1270 • When using PBPK modeling to predict the DDI potential of the investigational drug as an
1271 *enzyme substrate*, the sponsor should address the following questions (Vieira, Kim, et al.
1272 2014; Wagner, Pan, et al. 2015; Wagner, Pan, et al. 2016):
1273

1274 - Can the base PBPK model of the investigational substrate describe the available
1275 clinical PK data using different dosing regimens (e.g., a dose proportionality
1276 study) and dosing routes (e.g., intravenous or oral)?
1277

1278 - Are elimination pathways quantitatively assigned in the substrate's model
1279 according to available in vitro and in vivo data?
1280

1281 - Are index perpetrator models verified with regard to their modulating effect on
1282 enzyme activity in humans?
1283

1284 - Are there sensitivity analyses for parameters exhibiting a high level of
1285 uncertainty?
1286

1287 - If complex metabolic and transport mechanisms are expected, do the substrate and
1288 modulator models include the major disposition and interaction mechanism and
1289 are they verified in a step-wise manner? (see also 2.b below for transporters)
1290

1291 The sponsor may use PBPK models to predict the effects of enzyme modulators on the
1292 PK of an investigational substrate if the models can describe the available data on DDIs
1293 for a strong enzyme inhibitor or inducer (Wagner, Zhao, et al. 2015; Wagner, Pan, et al.
1294 2015; Wagner, Pan, et al. 2016).
1295

1296 • When using PBPK modeling to predict the drug interaction potential of an investigational
1297 drug as an *enzyme perpetrator*, the sponsor should address the following questions
1298 (Vieira, Zhao, et al. 2012; Wagner, Pan, et al. 2015; Wagner, Pan, et al. 2016):
1299

1300 - Can the base PBPK model of the investigational perpetrator describe the available
1301 clinical PK data using different dosing regimens (e.g., a dose proportionality
1302 study) and dosing routes (e.g., intravenous or oral)?
1303

1304 - Are index substrate models verified with regard to the effect of altered enzyme
1305 activity on its PK in humans?
1306

1307 - Were inhibition and induction mechanisms separately considered to ensure a
1308 conservative prediction of in vivo enzyme inhibition or induction?

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- 1309
1310 - Did the simulation use the highest clinical dose of the investigational perpetrator?
1311
1312 - Are there sensitivity analyses for parameters exhibiting high levels of uncertainty?
1313

1314 2. *General Considerations When Using Predictive Models to Evaluate*
1315 *Transporter-Mediated DDIs*

- 1316
1317 a. Using basic models to predict the effect of a drug as a transporter inhibitor
1318

1319 Evaluating a drug as a potential transporter inhibitor begins with the use of a basic model,
1320 applying the same concepts as for metabolizing enzymes (see section IV.B). The predictions
1321 and predefined cutoff criteria from this basic model approach determine the need to further
1322 evaluate transporter inhibition in vivo. Generally, the sponsor should assume reversible
1323 inhibition and use the IC₅₀ as a practical substitute for the K_i. The basic models described in
1324 this document use the gut luminal concentration, the maximal unbound plasma concentration,
1325 and/or the estimated maximal unbound concentration at the hepatic inlet of the interacting
1326 drug for [I]. The sponsor should compare the calculated R or [I]/IC₅₀ values to predefined
1327 cutoff criteria to determine whether it is possible to rule out the potential for a DDI. If the
1328 basic model does not rule out the potential for DDIs, the sponsor should further evaluate the
1329 DDI potential of the investigational drug.

- 1330
1331 b. Using PBPK models to predict transporter-based DDIs
1332

1333 PBPK models can include ADME processes mediated by transporters as well as passive
1334 diffusion and metabolism. Compared to CYP enzymes, the predictive performance of PBPK
1335 modeling for transporter-based DDIs has not been established (Wagner, Zhao, et al. 2015).
1336 This is largely due to knowledge gaps in transporter biology and limited experience in
1337 determining and modeling the kinetics of transporters (Pan, Hsu, et al, 2016). Recent
1338 applications of PBPK models to evaluate the interplay between transporters and enzymes
1339 suggest that a model of an investigational drug as a substrate of multiple transporters and
1340 enzymes is only adequate for confident predictions of untested DDI scenarios after the model
1341 has been verified with clinical data from a wide range of DDI or pharmacogenetic studies for
1342 the applicable enzyme or transporter (Varma, Lai, et al. 2012; Gertz, Cartwright, et al. 2013;
1343 Varma, Lai, et al. 2013; Varma, Lin, et al. 2013; Jamei, Bajot, et al. 2014; Varma, Scialis, et
1344 al. 2014; Snoeys, Beumont, et al. 2015). For drugs that are potential transporter inhibitors,
1345 the sponsor should establish and verify models for transporter substrates (Gertz,
1346 Tsamandouras, et al. 2014; Tsamandouras, Dickinson, et al. 2015; Snoeys, Beumont, et al.
1347 2015).

