

21 July 2022
EMA/CHMP/ICH/652460/2022
Committee for Medicinal Products for Human Use

ICH Guideline M12 on drug interaction studies Step 2b

Transmission to CHMP	06 July 2022
Adoption by CHMP	21 July 2022
Release for public consultation	21 July 2022
Deadline for comments	21 November 2022

Comments should be provided using this [template](#). The completed comments form should be sent to ich@ema.europa.eu



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INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL
REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

DRUG INTERACTION STUDIES
M12

Draft version

Endorsed on 24 May 2022

Currently under public consultation

At Step 2 of the ICH Process, a consensus draft text or guideline, agreed by the appropriate ICH Expert Working Group, is transmitted by the ICH Assembly to the regulatory authorities of the ICH regions for internal and external consultation, according to national or regional procedures.

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M12
Document History

Code	History	Date
M12	Endorsement by the Members of the ICH Assembly under <i>Step 2</i> and release for public consultation.	24 May 2022

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ICH HARMONISED GUIDELINE
DRUG INTERACTION STUDIES
M12
ICH Consensus Guideline

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148 1. INTRODUCTION

149 1.1 Objective

150 This guideline provides recommendation to promote a consistent approach in designing,
151 conducting, and interpreting enzyme- or transporter-mediated in vitro and clinical drug-drug
152 interaction (DDI) studies during the development of a therapeutic product. A consistent
153 approach will reduce uncertainty for pharmaceutical industry to meet the requirement of multiple
154 regulatory agencies and lead to more efficient utilization of resources.

155 1.2 Background

156 In clinical practice, patients are often prescribed more than one drug which can result in a DDI.
157 Some patients, in particular fragile older patients or patients with serious or multiple health issues,
158 can be prescribed a large number of different drugs (i.e., polypharmacy). The occurrence of DDIs
159 is a common clinical problem that can increase the risk of adverse events, sometimes leading to
160 hospital admissions. Alternatively, some DDIs can reduce treatment efficacy. Hence, it is
161 important to consider an investigational drug's potential to interact with other drugs.

162 Regional guidelines for investigations of DDIs have been available for decades and have
163 undergone several updates as scientific progress has been made. In general, the proposed approach
164 to the investigation of interaction potential of investigational new drugs has been similar between
165 regions, but despite harmonization initiatives, some differences have remained. This ICH guideline
166 aims to harmonize recommendations for in vitro and clinical evaluation of DDIs.

167 This guideline provides general recommendations on how to evaluate the DDI potential of an
168 investigational drug. It is recognized that the DDI evaluation is generally tailored based on the
169 specific drug, intended patient population, and therapeutic context. Alternative approaches can be
170 used if they satisfy the requirements of the applicable statutes and regulations. The focus of the
171 guideline is the development of new drugs, but if new scientific information regarding the potential
172 for DDIs is obtained after drug approval, additional DDI evaluation should be considered.

173 1.3 Scope

174 The scope of the guideline is limited to pharmacokinetic interactions, with a focus on enzyme- and
175 transporter-mediated interactions. These aspects in general apply to the development of small
176 chemical molecules. DDI evaluation of biologics is only covered briefly, with focus on monoclonal
177 antibodies and antibody-drug conjugates. Guidance is provided on how to investigate interactions
178 mediated by inhibition or induction of enzymes or transporters, both in vitro and in vivo, and on
179 how to translate the results to appropriate treatment recommendations. The guideline also includes
180 recommendations on how to address metabolite-mediated interactions. The use of model-based
181 data evaluation and DDI predictions are also covered.

182 Other types of pharmacokinetic interactions, e.g., regarding impact on absorption (e.g., gastric pH
183 change, gastric motility change, formation of chelation or complexation, etc.), food effects, or
184 protein binding displacement, are not part of this document and may be covered by regional
185 guidelines. Similarly, DDIs that are a result of pharmacodynamic interactions are beyond the scope
186 of this guideline.

187 **1.4 General Principles**

188 The potential for an investigational drug to cause DDIs should be investigated in a stepwise manner
189 during drug development. The potential for a new drug to cause pharmacokinetic interactions both
190 as a *victim* (effect of other drugs on the investigational drug) and as a *perpetrator* (effect of the
191 investigational drug on concomitant drugs) should be evaluated. All aspects mentioned below are
192 further expanded and discussed later in the document.

193 Evaluating the potential of an investigational drug as a *victim* of a metabolic enzyme- or
194 transporter-mediated DDI involves identification of the principal routes of the drug's elimination.
195 For drugs that are not eliminated predominantly unchanged in urine or that are not biologics
196 eliminated through unspecific catabolism, the keystone of the identification of principal
197 elimination routes is a well performed clinical mass balance study. In some instances, e.g., if a
198 large part of the dose is found as unchanged drug in feces, an absolute bioavailability study can
199 also be a useful complement to aid interpretation. Using data from the mass balance study, the
200 quantitative contributions of the different elimination pathways should be estimated based on the
201 amount of dose excreted as primary and secondary metabolites along specific routes. For
202 quantitatively important elimination pathways, *in vitro* and clinical studies should be used to
203 identify the main enzymes or transporter proteins involved in these pathways. The ability to predict
204 interactions affecting the investigational drug is dependent on the identification of these proteins.

205 Evaluating the DDI potential of an investigational drug as a *perpetrator*, involves characterizing
206 the effect of the drug on enzymes and transporters. This evaluation often starts with *in vitro*
207 experiments to elucidate potential DDI mechanisms. Identification of DDI risks should then be
208 followed by clinical DDI studies based on mechanistic knowledge, and the results should be
209 translated to appropriate clinical management recommendations for drugs as a *victim* and
210 *perpetrator* of DDIs.

211 The results of DDI evaluations inform the protocols for clinical studies in patients regarding the
212 use of concomitant drugs. Information about the interaction potential should be gained as early in
213 drug development as practically possible to assure safety and avoid unnecessary restrictions of
214 concomitant medications and/or exclusion of patients who require the concomitant medications in
215 clinical studies, typically phase 2/3 studies. The timing of the different non-clinical and clinical
216 studies is dependent on the context and type of product; some general recommendations are given
217 below. Predictive modeling (see Section 7.3) can also assist evaluation of the DDI potential.

- 218 • In vitro data on the investigational drug as a substrate of metabolic enzymes generally
219 should be obtained before starting phase 1 (first-in-human) to evaluate metabolic stability
220 and identify the potential main metabolic pathway(s) and enzyme(s) that metabolize the
221 investigational drug (reaction phenotyping studies). If in vitro studies suggest the
222 possibility of clinically significant interaction with inhibitors or inducers of a metabolic
223 enzyme, it is preferable that dedicated clinical DDI studies be conducted prior to studies in
224 patients. Until studies are conducted, a conservative strategy, such as excluding patients on
225 certain concomitant drugs that are inhibitors or inducers, may be needed.
- 226 • The results of the mass balance study should generally be available before starting phase
227 3. Based on results of the mass balance study and in vitro studies, clinical studies with
228 strong index enzyme inhibitors and inducers should be considered to confirm and quantify
229 the main metabolism pathways and define the risk for clinically significant DDIs.
- 230 • ADME (absorption, distribution, metabolism, and excretion) properties determine whether
231 in vitro data of the investigational drug as a substrate for transport proteins should be
232 collected. If a drug has limited absorption or is expected to undergo significant active
233 hepatic uptake, biliary excretion or active renal secretion as unchanged drug, the relevant
234 transporters should be identified in vitro before initiating clinical studies in patients to
235 avoid protocol restrictions.
- 236 • In vitro data on the effects of the investigational drug as a perpetrator on the major
237 cytochrome P450 (CYP) enzymes and transporters should generally be available before
238 administering the drug to patients.
- 239 • The pharmacokinetic DDI potential of metabolites with significant plasma exposure or
240 pharmacological activity should be considered similarly as for the parent drug, but these
241 investigations can generally be completed later in development when more knowledge
242 about the exposure and activity of metabolites is available.

243 2. IN VITRO EVALUATION

244 2.1 Evaluation of Metabolism-Mediated Interactions

245 In vitro studies are important first steps to identify risks for a drug to be a *victim* or *perpetrator* for
246 DDIs through inhibition or induction of drug metabolizing enzymes.

247 2.1.1 Drug as a Substrate of Metabolizing Enzymes

248 Typically, an in vitro screening to identify the main enzymes responsible for the metabolism of a
249 new drug is performed early in drug development. If the mass-balance study suggests metabolism
250 as an important elimination mechanism for the drug, enzymes involved in metabolic pathways
251 which based on the mass-balance study are estimated to contribute to $\geq 25\%$ of drug elimination
252 should normally be identified. This applies to CYP enzymes as well as non-CYP enzymes.

253 If oxidative metabolism is important, the identification of catalyzing enzymes usually starts by
254 determining whether the investigational drug is an in vitro substrate for the most common CYP

255 enzymes involved in drug metabolism: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19,
256 CYP2D6, and CYP3A using in vitro phenotyping experiments. If the drug is not found to undergo
257 significant metabolism by these major CYP enzymes, other enzymes can be investigated. These
258 additional enzymes can include, but are not limited to:

- 259 • Other CYP enzymes, including CYP2A6, CYP2E1 CYP2J2, and CYP4F2 Other phase 1
260 enzymes, including alcohol/aldehyde dehydrogenase (ADH/ALDH), aldehyde oxidase
261 (AO), carboxylesterase (CES), flavin monooxygenase (FMO), monoamine oxidase
262 (MAO), and xanthine oxidase (XO).
- 263 • Phase 2 enzymes: The most frequently evaluated, Uridine 5'-diphospho-
264 glucuronosyltransferase (UDP-glucuronosyl transferases (UGTs)), are responsible for
265 glucuronide conjugation of drugs and metabolites. A phenotyping study is recommended
266 for an investigational drug if it is mainly eliminated by direct glucuronidation. The
267 following UGTs play a role in metabolism of certain drugs: UGT1A1, 1A3, 1A4, 1A6,
268 1A9, 1A10, 2B4, 2B7, 2B10, 2B15, and 2B17 (1).
- 269 • Other phase 2 enzymes, including glutathione S-transferases (GSTs), N-acetyltransferases
270 (NATs), sulfotransferases (SULTs).

271 Details on the experimental setup for in vitro studies to identify enzymes catalyzing the main
272 elimination pathways are given in Sections 7.1.1 and 7.1.2.

273 When the candidate enzymes have been identified in vitro, the main metabolic pathways ($\geq 25\%$
274 of total elimination) generally require additional clinical characterization to determine and quantify
275 the risk of interaction with the investigational drug as a *victim*. This is normally done by
276 performing clinical DDI studies using a strong index inhibitor of the enzyme. For some enzymes,
277 pharmacogenetic studies can substitute for clinical DDI studies (refer to Section 4.1). A clinical
278 study with a strong inducer is also generally conducted, since inducers often up-regulate
279 expression of multiple enzymes and transporters (except CYP2D6, which is generally considered
280 not inducible by drugs).

281 **2.1.2 Drug as an Inhibitor of CYP Enzymes**

282 An investigational drug's potential to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19,
283 CYP2D6, and CYP3A in both a reversible manner (i.e., as reversible inhibitor) and time-dependent
284 manner (i.e., as time-dependent inhibitor (TDI)) should be evaluated. Investigation of potential
285 inhibition of UGT enzymes is further discussed in Section 2.1.3. For details on the experimental
286 setup for these experiments, refer to Sections 7.1.1 and 7.1.3.

287 **2.1.2.1 Reversible Inhibition**

288 In the reversible inhibition experiments, a K_i (inhibition constant) is usually determined
289 experimentally or estimated based on half-maximal inhibitory concentration (IC_{50}) (refer to
290 Section 7.1.3). If the initial experiments testing a sufficiently high concentration of the

291 investigational drug already indicate that the K_i will be markedly higher than the cutoffs given (see
292 below), the risk for clinical inhibition can normally be excluded without further data.

293 The risk for reversible enzyme inhibition can be excluded based on in vitro data (“basic method”)
294 if

$$295 \quad K_{i,u} > 50 \times C_{\max,u} \text{ (i.e., } \frac{C_{\max,u}}{K_{i,u}} < 0.02)$$

296 $K_{i,u}$ is the unbound inhibition constant.

297 $C_{\max,u}$ is the average unbound C_{\max} at the highest recommended dose at steady state.

298 Considering uncertainties in protein binding measurements for highly bound drugs, i.e., >99%
299 protein binding, $f_{u,p}$ (fraction unbound in plasma) should be set at 0.01 (i.e. 1%). It is understood
300 that there have been advances in methodologies to measure $f_{u,p}$ for highly protein bound drugs,
301 and this is an area of active research. Hence, in some situations, the measured $f_{u,p}$ can be used if
302 the accuracy and precision of measurement is demonstrated. Such a demonstration should include
303 full validation data of the protein binding assay including bioanalytical method with appropriate
304 positive controls (i.e., drugs with high binding to relevant plasma proteins). Demonstration of
305 reproducible findings with different assays (e.g., ultrafiltration, equilibrium dialysis,
306 ultracentrifugation) increases the reliability of the $f_{u,p}$ measurement and is preferred. This
307 consideration for $f_{u,p}$ applies in other contexts where basic method, mechanistic static, and dynamic
308 models (often referred as physiologically based pharmacokinetic (PBPK) modeling) can be used
309 to interpret the in vitro results of enzyme and transporter inhibition/induction experiments.

310 For orally administered drugs that are inhibitors of CYP3A, the risk of intestinal CYP3A inhibition
311 can be excluded if

$$312 \quad K_i > 0.1 \times \frac{\text{maximum clinical dose}}{250 \text{ mL}} \text{ (i.e., } \frac{\text{Dose}/250 \text{ mL}}{K_i} < 10)$$

313 If risk for clinical inhibition cannot be excluded using this basic method, mechanistic static and/or
314 PBPK models can be used to interpret the in vitro experiment results (refer to Section 7.3). If in
315 vitro data and modeling do not exclude the risk for clinical inhibition, a clinical DDI study with a
316 sensitive index substrate should be conducted.

317 If a clinical study using a substrate for an enzyme that was inhibited in vitro by an investigational
318 drug with a low K_i shows lack of inhibition, then the risk for clinical inhibition can be excluded
319 for other enzymes having a larger K_i . Such an inference should be made only for the enzymes that
320 are expressed at the same site and for which the inhibition potencies are determined in the same
321 experiment (rank order approach) (2, 3). Of note, an orally administered drug can inhibit intestinal
322 metabolic enzymes (e.g., CYP3A) in addition to hepatic enzymes. In such situations, the risk for
323 inhibition of CYP3A in the gastrointestinal (GI) tract should be considered even if systemic
324 inhibition of CYP3A can be excluded using the rank order approach based on a negative clinical
325 study on another CYP enzyme. In the presence of inhibitory metabolites of an investigational drug,

326 their contribution should also be considered when using rank order approach to determine if
327 clinical studies should be conducted.

328 **2.1.2.2 Time-Dependent Inhibition**

329 If an in vitro assay (described in Section 7.1.3) indicates an increased enzyme inhibition potential
330 with drug pre-incubation, the following equation can be used as the basic method to evaluate the
331 risk for TDI (4-6). The risk for in vivo inhibition can be excluded based on in vitro data if

$$332 \frac{(k_{obs} + k_{deg})}{k_{deg}} < 1.25$$

333

$$334 \text{ where } k_{obs} = \frac{(k_{inact} \times 5 \times C_{max,u})}{(K_{I,u} + 5 \times C_{max,u})}$$

335

336 *k_{obs}* is the apparent first-order inactivation rate constant of the affected enzyme.

337 *k_{deg}* is the apparent first-order degradation rate constant of the affected enzyme (refer to Table 5) ⁽⁷⁻¹⁰⁾.

338 *K_{I,u}* is the unbound inhibitor concentration causing half-maximal inactivation.

339 *k_{inact}* is the maximal inactivation rate constant.

340 *C_{max,u}* is the maximal unbound plasma concentration of the inhibitor drug at steady state. *f_{u,p}* should be set
341 to 1% if experimentally determined to be < 1% (also refer to Section 2.1.2.1).

342 Note: *C_{max,u}* and *K_{I,u}* should be expressed in the same unit (e.g., in a molar concentration unit).

343 If the above ratio is ≥ 1.25 , mechanistic static and/or PBPK models can be used to interpret the in
344 vitro experiment results (refer to Section 7.3). If in vitro data and modeling do not exclude the risk
345 for clinical inhibition, a clinical DDI study with a sensitive index substrate should be conducted.
346 The rank order approach, mentioned above for reversible inhibitors, does not apply to TDIs.

347 **2.1.3 Drug as an Inhibitor of UGTs**

348 It is recognized that a drug which is not a substrate of an enzyme can still be an inhibitor. However,
349 considering the generally limited magnitude of UGT inhibition-mediated DDIs, a routine
350 evaluation of investigational drugs to inhibit UGTs may not be warranted. If direct glucuronidation
351 is one of the major elimination pathways of an investigational drug, it is recommended to study in
352 vitro whether the drug can inhibit UGTs including UGT1A1 and UGT2B7. The evaluation is
353 usually performed using recombinant UGTs or human liver microsome (HLM) with relatively
354 selective substrates (refer to Table 8, Section 7.4.2.1 for an illustrative list of substrates). When an
355 investigational drug is to be used with another drug that is mainly metabolized by direct
356 glucuronidation, it is recommended to evaluate the in vitro potential inhibitory effect of the
357 investigational drug on the UGT isoform(s) responsible for the elimination of the other drug.

358 **2.1.4 Drug as an Inducer of CYP Enzymes**

359 An investigational drug's potential to induce enzymes via activation of nuclear receptors pregnane
360 X receptor (PXR), constitutive androstane receptor (CAR), aryl hydrocarbon receptor (AhR), and
361 if relevant other drug regulation pathways, should be evaluated. For technical advice on the
362 experiments, refer to Section 7.1.4.

363 To assess the DDI liability of a drug as an inducer, studies should be performed in human
364 hepatocytes from at least 3 individual donors and the extent of enzyme induction should be
365 measured at mRNA level. The enzymes CYP3A4, CYP2B6 and CYP1A2 should always be
366 included as markers of induction mediated via PXR/CAR (CYP3A4, CYP2B6) and AhR
367 (CYP1A2). Induction of other enzymes via these pathways can be studied in vitro but sometimes
368 it is challenging to obtain satisfactory sensitivity to get a conclusive result. For CYP2C19, the
369 mRNA responses to inducers are often limited (11, 12), and thus the activity should be measured
370 using a probe substrate to evaluate the CYP2C19 induction potential of the investigational drug.

371 If the in vivo induction potential of CYP3A4 enzymes by an investigational drug can be excluded
372 based on in vitro results, evaluating the induction potential of a drug on CYP2C enzymes is not
373 necessary because both CYP3A4 and CYP2C enzymes are induced via activation of the PXR, and
374 CYP2Cs are generally less inducible compared to CYP3A4.

375 If the investigational drug induces CYP3A4 in vitro, and the results suggest that a clinical study
376 should be conducted, the potential of the investigational drug to induce CYP2Cs should be
377 evaluated in vitro and/or in vivo. Alternatively, a negative clinical study with a sensitive CYP3A
378 substrate can be used to rule out the induction potential of an investigational drug on CYP2C
379 enzymes if the potential of CYP3A inhibition by the drug and its metabolite(s) can be excluded
380 via in vitro and/or in vivo evaluation.

