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May 21, 2012

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Docket No. FDA–2006-D-0036: Draft Guidance for Industry Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations

Dear Sir/Madam:

The Biotechnology Industry Organization (BIO) thanks the Food and Drug Administration (FDA) for the opportunity to submit comments on the "Draft Guidance for Industry Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations."

BIO represents more than 1,100 biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO members are involved in the research and development of innovative healthcare, agricultural, industrial and environmental biotechnology products, thereby expanding the boundaries of science to benefit humanity by providing better healthcare, enhanced agriculture, and a cleaner and safer environment.

GENERAL COMMENTS:

I. Nonclinical:

BIO applauds FDA for writing a Draft Guidance that is well thought through and written, and generally encompasses extensive detail that will be helpful to Sponsors. However,

we find that the Draft Guidance lacks sufficient detail in certain respects, which could make it difficult for the Sponsor to make a go/no-go decision on the conduct of a clinical Drug-Drug Interaction (DDI) study, and potentially lead to non-uniform application of the guidance and a wide range of interpretation for some DDIs (please see our Specific Comments section, below, for more information). For example, we recommend that FDA provide guidance on certain cut-off values for clinical actions and the appropriate concentrations for DDI assessments. We also request more detail with respect to certain of the criteria in the Decision Tree (Draft Guidance, p. 16).

We note that due to the heterogeneity of cell systems or other *in vitro* evaluation tools, the cut off values of I/Ki, I2/Ki or R value can vary dramatically from lab to lab. We recommend that FDA add a footnote to clarify how this variation should be handled.

The Guidance regarding the threshold for metabolites of ≥25% of parent area under the concentration curve (AUC) should be revisited, in consideration of compounds that are extensively metabolized, in order to avoid overwhelming number of DDI studies on scores of metabolites. We suggest that for drugs that are extensively metabolized, a different threshold of ≥25% of total AUC be used.

In addition to the recommendations above, we suggest that this Draft Guidance document provide more clarity on how modeling and simulation data can be used in product labeling statements, and be harmonized with the similar European Union (EU) guidance document so that it will be more useful to the global pharmaceutical industry (*e.g.*, the number of the main transporters listed in this FDA guidance document should be the same as those listed in EU guidance document).

II. Clinical:

It appears that several recommendations in the Draft Guidance (*e.g.*, use of total Cmax versus unbound Cmax for organic anion-transporting polypeptides (OATPs) versus organic cation transporter/organic aniton transporters (OCTs/OATs), use of mRNA as endpoint for enzyme induction, and equations proposed for mechanistic models to assess investigational drug as inhibitor or inducer) are based on one or more publications from a single group or laboratory. Confidence in broad implementation of these recommendations would be strengthened by confirmation of original findings in single or limited publications by additional groups or labs. We recommend indicating potential limitations associated with recommendations based on single publication/laboratory as that field continues to evolve. Also, please provide literature reference when a recommendation is based on a single or limited number of references.

We note that this Draft Guidance only addresses the *in vitro* and *in vivo* DDI studies that are expected as part of NDA/BLA submissions in support of regulatory review and labeling. It would be useful to include guidance on DDI risk management during drug development. Especially in therapeutic areas such as oncology, clinical development is often initiated in patient populations where polypharmacy is common. The principles offered in this guidance for risk assessment from *in vitro* DDI data and application of physiological based pharmacokinetic (PB-PK) models of DDIs are equally applicable to guide inclusion/exclusion criteria with respect to concomitant medications and/or

cautious use in patient studies when *in vivo* DDI information is not yet available. We suggest including risk management approaches for DDIs in clinical development prior to availability of *in vivo* clinical DDI results. For new molecular entities (NMEs) as substrates of interactions, the decision to exclude strong inhibitors/inducers of specific drug metabolizing enzymes or transporters versus allowing their cautious use may be determined based on the expected contribution of the particular enzyme/transporter to overall clearance. When the NME is expected to have clinically important toxicities or is a narrow therapeutic range (NTR) drug, a major contribution (*e.g.*, 50% or higher) may indicate exclusion of strong and moderate inhibitors/inducers, whereas a smaller contribution (*e.g.*, 25-50%) may only necessitate exclusion of strong inhibitors or inducers. For the NME as the interacting drug, *in vitro-in vivo* extrapolation using the approaches outlined in Figure 4 (using predicted human PK when clinical PK data are not available) may be used to estimate the level of DDI risk. If the predicted magnitude of interaction is not large, it may suffice to exclude concomitant use of NTR substrates only and recommend cautious use of non-NTR substrates. However, if the predicted interaction magnitude is large, it may be necessary to additionally exclude sensitive non-NTR substrates as well.

CONCLUSION:

BIO appreciates this opportunity to comment on the "Draft Guidance for Industry Drug Interaction Studies—Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations." Specific, detailed comments are included in the following chart. We would be pleased to provide further input or clarification of our comments, as needed.