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1350 **VIII. ABBREVIATIONS AND ACRONYMS**

- 1351
- 1352 ADME: absorption, distribution, metabolism, and/or excretion
- 1353 AP: apical
- 1354 ATP: adenosine triphosphate
- 1355 AUC: area under the plasma concentration-time curve
- 1356 AUC_{metabolite}: area under the plasma concentration-time curve of metabolite
- 1357 AUC_{parent}: area under the plasma concentration-time curve of parent drug
- 1358 AUCR: area under the plasma concentration-time curve ratio
- 1359 BL: basolateral
- 1360 B/P: blood to plasma ratio
- 1361 BCRP: breast cancer resistance protein
- 1362 CHO: Chinese hamster ovary cell
- 1363 Cl_{int}: intrinsic clearance
- 1364 CL_r: renal clearance
- 1365 C_{max}: total maximal concentration in plasma
- 1366 CYP: cytochrome P450
- 1367 d: scaling factor
- 1368 DDI: drug-drug interaction
- 1369 EC₅₀: concentration causing half maximal effect determined in vitro
- 1370 E_{max}: maximum induction effect determined in vitro
- 1371 ER: efflux ratio
- 1372 F_a: fraction absorbed
- 1373 F_g: intestinal availability
- 1374 f_m: fraction of systemic clearance of the substrate mediated by the CYP enzyme that is subject to
- 1375 inhibition/induction.
- 1376 FMO: flavin monooxygenase
- 1377 f_{u,p}: unbound fraction in plasma
- 1378 GFR: glomerular filtration rate
- 1379 HEK293: human embryonic kidney 293 cell
- 1380 [I]: concentration of the interacting drug
- 1381 IC₅₀: half-maximal inhibitory concentration
- 1382 I_{gut}: intestinal luminal concentration estimated as dose/250 mL
- 1383 I_{in,max}: estimated maximum plasma inhibitor concentration at the inlet to the liver
- 1384 I_{max,u}: maximal unbound plasma concentration of the interacting drug
- 1385 J_{max}: maximal flux rate
- 1386 k_a: absorption rate constant
- 1387 k_d: dissociation constant
- 1388 k_p: partition coefficient
- 1389 k_{deg}: apparent first-order degradation rate constant of the affected enzyme
- 1390 K_i: inhibition constant
- 1391 K_I: inhibitor concentration causing half-maximal inactivation
- 1392 k_{inact}: maximal inactivation rate constant

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1393	K_m : Michaelis-Menton constant
1394	k_{obs} : observed (apparent first order) inactivation rate constant of the affected enzyme
1395	LLC-PK1: Lewis-lung cancer porcine kidney 1 cell
1396	LogP: octanol-water partition coefficient
1397	MAO: monoamine oxidase
1398	MATE: multidrug and toxin extrusion
1399	MDCK: Madin-Darby canine kidney cell
1400	MDR1: multi-drug resistance 1 protein
1401	NADPH: nicotinamide adenine dinucleotide phosphate (reduced form)
1402	OAT: organic anion transporter
1403	OATP: organic anion transporting polypeptide
1404	OCT: organic cation transporter
1405	P_{app} : apparent permeability
1406	PBPK: physiologically-based pharmacokinetic
1407	PD: pharmacodynamics
1408	P-gp: P-glycoprotein
1409	PK: pharmacokinetic
1410	pKa: negative logarithm of the ionization constant (K_a) of an acid, a measure of the strength of
1411	an acid
1412	PXR: pregnane X receptor
1413	Q_{en} : blood flow through enterocytes
1414	Q_h : hepatic blood flow rate
1415	R: ratio of victim AUC in the presence and absence of perpetrators (inhibitors or inducers),
1416	predicted with basic models
1417	R_B : blood to plasma ratio
1418	S9: supernatants after 9000 g centrifugation
1419	SCH: sandwich cultured hepatocytes
1420	SLC: solute carrier
1421	TDI: time-dependent inhibition
1422	UGT: uridine diphosphate (UDP)-glucuronosyl transferase
1423	V_{max} : maximal rate
1424	V_{ss} : steady-state volume of distribution
1425	XO: xanthine oxidase

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