381 As described below, there are several methods that can be used to interpret mRNA data from in
382 vitro induction experiments and to assess the in vivo potential of a drug to induce enzymes. It is
383 recommended to first use the basic qualitative method (mRNA fold-change). If the basic method
384 indicates induction potential, the evaluation can continue using more quantitative approaches (e.g.,
385 correlation methods) provided it is possible to study a wide range of concentrations of the
386 investigational drug to determine induction parameters (e.g., E_{max} and EC_{50}). For the more
387 quantitative approaches, one well-performing, qualified batch of hepatocytes is sufficient. The
388 basic method only uses in vitro data from the investigational drug, whereas correlation methods
389 compare the induction response of the drug to that of multiple established clinical inducers of the
390 enzyme of interest.

391 In addition, mechanistic static or PBPK models can potentially be used (refer to Section 7.3). If a
392 risk for induction cannot be excluded based on in vitro data and modeling, clinical studies with
393 sensitive substrates of the enzymes of interest should be conducted.

394 **2.1.4.1 Basic 'mRNA Fold-Change' Method**

395 The induction results should be evaluated separately for each donor. The levels of mRNA should
396 be compared to the control (vehicle) incubations, and a fold-change over the vehicle control should
397 be calculated. In vivo induction potential cannot be excluded if the drug in hepatocytes from at
398 least one donor meets the following criteria, and further evaluation of the induction potential
399 should be conducted:

- 400 • increases mRNA expression of a CYP enzyme in a concentration-dependent manner; and
401 • the fold-change of CYP mRNA expression is ≥ 2 -fold at $15 \times C_{max,u}$ ($f_{u,p} = 0.01$, if
402 experimentally determined to be $< 1\%$; also refer to Section 2.1.2.1).

403 In addition, the induction potential cannot be ruled out for an investigational drug that increases
404 CYP enzyme mRNA less than 2-fold of the vehicle control but more than 20% of the response of
405 the positive control. Further evaluation is recommended when there is an inconclusive finding,
406 e.g., conducting in vitro testing with hepatocyte from another donor that has ≥ 6 -fold mRNA
407 increase of the CYP enzyme by a positive control.

408 To calculate the percent of the response to the positive control, the following equation should be
409 used:

$$410 \quad \% \text{ of positive control} = \frac{(\text{mRNA fold increase of test drug treated cells} - 1)}{(\text{mRNA fold increase of positive control} - 1)} \times 100$$

411 **2.1.4.2 Correlation Methods**

412 Correlation methods compare the induction effect of the investigational drug to that of established
413 clinical inducers of the enzyme of interest (13-15). The magnitude of a clinical induction effect
414 (e.g., area under the curve (AUC) ratio of sensitive substrate in the presence and absence of
415 inducers) of an investigational drug is predicted based on a calibration curve of relative induction
416 scores (RIS, see equation below) or $C_{max,u}/EC_{50}$ versus the in vivo induction effect for a set of
417 known inducers of the same enzyme (also refer to Section 7.1.4). If the predicted AUC ratio > 0.8 ,
418 the analysis can be used to exclude the risk for in vivo induction.

$$420 \quad RIS = \frac{E_{max} \times C_{max,u}}{EC_{50} + C_{max,u}}$$

419

421 *EC₅₀ is the concentration causing half the maximal effect.*

422 *E_{max} is the maximum induction effect.*

423 *C_{max,u} is the unbound maximum plasma concentration of a drug at steady state, and f_{u,p} is 0.01, if*
424 *experimentally determined to be $< 1\%$.*

425 Sometimes, E_{max} or EC₅₀ cannot be estimated due to an incomplete in vitro induction profile (e.g.,
426 limited by solubility or cytotoxicity of tested drug). An alternative correlation approach can be
427 used if the method is validated (16).

428 **2.1.4.3 Basic Kinetic Model**

429 Mechanistic models have been proposed to predict the sum of different interaction processes
430 (reversible inhibition, TDI, induction) systemically as well as in the GI tract (17). This approach
431 is further discussed in Section 7.3.

432 A limited version of this approach is described as below (18, 19). If $R > 0.8$, the analysis can be
433 used to exclude the risk for in vivo induction.

434

435

$$R = \frac{1}{1 + d \times \frac{(E_{max} \times 10 \times C_{max,u})}{(EC_{50} + 10 \times C_{max,u})}}$$

436

437 *R is predicted AUC ratio of a sensitive enzyme substrate with and without an inducer*

438 *$C_{max,u}$ is the unbound maximum plasma concentration in plasma, and $f_{u,p}$ is 0.01, if experimentally*
439 *determined to be < 1%.*

440 *d - scaling factor (20). If the scaling factor has not been determined in a calibrated hepatocyte batch (see*
441 *Section 7.1.4), $d=1$ should be used.*

442 If the above methods indicate that the investigational drug has the potential to induce metabolizing
443 enzymes (using specific cutoff values mentioned above or developed by individual laboratories
444 for these methods), the enzyme induction potential of the investigational drug should be further
445 investigated by conducting a clinical DDI study with a sensitive index substrate or using
446 mechanistic models (refer to Sections 7.3).

447 **2.1.4.4 Additional Considerations Related to Induction**

448 In vitro induction studies can also detect enzyme down-regulation. However, research in this area
449 is presently very limited, and the mechanisms behind these effects are unclear (11). If
450 concentration-dependent down-regulation is observed in vitro and is not attributable to
451 cytotoxicity, additional in vitro or clinical studies can be considered to understand the potential
452 clinical consequences.

453 **2.2 Evaluation of Transporter-Mediated Interactions**

454 **2.2.1 Drug as a Substrate of Transporters**

455 P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) are efflux transporters
456 expressed in the GI tract and can affect oral bioavailability of drugs. Thus, the possibility of being
457 a substrate of P-gp and/or BCRP is often evaluated in vitro for investigational drugs given orally.
458 Because P-gp and BCRP are also expressed in the liver and kidneys, in vitro study should be
459 considered for a drug if biliary excretion or active renal secretion is likely to be a major elimination
460 pathway of the drug. In addition, if the pharmacological target of the drug is in the brain, evaluating
461 the drug as a substrate of P-gp and BCRP can help determine whether the drug penetrates into the
462 brain (21).

463 Organic anion transporting polypeptide (OATP)1B1 and OATP1B3 are important hepatic uptake
464 transporters that often mediate transport of compounds containing anionic group under
465 physiological pH of systemic circulation. Examination of whether an investigational drug is a

466 substrate for OATP1B1 and 1B3 should be considered if hepatic metabolism or biliary excretion
467 accounts for $\geq 25\%$ of elimination of a drug or if the pharmacological target of a drug is in the liver.

468 Organic anion transporter (OAT)1, OAT3, and Organic cation transporter (OCT)2 are renal uptake
469 transporters. Multidrug and toxin extrusion protein (MATE)1 and MATE2-K are renal efflux
470 transporters. These transporters are often involved in active renal secretion of drugs. In vitro
471 studies to evaluate a drug as substrate of these transporters should be considered if a drug has renal
472 toxicity or the drug clearance by renal active secretion is $\geq 25\%$ of its systemic clearance. Assuming
473 there is no reabsorption (e.g., passive reabsorption is equal to passive secretion and there is no
474 active reabsorption), active secretion can be calculated as $(CL_r - (f_{u,p} \times GFR))$, where GFR is
475 glomerular filtration rate and CL_r is renal clearance. If pharmacokinetic data following intravenous
476 administration are not available, systemic clearance can be derived by multiplying apparent total
477 clearance by estimated bioavailability.

478 Besides the above-mentioned transporters, the importance of in vitro evaluation of a drug as
479 substrate of additional transporters can be decided on a case-by-case basis. For example, multidrug
480 resistance-associated protein (MRP)2 is also an efflux transporter in similar locations as P-gp and
481 BCRP; OATP2B1 is an uptake transporter present in the intestines and is responsible for
482 absorption of certain drugs; and OCT1 is a hepatic transporter mediating the uptake of some drugs
483 into the liver. The decision to evaluate additional transporters can take into consideration the site
484 of action, passive permeability, and knowledge about absorption and elimination pathways of a
485 drug.

486 ***2.2.1.1 Data Analysis and Interpretation***

487 When examining the possibility that an investigational drug is a substrate of transporters, in vitro
488 studies should be performed using experimental systems with the transporter activity confirmed
489 using probe substrates and inhibitors (refer to Tables 10 and 11, Section 7.4.3 for some examples).
490 Further details about considerations when performing in vitro studies are described in Sections
491 7.2.1 and 7.2.2.

492 For uptake studies, if there is significant uptake of a tested drug in transporter-expressed cells
493 relative to the vehicle control-transfected cells (e.g., ≥ 2 -fold than controls) and the uptake in
494 transporter-expressed cells can be inhibited by more than 50% by a known inhibitor of the
495 transporter, the tested drug can be considered a substrate of the transporter examined.

496 For bidirectional efflux studies, if there is significant directional transport of a tested drug in
497 transporter-expressed cells relative to un-transfected or parental cells (e.g., net efflux ratio ≥ 2) or
498 Caco-2 cells (e.g., efflux ratio ≥ 2), and the efflux ratio can be inhibited by more than 50% by a
499 known inhibitor of the transporter, the tested drug can be considered as a substrate of the
500 transporter examined.

501 A cutoff other than 2 or a specific relative ratio to positive controls can be used if prior experience
 502 with the cell system used justifies these alternative methods. Sponsors can also propose criteria for
 503 vesicle assays based on prior experience and internal data.

504 If in vitro studies indicate that a drug is a substrate of a transporter, clinical studies should be
 505 considered. Refer to Section 3.2.5.1 for more details.

506 **2.2.2 Drug as an Inhibitor of Transporters**

507 Studies should be conducted to evaluate whether an investigational drug is an inhibitor of P-gp,
 508 BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K. Sponsors can
 509 consider evaluating the inhibition potential of a drug on other transporters such as BSEP (bile salt
 510 export pump, a hepatic efflux transporter responsible for excretion of bile acids and involved in
 511 bile acid homeostasis), MRP2, OCT1, and OATP2B1 on a case by case basis. In vitro studies
 512 should be performed using an experimental system whose transport activity is confirmed using
 513 probe substrates and inhibitors (see Section 7.4.3 for more details). Considerations about how in
 514 vitro studies should be conducted are described in Sections 7.2.1 and 7.2.3.

515 The risk for transporter inhibition by an investigational drug in humans can be excluded based on
 516 in vitro data using the following basic methods (22-24) . The contribution of drug metabolites to
 517 transporter inhibition should also be considered (see Section 2.3.2).

518 **Table 1: Recommended ratio and cut-off value for drug as inhibitor of transporters**

P-gp or BCRP	K_i or $IC_{50} > 0.1 \times (\text{Dose}/250 \text{ mL})$ (i.e., $(\text{Dose}/250 \text{ mL})/K_i$ or $IC_{50} < 10$) for orally administered drugs
OATP1B1 or OATP1B3	K_i or $IC_{50} > 10 \times C_{\text{max, inlet,u}}$ (i.e., $C_{\text{max,inlet,u}}/K_i$ or $IC_{50} < 0.1$)
OAT1 or OAT3	K_i or $IC_{50} > 10 \times C_{\text{max,u}}$ (i.e., $C_{\text{max,u}}/K_i$ or $IC_{50} < 0.1$)
OCT2	K_i or $IC_{50} > 10 \times C_{\text{max,u}}$ (i.e., $C_{\text{max,u}}/K_i$ or $IC_{50} < 0.1$)
MATE1/MATE2-K	K_i or $IC_{50} > 50 \times C_{\text{max,u}}$ (i.e., $C_{\text{max,u}}/K_i$ or $IC_{50} < 0.02$)

519 $C_{\text{max,u}}$ is unbound maximal plasma concentration of an inhibitor at steady state after therapeutic dose.

520 $C_{\text{max,inlet,u}}$ is estimated unbound maximum plasma concentration of an inhibitor at liver inlet.

521 The $f_{u,p}$ should be set to 1% if experimentally determined to be < 1% (also refer to Section 2.1.2.1).

522 The recommended ratio and cut-off value for P-gp or BCRP is for orally administered drugs. If
 523 the investigational drug is administered parenterally or if it is a metabolite formed post-absorption
 524 that inhibits P-gp or BCRP, K_i or $IC_{50} > 50 \times C_{\text{max,u}}$ (i.e., $C_{\text{max,u}}/K_i$ or $IC_{50} < 0.02$) can be used.

525 The cut-off values in Table 1 were determined based on in vitro-to-in vivo extrapolation analyses.
 526 Since the majority of the in vitro inhibitory potency data in those analyses were IC_{50} , both IC_{50}
 527 and K_i values can be used when applying the basic methods above. However, if the potential for
 528 an interaction is studied further with modeling approaches, K_i should be determined and used. It
 529 is recommended to use substrate concentrations less than K_m for in vitro transporter inhibition

530 experiments. Assuming competitive inhibition, the K_i of an inhibitor approaches IC_{50} when
531 substrate concentration is much less than K_m .

532 The cut-off values described above are based on limited published data. Other cut-off values can
533 be proposed if justified based on in vitro to in vivo extrapolation and a calibration of the specific
534 in vitro systems with known inhibitors and non-inhibitors of these transporter systems.

535 If the above analysis indicates that a drug inhibits a transporter, a clinical study should be
536 considered based on whether the likely concomitant medications used in the indicated patient
537 populations are known substrates of the inhibited transporter and the safety profiles of those
538 substrates. Alternatively, the inhibition potential of a drug can be evaluated using mechanistic
539 static models, PBPK modeling, or endogenous biomarkers. These approaches should be supported
540 by submission of evidence supporting validity of the methods.

541 ***2.2.3 Drug as an Inducer of Transporters***

542 Currently, in vitro methods to evaluate transporter induction are not well established. If an
543 investigational drug has been observed to be an inducer of CYP enzymes via activation of nuclear
544 receptors such as PXR or CAR, it is likely that transporters regulated through these receptors will
545 be induced, such as P-gp. Refer to Section 3.2.5 which describes conducting clinical DDI studies
546 mediated by transporters for more considerations.

547 **2.3 DDI Potential of Metabolites**

548 The assessment of DDI liability of an investigational drug's metabolites often starts with in vitro
549 experiments and generally uses the same strategies as those for parent drugs. As described below,
550 evaluation of the DDI potential of metabolites with significant plasma exposure or
551 pharmacological activities should be considered.

552 ***2.3.1 Metabolite as a Substrate***

553 The risk of DDIs through altered formation or elimination of a metabolite should be investigated
554 if available data indicate that change in metabolite exposure can result in clinically meaningful
555 alteration of efficacy or safety of a drug ("target" as well as "off-target" effects). The enzymes
556 responsible for formation and elimination of a metabolite should be identified in vitro if the
557 metabolite contributes to an in vivo target effect to a similar or greater extent than the parent drug.
558 The contribution to efficacy should be estimated by taking into account unbound metabolite and
559 parent drug exposures (e.g., AUC expressed in molar units) in humans, pharmacological potency
560 (e.g., receptor binding affinity, enzyme inhibitory potency), and if available, data related to target
561 tissue distribution. If the plasma protein binding of the parent drug and the metabolite is high, it is
562 preferred to determine their protein binding in the same study to reduce inter-study variability. In
563 addition, if a metabolite is suspected to cause significant adverse effects based on available
564 nonclinical or clinical information, major enzymes involved in the formation and elimination of
565 that metabolite should be identified if possible. Similar to metabolic phenotyping for parent drugs,

566 the characterization of enzymes involved in metabolite formation and metabolism should also start
567 with major CYP enzymes and can examine other enzymes when appropriate.

568 The general principles described above can also be applied to characterization of a metabolite as a
569 substrate of major transporters, with consideration of the relevance of transporter-mediated
570 distribution or elimination in the disposition of a metabolite.

571 Whether a sponsor should conduct a clinical DDI study with an inhibitor or inducer of an enzyme
572 or a transporter depends on the estimated fraction of formation or elimination of a metabolite
573 mediated by an enzyme or transporter, how much the metabolite contributes to the clinical effect,
574 the exposure-response relationship of the metabolite if known, and likely concomitant medications
575 that affect the enzyme or transporter.

576 ***2.3.2 Metabolite as an Inhibitor***

577 If in vitro assessments suggest that the parent drug inhibits major CYP enzymes and transporters
578 and clinical DDI studies are planned, in vitro assessments of metabolites as enzyme or transporter
579 inhibitors may not be needed because the inhibition potential of metabolites would be implicitly
580 reflected in a clinical DDI study along with the parent drug, unless clinically relevant exposures
581 of the metabolite cannot be adequately represented in the clinical DDI study (i.e., the study
582 duration does not allow the metabolite to accumulate). It is noted that in vitro assessments of
583 metabolites can become useful in interpreting the results of DDI studies.

584 If in vitro assessments suggest that the parent drug alone does not inhibit major CYP
585 enzymes/transporters or is not expected to inhibit enzymes/transporters clinically, DDI liability
586 due to metabolites as inhibitors can still exist. As a pragmatic rule, it is recommended to investigate
587 the CYP enzyme and transporter inhibitory potential of metabolites that have $AUC_{\text{metabolite}} \geq 25\%$
588 of AUC_{parent} and also account for at least 10% of drug-related material in circulation (i.e.,
589 considered as major metabolite often determined based on radioactivity data).

590 Based on the results of in vitro DDI assessments of a metabolite, the determination of whether to
591 conduct a clinical DDI study follows the same approaches as those for the parent drug, except that
592 some metabolites could be irrelevant for the evaluation of intestinal CYP or transporter inhibition.
593 If basic methods suggest that the metabolite(s) could have in vivo DDI liability, and a mechanistic
594 static or PBPK model is then used to evaluate the DDI risk of a drug, metabolite(s) should be
595 incorporated in those models.

596 ***2.3.3 Metabolite as an Inducer***

597 While metabolites can induce CYP enzymes, the in vitro evaluation of the parent drug as a
598 potential inducer could also reflect induction by metabolites because metabolites can be generated
599 during incubation of the parent drug with hepatocytes. However, when the drug is a prodrug or a
600 metabolite is mainly formed extra-hepatically, in vitro evaluation of a metabolite's induction
601 potential on CYP enzymes is recommended if the metabolite is a major metabolite and has

602 $AUC_{\text{metabolite}}/AUC_{\text{parent}} \geq 25\%$. Based on the results of in vitro assessments of the metabolite, the
603 determination of whether to conduct a clinical DDI study follows the same approach as for the
604 parent drug.

605 **3. CLINICAL EVALUATION**

606 **3.1 Types of Clinical DDI Studies (Terminology)**

607 There are different study types that can be conducted to determine the presence or absence of a
608 clinical DDI and the magnitude of the DDI if one exists. The study types described in this section
609 are not mutually exclusive. The specific goal of a study should be considered when determining
610 the type of study to conduct.

611 Regulatory decision-making generally relies upon prospective studies specifically designed to
612 evaluate the potential for DDIs. Retrospective evaluation of drug concentrations from studies not
613 designed to evaluate DDIs rarely includes sufficient accuracy and precision to provide an adequate
614 assessment. DDIs identified or ruled out using a retrospective analysis may need to be confirmed
615 using a prospective evaluation.

616 In some situations, predictive modeling approaches (mechanistic static or PBPK) can be used to
617 translate in vitro results to the clinical setting, without a clinical DDI study. The scenarios and best
618 practice considerations are described in Section 7.3.