Sincerely,

/S/

Andrew J. Emmett
Managing Director, Science and Regulatory Affairs
Biotechnology Industry Organization (BIO)

SPECIFIC COMMENTS

SECTION	ISSUE	PROPOSED CHANGE
I. INTRODUCTION		
	The Draft Guidance does not include reference to transporter-independent DDI mechanisms that may impact absorption of investigational drug or concomitantly administered drugs (*e.g.*, pH-dependent changes in solubility, cation complexation).	Please include a reference to PK-based DDI mechanisms unrelated to drug metabolizing enzymes or transporters in the Introduction section.
Lines 91-92, as well as 751, 975 :	The text reads: "The potential for drug interactions with metabolites of investigational drugs (metabolites present at ≥25% of parent drug AUC) should be considered (see section IV.A.3)."	Please revise to: "≥25% of total AUC (e.g. radioactivity) in the event of extensive metabolism, as evidenced by ≤10% of the systemic AUC represented by parent compound."
Lines 94-96:	This bullet has been included in the first bullet in general. If this bullet point is included, please add "Transporter-mediated DDI should also be explored for the investigational drugs that are eliminated mainly by metabolism…."	Please add: Transporter mediated DDI should also be explored for the investigational drugs that are eliminated mainly by metabolism...
Lines 101-105:	Criteria for no effect boundary should consider exposure-response relationship and	We suggest indicating factors (exposure-response relationships for safety and efficacy) that a Sponsor should consider in establishing no

	population PK variability.	effect boundary range for deciding whether *in vivo* studies are needed.
Lines 113-115:	Additional guidance on submission requirements for PB-PK modeling results would be useful.	Please provide additional detail on suggested content and format of PB-PK modeling report(s) that are needed to support the decision to waive an *in vivo* DDI study.
Lines 125-127:	This information is not mentioned in later induction sections (Section IV.A.1-b). *In vivo* DDI studies for most oncology drugs cannot be conducted in healthy volunteers, and, therefore, it is more difficult to conduct *in vivo* DDI studies to assess potential induction of oral contraceptives by oncology drugs that are potential teratogens.	Given the importance of this information, please also include it in the main induction section. Instead of evaluating the effects of an oncology investigational drug on oral contraceptives, an alternative risk management strategy is requiring that patients taking concomitant hormonal contraceptives use a second non-hormonal contraceptive method with a similar pregnancy prevention rate. This information can be included in the drug label.
II. BACKGROUND		
Lines 290-292:	This sentence does not address DDI evaluations that may be needed to assess whether absorption of investigational drug is affected by other drugs via non-enzymatic or non-transporter-based mechanisms (*e.g.*, pH changes, cation complexation).	Please revise sentence to include "absorption."
Lines 438:	Footnote "b" cannot be found in the table.	Please add footnote "b".
Lines 446-448:	The Draft Guidance states: "Therapeutic proteins (TPs) typically do not undergo metabolism or transport as their	For further clarification please add the text: Thus, standard assessments of TP as victim, *e.g.*, with Ketoconazole or rifampin in the clinic, are not required.

	clearance pathway, therefore the potential is limited for small molecule drugs (terme "drug" in this document) to affect TPs through metabolism or transport pathways."	
Lines 446-451:	The Agency is suggesting the PK of TPs can be affected by small molecules in this paragraph, but no further comments are made on the triggers for clinical investigations or study timings.	Please add text on the triggers for clinical investigations or study timings.
Lines 454-455:	Rather than a direct inhibitory effect at the enzyme level, cytokines repress CYP isoform expression at the transcriptional level.	Please replace "inhibition" with "repression" so the statement reads: For example, cytokines such as IL-6 can produce a concentration-dependent ~~inhibition~~ repression on various CYP isoforms…
III. GENERAL STRATEGIES		
Section IVA2, *In Vitro* Transporter Studies:	For *in vitro* evaluation of an investigational drug as a transporter substrate or inhibitor, no guidance is provided with respect to *in vitro* methodology, experimental design considerations, choice of test systems, and preferred chemical substrates and inhibitors for *in vitro* experiments.	Please consider including an appendix that includes scientific considerations for *in vitro* transporter experiments, similar in concept to the appendix that was provided in the 2006 Draft Guidance for drug metabolizing enzymes.
Lines 504-506:	Appendix C-1 of 2006 Draft Guidance provided useful information regarding *in vitro* drug metabolizing enzyme identification. Similarly, Appendices C-2 and C-3 provided useful information regarding *in vitro*	Please include appendices with information regarding experimental considerations for *in vitro* studies evaluating drug metabolizing enzyme identification, inhibition, and induction.