619 ***3.1.1 Standalone and Nested DDI Studies***

620 A stand-alone DDI study is a clinical study with the primary objective of determining the presence
621 or absence of a clinical DDI and the magnitude of the DDI. Alternatively, DDIs can be evaluated
622 as part of larger studies in patients (e.g., phase 2/3) for which DDI evaluation is not the primary
623 objective, if the DDI evaluation is prospectively planned and appropriately designed. As such, the
624 DDI evaluation is nested within a larger study (refer to Section 3.2.2 for more details).

625 ***3.1.2 DDI Studies with Index Perpetrators and Index Substrates***

626 *Perpetrators* (inhibitors or inducers) and substrates (*victims*) with well-understood and predictable
627 pharmacokinetic and DDI properties with regard to level of inhibition, induction, or metabolic
628 pathway are known as “index drugs”. The most common purpose of studies conducted with these
629 drugs is to estimate the greatest magnitude of interaction for the studied pathway. For drugs that
630 are evaluated as *victims* of a DDI, the greatest magnitude of interaction generally results from
631 concomitant administration of a strong index inhibitor or inducer of the drug's metabolic
632 pathway(s). For drugs evaluated as *perpetrators* of DDIs, the greatest magnitude of interaction
633 generally results from concomitant administration of the drug with a sensitive index substrate.

634 A distinctive feature of index studies is that the results usually can be extrapolated to other drug
635 combinations. Thus, after conducting a study with an index inhibitor, one can assume that other

636 inhibitors of equal strength for that metabolic pathway will generally have a similar DDI effect
637 size. Additionally, if one concludes that the change in drug exposure following a concomitant
638 strong index inhibitor is not clinically relevant, the same can be concluded for all other inhibitors
639 for that particular metabolic pathway without additional studies. Results from DDI studies with
640 index *perpetrators* or substrates are also used to help design DDI studies with commonly used
641 concomitant medications in the investigational drug's target population.

642 A list of index drugs (either as substrates, inhibitors, or inducers) is presented in Section 7.5.1.

643 Index substrates or *perpetrators* have not been identified for transporters and several metabolic
644 pathways (e.g., CYP2B6, UGTs). The lack of index substrates or *perpetrators* is mainly due to
645 selectivity issues. However, information similar to that provided by studies with index
646 *perpetrators* or substrates (i.e., the likelihood of a DDI due to a specific pathway) is often
647 important. Although index substrates and *perpetrators* have not been identified, Sections 7.5.2 and
648 7.5.3 list drugs that can be useful for DDI studies because they provide informative results and
649 explain the limitations of the drugs. However, extrapolation of results of these studies can be more
650 difficult than extrapolation of results from studies with index drugs.

651 **3.1.3 DDI Studies with Expected Concomitant Drugs**

652 It can be informative to conduct studies that investigate DDIs between the investigated drug and
653 drugs likely to be administered to the target population. These studies can also be considered when
654 a drug is used as an add-on to other therapies or as part of a fixed dose combination. When choosing
655 drugs to evaluate in these studies, sponsors should consider the mechanistic understanding of the
656 potential for DDIs and the relative frequency of co-administration. Results of studies with index
657 drugs can help determine what additional studies should be conducted.

658 Because of a general lack of index substrates or *perpetrators* for transporter-mediated pathways
659 and several metabolic pathways (UGTs; CYP2B6), the choice of transporter substrates or
660 *perpetrators* for DDI evaluation is often based on the likelihood of co-administration.

661 Although these studies are often informative to patients and medical professionals, the results
662 could be difficult to extrapolate to other drugs.

663 **3.1.4 Cocktail Approach**

664 A cocktail study includes the simultaneous administration of substrates of multiple enzymes and/or
665 transporters to study subjects. A cocktail approach can simultaneously evaluate a drug's inhibition
666 or induction potential for multiple enzymes and transporters if the study is properly designed and
667 conducted (refer to Section 3.2.6 for additional details).

668 **3.2 Study Planning and Considerations for Clinical DDI Studies**

669 The objective of most DDI studies is to determine the ratio of a measure of substrate drug exposure

670 (e.g., AUC ratio) in the presence and absence of a *perpetrator* drug. The following considerations
671 are important when designing prospective clinical DDI studies to unambiguously determine this
672 ratio.

673 **3.2.1 Study Design**

674 **3.2.1.1 Study Population and Number of Subjects**

675 Most clinical DDI studies can be conducted using healthy subjects, under the assumption that
676 findings in healthy subjects translate to findings in the intended patient population. However,
677 safety considerations can prevent the use of healthy subjects in studies of certain drugs. For some
678 drugs, use of the intended patient population in DDI studies can allow for evaluation of
679 pharmacodynamic endpoints that cannot be studied in healthy subjects, in addition to PK
680 endpoints.

681 The number of subjects included in a DDI study should be sufficient to provide a reliable estimate
682 of the magnitude and variability of the interaction.

683 **3.2.1.2 Dose**

684 For studies intended to identify the interaction of greatest magnitude, the doses of the *perpetrator*
685 drug used in DDI studies should maximize the possibility of identifying a DDI. Thus, the
686 maximum dose and the shortest dosing interval of the *perpetrator* under the intended conditions
687 of use should generally be evaluated.

688 If the *victim* drug has dose-proportional pharmacokinetics, sponsors can study any dose in the
689 range where exposure to the drug increases in a dose-proportional manner. If the *victim* drug has
690 dose-dependent pharmacokinetics, the therapeutic dose most likely to demonstrate a DDI should
691 be used. When there are safety concerns, lower doses of the *victim* drug can be used.

692 For studies with anticipated concomitant drugs when a clinically significant DDI is anticipated, it
693 can be informative to build a dose adjustment of the *victim* drug into the study to allow
694 identification of doses that can be administered together in clinical practice. In such a scenario, a
695 clinically relevant dose of the *perpetrator* should be used.

696 **3.2.1.3 Single or Multiple Doses**

697 The *perpetrator* drug is often administered in a multiple dose regimen in DDI studies. However,
698 sponsors can evaluate single-dose administration of a perpetrator if the interaction potential is only
699 relevant during absorption (e.g., inhibition of intestinal P-gp or BCRP).

700 In addition, DDI studies can evaluate single-dose administration of a *perpetrator* if the exposure
701 of *perpetrator* following a single dose is representative of exposure at steady-state and if the
702 *perpetrator* is not a potential inducer or time-dependent inhibitor. When studied with a substrate
703 with a long half-life, it may be necessary to administer a *perpetrator* multiple times to cover the

704 full time-course of the substrate exposure. The duration of the treatment with the *perpetrator*
705 should be long enough to cover at least 90% of the plasma concentration-time curve of the *victim*.
706 However, if the *victim* has a very long terminal half-life that does not allow dosing with the
707 *perpetrator* to cover the full AUC, population PK analysis or PBPK analysis can be used to
708 estimate the full interaction effect on the exposure of the *victim*.

709 If a metabolite of the *perpetrator* has demonstrated time-dependent inhibition of the enzyme being
710 evaluated in the DDI study, the duration of the treatment with the parent drug should be sufficient
711 for steady state of the metabolite to be reached.

712 Inducers should be administered as multiple doses to ensure the maximal induction of a specific
713 pathway. It may take about 2 weeks of daily drug administration to achieve the maximum level of
714 induction in a specific pathway. Shorter treatment duration of *perpetrators* can be used with
715 appropriate justification. When there are multiple mechanisms of interactions for a specific
716 *perpetrator*, single-dose administration can be appropriate in certain situations (e.g., evaluation of
717 rifampin as an inhibitor of OATP1B1), while multiple-dose administration can be appropriate in
718 other situations (e.g., evaluation of rifampin as a CYP3A inducer).

719 If the substrate does not demonstrate time-dependent pharmacokinetics, the substrate can be
720 administered as single doses, and the observed magnitude increase in exposure can be extrapolated
721 to steady-state conditions. If the substrate demonstrates time-dependent pharmacokinetics,
722 multiple-dose administration of the substrate and a *perpetrator* should be evaluated.

723 **3.2.1.4 Formulations and Route of Administration**

724 The route of administration of the investigational drug evaluated in DDI studies should generally
725 be the one planned for routine clinical use. When multiple routes of administration are developed
726 for clinical use, the route of drug administration for DDI studies should be selected based on the
727 expected mechanisms of the DDIs and the similarity of the concentration-time profiles for the
728 parent drug and metabolites after different routes of administration.

729 Formulation-related differences in DDIs may also occur. There are several examples of excipients
730 resulting in altered DDIs (25, 26). The possibility of formulation differences in interaction
731 potential should be considered when extrapolating interaction results between formulations (27,
732 28). In general, DDI potential can be extrapolated between formulations by comparing their rate
733 and extent of absorption.

734 **3.2.1.5 Parallel Versus Crossover Studies**

735 Crossover studies (one-sequence or randomized) are preferred over parallel study designs in order
736 to reduce variability. Duration of the washout period should be based on the pharmacokinetics of
737 the substrate and the *perpetrator*, the anticipated impact on the substrate's half-life, and the
738 duration necessary for enzyme activity to return to baseline or for potential pharmacodynamic

739 effects to return to pre-treatment levels (if pharmacodynamic effects are also assessed). In some
740 situations, additional crossover periods can be informative (e.g., to evaluate the time it takes for
741 enzyme activity to return to normal following removal of an inducer or time-dependent inhibitor,
742 to evaluate two drugs that may affect each other (each drug alone and in combination), or to
743 evaluate the effects of acute and chronic treatment of a drug).

744 Parallel, two-arm studies can be appropriate when a crossover study design is not feasible, such as
745 when one of the drugs has a long half-life. Typically, parallel-design studies call for larger sample
746 sizes than crossover studies and subjects should be matched for intrinsic factors likely to affect
747 pharmacokinetics.

748 **3.2.1.6 Timing of Drug Administration**

749 In most DDI studies, the *perpetrator* and *victim* drugs can be administered at the same time.
750 However, the timing of administration of the *perpetrator* is critical if it is both an inhibitor and an
751 inducer. For example, rifampin is an inducer of multiple enzymes and transporters, and also an
752 inhibitor of transporters (e.g., OATP1B and P-gp). If rifampin, after a pre-treatment period, is co-
753 administered with a drug that is a substrate of an inducible enzyme and also OATP1B1 and/or P-
754 gp, the observed exposure change of the *victim* reflects the net effect and underestimates the effects
755 of other inducers that do not inhibit OATP1B1 and/or P-gp. To determine the impact of induction,
756 staggered administration of rifampin with the *victim* (e.g., separated by 24 hours) is recommended.

757 If a large part of an interaction occurs during absorption or first pass, staggered dosing schedules
758 can be studied (clinical study or PBPK) to understand whether such a method is a viable mitigation
759 strategy for the DDI.

760 When evaluating the interaction between drugs that require different food conditions for optimal
761 absorption, the timing of drug administration should be adjusted to maximize the potential to detect
762 an interaction (i.e., index studies) and/or to reflect the clinically relevant conditions (i.e.,
763 concomitant use studies).

764 **3.2.1.7 Co-Medications and Other Extrinsic Factors Affecting DDIs**

765 To reduce variability in the magnitude of DDIs, use of the following should be excluded to the
766 extent possible during DDI studies: other medications, dietary/nutritional supplements, tobacco,
767 alcohol, foods, and fruit juices that may affect the expression or function of enzymes and
768 transporters. The exclusion should begin for a sufficient time before subjects enter the study and
769 continue for the duration of the study.

770 **3.2.1.8 Sample and Data Collection**

771 PK sampling times should be sufficient to characterize the AUC_{0-inf} (for single-dose studies) or the
772 AUC_{0-tau} (for multiple-dose studies) and C_{max} of the substrate drug administered alone and under
773 conditions of the anticipated interaction. Data on additional pharmacokinetic parameters should

774 be collected based on the pharmacokinetic or pharmacological relevance for the proposed
775 indication (e.g., the minimum concentration (C_{\min}), partial AUC). The sampling times for single-
776 dose studies should be planned so that the mean difference between the AUC_{0-t} and the $AUC_{0-\infty}$
777 is less than 20 percent. Samples collected should contain the moieties needed to interpret study
778 results; in most cases, the moiety needed to interpret results will be the parent drug. Metabolite
779 concentrations should be determined if they provide information about the effect of a DDI on
780 safety or efficacy, or if the data inform the mechanism of the drug interaction.

781 **3.2.1.9 Pharmacodynamic Endpoints**

782 When in vitro data provide a plausible DDI mechanism that cannot be evaluated with systemic
783 drug exposure, collection and analysis of pharmacodynamic data can be informative. One possible
784 scenario where this could occur is when transporter inhibition alters access of the drug to specific
785 organs or tissues. In such scenarios, clinical consequences, such as altered efficacy or increased
786 toxicity resulting from altered tissue distribution of a substrate drug, can be measured as
787 pharmacodynamic endpoints, and in vitro evidence of a drug's interaction potential can support
788 data interpretation.

789 **3.2.2 Specific Considerations for Nested DDI Studies**

790 Nested DDI studies are clinical DDI investigations that are part of other studies (e.g., phase 2/3)
791 in which the assessment of DDI is not the primary objective. However, these trials are designed
792 prospectively to investigate DDIs as an exploratory or secondary objective. Nested DDI studies
793 are usually used to evaluate the drug as a *victim* of concomitant drugs and sometimes can also be
794 used to assess the drug as a *perpetrator*. The results of such analyses can be informative, and
795 sometimes conclusive, when the clinical studies are adequately designed to detect significant
796 changes in drug exposure due to DDIs. An advantage of nested DDI studies is the fact that they
797 are conducted in a patient population and may more closely represent the anticipated clinical
798 setting. However, nested DDI studies can also be challenging because they call for careful attention
799 to study design and data collection. In some cases, PBPK modeling can assist the design of nested
800 DDI studies (refer to Section 7.3.2). If large interactions are anticipated that would result in
801 clinically unacceptable risks to subjects, including increased toxicity or decreased efficacy,
802 sponsors should consider whether another approach to DDI evaluation is more appropriate (29).

803 A nested DDI study can evaluate the effect of concomitant drugs that are used for the full duration
804 of the clinical trial or those that are added in response to the patient's condition during the trial.
805 Concomitant drugs to be evaluated should be prespecified. The drugs are typically selected
806 because there is a mechanistic reason to anticipate an interaction. Relevance in the patient
807 population is also a consideration. The study design can specify individual drugs or a grouping,
808 based on mechanism (e.g., strong CYP3A inhibitors) (30). However, if a grouping is evaluated it
809 is important to consider the potential for differences in the effect of different drugs in the group
810 and the effect of the potential variability on data analysis and translation of the findings.

811 Simulations can be used to determine the appropriate number of PK samples and to assist in the
812 selection of sampling times. A power analysis can also be performed to estimate the minimum
813 effect size that is likely to be detected with acceptable precision in a study using a given number
814 of patients on a concomitant drug.

815 Collection of the following data is critical to ensure interpretable results: timing of drug
816 administration (investigational drug and concomitant drug), drug dose, timing relative to food
817 (when relevant), other concomitant drugs, and PK sampling date and time (actual, not scheduled).
818 It is also important to document the start date of the concomitant drug relative to when an
819 interaction will be observed, particularly when the concomitant drug is an inducer or time-
820 dependent inhibitor.

821 Nested DDI studies are typically evaluated using population PK analysis, which should be
822 performed according to well-established scientific practice using a model that is validated in
823 relation to its purposes. The population PK analysis plan for the DDI assessment should be
824 established prior to conduct of the study. In general, the standard analysis approach is a binary
825 evaluation that includes the concomitant drug as a static categorical covariate. Sponsors should
826 consider whether their selected analysis methods will provide the desired level of precision in DDI
827 evaluation. Regardless of analysis method, all assumptions should be stated.

828 In some instances, unplanned analyses of potential DDIs in phase 2/3 trials are conducted to
829 explain clinical study results, such as safety or efficacy issues in a group of patients, or to screen
830 for potential DDIs not anticipated at the time the trials were designed. If the data collected meet
831 the criteria described above, it can be possible to draw conclusions about the presence or absence
832 of an interaction. In situations where the data do not permit an accurate assessment of a DDI, a
833 confirmatory evaluation of the DDI should be conducted.

834 ***3.2.3 Considerations for CYP-Mediated Interactions***

835 ***3.2.3.1 The Investigational Drug as a Substrate for CYP Enzymes***

836 When evaluating the investigational drug as a substrate, the first clinical DDI studies should, in
837 general, determine the effects of a strong index inhibitor and a strong index inducer on the
838 investigational drug. Moderate index inhibitors or inducers can be used if strong index inhibitors
839 or inducers are not available for a particular enzyme. Some of these inhibitors and inducers can
840 also affect other metabolism and/or transporter pathways; thus, when selecting index inhibitors
841 and inducers for prospective DDI studies, all metabolic and transport pathways of the
842 investigational drug should be considered. Studies with other strong inhibitors and inducers of
843 CYP enzymes can also be appropriate, considering the criteria listed in Section 7.5.1. If the
844 investigational drug is a substrate for multiple enzymes and/or transporters, measuring metabolites
845 can, in some cases, help with the interpretation of study results and interacting mechanisms.

846 If a DDI study with a strong index inhibitor or inducer indicates no DDI is present, additional
847 clinical studies with other inhibitors or inducers of the same enzyme are not needed. However, as
848 a negative DDI study may reveal that the enzyme proposed to be the major metabolizing enzyme
849 based on in vitro data is not contributing to the elimination of the drug, this may instead indicate
850 that further clinical investigations with strong inhibitors of alternative candidate enzymes should
851 be conducted.

852 If a DDI study with strong index inhibitors or inducers indicates that there is a clinically relevant
853 interaction, evaluating the impact of moderate inhibitors or inducers can be useful to gain a full
854 understanding of the investigational drug's DDI potential. The evaluated moderate inhibitors and
855 inducers may be anticipated concomitant medications in the intended patient population. The effect
856 of the additional inhibitors and inducers can be evaluated in a clinical interaction study, or, in some
857 cases, modeling approaches can provide additional information (refer to Section 7.3). If it is
858 anticipated that co-administration with strong inducers or inhibitors should be avoided, a DDI
859 study with a moderate inducer or inhibitor may be preferable as the initial study.

860 If the investigational drug is subject to significant metabolism by a genetically polymorphic
861 enzyme for which a well-defined poor metabolizer phenotype exists that results in non-functional
862 enzyme activity, a comparison of the pharmacokinetic parameters of the drug in individuals with
863 the poor metabolizer phenotype versus those with a normal metabolizer phenotype can substitute
864 for an interaction study for that particular pathway (refer to Section 4.1).

865 ***3.2.3.2 The Investigational Drug as an Inhibitor or an Inducer of CYP Enzymes***

866 When studying an investigational drug as a potential inhibitor or inducer of a CYP enzyme, the
867 index substrate selected for the initial clinical studies should be sensitive to changes in activity or
868 amount of the CYP enzyme being evaluated (refer to Section 7.5.1). Because some substrates are
869 not specific for one CYP enzyme and sometimes are also substrates of transporters, the most
870 appropriate substrate should be selected considering the inhibitor/inducer characteristics of the
871 investigational drug, based on available in vitro and clinical data. Other CYP enzyme substrates
872 can also be appropriate. If the substrate drug is metabolized by more than one enzyme, measuring
873 metabolites sometimes can help with interpretation of study results.

874 If the initial study with the most sensitive index substrates is negative, studies with less sensitive
875 substrates of the enzyme are not needed. If an initial study determines that an investigational drug
876 either inhibits or induces the metabolism of sensitive index substrates, further studies using other
877 substrates (e.g., relevant co-medications) can be useful. The magnitude of the effect of the
878 investigational drug on the sensitive index substrate and the potential for concomitant use with
879 other drugs that are substrates of the same enzyme should be considered.

880 If the investigational drug is both an inducer and an inhibitor of an enzyme, the net effect of the
881 drug on enzyme function may be time dependent. The timing of pharmacokinetic endpoints should

882 permit an understanding of the changes in effects over time, when relevant (31). To achieve this
883 understanding, the pharmacokinetics of the *victim* drug should be evaluated at early and late time
884 points during the administration of the investigational drug in the test period. The effect of
885 reversible inhibition may be more pronounced in the beginning of the treatment and the induction
886 may be most pronounced after ending the treatment.

887 ***3.2.4 Considerations for Evaluation of UGT-Mediated Interactions***

888 ***3.2.4.1 Investigational Drug as a Substrate of UGTs***

889 Based on limited literature evidence, the magnitude of DDI mediated through inhibition of UGTs
890 (reflected by AUC ratio of a substrate in the presence of an inhibitor compared to no inhibitor)
891 rarely exceeds 3-fold and is often around 2-fold or less (32). For an investigational drug that is
892 mainly eliminated by direct glucuronidation, clinical DDI studies with UGT inhibitors should be
893 conducted on a case-by-case basis, considering the safety profile of the drug and the likelihood of
894 its concomitant use with inhibitors of that UGT isoform (refer to Table 16, Section 7.5.2 for some
895 examples of UGT inhibitors). Some UGT substrates are also substrates of other enzymes or
896 transporters, and the interaction with a UGT inhibitor may involve other mechanisms when the
897 UGT inhibitor also affects those enzymes or transporters. Thus, it may be valuable to also measure
898 the glucuronide conjugate concentrations in addition to the UGT substrate itself. The change of
899 glucuronide metabolite relative to the parent drug may provide insight into the underlying
900 mechanism of interaction. In addition, some glucuronide metabolites are active or reactive and
901 may significantly contribute to efficacy or safety of a drug. In such cases, the concentrations of
902 glucuronide conjugates should be measured in addition to parent drug concentrations.

903 Genetic variation in certain UGT enzymes (for example, UGT1A1, UGT2B7, and UGT2B15) has
904 been reported to contribute to variation in the pharmacokinetics of drugs metabolized by UGTs.
905 In certain cases, comparative PK data in subjects with various UGT genotypes can be used to
906 identify the importance of the UGT pathway(s) in the elimination of a drug in vivo and to estimate
907 the extent of DDI with inhibitors of UGT.

908 In addition, UGTs can also be induced, for example, by certain PXR agonists (e.g., moderate or
909 strong CYP3A inducers). The impact of inducers on an investigational drug that is mainly
910 metabolized by UGTs should also be considered and evaluated depending on the likelihood of its
911 concomitant use with UGT inducers and the dose/exposure-efficacy relationship of the
912 investigational drug.

913 ***3.2.4.2 Investigational Drug as an Inhibitor of UGTs***

914 Due to the limited availability of data from clinical DDI studies that evaluate inhibition of UGT
915 isoenzymes, cutoffs for determining DDI risk using basic models like those for CYP enzymes have
916 not been established. This is an area of ongoing research, and in the interim, sponsors can consider
917 the same criterion as the one applied to CYPs (i.e., compare $C_{\max,u}/K_{i,u} < 0.02$), or propose an

918 alternative with justification. A decision on whether to perform a clinical DDI study to evaluate
 919 the effect of a drug as a UGT inhibitor should also take into consideration the likelihood of the
 920 drug's concomitant use with known substrates of the UGT isoform (refer to Table 15, Section 7.5.2
 921 for examples) and the safety profiles of those substrates.

922 **3.2.4.3 Investigational Drug as an Inducer of UGTs**

923 There is limited understanding about gene expression of UGTs. However, limited clinical DDI
 924 studies indicate certain UGTs may be induced by agonists of PXR and/or CAR, which also regulate
 925 CYP3A4 expression. UGTs are less inducible than CYP3A4. Thus, for a drug found to induce
 926 CYP3A4 in vitro and further evaluated with a clinical DDI study, the effect of the drug on CYP3A4
 927 substrates may inform its potential induction effect on UGTs. If a drug reduces the AUC of a
 928 sensitive substrate of CYP3A by $\geq 50\%$, a further clinical DDI study can be conducted with the
 929 drug and a UGT substrate, depending on the magnitude of exposure change of the CYP3A
 930 substrate, the likelihood of concomitant use of the investigational drug with UGT substrates,
 931 whether there are other enzymes/transporters involved in the pharmacokinetics of UGT substrates
 932 which can also be regulated by PXR/CAR agonists, and the dose or exposure-efficacy relationship
 933 of those UGT substrates. It is noted that some CYP3A4 inducers have their induction effect
 934 overridden by their inhibition effect on CYP3A. Thus, while those drugs inhibit CYP3A4 in
 935 clinical studies, they may exhibit induction effects on UGTs.

936 **3.2.5 Considerations for Evaluation of Transporter-Mediated Interactions**

937 **3.2.5.1 Investigational Drug as a Substrate of Transporters**

938 If in vitro studies indicate that the investigational drug is a transporter substrate, sponsors should
 939 determine whether to conduct clinical DDI studies based on the drug's passive permeability, route
 940 of administration, in vivo absorption and elimination, putative site of action, safety profile, dose
 941 or exposure-response (efficacy and safety) relationship, and likely concomitant drugs that are
 942 known inhibitors or inducers of the transporters. The following general guidelines in Table 2 help
 943 to determine when a clinical DDI study is generally recommended for investigational drugs that
 944 are transporter substrates in vitro:

945
 946 **Table 2: Consideration for clinical evaluation of drug as substrate of transporters**

Transporters	When a clinical DDI study is generally recommended
P-gp and BCRP	When intestinal absorption is limited, or biliary excretion/active renal secretion is a major elimination pathway.
OATP1B1 and OATP1B3	When hepatic/biliary elimination is a significant clearance pathway ($\geq 25\%$) for the investigational drug or the action site of the drug is in liver, and the drug's properties support the importance of active uptake of the drug into the liver.

OAT1 and OAT3, OCT2, MATE1, and MATE2-K	When the investigational drug undergoes significant active renal secretion (i.e., accounting for $\geq 25\%$ of systemic clearance) or there are concerns about renal toxicity
---	--

947 When evaluating an investigational drug as a *victim* in transporter-mediated DDIs, the selected
948 *perpetrator* drug should be a known inhibitor of the transporter under investigation. Because of a
949 general lack of index *perpetrators* for transporter-mediated pathways, the choice of transporter
950 *perpetrator* is typically based on the likelihood of concomitant use (e.g., to obtain clinically
951 relevant DDI information that can inform labeling regarding the management of a DDI).

952 Transporter inhibitors can be used to understand the underlying mechanisms of DDIs or to
953 determine the anticipated largest magnitude DDI. If in vitro studies indicate a drug is a substrate
954 of multiple transporters, a clinical study can be conducted with a broad inhibitor of multiple
955 transporters to determine the anticipated largest magnitude DDI. For example, cyclosporine, which
956 inhibits intestinal P-gp and BCRP and hepatic OATPs, can be used as the inhibitor in a DDI study.
957 Negative results from this kind of study may rule out the need to further evaluate the drug as a
958 substrate for any of the individual transporters. If the study result is positive, additional studies
959 with more selective inhibitors of specific transporter pathways can be conducted to determine the
960 impact of inhibition of each transporter on the disposition of the substrate drug. The same paradigm
961 can apply to an investigational drug that is a substrate for both transporters and metabolic enzymes
962 (e.g., CYP3A and P-gp).

963 If the goal of the study is to determine the role of a specific pathway in the pharmacokinetics of a
964 substrate drug and resulting DDIs due to that pathway, then a more selective inhibitor should be
965 used. Use of these inhibitors in clinical studies can provide a mechanistic understanding of
966 transporter-mediated DDIs. Some transporters, including OATP1B1 and BCRP, are encoded by
967 genetically polymorphic genes (SLCO1B1 and ABCG2, respectively) for which phenotypes with
968 reduced functionality exist. Similar to drugs that are substrates of CYPs encoded by polymorphic
969 genes, the relative contribution of a specific transporter to the disposition of the investigational
970 drug can be evaluated in subjects with different transporter genotypes (refer to Section 4.1).

971 Examples of transporter inhibitors are listed in Section 7.5.3.2. Many of them not only inhibit the
972 specified transporters but also can inhibit other transporters and/or CYP enzymes. Thus,
973 extrapolation of results from transporter inhibition studies to other drugs can be challenging.
974 Interpretation of the study results should consider the knowledge of transport and metabolic
975 pathways for the investigational drug.

976 **3.2.5.2 Investigational Drug as an Inhibitor of Transporters**

977 If in vitro studies indicate that the investigational drug is a transporter inhibitor, the determination
978 of whether to conduct a clinical DDI study should be based on likely concomitant drugs and safety
979 considerations. When studying the investigational drug's potential to act as an inhibitor drug for a
980 transporter, a substrate drug whose pharmacokinetic profile is markedly altered by

981 coadministration of known inhibitors of that transporter and is also a likely concomitant drug is
982 preferred. Some examples of transporter substrates that can be used in DDI studies are listed in
983 Section 7.5.3.1. Because many drugs are substrates of multiple transporters and/or enzymes, the
984 observed clinical interactions can be a result of the modulation of multiple pathways if the
985 investigational drug is also an inhibitor or inducer for those pathways. Extrapolation of results
986 from these studies to other drugs can thus be challenging. The choice of substrates can be
987 determined by the therapeutic area of the investigational drug and the likely concomitant drugs
988 that are known substrates of the transporters.

989 In some cases, an alteration in drug transport may not be fully reflected by changes in plasma
990 concentrations alone. Therefore, measurement of metabolite or pharmacodynamic markers to
991 reflect altered distribution to the organs expressing the transporter should be included to interpret
992 the potential for an interaction.

993 Recent literature reports indicate potential utility of endogenous substrates for some drug
994 transporters (33-37). Evaluating the change in exposure of the endogenous substrate when the
995 investigational drug is administered may provide information regarding the drug's potential as a
996 transporter inhibitor.

997 ***3.2.5.3 Investigational Drug as an Inducer of Transporters***

998 Since P-gp is co-regulated with CYP3A, for example by agonists of PXR and/or CAR, but is less
999 inducible than CYP3A (38, 39), if an investigational drug reduces the AUC of a sensitive substrate
1000 of CYP3A by 50% or more (i.e., being a moderate or strong inducer), a further clinical study to
1001 evaluate potential induction effect of the drug on P-gp substrates should be considered, taking into
1002 account the following factors: the magnitude of CYP3A substrate AUC change by the
1003 investigational drug, the likelihood of concomitant use of the drug with P-gp substrates, whether
1004 there are other enzymes/transporters involved in the pharmacokinetics of P-gp substrates which
1005 can also be regulated by PXR and/or CAR agonists, and the dose or exposure-efficacy relationship
1006 of P-gp substrates. It is noted that some CYP3A4 inducers have their induction effect overridden
1007 by their inhibition effect on CYP3A. Thus, while those drugs inhibit CYP3A4 in clinical studies,
1008 they may exhibit induction effects on P-gp. Sponsors should also consider whether to conduct
1009 clinical DDI studies to evaluate the potential effect of a drug on other transporters regulated
1010 through the same pathways as CYP3A.

1011 ***3.2.6 Cocktail Studies-Considerations for CYP or Transporter Cocktail Studies***

1012 A cocktail approach can simultaneously evaluate a drug's inhibition or induction potential for
1013 multiple CYPs and transporters if the study is properly designed. Ideal conditions for the cocktail
1014 study are: (1) the substrates are specific for individual CYP enzymes or transporters; (2) there are
1015 no interactions among the substrates; and (3) the study is conducted with a sufficient number of
1016 subjects. If the first two conditions are not met, the lack of specificity or the interaction among
1017 substrates should be understood and incorporated into the study results interpretation. Negative

1018 results from a well-conducted cocktail study can eliminate the need for further evaluation of
1019 particular CYP enzymes or transporters. Positive results from a well-conducted cocktail study that
1020 includes all elements of a prospective DDI study can be interpreted the same way as positive results
1021 from any other well-conducted DDI study. It should be noted that findings obtained with a
1022 microdose of a substrate cannot always be extrapolated to a therapeutic dose of that substrate.

1023 **4. OTHER TOPICS**

1024 **4.1 Pharmacogenetics**

1025 Pharmacogenetic variations in genes encoding drug metabolizing enzymes or drug transporters
1026 can affect the pharmacokinetics of a drug, increase interindividual variability in drug exposure,
1027 affect safety or efficacy, and alter the magnitude of DDIs. Important pharmacogenes include those
1028 that encode phase 1 (e.g., CYP2C9, CYP2C19, CYP2D6) and phase 2 (e.g., NAT2, UGT1A1)
1029 drug metabolizing enzymes as well as genes that encode drug transporters (e.g., BCRP,
1030 OATP1B1). Polymorphisms in metabolizing enzymes can lead to increased, normal, decreased, or
1031 absent enzyme activity resulting in ultra-rapid (UM), normal or extensive (NM or EM, hereafter
1032 referred to as NM), intermediate (IM), and poor (PM) metabolizers, respectively. Polymorphisms
1033 in drug transporters can increase or decrease transport of a drug across membranes. These drug
1034 metabolizing enzyme and transporter polymorphisms can affect the systemic or tissue
1035 concentrations of a drug and/or its metabolite(s).

1036 The scope of this section is limited to the evaluation of the impact of pharmacogenetics on DDIs
1037 and on DDI evaluation. While the considerations described below use metabolizing enzymes as
1038 examples, the concept can also be applicable to transporters with polymorphisms.

1039 If an investigational drug is a substrate/inhibitor for a polymorphic enzyme and a DDI study with
1040 an index inhibitor/substrate is conducted to evaluate pharmacokinetic changes, it is recommended
1041 to prospectively characterize the subject's genotype. Exclusion of PMs is recommended, to allow
1042 characterization of the greatest magnitude of interaction. If PMs are not excluded, the DDI effect
1043 should be evaluated separately in subjects with different phenotypes (e.g., PM, IM, and NM), as
1044 relevant.

1045 If an investigational drug is subject to significant metabolism by an enzyme with a well-defined
1046 PM phenotype (for example, CYP2D6, CYP2C19), exposure in PM is expected to be similar to
1047 the effect of a strong inhibitor of that pathway. A comparison of the pharmacokinetic parameters
1048 of the drug in individuals with the PM phenotype with those with a NM phenotype can substitute
1049 for a DDI study of that pathway with a strong inhibitor. Similarly, the exposures in subjects with
1050 a polymorphic PM phenotype could be estimated using the results of an in vivo DDI study with a
1051 strong inhibitor. If there is a significant difference in exposure between individuals with the PM
1052 and NM phenotypes, further studies to evaluate the DDI potential with moderate inhibitors or
1053 inducers of the specific enzyme should be considered.

1054 When an enzyme encoded by a polymorphic gene is one of two major elimination routes of an
1055 investigational drug, the interaction effects of inhibiting the other enzymes is expected to vary in
1056 different phenotypes of the polymorphic enzyme. In a DDI study evaluating the impact of
1057 inhibitors of the other enzyme, prospective genotyping and enrichment of subjects with absent or
1058 decreased function of the polymorphic gene besides NM subjects can help assess the interaction
1059 effects in the various phenotypes. Because the DDI magnitude may become large in PMs or IMs
1060 of the polymorphic enzyme when combined with an inhibitor of a parallel pathway, depending on
1061 the safety profile of the drug, different doses should be considered in those subjects. PBPK
1062 modeling can be useful to supplement such studies or to extrapolate the interaction effects in
1063 different genotypes (refer to Section 7.3.2).

1064 A retrospective pharmacogenetic analysis can help elucidate reasons for a high variability in a DDI
1065 study. When study enrollment is not based on the genotype of a polymorphic metabolizing enzyme
1066 or transporter, a retrospective analysis of the metabolizing enzyme or transporter of interest can
1067 help to characterize differences in the magnitude of the DDI across genotype groups and explain
1068 why some subjects have unanticipated increases or decreases in drug concentrations.

1069 Guidance on DNA sample collection for prospective and retrospective pharmacogenetic analysis
1070 can be found elsewhere (40, 41). As the frequency of certain pharmacogenetic variations can vary
1071 across populations, when performing pharmacogenetic analysis, an individual's race/ethnicity
1072 should be considered. In addition, regional regulations on sampling and analyzing human derived
1073 materials need to be followed.

1074 **4.2 Therapeutic Protein DDIs**

1075 In general, the risk of pharmacokinetic DDIs is lower for proteins. The in vitro assays that are
1076 applicable for small molecules are generally not applicable to proteins.

1077 When evaluating the potential for a DDI between monoclonal antibodies and small molecules or
1078 between monoclonal antibodies, the mechanisms of a potential DDI should be considered, taking
1079 into account the pharmacology and clearance of the monoclonal antibodies as well as any co-
1080 administered medications in the patient population.

1081 ***4.2.1 Proinflammatory Cytokine-Related Mechanism***

1082 Certain therapeutic proteins may exert an indirect effect on expression of CYP enzymes and thus
1083 affect the pharmacokinetics of small molecules. Therapeutic proteins that are proinflammatory
1084 cytokines (e.g., peginterferon) or that can increase cytokine levels can down-regulate the
1085 expression of CYP enzymes, thereby decreasing the metabolism of drugs that are CYP substrates
1086 and increasing their exposure levels. The increase in cytokine levels as a result of drug treatment
1087 can be transient or persistent; sponsors should consider this increase when determining whether to
1088 conduct a DDI study as well as the design of that study.

1089 Conversely, therapeutic proteins that reduce the elevated cytokine levels (e.g., inhibitors of tumor
1090 necrosis factor) can relieve the CYP down-regulation from an inflammatory environment (e.g.,
1091 rheumatoid arthritis), thereby increasing CYP expression and activity and reducing exposure for
1092 CYP substrates.

1093 If the investigational drug is a cytokine or a cytokine modifier, sponsors should consider whether
1094 to perform a clinical DDI study to evaluate the effects of the investigational therapeutic protein on
1095 sensitive substrates for CYP enzymes. Known drug effects on metabolism in disease states with
1096 similar or higher inflammatory burden, differences in exposure levels of sensitive CYP substrates
1097 in healthy subjects versus patients in the indicated population, and the magnitude of the drug effect
1098 on cytokine levels should be considered when determining whether to conduct a clinical study. In
1099 some cases, a DDI study in the relevant indicated population should be conducted to further inform
1100 instructions for use of the drug. Important design aspects include the disease type and severity in
1101 the included patients and the dose and treatment time of the *perpetrator* drug.

1102 **4.2.2 Antibody-Drug Conjugates**

1103 For antibody-drug conjugates (ADCs), the small molecule drug component conjugated to the
1104 antibody component can be released in unconjugated form. Therefore, the DDI potential of both
1105 the antibody and the small molecule drug component should be considered. In general, for the
1106 small molecule component, the potential to inhibit or induce enzymes and transporters should be
1107 addressed in line with what is described elsewhere in this guideline. In many cases, however, the
1108 systemic concentration of free drug might be too low to act as a *perpetrator* in vivo.

1109 It is important to understand the formation, distribution and elimination kinetics of the small
1110 molecule and to assess the systemic exposure of the small molecule drug component of the ADC.
1111 It might be necessary to evaluate the small molecule component (administered as an ADC) as a
1112 *victim* drug, in particular if increased levels of free drug may be associated with safety concerns.
1113 Understanding the exposure-response relationship of the various moieties is important in
1114 determining whether to conduct DDI studies and their significance.

1115 **5. REPORTING AND INTERPRETING CLINICAL DDI STUDY RESULTS**

1116 A DDI study report should include and justify the study design and data analysis method based on
1117 what is known about the mechanism of the DDI and the PK properties of the *perpetrator* and *victim*
1118 drugs. Data analysis of pharmacokinetic parameters (and pharmacodynamic parameters, when
1119 relevant) should include all subjects enrolled in the study who have evaluable PK and/or
1120 pharmacodynamic data. If a subject is dropped from the study or has incomplete plasma
1121 concentration sampling during a treatment period, the possibility that the observation is due to an
1122 interaction should be considered. When indicated, the interaction effect should be presented with
1123 and without the individuals proposed for exclusion.

1124 **5.1 Pharmacokinetic Data Analysis**

1125 **5.1.1 Non-Compartmental Analysis (NCA)**

1126 The following exposure measures should be determined for each subject: $AUC_{0-\infty}$, AUC_{0-t} , the
1127 percent extrapolated from AUC_{0-t} to $AUC_{0-\infty}$, C_{max} , and time to C_{max} (T_{max}). For multiple-dose
1128 studies, C_{max} , C_{min} , AUC_{TAU} at steady-state should also be reported. Additional parameters can help
1129 to interpret the PK results: clearance, half-life, and volume of distribution. Parameters for
1130 metabolites, when measured, should also be presented. NCA can be used to evaluate DDI studies
1131 conducted to evaluate the investigational drug as a *victim* or *perpetrator*.

1132 **5.1.2 Population PK Analysis**

1133 PK data collected in nested DDI studies should typically be evaluated using population PK
1134 methods. DDIs should be evaluated using all plausible structural elements of the PK model (e.g.,
1135 clearance (CL or CL/F), relative bioavailability, rate of absorption). Population PK analyses should
1136 derive PK parameters appropriate for the study design and PK properties of the drug, such as AUC
1137 and C_{max} . For multiple-dose studies, C_{max} , C_{min} and AUC_{0-TAU} at steady-state should be reported.

1138 **5.2 Reporting DDI Results**

1139 Typical pharmacokinetics endpoints for DDI studies should include changes in drug exposure
1140 parameters for the *victim* drug, such as AUC, C_{max} , and in some situations, C_{min} . Pharmacokinetic
1141 results of DDI studies should be reported as the geometric mean ratio of the observed
1142 pharmacokinetic exposure measures with and without the *perpetrator* drug and the associated 90
1143 percent confidence interval. Measures of the observed variability of the interaction, such as the
1144 range of AUC or C_{max} ratios for individuals in a cross-over study, should be reported. A comparison
1145 of the individual pharmacokinetic parameters with and without concomitant medication should
1146 also be presented graphically, e.g., as spaghetti-plots.

1147 If pharmacodynamic endpoints are also assessed in the DDI study, the results should be reported
1148 and summarized.

1149 **5.3. Interpreting DDI Study Results**

1150 **5.3.1 Investigational Drug as a Victim of DDIs: Determination of No-Effect Boundaries**

1151 The results of a DDI study should be interpreted based on the no-effect boundaries for the *victim*
1152 drug. No effect-boundaries represent the interval within which a change in systemic exposure
1153 measure is considered not significant enough to warrant clinical action (e.g., avoiding
1154 coadministration, dose or schedule adjustment, or additional therapeutic monitoring).

1155 It is preferable for no-effect boundaries to be developed based on exposure-response relationships
1156 derived from clinical trials, as well as other relevant information for the *victim* drug (e.g., safety

1157 data and the maximum-tolerated dose). A good understanding of exposure-response relationships
1158 for desirable and undesirable drug effects, as well as knowledge of the variability of exposures in
1159 the indicated population, facilitates data interpretation.

1160 In general, the point estimate for the ratio between the exposure of the *victim* with and without the
1161 *perpetrator* can be used to evaluate the magnitude of the interaction and to determine whether
1162 interventions such as dose adjustments should be considered. Sponsors should also consider the
1163 variability of the interaction. As indicated in 3.2.1.1, the number of subjects included in the study
1164 should be sufficient to provide a reliable estimate of the magnitude and variability of the
1165 interaction. If the 90 percent confidence interval for the measured changes in systemic exposures
1166 in the DDI study falls completely within the chosen no-effect boundary, no clinically relevant DDI
1167 is present. However, because DDI studies are not typically powered for the 90 percent confidence
1168 interval to fall within the chosen no-effect boundary, a strict statistical interpretation of the DDI
1169 study may not be applicable. A method that determines the proportion of subjects that extend
1170 beyond the no-effect boundary can also be used to interpret the results.

1171 If the 90% confidence interval for the measured changes in systemic exposure in the DDI study
1172 falls within 80-125%, the study can be interpreted as negative, unless the *victim* drug's safety or
1173 efficacy is affected by small changes in exposure. Because this range is typically overly
1174 conservative, it is not the selected no-effect boundary in most cases. In the absence of a defined
1175 exposure-response relationship, the totality of evidence should be considered when determining
1176 the clinical impact of a DDI.

1177 **5.3.2 Investigational Drug as a Perpetrator of DDIs: Classification System**

1178 The classification system assists in the extrapolation of DDI study results to drugs that have not
1179 been evaluated in a clinical DDI study.

1180 If an investigational drug is a CYP inhibitor, it can be classified as a strong, moderate, or weak
1181 inhibitor based on its effect on an index CYP substrate. The convention is to categorize CYP
1182 inhibition in the following way:

- 1183 • A strong inhibitor increases the AUC of a sensitive index CYP substrate ≥ 5 -fold.
- 1184 • A moderate inhibitor increases the AUC of a sensitive index CYP substrate by ≥ 2 - to < 5 -
1185 fold.
- 1186 • A weak inhibitor increases the AUC of a sensitive index CYP substrate by ≥ 1.25 - to < 2 -
1187 fold.

1188 If an investigational drug is a CYP inducer, it can be classified as a strong, moderate, or weak
1189 inducer based on its effect on an index CYP substrate. The convention is to categorize CYP
1190 induction in the following way:

- 1191 • A strong inducer decreases the AUC of a sensitive index CYP substrate by ≥ 80 percent.

- 1192 • A moderate inducer decreases the AUC of a sensitive index CYP substrate by ≥ 50 to < 80
1193 percent.
- 1194 • A weak inducer decreases the AUC of a sensitive index CYP substrate by ≥ 20 to < 50
1195 percent.

1196 These categories generally describe the effect of the investigational drug when given at the highest
1197 clinical dose and the shortest dosing interval within its therapeutic dose range/dosing regimen. It
1198 is noted that the effects of some inhibitors or inducers are dose dependent.

1199 Although CYP inhibitor and inducer classifications are typically based on DDI studies with index
1200 substrates, if the metabolic properties of a sensitive substrate are well understood, it can be possible
1201 to classify the investigational drug based on a study with the alternative substrate.

1202 Currently, there are no classification systems for transporters or non-CYP enzymes, because the
1203 magnitude of DDIs mediated by transporters or non-CYP enzymes (e.g., UGTs) has a more limited
1204 range. Inhibition of these pathways often results in AUC increases around 3-fold or less, and the
1205 interacting mechanisms may involve other transporters and/or enzymes, making it challenging to
1206 classify inhibitors using the same criteria as those for CYP enzymes.

1207 ***5.3.3 Extrapolating Study Results***

1208 Clinical evaluation of all possible combinations of drugs is not feasible. When possible, results
1209 from DDI studies should be extrapolated to other drugs and clinical situations. Results from DDI
1210 studies with index drugs generally represent the largest magnitude interaction by a specific
1211 mechanism and can be used to predict the magnitude of other interactions by the same mechanism.
1212 The classification system for CYP inhibitors and inducers assists the extrapolation. For example,
1213 if there is no effect on the exposure of an investigational drug when co-administered with a strong
1214 CYP3A index inhibitor, then one can generally assume that there is no effect when other strong,
1215 moderate, or weak CYP3A4 inhibitors are co-administered with the investigational drug. If
1216 administration of a strong CYP2D6 index inhibitor results in a significant increase in exposure of
1217 the investigational drug, these results can be directly extrapolated to other strong CYP2D6
1218 inhibitors. In some cases, extrapolation of positive findings to moderate and weak inhibitors can
1219 be possible using mechanistic modeling.

1220 Because of the lack of specific transporter substrates and inhibitors and the possible interplay with
1221 metabolism, it is generally challenging to extrapolate results from DDI studies evaluating
1222 transporter-mediated DDIs or transporter-metabolism interactions from one drug to other drugs.
1223 However, if the ADME properties of the investigational drug and potential concomitant drugs are
1224 well understood, it is possible to estimate transporter-mediated interactions with other concomitant
1225 drugs.

1226 **5.3.3.1 Extrapolating Complex Scenarios**

1227 Most DDI studies evaluate the interaction between two drugs and consider the effect on single
1228 transporters or enzymes. However, DDIs for a specific drug may result from a combination of
1229 mechanisms, and patients may receive more than two potentially interacting drugs. Some of the
1230 resulting “complex DDI scenarios” are listed below:

- 1231 • Concurrent inhibition of an enzyme and a transporter by a drug.
- 1232 • Concurrent inhibition and induction of a drug’s metabolic pathways, involving one or
1233 more enzymes.
- 1234 • Increased inhibition of drug elimination by use of inhibitors of more than one enzyme
1235 that metabolizes the drug.
- 1236 • Inhibition of an enzyme other than the genetic polymorphic enzyme in poor
1237 metabolizers taking a substrate that is metabolized by both enzymes.
- 1238 • Effect of enzyme/transporter inhibitors in subjects with varying degrees of impairment
1239 of drug eliminating organs (e.g., liver or kidney).
- 1240 • The two drugs affect one another’s PK (both act as *perpetrator* and *victim*).

1241 When there are multiple factors that affect the absorption and disposition of an investigational drug
1242 as well as multiple mechanisms of DDIs, sponsors should consider evaluating the effect of the
1243 combination of mechanisms and/or individual factors on drug exposure. The complex scenarios
1244 can be evaluated by integrating knowledge from the relevant in vitro and clinical studies. PBPK
1245 models can be used to: (1) integrate the information from multiple studies; (2) determine whether
1246 a clinical study would be informative; and (3) inform the design of clinical studies.

1247 **6. RISK ASSESSMENT AND MANAGEMENT**

1248 Risk assessment should inform the use of DDI management strategies. A DDI is clinically relevant
1249 if concomitant use of the drugs leads to safety, effectiveness, or tolerability concerns greater than
1250 those present when the drugs are administered alone.

1251 In general, DDI prevention and risk minimization strategies should result in drug concentrations
1252 of the *victim* drug falling within the no-effect boundaries. The risk assessment and development
1253 of risk minimization strategies should consider the following factors:

- 1254 • The exposure-response relationships for safety and efficacy.
- 1255 • The variability of the observed DDI data, if available.
- 1256 • The expected duration of concomitant drug use (e.g., acute, short-term, or chronic use
1257 of one or both drugs).
- 1258 • The anticipated timing of the introduction of the concomitant medication.
- 1259 • The mechanism of the DDI (e.g., reversible or time-dependent inhibition, induction,
1260 combined inhibition and induction).

- 1261 • The availability of monitoring parameters (e.g., therapeutic drug monitoring, laboratory
1262 tests).
- 1263 • The ability to interrupt the investigational drug or concomitant interacting medication
1264 and the availability of other therapeutic options for either drug.
- 1265 • The clinical importance of the relevant adverse outcome relative to the clinical benefit
1266 of the drugs.

1267 In addition to the above considerations, DDI risk minimization and prevention strategies can
1268 include the following: (Note that there may be regional regulatory differences in how
1269 recommendations are worded in labeling.)

- 1270 • Contraindicating or avoiding concomitant use.
- 1271 • Temporarily discontinuing one of the interacting drugs.
- 1272 • Modifying the dosage of one of the drugs.
- 1273 • Staggering drug administration (e.g., administer the investigational drug at a different
1274 time than a concomitant drug).
- 1275 • Implementing specific monitoring strategies (e.g., therapeutic drug monitoring,
1276 laboratory testing).
- 1277 • Replacing one of the interacting drugs with a drug not expected to interact.

1278 **7. APPENDICES**

1279 **7.1 In Vitro Evaluation of Metabolism-Based DDIs**

1280 ***7.1.1 In Vitro Systems***

1281 Various hepatic in vitro systems can be used to evaluate the risk for enzyme-mediated interactions
1282 for an investigational drug, including:

- 1283 • Subcellular human liver tissue fractions such as microsomal systems (human liver
1284 microsomes (HLM); containing CYP450 and UGT enzymes), supernatants after 9000 g
1285 centrifugation of liver homogenate (S9; containing microsomal as well as cytosolic
1286 enzymes such as sulfotransferases, glutathione transferases, aldehyde dehydrogenase,
1287 aldehyde oxidase and alcohol dehydrogenase), and cytosol (adding co-factors as
1288 appropriate). For HLM, a pool of at least 10 donors is suggested.
- 1289 • Recombinant human CYP and UGT enzymes. These systems usually express only one
1290 single enzyme.
- 1291 • Human liver tissues, including freshly prepared or cryopreserved hepatocytes that
1292 preserve enzyme architecture and contain the full complement of phase 1 and 2 drug
1293 metabolizing enzymes. For phenotyping and inhibition experiments, hepatocytes pooled
1294 from at least 10 donors is suggested, whereas for induction experiments usually
1295 hepatocytes from at least 3 individual donors should be used.

1296 The *in vitro* systems used should be robust and reproducible.

1297 Microsomal protein concentrations should be minimised, and standardised assay conditions (e.g.,
1298 buffer strength, type, and pH) should be used. An incubation time and an enzyme amount that
1299 result in linear formation of the metabolite (at an initial rate of the metabolite formation) is
1300 recommended.

1301 For phenotyping experiments, the system should be characterized with *in vitro* probe substrates to
1302 prove the activity of each enzyme. In general, a probe substrate should be selective (e.g.,
1303 predominantly metabolized by a single enzyme), or a specific metabolite of a probe substrate is
1304 primarily formed by a single enzyme. A list of examples of probe substrates with their marker
1305 reactions and literature reported K_m values can be found in Table 4, Section 7.4.1.1. For studies of
1306 time-dependent inhibition or induction, appropriate inhibitors or inducers should be included as
1307 positive controls (refer to Section 7.4.1 for more details).

1308 For enzyme inhibition studies, if the investigational drug is metabolized by the enzymes present
1309 in the incubation, the probe substrate should, if possible, have a markedly faster metabolism rate
1310 than the investigational drug to minimize the influence of investigational drug metabolism
1311 (decreasing concentrations) on the estimation of inhibitory parameters.

1312 Robust analytical methods should be used to quantify an investigational drug and its relevant
1313 metabolite(s) in phenotyping experiments, as well as probe substrates and/or their relevant
1314 metabolites in inhibition and induction experiments (when enzyme activities are measured). Good
1315 laboratory practice (GLP) standard is not required, but a full description of the analytical methods
1316 employed, including validation of the analytical parameters, should be provided (42).

1317 It is recognized that obtaining high drug concentrations in the *in vitro* studies of enzyme inhibition
1318 or induction may not be possible in some circumstances due to poor aqueous solubility or cell
1319 toxicity. If limited by solubility, co-solvents can be used to reach the highest concentration
1320 possible. Any organic solvents should be used at low concentrations (<1% volume/volume and
1321 preferably < 0.5%) because some solvents can inhibit or activate enzymes. The experiment should
1322 include a solvent (vehicle) control, and when appropriate, also a no-solvent control to evaluate
1323 potential effect of solvent on enzyme reaction. There is at present much uncertainty regarding how
1324 to interpret *in vitro* inhibition and induction data when sufficiently high concentrations cannot be
1325 tested; thus the general recommendation is to test the DDI potential of these compounds *in vivo*,
1326 unless *in vitro* testing is sufficiently justified.

1327 Limited drug stability or non-specific binding in the incubations (e.g., with apparatus, microsomes
1328 or hepatocytes) can also create experimental challenges in *in vitro* studies of enzyme inhibition or
1329 induction. Actual unbound concentrations of the drug in the *in vitro* system (e.g., incubation
1330 medium) should in general be used for extrapolating *in vitro* results to *in vivo* scenarios. When
1331 non-specific binding or metabolic instability is encountered, sponsors should consider whether to
1332 adjust experimental conditions or correct for non-specific binding or instability when interpreting

1333 the data (e.g., derive $K_{i,u}$ from K_i). Non-specific binding can be measured experimentally (e.g.,
1334 using equilibrium dialysis or ultrafiltration) or predicted using in silico methods (43, 44). For
1335 highly lipophilic drugs, it is preferred to experimentally determine non-specific binding (45).

1336 For induction experiments, sponsors are encouraged to measure concentrations of the parent drug
1337 in the medium on the last day of incubation with hepatocytes and protein binding should also be
1338 considered. When measured concentrations are substantially lower than nominal concentrations,
1339 sponsors should discuss the potential impact of the discrepancy on data interpretation (46, 47).

1340 ***7.1.2 Investigational Drug as an Enzyme Substrate: Reaction Phenotyping***

1341 Drug metabolizing enzyme identification studies, often referred to as reaction phenotyping studies,
1342 identify the specific enzymes contributing to the main elimination pathways of a drug. Along with
1343 other information (e.g., in vivo pharmacokinetics, mass-balance study, pharmacogenetic data or
1344 available DDI data), in vitro phenotyping data are often used to identify and quantify elimination
1345 pathways of an investigational drug.

1346 Although the main focus of this guideline is on hepatic CYP involved metabolism, in order to
1347 identify the metabolic pathways for the individual investigational drug, non-CYP enzyme-based
1348 metabolism and metabolism occurring in extra-hepatic tissues should also be considered for certain
1349 drugs.

1350 ***7.1.2.1 Metabolic Pathway Identification***

1351 Metabolic pathway identification experiments should be performed early in drug development to
1352 identify the number and structures of metabolites formed when a drug is metabolized, and to
1353 determine whether the metabolic pathways are parallel or sequential. These experiments use HLM,
1354 intact human liver systems (e.g., hepatocytes), or recombinant enzyme systems. Data obtained
1355 from metabolic pathway identification experiments help to determine whether and how to conduct
1356 a reaction phenotyping study.

1357 ***7.1.2.2 Metabolic Enzyme Identification***

1358 Reaction phenotyping can be done either in HLM or hepatocytes using selective enzyme inhibitors
1359 (chemicals or antibodies) or in human recombinant enzymes. When using individual human
1360 recombinant enzymes, the difference in the amount and enzyme activity of CYPs between the
1361 recombinant CYP enzyme systems and the human liver should be considered. Whenever possible,
1362 all experiments should be conducted with drug concentrations relevant to the clinical setting, and
1363 under initial rate conditions (e.g., linearity of metabolite production rates with respect to time and
1364 enzyme concentrations).

1365 The contribution of individual enzymes to the overall metabolism of an investigational drug can
1366 be examined by measurement of parent drug depletion or measurement of metabolite formation.
1367 For the latter method, all of the major metabolites should have been identified and quantified in
1368 metabolite formation experiments. The use of a radiolabelled drug substrate is advantageous

1369 because samples can be analysed using liquid chromatography coupled with a radioactivity
1370 detector and a mass spectrometer to identify and quantify drug-related species. Evaluation of
1371 individual isomers of racemic drugs is recommended when it is important to understand the
1372 different disposition characteristics of each isomer (e.g., when two isomers have different
1373 pharmacological activities).

1374 Some chemical inhibitors are not specific for an individual CYP enzyme. The selectivity and
1375 potency of inhibitors should be verified in the same experimental conditions using probe substrates
1376 for each CYP enzyme (see Section 7.4.1.1 for more details). If specific antibodies are used instead
1377 of inhibitors, the inhibitory effect of an antibody to a CYP enzyme should be tested at sufficiently
1378 low and high concentrations to establish a titration curve and ensure the maximal inhibition of a
1379 particular pathway (ideally resulting in greater than 80 percent inhibition). The effect of an
1380 antibody should be verified using probe substrates of each CYP isoform in the same experimental
1381 conditions.

1382 For UGT enzymes, in vitro studies are most commonly performed with HLM or recombinant UGT
1383 enzymes as the enzyme source. When HLM used as enzyme source, either addition of alamethicin
1384 or sonication is necessary for activating HLM (1). Determination of the contribution of each UGT
1385 isoform to the overall elimination is sometimes challenging due to lack of selective inhibitors,
1386 variability of results depending on experiment conditions, and instability of glucuronide metabolite
1387 in feces from a mass balance study (48).

1388 **7.1.3 Investigational Drug as an Enzyme Inhibitor**

1389 The potential of an investigational drug to inhibit CYP enzymes is usually investigated using
1390 selective probe substrates to determine the type of inhibition (e.g., reversible inhibition or time-
1391 dependent inhibition (TDI)) and measure of inhibition potency (e.g., K_i for reversible inhibition,
1392 and K_i and k_{inact} for TDI). The in vitro systems used for these studies include pooled HLM,
1393 microsomes obtained from recombinant CYP-expression systems, or pooled human hepatocytes.

1394 For reversible inhibition, experiments with a high concentration of test drug can be performed first
1395 to study its inhibition potential on a particular enzyme (e.g., $50 \times C_{max,u}$ or $0.1 \times \text{Dose}/250 \text{ mL}$,
1396 refer to Section 2.1.2.1). If clinical interaction cannot be excluded at the high concentration, lower
1397 drug concentrations should be tested to estimate the drug's IC_{50} or K_i value; it is recommended to
1398 examine at least four different concentrations of the investigational drug. Experiments with
1399 varying concentrations of both the inhibitor and substrate concentrations to cover ranges above
1400 and below the substrate's K_m , should be tested to determine the K_i for inhibition. For competitive
1401 inhibition or uncompetitive inhibition, $IC_{50}/2$ can be used as an estimate for K_i if the substrate
1402 concentration in the incubation is the same as its K_m value (49). If the substrate concentration is
1403 much less than the K_m value, then the IC_{50} value will approximate the K_i value for a competitive
1404 inhibitor. More accurate estimation of the K_i value can be derived from the IC_{50} value using the
1405 Cheng-Prusoff equation (50). For non-competitive inhibition, K_i value is equal to IC_{50} regardless
1406 of substrate concentration used (51). Thus, $IC_{50}/2$ can still be used as a conservative estimate.

1407 There are various assays to identify TDI of CYP enzymes. For example, TDI can be detected by
1408 assessing a difference in IC₅₀ curves generated with and without a pre-incubation with
1409 nicotinamide adenine dinucleotide phosphate (NADPH) (i.e., IC₅₀ shift), decreases in enzyme
1410 activity (measurement of the pseudo first-order rate constant, k_{obs}) or percent activity loss with the
1411 inactivator over time (also called standard dilution methods). In the IC₅₀ shift assay, pooled HLM
1412 should typically be pre-incubated for 30 min with the investigational drug at concentrations that
1413 surround 10-fold (or greater, depending on the dilution factor) of their reversible IC₅₀ values with
1414 or without NADPH. The pre-incubation samples should then be diluted (10-fold or greater) into
1415 an incubation containing probe substrate (at a concentration around its K_m for the reaction) and
1416 NADPH. A left shift of the IC₅₀ curve (e.g., ≥ 1.5- or 2-fold) from the samples pre-incubated with
1417 NADPH compared to those without, suggests a potential for enzyme inactivation by the
1418 investigational drug. The degree of the fold-shift to establish a positive result would be dependent
1419 upon the demonstrated sensitivity of the experimental system used to detect known TDI
1420 compounds, particularly at least one with a lower fold-shift (e.g. ritonavir) (52).

1421 To rule out a TDI, the decreases in CYP enzyme activity with time can also be evaluated at a single
1422 concentration of the investigational drug (e.g., k_{obs} or percent activity loss). When such a method
1423 is used, the test compound should be pre-incubated with pooled HLM with and without NADPH
1424 typically for 30 min, the reaction should then be diluted appropriately (10-fold or greater to dilute
1425 out the test compound). A vehicle control should be included to correct for potential enzyme
1426 activity loss over the time of the study. The remaining CYP activity should be determined by
1427 measurement of a select CYP probe substrate metabolism (in this case, a high concentration of
1428 substrate can be used to help with the dilution of the test compound). A reduction in CYP enzyme
1429 activity greater than a pre-defined threshold for the assay (e.g., of >20% reduction in activity or a
1430 k_{obs} value of >0.01 min⁻¹) can be used to define a positive result.

1431 When a drug is identified as a TDI with initial assessment as described above, definitive in vitro
1432 studies should be performed to obtain TDI parameters (i.e., k_{inact} and K_i) in pooled HLM for DDI
1433 predictions (4). Human hepatocytes and rhCYP can also be considered for TDI assessment.

1434 ***7.1.4 Investigational Drug as an Enzyme Inducer***

1435 The potential of an investigational drug to act as an inducer of CYP enzymes is normally
1436 investigated in plateable, cryopreserved or freshly isolated, human hepatocytes. Alternative in
1437 vitro systems such as immortalized hepatic cell lines and cell receptor assays can be used, but the
1438 results from these studies are generally considered supportive rather than definitive in nature. If an
1439 alternative in vitro system is used as the main method, the sponsor should provide a justification
1440 supporting the appropriateness of the in vitro system as well as data interpretation.

1441 It is recommended to measure the extent of enzyme induction at the mRNA level. Enzyme activity
1442 can also be measured, measuring only the enzyme activity is usually not recommended as the
1443 induction could be masked in the presence of concomitant inhibition. For CYP2C19, enzyme

1444 activity should be measured, since its mRNA change is often limited even in response to positive
1445 control (53).

1446 Regardless of which in vitro system and endpoint are chosen, the system should be validated to
1447 show that all major CYP enzymes are functional and inducible with positive controls. The response
1448 of positive controls (measured as mRNA fold change) is normally at least a 6-fold increase for
1449 CYP1A2, 2B6, and 3A4, which is considered indicative of satisfactory sensitivity of hepatocyte
1450 lots (refer to Section 2.1.4.1) (54). It is more difficult to obtain satisfactory sensitivity of
1451 hepatocytes for some other enzymes (e.g., CYP2C8, CYP2C9, CYP2C19) that are less inducible
1452 (11, 12). Limited fold of increase in mRNA of these enzymes to positive controls (e.g., rifampin)
1453 pose a challenge to get a conclusive interpretation of results.

1454 Incubation of an investigational drug usually lasts for 48-72 hours to allow complete induction to
1455 occur. Justification should be provided for shorter incubation time. Incubations normally include
1456 a daily addition of the investigational drug, and the medium containing the drug should be changed
1457 regularly. More frequent addition of a drug can be considered if its stability is low. The optimal
1458 time course for incubation should allow detection of enzyme induction without causing cell
1459 toxicity. If cytotoxicity occurs, reduced incubation durations can be used if adequate sensitivity of
1460 the assay can be demonstrated.

1461 Culture quality should be verified and documented by cell morphology and biochemistry tests. A
1462 suitable viability assessment is normally performed before and at the end of the incubation period
1463 to certify that cell toxicity is not influencing the induction response. If toxicity/loss of viability is
1464 observed, influence on the study results should be discussed in the study report and in vivo studies
1465 may be considered.

1466 If hepatocytes from a donor (a) do not respond satisfactorily to the positive induction controls, (b)
1467 demonstrate viability <80% at the start of the incubation, or (c) demonstrate viability at the end of
1468 the incubation that deviates markedly from the viability at the beginning of the experiment, the
1469 cells can be replaced by hepatocytes from a new donor.

1470 To rule out that the investigational drug is an in vitro inducer, an induction study including 3 donor
1471 hepatocytes can be run with at least 3 replicates of 3-5 different concentrations of the test
1472 investigational drug, encompassing $15 \times C_{\max,u}$. The basic mRNA fold-change method can be used
1473 to evaluate in vivo induction potential based upon the criteria described earlier (refer to Section
1474 2.1.4.1).

1475 If there is an induction signal, the sponsor can further use the correlation method or mechanistic
1476 static models to predict the magnitude of a clinical induction effect of an investigational drug.
1477 These methods utilize full concentration-response curves for induction, to estimate E_{\max} and EC_{50}
1478 of the investigational drug. In addition, to use these methods, a batch of hepatocytes should be
1479 “calibrated” (13). For the correlation method, a large set of inducers ($n \geq 8$) covering the full in
1480 vivo induction potency range and including at least 2 weak inducers, are recommended for

1481 calibration. E_{max} and EC_{50} are determined for all inducers and a correlation is established between
1482 a certain matrix (incorporating E_{max} and/or EC_{50} and clinical concentrations of inducers) and in
1483 vivo change in the AUC of a sensitive substrate of a specific enzyme (e.g., midazolam for CYP3A)
1484 for each inducer. For the mechanistic method, an empirical calibration factor, 'd' factor to enable
1485 in vitro to in vivo induction scaling, should be determined for a hepatocyte batch. The 'd' factor
1486 can be estimated by correlating the predicted and observed induction effects (i.e., AUC ratio of a
1487 sensitive substrate of a particular enzyme) of a set of known inducers and performing a linear
1488 regression to identify a 'd' value that can minimize the prediction error (20). If the 'd' factor is not
1489 estimated, it should be set as a default value of 1.

1490 For the correlation or mechanistic static methods, sponsors can use only one hepatocyte donor.
1491 The calibration can be established once for that batch of hepatocytes rather than multiple times for
1492 each experiment with investigational drugs. When performing the in vitro study evaluating the
1493 induction potential of an investigational drug, a criteria for acceptable assay variability should be
1494 established. At least 2 of the inducers (weak and strong) of the calibration set should be included
1495 as controls with responses falling within the defined assay variability, in order to utilize the
1496 calibration set of that hepatocyte batch. If this method is used, both the calibration data
1497 set/calibration report and the data on the investigational new drug should be submitted.

1498 **7.2 In Vitro Evaluation of Transporter-Based DDIs**

1499 **7.2.1 In Vitro Systems**

1500 Various in vitro transporter assays can be used to evaluate the risk for transporter-mediated
1501 interactions of an investigational drug. Selecting the in vitro model can depend on the purpose of
1502 the study and the questions to be addressed. Available in vitro systems include:

- 1503 • Membrane vesicles

1504 In vitro systems using inside-out membrane vesicles from cells transfected with a
1505 transporter can be used to evaluate whether an investigational drug is a substrate or
1506 inhibitor of efflux transporters such as P-gp or BCRP but may fail to identify highly
1507 permeable drugs or highly non-specific binding drugs as substrates. Vesicles can also be
1508 used for MATE transporters evaluation.

1509 P-gp and BCRP assays using membrane vesicles should directly measure the adenosine
1510 triphosphate (ATP)-dependent, transporter-mediated uptake of drugs with control (non-
1511 transfected) vesicles for comparisons.

- 1512 • Bi-directional transport assays with cell-based systems.

1513 Bidirectional assays can be used to evaluate whether an investigational drug is a substrate
1514 or inhibitor of efflux transporters such as P-gp or BCRP.

1515 The permeability of the drug should be investigated in both directions, preferably under
1516 sink conditions (the concentration on the receiver side is less than 10% of the concentration
1517 on the donor side) unless the absence of sink conditions is compensated for in the
1518 calculations. The apparent permeability (P_{app}) of the drug in both the AP→BL (absorption:
1519 apical to basolateral) and BL→AP (efflux: basolateral to apical) directions can be
1520 calculated, as well as an efflux ratio (ER) of BL→AP to AP→BL.

$$ER = \frac{P_{app,BL-AP}}{P_{app,AP-BL}}$$

1521
1522
1523 When using transfected cell lines, efflux ratios of the transfected cell line should be
1524 compared with appropriate control conditions to account for endogenous transporter
1525 activity and non-specific binding. One approach is to compare the efflux ratios from
1526 transfected cell line to the parental or empty vector-transfected cell line.
1527
1528

$$Net\ ER = \frac{ER_{transfected}}{ER_{parental}}$$

1529
1530
1531 The integrity of monolayer membrane should be measured before and after experiments
1532 by examining whether transepithelial/transendothelial electrical resistance (TEER) values
1533 or permeability of paracellular markers fall within the pre-defined acceptance range.

1534 • Uptake assays with cell-based systems:

1535 Uptake assays can be used to evaluate whether an investigational drug is a substrate or
1536 inhibitor of solute carrier (SLC) transporters such as OCTs, OATs, OATPs and MATEs,
1537 but can also be used to investigate efflux transporters.

1538 When transfected cell lines are used to evaluate whether a drug is a substrate of a
1539 transporter, the drug uptake in the transfected cell line should be compared to the parental
1540 or empty vector-transfected cell line, or a comparison of the uptake with or without an
1541 inhibitor of the transporter should be performed. When assessing a drug as an inhibitor of
1542 a transporter, evaluation of the uptake of a known probe substrate using transporter-
1543 transfected cell lines alone can be sufficient. Besides transfected cell lines, human
1544 hepatocytes or hepatic cell lines in suspension or plated can be used.

1545 The model system and experimental conditions should be validated, including culture and transport
1546 assay conditions. Transport studies should be performed under linear transport rate conditions
1547 (probe substrate concentration used is usually below its K_m for the transporter). Appropriate
1548 positive controls should be included in the test study to ensure the validity of the study's results.
1549 The assays should be optimized to ensure consistent transporter function (e.g., uptake, efflux) with
1550 control experiments (e.g., positive and negative controls for substrates/inhibitors (refer to Tables

1551 10 and 11, Section 7.4.3 for some examples), non-transfected control cells). The following
1552 conditions should be considered whenever applicable: the source of the membrane vesicles or
1553 cells, cell culture conditions (e.g., cell passage number, seeding density, monolayer age), probe
1554 substrate/inhibitor concentrations, incubation time, buffer/pH conditions, sampling interval, and
1555 methods for estimating parameters such as the IC_{50} , K_i , and K_m . In addition, adding serum or
1556 plasma proteins to the media can also affect transport activity.

1557 Laboratory acceptance criteria for study results should be established (e.g., monolayer integrity,
1558 passive permeability, efflux or uptake of probe substrates, K_m for a probe substrate, IC_{50} for a
1559 probe inhibitor). The K_m value of a probe substrate or the IC_{50} value of a probe inhibitor should be
1560 comparable to literature-reported values.

1561 The substrate should be readily measured with no interference from the assay matrix.

1562 Any organic solvents should only be used at low concentrations (< 1% volume/volume and
1563 preferably < 0.5%) because some solvents can affect cell integrity or transporter function. The
1564 experiment should include a solvent (vehicle) control, and when appropriate, also a no-solvent
1565 control.

1566 For both substrate and inhibitor studies, the sponsor should demonstrate sufficient total recovery
1567 of the drugs (e.g., 80% (55)).

1568 ***7.2.2 Investigational Drug as a Transporter Substrate***

1569 The concentration range of an investigational drug should be relevant to the site of transport and
1570 should be based on the expected clinical concentration range. For transporters expressed in
1571 multiple organs (e.g., P-gp, BCRP), the sponsors should provide justification for the choice of
1572 concentrations taking into consideration the sites where the transporter is likely to play a role for
1573 drug disposition. When a range of drug concentrations is relevant, it is important to assure that low
1574 concentrations are included, as high concentrations may saturate transporters that are still active at
1575 lower drug concentrations.

1576 If the in vitro system expresses multiple transporters (e.g., Caco-2 cells, hepatocytes), the sponsor
1577 should conduct additional experiments to confirm the findings with two or more known potent
1578 inhibitors, including the ones that are relatively specific for individual transporters.

1579 If active transport is concluded, the passive permeability in the absence of transporters is one of
1580 the factors that could be taken into account to estimate the clinical importance of the transporter.
1581 For intestinal transporters, the role of these transporters may be limited if the permeability in the
1582 absence of transporters is high (\geq the permeability constant of the highly permeable control drug).
1583 In this case, the effect of active drug transport may be negligible as compared to the passive,
1584 concentration-gradient driven absorption of the drug. To estimate the permeability of a drug in the
1585 absence of transporters, for bi-directional assays (e.g., Caco-2 cells) the permeability constant can,
1586 for example, be determined at concentrations high enough to completely saturate the transporters

1587 (assessed as an ER ratio of 0.5 – 2). If this approach is used, it should be established that the cell
1588 monolayer is unaffected. Alternatively, the permeability of a drug can be measured in the presence
1589 of a broad inhibitor of transporters. The investigation should include a well validated, high and
1590 low permeable reference substance (for example, metoprolol and mannitol; refer to (55) for more
1591 details).

1592 **7.2.3 Investigational Drug as a Transporter Inhibitor**

1593 Normally the investigation of transporter inhibition starts with testing a high concentration of the
1594 test drug, for example, $10 \times C_{\max,u}$ for OAT1/3 and OCT2, $50 \times C_{\max,u}$ for MATEs, $10 \times$ liver inlet
1595 $C_{\max,u}$ for OATP1B1/3, and $0.1 \times$ the highest therapeutic dose/250 mL for orally administered P-
1596 gp or BCRP inhibitors. The drug concentration should, however, not exceed the drug's solubility
1597 limits or cause deleterious effects (e.g., cytotoxicity) in the cells. There is at present much
1598 uncertainty regarding how to extrapolate in vitro results to in vivo when sufficiently high
1599 concentrations cannot be tested, thus the general recommendation is to test the DDI potential of
1600 these compounds in vivo, unless in vitro results are sufficiently justified.

1601 Several factors may cause actual drug concentrations in the in vitro assays to deviate from nominal
1602 concentrations, including poor aqueous solubility, non-specific binding, and instability. Correction
1603 for binding or stability or solubility issues should be conducted when interpreting the data.
1604 Sponsors are encouraged to measure unbound drug concentrations in the medium.

1605 If the test drug demonstrates inhibitory activity at the recommended cut-off concentration, the
1606 sponsor should test additional concentrations to estimate IC_{50} or K_i values. The sponsor should
1607 evaluate at least four concentrations of the investigational drug with the probe substrate. The
1608 sponsor can then compare IC_{50} or K_i values to clinical plasma or estimated intestinal concentrations
1609 of a drug to predict the potential for DDIs.

1610 For some transporters (e.g., OATP1B1 and OATP1B3) and experimental systems, it can be
1611 relevant to determine IC_{50} or K_i following pre-incubation with the investigational drug, since some
1612 inhibitors demonstrated more inhibition potency after pre-incubation (56-60). This is an area of
1613 emerging information, and sponsors are encouraged to follow current literature for information on
1614 transporters of interest and relevant experimental protocols.

1615 **7.3. Predictive Modeling**

1616 This section describes how modeling approaches can be used to: (1) characterize the potential for
1617 DDIs, (2) indicate whether a dedicated clinical DDI study should be conducted, and (3) support
1618 clinical recommendations in the absence of a clinical DDI study. The modeling approaches
1619 discussed are static mechanistic models and dynamic mechanistic models (also known as PBPK
1620 models).

1621 Various mathematical and mechanistic modeling approaches can help translate in vitro
1622 observations into predictions of potential clinical DDIs. In some cases, findings from in vitro and

1623 early clinical studies, in conjunction with model-based predictions, can be used to determine
1624 whether initial or additional clinical investigations of a drug's DDI potential as a *victim* or
1625 *perpetrator* of CYP enzyme- or transporter-mediated interaction should be conducted.

1626 Section 3 of this guideline describes the evaluation of in vitro metabolism and transporter studies
1627 to determine whether further evaluation of a drug as a *victim* or *perpetrator* of CYP enzyme- or
1628 transporter-mediated interactions should be conducted. If those assessments indicate further
1629 evaluations should be conducted, they may be conducted using mechanistic static models or PBPK
1630 models (if adequate data are available, as described below) or by conducting a clinical DDI study.
1631 For each drug development program, multiple approaches for assessing DDI risk may be feasible.

1632 Depending on the results of the mechanistic static or PBPK modeling, follow-up clinical DDI
1633 studies could be needed.

1634 The use of appropriate in vitro experimental conditions is critical to any model used for a
1635 quantitative prediction.

1636 ***7.3.1 Using Mechanistic Static Models for DDI Predictions***

1637 A mechanistic static model incorporates detailed drug disposition and drug interaction mechanisms
1638 for both interacting and substrate drugs (61, 62). The model includes the effect of reversible and
1639 time dependent enzyme inhibition, as well as enzyme induction. Thus, the model can estimate the
1640 effect of several interaction processes. The overall effect of the *perpetrator* drug on the substrate
1641 drug is represented as AUCR (ratio of the AUC of the substrate drug in the presence and absence
1642 of the *perpetrator* drug) and is given by the equation below.

1643 ***7.3.1.1. Evaluation of an Investigational Drug as a DDI Perpetrator***

1644 For a drug that is both an inhibitor and an inducer of an enzyme, in addition to the combination of
1645 inhibition and induction, a drug's inhibition potential alone (A and B only, assuming C is equal to
1646 1 in the equation below), and induction potential alone (C only, assuming A and B are equal to 1
1647 in the equation below) should be conducted. Concurrent prediction can lead to a false negative
1648 prediction if the inhibition potential is over-predicted, thus masking the induction effect (63). If
1649 the induction potential is over-predicted, it will mask the inhibition effect.

1650 ***7.3.1.2. Evaluation of Investigational Drug as a Victim of CYP-Mediated DDIs***

1651 In principle, mechanistic static models can be used to predict DDI effects with a less potent
1652 *perpetrators* after the model has been confirmed with index *perpetrators*.

1653 ***7.3.1.3. Evaluation of The Potential for Transporter-Mediated DDIs***

1654 Although there are fewer examples, with adequate data about transporters involved and the fraction
1655 of drug transported at various tissues (ft), the mechanistic static models as noted below and in
1656 Table 3 can be used to evaluate transporter-mediated DDIs (64-68). The potential applications and

1657 considerations listed for PBPK modeling (refer Section 7.3.2.2) are also relevant for mechanistic
 1658 static modeling.

1659 **Equation to calculate AUCR of the substrate drugs (AUC plus investigational drug/AUC
 1660 minus investigational drug)**

1661
$$AUCR = \left(\frac{1}{[A_g \times B_g \times C_g] \times (1 - F_g) + F_g} \right) \times \left(\frac{1}{[A_h \times B_h \times C_h] \times f_m + (1 - f_m)} \right)$$

1662 *The equation assumes that the drug has negligible extrahepatic clearance.*

1663 *A is the effect of reversible inhibitions.*

1664 *B is the effect of TDI.*

1665 *C is the effect of induction.*

1666 *F_g is the fraction available after intestinal metabolism.*

1667 *f_m is the fraction of hepatic clearance of the substrate mediated by the CYP enzyme that is subject to
 1668 inhibition/induction.*

1669 *Subscripts ‘h’ denote liver.*

1670 *Subscripts ‘g’ denote gut.*

1671

1672 **Table 3: Equations to calculate AUCR of the substrate drug for reversible and time-
 1673 dependent inhibition**

	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	$C_g = 1 + \frac{d \times E_{max} \times [I]_g}{[I]_g + EC_{50}}$	$C_h = 1 + \frac{d \times E_{max} \times [I]_h}{[I]_h + EC_{50}}$

1674

1675 Each value can be estimated with the following equations:

1676 $[I]_h = f_{u,p} \times (C_{max} + (Fa \times Fg \times ka \times Dose) / Qh / R_B)$ (69).

1677 $[I]_g = Fa \times ka \times Dose / Qen$ (70).

1678 *f_{u,p} is the unbound fraction in plasma. The f_{u,p} should be set to 1% if experimentally determined to be < 1%
 1679 (also refer to Section 2.1.2.1). Since the potential impact of f_{u,p} on the prediction of DDI is high, sensitivity
 1680 analyses for f_{u,p} should be provided for highly protein bound drugs.*

1681 *C_{max} is the maximal total (free and bound) inhibitor concentration in the plasma at steady state.*

1682 *Fa is the fraction absorbed after oral administration; a value of 1 should be used when the data are not
 1683 available.*

1684 *Fg is the fraction available after intestinal metabolism; a value of 1 should be used when the data are not
 1685 available.*

1686 *ka is the first order absorption rate constant in vivo; a value of 0.1 min⁻¹ (69) can be used when the data
 1687 are not available.*

1688 *Qen is the blood flow through enterocytes (e.g., 18 L/hr/70 kg (71)).*

1689 *Qh is the hepatic blood flow (e.g., 97 L/hr/70 kg (72)).*

1690 *R_B is the blood-to-plasma concentration ratio.*

1691 *d* is a scaling factor determined in a calibrated hepatocyte batch based on positive control inducers (20,
1692 61, 63). If not determined, it is assumed to be 1 (20, 63). A different value can be used if supported by prior
1693 experience with the system used (18).

1694 Reports of modeling exercises and results should provide support for input parameters based on
1695 data and/or scientific literature.

1696 If the model estimates AUCR between 0.80 to 1.25, the risk of a clinically relevant interaction is
1697 low, and additional evaluations of the drug as a *perpetrator* for the studied enzyme are not needed.
1698 If AUCR is outside 0.80 to 1.25, further evaluation should be conducted to quantify the effect.
1699 Alternatively, sponsors should provide sufficient justification(s) if no further assessments are
1700 planned.

1701 Mechanistic static models are currently used to determine whether the potential for a DDI can be
1702 ruled out. This use, along with the current equations used for drug concentrations in the gut and
1703 liver (above), can be overly conservative and thus result in false positive results. There are ongoing
1704 efforts to determine the most relevant drug concentrations in gut and liver (6, 62). The results of
1705 these efforts could lead to the use of mechanistic static models to provide quantitative estimates of
1706 interactions due to CYPs and/or transporters. If additional research supports the use of the models
1707 in a more quantitative manner, reports of results should include justifications for both system- and
1708 drug-dependent parameters and sensitivity analyses when relevant.

1709 **7.3.2 Using PBPK Models to Predict Enzyme or Transporter-Based DDIs**

1710 PBPK models can assist in the evaluation of the DDI potential of an investigational drug and/or a
1711 metabolite as a *victim* or *perpetrator* of enzyme or transporter-mediated interactions. Compared
1712 with a mechanistic static model, since a PBPK model considers changes in concentration over time,
1713 information regarding time-dependent interactions can be obtained in more detail. When PBPK
1714 modeling is used to support drug development and regulatory decisions, it is important to justify
1715 any model assumptions, the physiological and biochemical plausibility of the model, variability,
1716 and uncertainty measures. PBPK analysis reports should include a description of the context of
1717 use for the model, model structure and development plan, the sources and justifications for both
1718 system- and drug-dependent parameters, and an adequate sensitivity analysis plan. When using
1719 predefined models (structural and error) from commercially available software, the software
1720 version and any deviations from predefined models should be described (73). In some scenarios,
1721 simulation data from a robust PBPK model can be used to conclude the DDI potential of an
1722 investigational drug instead of a dedicated clinical DDI study.

1723 In general, broad recommendations for PBPK model verification, validation and the reporting of
1724 the results are beyond the scope of this guideline (refer to (74-78) for guidance on these topics).
1725 Instead, this guideline describes the utility of PBPK modeling for the evaluation of DDIs, with the
1726 understanding that models should be demonstrated as fit-for-purpose. Specific best practice
1727 considerations for use of PBPK modeling for the evaluation of DDIs are also described below.

1728 **7.3.2.1 Potential Applications of PBPK to the Evaluation of CYP-Mediated DDIs**

1729 Related to evaluation of CYP-mediated DDIs, PBPK models can help select key DDI studies for
1730 a development program and support the study design for clinical DDI studies. They can also be
1731 used to explain PK observations, such as observed PK differences that are due to genetic
1732 polymorphism.

1733 When evaluating a drug as a potential *victim* of CYP-mediated DDIs, PBPK models can be used
1734 to predict DDI effects with a less potent perpetrator after the model has been confirmed with index
1735 *perpetrators*. They can also predict clinically relevant DDI scenarios, such as the effect following
1736 multiple dose administration of the substrate drug if only single dose administration is evaluated
1737 in a clinical DDI study.

1738 When evaluating a drug as a potential *perpetrator* of CYP-mediated DDIs, PBPK models can be
1739 used to support the lack of clinical DDI potential and to predict DDI effects under different dosing
1740 regimens after the model has been confirmed with an index substrate.

1741 **7.3.2.1.1 Modeling Considerations - PBPK for Evaluation of CYP Interactions for Drugs as**
1742 **Substrates**

1743 Sponsors should consider the following when using PBPK modeling to predict the DDI potential
1744 of the investigational drug (including clinically relevant metabolite(s)) as a CYP enzyme substrate:

- 1745 • The base PBPK model of the investigational drug should describe the available clinical PK
1746 data using different dosing regimens (e.g., a dose proportionality study, repeated dosing)
1747 and dosing routes (e.g., intravenous or oral).
- 1748 • The major metabolic and other elimination pathways should be quantitatively assigned in
1749 the investigational drug's model according to available in vitro and clinical data.
- 1750 • The uncertainty of the PBPK model parameters should be assessed using sensitivity
1751 analysis. For example, since the potential impact of $f_{u,p}$ on the prediction of DDI is high,
1752 sensitivity analyses for $f_{u,p}$ is expected for highly protein bound drugs.
- 1753 • The index *perpetrator* models should describe the available clinical PK data using different
1754 dosing regimens (e.g., a dose proportionality study) and, as appropriate, different dosing
1755 routes (e.g., intravenous or oral).
- 1756 • The acceptability of index *perpetrator* models should be independently confirmed with
1757 regard to their modulating effect on the PK of sensitive enzyme substrates in humans.
- 1758 • If complex metabolic and transport mechanisms are expected, the substrate and *perpetrator*
1759 models should include the relevant disposition and interaction mechanisms and should be
1760 deemed fit-for-purpose.

1761 **7.3.2.1.2 Modeling Considerations - PBPK for Evaluation of CYP Interactions for Drugs as**
1762 **Perpetrators**

1763 Sponsors should consider the following when using PBPK modeling to predict the drug interaction
1764 potential of an investigational drug (including clinically relevant metabolite(s)) as a CYP enzyme
1765 *perpetrator*:

- 1766 • The base PBPK model of the investigational *perpetrator* (and its metabolites, when
1767 relevant) should describe the available clinical PK data using different dosing regimens
1768 (e.g., a dose proportionality study, repeated dosing) and, as appropriate, dosing routes (e.g.,
1769 intravenous or oral).
- 1770 • The DDI parameters should be assigned in the *perpetrator*'s model according to available
1771 in vitro and clinical data such as clinical DDI study(ies).
- 1772 • For *perpetrators* that exhibit both inhibition and induction, the inhibition and induction
1773 mechanisms should be separately considered, in addition to the combination of inhibition
1774 and induction, to ensure a conservative prediction of in vivo enzyme inhibition or induction.
1775 In most cases, the clinically relevant effect of interest is the combined effect.
- 1776 • The index substrate models should describe the available clinical PK data using different
1777 dosing regimens (e.g., a dose proportionality study) and as appropriate, different dosing
1778 routes (e.g., intravenous or oral).
- 1779 • Sensitive index substrate models should be independently confirmed with regard to the
1780 effect of a strong index *perpetrator*-mediated altered enzyme activity on its PK in humans.
- 1781 • The simulation should include the highest clinical dose and shortest dosing interval of the
1782 investigational *perpetrator*. The PK and modulating effect of the highest dose should be
1783 confirmed before use in the simulation.
- 1784 • Sensitivity analyses should be conducted for parameters exhibiting high levels of
1785 uncertainty.

1786 **7.3.2.2 Potential Applications of PBPK to the Evaluation of Transporter-Mediated DDIs**

1787 Related to evaluation of transporter-mediated DDIs, PBPK models can be used to support the
1788 initial study design for clinical DDI studies when a DDI liability is identified.

1789 When evaluating a drug as a potential *victim* of transporter-mediated DDIs, PBPK models can be
1790 used to explain PK observations, such as PK differences that are due to genetic polymorphism
1791 (e.g., OATP1B1). PBPK models can also be used to explore involvement of specific transporters
1792 in a drug's ADME.

1793 When evaluating a drug as a potential inhibitor of transporter-mediated DDIs, PBPK models can
1794 support negative DDI prediction when the drug is an in vitro inhibitor for a basolateral uptake

1795 transporter. They can also be used to evaluate the effect of an investigational drug on the PK of a
1796 transporter substrate with a well characterized pathway.

1797 ***7.3.2.2.1 Modeling Considerations - Drug as a Transporter Substrate***

1798 In general, quantitatively confirming the model regarding the involvement of the specific
1799 transporter in the relevant organ(s) is challenging. Comprehensive model exploration and/or
1800 clinical studies should be conducted for quantitative model confirmation.

1801 ***7.3.2.2.2 Modeling Considerations - Drug as a Transporter Inhibitor***

1802 In general, when using PBPK models to evaluate a drug as a transporter inhibitor, the substrate
1803 model should be confirmed for the relevant transporter(s). Further, the analysis report should
1804 include a sensitivity analysis for the inhibition constant.

1805 **7.4. List of Drugs that can be used in In Vitro Studies**

1806 ***7.4.1 CYP Enzymes***

1807 ***7.4.1.1 CYP Enzyme Substrates for In Vitro Studies***

1808 Probe substrates are used to measure *perpetrator* properties of a drug candidate on individual CYP
1809 enzymes (see Table 4 for examples of substrates). The substrates should be selective, or the
1810 formation of a specific metabolite is selective for a CYP enzyme. Concentration of the substrate
1811 should be at or below its K_m for the reaction.

1812 **Table 4: Examples of probe substrates for CYP enzymes (in vitro studies)**

CYP Enzyme	Probe substrate	Marker reaction
CYP1A2	Phenacetin 7-Ethoxyresorufin	Phenacetin O-deethylation 7-Ethoxyresorufin-O-deethylation
CYP2B6	Bupropion Efavirenz	Bupropion hydroxylation Efavirenz hydroxylation
CYP2C8	Paclitaxel Amodiaquine	Paclitaxel 6 α -hydroxylation Amodiaquine N-deethylation
CYP2C9	S-warfarin Diclofenac	S-warfarin 7-hydroxylation Diclofenac 4'-hydroxylation
CYP2C19	S-Mephenytoin	S-Mephenytoin 4'-hydroxylation
CYP2D6	Bufuralol Dextromethorphan	Bufuralol 1'-hydroxylation Dextromethorphan O-demethylation
CYP3A (recommend using two structurally different substrates)	Midazolam Testosterone	Midazolam 1'-hydroxylation Testosterone 6 β -hydroxylation

1813 **7.4.1.2 CYP Enzymes Perpetrators for In Vitro Studies**

1814 The enzyme inhibitors and inducers are used to phenotype individual CYP enzymes involved in
 1815 the drug candidate metabolism in vitro. In general, the inhibitors/inducers should be selective at
 1816 the concentration used. The following tables are provided to help sponsors design in vitro studies
 1817 and to evaluate the interaction potential (Tables 5-7). These tables are not exhaustive, and sponsors
 1818 can use other inhibitors/inducers with appropriate justification.

1819 **Table 5: Examples of inhibitors for CYP enzymes (in vitro studies)**

CYP Enzyme	Inhibitor
CYP1A2	α -Naphthoflavone, Furafylline*
CYP2B6	Clopidogrel*, Ticlopidine*, Thiotepa*
CYP2C8	Gemfibrozil glucuronide*, Montelukast, Phenelzine*
CYP2C9	Sulfaphenazole, Tienilic acid*
CYP2C19	Loratadine, Ticlopidine*
CYP2D6	Paroxetine*, Quinidine
CYP3A	Azamulin*, Itraconazole, Ketoconazole, Troleandomycin*

1820 * Designated as time dependent inhibitor. When used, those inhibitors should be pre-incubated with the
 1821 experimental system.

1822 **Table 6: The turnover rate constant (K_{deg}) and half-life ($t_{1/2}$) of major CYP enzymes to aid**
 1823 **in the assessment of time-dependent inhibition**

Enzymes (hepatic)	$t_{1/2}$ (hr)	K_{deg} (/min)
CYP1A2 (79)	38	0.00030
CYP2B (80)	32	0.00036
CYP2C8 (81)	22	0.00053
CYP2C9 (80)	104	0.00011
CYP2C19 (80)	26	0.00044
CYP2D6 (82, 83)	51	0.00023
CYP3A4 (10)	36	0.00032
CYP3A4 (intestinal) (84, 85)	24	0.00048

1824 **Table 7: Examples of inducers for CYP enzymes (In Vitro Studies)**

CYP Enzyme	Inducer
CYP1A2	Omeprazole
CYP2B6	Phenobarbital
CYP2C8	Rifampicin
CYP2C9	Rifampicin
CYP2C19	Rifampicin
CYP3A4	Rifampicin

1825 **7.4.2 UGTs**

1826 **7.4.2.1 UGT Substrates for In Vitro Studies**

1827 The list provided in Table 8 is not exhaustive, and sponsors can use other substrates with
 1828 appropriate justification.

1829 **Table 8: Examples of substrates for UGTs (In Vitro Studies)**

UGT enzyme	Substrate
UGT1A1	β -Estradiol, PF-06409577
UGT1A3	Telmisartan
UGT1A4	Trifluoperazine, 1'-Hydroxymidazolam
UGT1A6	Deferiprone, 5-Hydroxytryptophol, Serotonin
UGT1A9	Mycophenolic acid, Propofol
UGT2B7	Morphine, Zidovudine
UGT2B10	Cotinine, RO5263397
UGT2B15	S-Oxazepam
UGT2B17	Testosterone

1830 **7.4.2.2 UGT Inhibitors for In Vitro Studies**

1831 There is a lack of relatively selective inhibitors for UGT1A3, UGT1A6, UGT2B7, and UGT2B15.
1832 In the absence of selective inhibitors, a combination of methods including use of recombinant UGT
1833 isoform, HLM expressing polymorphic variants of UGT isoform (where appropriate), the relative
1834 activity factor (RAF) or relative expression factor (REF) approach, and activity correlation
1835 approach can be employed. Comparative studies with multiple inhibitors can also help assess the
1836 involvement of a particular isoform. When using individual recombinant enzyme preparations, the
1837 difference in the amount and enzyme activity of UGTs between the recombinant enzyme systems
1838 and the human liver should be considered.

1839 The list provided in Table 9 is not exhaustive, and sponsors can use other inhibitors with
1840 appropriate justification.

1841 **Table 9: Examples of inhibitors for UGTs (In Vitro Studies)**

UGT enzyme	Inhibitor
UGT1A1	Nilotinib, Regorafenib
UGT1A3	-
UGT1A4	Hecogenin
UGT1A6	-
UGT1A9	Magnolol, Niflumic acid
UGT2B7	16 α - and 16 β -Phenyllogifolol*, fluconazole**
UGT2B10	Desloratadine
UGT2B15	-
UGT2B17	Imatinib

1842 *16 α - and 16 β -Phenyllogifolol also inhibit UGT2B4. Their effects on UGT2B10 remains unknown.

1843 ** Fluconazole also inhibits UGT2B10 and UGT2B17.

1844 **7.4.3 Transporters**

1845 Some substrates are not specific for an individual transporter. When an experimental system
1846 expressing multiple transporters is used, a more specific substrate is preferred. The following tables
1847 provide examples of transporter substrate and inhibitors for in vitro studies (Tables 10 and 11).

1848 **Table 10: Examples of substrates for transporters (In Vitro Studies)**

Transporter	Substrate
P-gp	Digoxin, <i>N</i> -methyl-quinidine (NMQ), Quinidine, Vinblastine
BCRP	Estrone-3-sulfate, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), Prazosin, Rosuvastatin, Sulfasalazine
OATP1B1, OATP1B3	Cholecystokinin octapeptide (CCK-8, selective for OATP1B3), Estradiol-17 β -glucuronide, Pitavastatin, Pravastatin, Rosuvastatin
OAT1	Adefovir, Cidofovir, <i>p</i> -aminohippurate (PAH), Tenofovir
OAT3	Benzylpenicillin, Estrone-3-sulfate, Methotrexate
MATE1, MATE2-K	Creatinine, Metformin, 1-methyl-4-phenylpyridinium (MPP+), Tetraethylammonium (TEA)
OCT2	Creatinine, Metformin, Tetraethylammonium (TEA)

1849

1850 **Table 11: Examples of inhibitors for transporters (In Vitro Studies)**

Transporter	Inhibitor
P-gp	GF120918 (dual P-gp/BCRP inhibitor), Verapamil, Valspodar (PSC833), Zosuquidar (LY335979)
BCRP	Fumitremorgin C, GF120918 (dual P-gp/BCRP inhibitor), Ko143, Novobiocin
OATP1B1, OATP1B3	Bromosulphophthalein (BSP), Cyclosporine, Rifampin, Rifamycin SV
OAT1, OAT3	Benzylpenicillin*, Probenecid
MATE1, MATE2-K	Cimetidine, Pyrimethamine, Quinidine
OCT2	Cimetidine, Clonidine

1851 * *Relatively selective inhibitor for OAT3.*

1852 7.5 List of Drugs that can be used in Clinical Studies

1853 7.5.1 CYPs Enzymes

1854 7.5.1.1 CYP Enzyme Substrates for Clinical Studies

1855 Ideally, drugs selections should be based on sensitivity, specificity, safety profiles, and reported
 1856 clinical DDI studies with inhibitors, as well as an absence of studies that indicate the drug does
 1857 not meet the criteria.

- 1858 • Index substrates predictably exhibit exposure increase due to inhibition of a given
 1859 metabolic pathway and results are available from prospective clinical DDI studies. These
 1860 drugs can be safely administered with potential inhibitors, sometimes with a dose
 1861 reduction.

1862 • Sensitive index substrates are index drugs that demonstrate an increase in AUC of ≥ 5 -fold
 1863 with strong index inhibitors of a given metabolic pathway in clinical DDI studies.

1864 • Moderately sensitive substrates are drug that demonstrate an increase in AUC of ≥ 2 - to < 5 -
 1865 fold with strong index inhibitors of a given metabolic pathway in clinical DDI studies.

1866 Sponsors are encouraged to consider the unique characteristics of each drug when designing DDI
 1867 studies. For example, a drug could be a substrate for multiple CYPs or a CYP plus a transporter.
 1868 In such a case, the selection of an index drug for a study should take into consideration the
 1869 knowledge about the potential *perpetrator* (enzymes and/or transporters which it could inhibit).

1870 The drugs listed in Table 12 below have been identified as appropriate index substrates for clinical
 1871 DDI studies. Other drugs can be proposed, considering the criteria above.

1872 **Table 12: Examples of index substrates for CYP enzymes (Clinical studies)**

CYP Enzyme	Sensitive index substrate (unless otherwise noted)	Comments
CYP1A2	Caffeine	
CYP2B6	Bupropion	Bupropion is metabolized by CYP2B6 and non-CYP enzymes. Thus, by itself is not a sensitive substrate. Hydroxybupropion should also be measured, since it is primarily formed by CYP2B6. Hydroxybupropion concentration changes should be considered when determining clinical significance, since it is the major active moiety.
CYP2C8	Repaglinide	Also metabolized by CYP3A though to a lesser extent. Transported by OATP1B1.
CYP2C9	S-warfarin, Flurbiprofen	Moderately sensitive substrate
CYP2C19	Omeprazole	Also metabolized by CYP3A though to a lesser extent. Measurement of metabolite concentrations should be considered when there are multiple interacting mechanisms involved.
CYP2D6	Desipramine, Dextromethorphan, Nebivolol	
CYP3A	Midazolam, Triazolam	

1873 **7.5.1.2 CYP Enzyme Inhibitors for Clinical Studies**

1874 Index inhibitors predictably inhibit metabolism via a given pathway, and results are available from
 1875 prospective clinical DDI studies. Strong and moderate inhibitors are drugs that increase the AUC
 1876 of sensitive index substrates of a given metabolic pathway ≥ 5 -fold and ≥ 2 - to < 5 -fold, respectively.

1877 Ideally, index inhibitors should be selected based on potency and selectivity of inhibition, safety
 1878 profiles, availability of reported clinical DDI studies with different in vivo substrates, as well as
 1879 an absence of studies that indicate the drug does not meet the criteria.

1880 Sponsors are encouraged to consider the unique characteristics of each drug when designing DDI
 1881 studies. For example, a drug could inhibit multiple CYPs or a CYP plus a transporter. Sponsors
 1882 should select an index inhibitor for a study based on knowledge about the potential CYPs and
 1883 transporters involved with the substrate's disposition.

1884 The drugs listed in Table 13 below have been identified as appropriate index inhibitors for clinical
 1885 DDI studies. Other drugs can be proposed, considering the criteria described above.

1886 **Table 13: Examples of index inhibitors for CYP enzymes (Clinical Studies)**

CYP Enzyme	Strong index inhibitors	Comments
CYP1A2	Fluvoxamine	Also strong inhibitor of CYP2C19; moderate inhibitor of CYP3A; weak inhibitors of CYP2C9 and CYP2D6.
CYP2B6		Ticlopidine can be used as a CYP2B6 inhibitor. It decreases hydroxybupropion formation by more than 80%.
CYP2C8	Gemfibrozil	Also inhibits OATP1B1 and OAT3.
CYP2C9	Fluconazole (moderate inhibitor)	Also strong inhibitor of CYP2C19; moderate inhibitor CYP3A.
CYP2C19	Fluvoxamine Fluconazole	Fluvoxamine: Also strong inhibitor of CYP1A2; moderate inhibitor of CYP3A; weak inhibitor of CYP2C9 and CYP2D6 Fluconazole: Also moderate inhibitor of CYP2C9 and CYP3A.
CYP2D6	Fluoxetine Paroxetine	Fluoxetine: Also strong inhibitor of CYP 2C19.
CYP3A	Clarithromycin Itraconazole	Clarithromycin and itraconazole both inhibit P-gp.

1887 **7.5.1.3 CYP Enzyme Inducers for Clinical Studies**

1888 Inducers in Table 14 below were selected based on potency of induction, safety profiles, and
 1889 availability of clinical DDI studies with different clinical substrates. Due to the mechanisms of
 1890 induction, inducers usually regulate the expression of multiple enzymes and transporters.

1891 Strong and moderate inducers decrease the AUC of sensitive index substrates of a given metabolic
 1892 pathway by $\geq 80\%$ and $\geq 50\%$ to $< 80\%$, respectively.

1893 **Table 14: Examples of inducers for CYP enzymes (Clinical Studies) - the list is not exhaustive**
 1894 **and other inducers can be used**
 1895

CYP Enzyme	Strong inducers	Moderate inducers
CYP1A2*		Phenytoin, Rifampin, Smoking
CYP2B6	Carbamazepine	Rifampin, Efavirenz
CYP2C8		Rifampin
CYP2C9		Rifampin
CYP2C19	Rifampin	
CYP3A	Carbamazepine, Phenytoin, Rifampin,	Efavirenz

1896 *CYP1A2: Phenytoin, rifampin, and cigarette smoking are weak-to-moderate inducers based on limited number of
 1897 clinical DDI studies conducted with caffeine, tizanidine, and theophylline.

1898 **7.5.2 UGTs**

1899 UGT substrates and perpetrators that are useful for clinical DDI studies are listed below (Tables
 1900 15-17). These lists are not exhaustive, other substrates/perpetrators can be used with appropriate
 1901 justifications.

1902 **Table 15: Examples of substrates for UGTs (Clinical Studies)**

UGT enzyme	Substrates
UGT1A1	Bictegravir, Cabotegravir, Dolutegravir, SN-38 (active metabolite of irinotecan)
UGT1A4	Lamotrigine (also by UGT2B7), Pexidartinib
UGT1A9	Canagliflozin, Dapagliflozin, Ertugliflozin
UGT2B7	Bempedoic acid, Indomethacin, Naproxen, Zidovudine
UGT2B15	Lorazepam, Oxazepam

1903

1904 **Table 16: Examples of inhibitors for UGTs (Clinical Studies)**

UGT enzyme	Inhibitors
UGT1A1	Atazanavir*
UGT1A4	Probenecid**, Valproic acid (also inhibit UGT2B7)
UGT1A9	Mefenamic Acid
UGT2B7	Probenecid
UGT2B15	Probenecid

1905 * Atazanavir is also an inhibitor of CYP3A.

1906 ** Probenecid is an inhibitor of OAT1 and OAT3 transporters.

1907 **Table 17: Examples of inducers for UGT (Clinical Studies)**

UGT enzyme	Inducers
UGT1A1	Carbamazepine, Efavirenz, Phenobarbital, Rifampin, St. John's wort, Tipranavir combined with ritonavir
UGT1A4	Carbamazepine, Lopinavir combined with ritonavir, Phenobarbital, Phenytoin, Rifampin
UGT1A9	Rifampin
UGT2B7	Rifampin
UGT2B15	Rifampin, Phenytoin

1908 **7.5.3 Transporters**

1909 **7.5.3.1 Transporter Substrates for Clinical Studies**

1910 Transporter substrates that are useful for clinical DDI studies are listed in Table 18 below. Many
 1911 of them are substrates of multiple transporters and/or enzymes. Thus, the extrapolation of results
 1912 from these studies to other drugs can be challenging and as indicated earlier (refer to main text),
 1913 index substrates are not available for transporters. Interpretation of the study results should take
 1914 into consideration the knowledge of the transporter inhibition properties for the investigational
 1915 drug as well as its effect on metabolic enzymes. It is most useful to select a transporter substrate
 1916 that is likely to be administered in the intended patient population for the investigational drug.

1917 The listed substrates exhibit markedly altered PK profiles following co-administration of known
 1918 inhibitors of the transporter, meeting the criteria below. In addition, they are generally safe for use
 1919 in clinical DDI studies.

1920 Criteria

1921 The criteria below were used to select recommended transporter substrates for use in DDI studies
 1922 to characterize a drug's transporter inhibition properties. Results from studies conducted with
 1923 clinically relevant doses were used for selection of drugs. When possible, drugs most relevant for
 1924 global drug development programs were selected.

- 1925 • P-gp: (1) AUC fold-increase ≥ 2 with itraconazole, quinidine, or verapamil co-
 1926 administration, (2) in vitro transport by P-gp expression systems, and (3) not extensively
 1927 metabolized in vivo.
- 1928 • BCRP: (1) AUC fold-increase ≥ 2 with pharmacogenetic alteration of ABCG2 (421C>A)
 1929 and (2) in vitro transport by BCRP expression systems.
- 1930 • OATP1B1/OATP1B3: (1) AUC fold-increase ≥ 2 with rifampin (single dose) or
 1931 cyclosporine co-administration, or pharmacogenetic alteration of SLCO1B1 (521T>C) and
 1932 (2) in vitro transport by OATP1B1 or OATP1B3 expression systems.
- 1933 • OAT1/OAT3: (1) AUC fold-increase ≥ 2 with probenecid co-administration, (2) fraction
 1934 excreted into urine as an unchanged drug ≥ 0.5 , and (3) in vitro transport by OAT1 and/or
 1935 OAT3 expression systems.

- 1936 • OCT2/MATEs: (1) AUC fold-increase ≥ 2 with dolutegravir or pyrimethamine; (2) fraction
 1937 excreted into urine as an unchanged drug ≥ 0.5 , and (3) in vitro transport by OCT2 and/or
 1938 MATEs expression system.

1939 Note: The list is not exhaustive and sponsors can use substrates that are not listed in the table if
 1940 the drug's transport properties are well understood and similar to the criteria above.

1941 **Table 18: Examples of substrates for transporters (Clinical Studies)**

Transporter	Substrates	Comments*
P-gp	Dabigatran etexilate Digoxin Fexofenadine	Dabigatran etexilate** – only affected by intestinal P-gp. Fexofenadine – also substrate for OATP1B1, 1B3 and 2B1.
BCRP	Rosuvastatin Sulfasalazine	Rosuvastatin – also a substrate for OATP1B1, 1B3, 2B1, and OAT3. Sulfasalazine – only affected by intestinal BCRP.
OATP1B1, OATP1B3	Atorvastatin Bosentan Pitavastatin Pravastatin Rosuvastatin Simvastatin acid	Atorvastatin – also a substrate of BCRP, P-gp, and CYP3A. Pravastatin – also a substrate of MRP2 and OAT3. Rosuvastatin – also a substrate for BCRP, OAT3, and OATP2B1. Simvastatin – also a substrate of CYP3A.
OAT1 OAT3	Adefovir Baricitinib Cefaclor Furosemide Oseltamivir carboxylate	Adefovir – Higher contribution of OAT1 than OAT3. Baricitinib, cefaclor and Penicillin G – Higher contribution of OAT3 than OAT1. Furosemide – dual substrate of OAT1/OAT3 is also a substrate of BCRP, OATP2B1, and UGT.
MATE1, MATE2-K, OCT2	Metformin	

1942 *Due to the evolving nature of the understanding, some of the drugs listed in the table could be substrates of other
 1943 transporters that are not listed here.

1944 ** Dabigatran etexilate is a pro-drug and converted by carboxylesterase (CES) to dabigatran which is the measured
 1945 moiety (dabigatran is not a substrate of P-gp). Thus, for correct interpretation of clinical DDI results, preassessment
 1946 of the inhibitory effects of an investigational drug on CES activity should be considered.

1947 *** Adefovir is the active moiety of its pro-drug, adefovir dipivoxil, which is a substrate of P-gp.

1948 7.5.3.2. Transporter Inhibitors for Clinical Studies

1949 Transporter inhibitors that are useful for clinical DDI studies are listed in Table 19 below. Many
 1950 of them not only inhibit the specified transporters but also inhibit some other transporters and/or
 1951 CYP enzymes. Thus, extrapolation of results from these studies to other drugs can be challenging
 1952 as indicated earlier (refer to main text), index inhibitors are not available for transporters.

1953 Interpretation of the study results should take into consideration the knowledge of transport and
 1954 metabolic/elimination pathways for the investigational drug. It is most useful to select a transporter
 1955 inhibitor that is likely to be administered in the intended patient population for the investigational
 1956 drug.

1957 The listed inhibitors lead to markedly altered PK profiles of known substrates of the transporter
 1958 following co-administration, meeting the criteria below. In addition, they are generally safe for use
 1959 in clinical DDI studies.

1960 Criteria

1961 The criteria below were used to select recommended transporter inhibitors for use in DDI studies
 1962 to characterize a drug's properties as a transporter substrate. Results from studies conducted with
 1963 clinically relevant doses were used for selection of drugs. When possible, drugs most relevant for
 1964 global drug development programs were selected.

- 1965 • P-gp: (1) AUC fold-increase of digoxin, dabigatran, or fexofenadine ≥ 2 with co-
 1966 administration and (2) in vitro inhibitor.
- 1967 • BCRP: (1) AUC fold-increase of rosuvastatin ≥ 2 or close to 2-fold with co-administration
 1968 and (2) in vitro inhibitor.
- 1969 • OATP1B1/OATP1B3: (1) AUC fold-increase ≥ 2 for at least one of the clinical substrates
 1970 with co-administration and (2) in vitro inhibitor.
- 1971 • OAT1/OAT3: (1) AUC fold-increase ≥ 2 for at least one of clinical the substrates with co-
 1972 administration and (2) in vitro inhibitor.
- 1973 • OCT2/MATE: (1) AUC fold-increase of metformin ≥ 2 with co-administration and (2) in
 1974 vitro inhibitor.

1975 Note: The list is not exhaustive and sponsors can use inhibitors that are not listed in the table if the
 1976 drug's transporter inhibition properties are well understood and similar to the criteria above.

1977 **Table 19: Examples of inhibitors for transporters (Clinical Studies)**

Transporter	Inhibitor	Comments
P-gp	Itraconazole Quinidine Verapamil	Itraconazole – also inhibits BCRP and CYP3A Verapamil – also inhibit CYP3A
BCRP	Cyclosporine Darolutamide Fostamatinib	Cyclosporine – also inhibits OATP1B1, 1B3, MRP2, and P-gp. Fostamatinib – also inhibits P-gp
OATP1B1, OATP1B3	Rifampin (single dose) Cyclosporine	Rifampin – also inhibits P-gp Cyclosporine – also inhibits MRP2, P-gp and BCRP
OAT1, OAT3	Probenecid	Probenecid – also inhibits OATP1B1.

MATE1, MATE2-K, OCT2	Dolutegravir Pyrimethamine	Dolutegravir – a relatively specific inhibitor for OCT2 Pyrimethamine – a relatively specific inhibitor of MATEs.
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1978

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