	evaluation of inhibition and induction, respectively. Topics included experimental design considerations, choice of test systems, *in vitro* methods and testing conditions, and preferred and acceptable chemical substrates and inhibitors for *in vitro* experiments. These topics are not discussed in detail in the current Draft Guidance.	
Lines 513-515, Figure 2:	The "No" answer will not only be based on *in vitro* data, but it can be determined by PB-PK modeling as well.	Please label as such based on *in vitro* data and/or rational DDI simulation modeling.
Lines 513:	Recombinant enzymes expressed in non-human tissue (such as insect cells) may be used to evaluate investigational drugs as enzyme substrates.	Please consider replacing "in Human Tissues" with "with biomaterials expressing human-specific enzymes."
Lines 513:	Figure 2 includes specific reference to UDP-glucuronosyltransferase (UGT) enzymes. This implies that investigational drug should be evaluated as a substrate or as an interacting drug of these enzymes. However, Section IV-A1 only provides recommendations on the evaluation of investigational drug as a substrate of UGT enzymes thought to play role in human drug metabolism. In addition, Figure 4 only applies to evaluation of the investigational drug as an inhibitor or inducer of CYP enzymes, but not UGT enzymes.	Please clarify in Figure 2 whether reference to Phase II enzymes (UGTs specifically) only applies to evaluation of investigational drug as substrate of UGTs.

Lines 513:	Clearance criterion is based on systemic clearance, which can be determined only after IV administration. It is not feasible or possible to administer some drugs via IV route of administration.	Please clarify whether clearance criterion can be based on apparent oral clearance, which is generally a reflection of hepatic intrinsic clearance assuming complete oral absorption and lack of extrahepatic metabolism.
Lines 518-520:	It is unclear how biliary clearance can be determined with orally administered drug. Without IV administration, it is difficult to distinguish whether drug in feces following oral administration represents unabsorbed drug or direct biliary excretion.	Please provide clarity on expectations for determination of biliary clearance in humans after oral administration, as the statement suggests that both IV and oral administrations would be required for clinical use for all drugs.
Lines 530:	The Draft Guidance states: "The in vitro systems include human liver tissues such as liver microsomes, microsomes expressing recombinant enzymes, or freshly isolated or cryopreserved human hepatocytes."	Please consider additional systems available for these assessments.
Lines 576-577:	The aldehyde oxidase has been also been shown to contribute significantly to drug metabolism for some drugs.	Please add "aldehyde oxidase (AO) "in the non-CYP Phase I enzyme list.
Lines 599-601:	It is recommended to determine whether an investigational drug is a substrate of UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15 using recombinant enzymes, but no recommendations are given for positive controls to confirm functional activity of	Please include a table of known substrates for UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15 that can be used as *in vitro* positive controls for UGT recombinant enzyme experiments.

	recombinant CYPs.	
Lines 604-607:	UGT1A1 is provided as an example of a UGT isoform exhibiting polymorphic variation. It would be helpful to provide other UGT isoforms where comparison of PK among different genotypes can be recommended in lieu of an *in vivo* human inhibition study.	Please include a table of UGT isoforms with known PK differences between different genotypes.
Lines 613-615 Figure 3:	UGT should be ≥25% of total clearance, not metabolism.	Please correct so that UGT is ≥25% of total clearance, not metabolism.
Lines 613:	It is unclear what step(s) should be taken if glucuronidation has been shown to be responsible for ≥ 25% of total metabolism, but no appreciable metabolism is observed with the indicated recombinant UGT enzymes believed to play key role in drug metabolism.	Please provide recommendations on whether additional UGT isoforms (besides UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15) should be evaluated, and if an *in vivo* inhibition study with a general inhibitor (probenecid, valproic acid) is required in this situation.
Lines 668-671:	Additional guidance on submission requirements for PB-PK modeling results would be useful.	Please provide additional detail on suggested content and format of PB-PK modeling report(s) that are needed to support the decision to waive an *in vivo* DDI study.
Lines 675-677:	Only the mRNA analysis is mentioned. Only one paper that showed CYP3A4 mRNA induction using simply three human liver donors has been used for generating this guidance for assessing CYP3A inducers. Based on limited testing and information, it is premature to use mRNA data alone to determine a CYP inducer for CYP3A, 2B6	Measurements of mRNA, protein and/or activity by investigational drugs in cultured human hepatocytes from >3 donors should be allowed for inducer qualification. For CYP 3A, 1A2, and 2B6, the investigational drug that produces a change that is equal to or greater than 40% of the positive control can be considered as an enzyme inducer and *in vitro* and *in vivo* evaluation is warranted.

	and 1A2. It should also be noted that 4-fold induction over control, as sited in the reference provided, led to 43% false positive, a rate too high for the method to be sufficiently predictive. It should be noted that both mRNA and activity are needed to assess the potential for concomitant enzyme inhibition and induction. Emax is used in Basic model equation and Emax is "fold-induction." When the cryopreserved hepatocytes were used, calculation of "fold-induction" is impossible because the control activity is very low and highly variable.	Please also allow use of hepatic inlet concentration for induction assessments.
Lines 675-677:	I or Cmax in the decision trees is not clearly defined.	Please clearly define the Cmax or I.
Lines 675:	Figure 4: General Scheme of Model-Based Prediction: The Investigational Drug (and Metabolite Present at ≥25% of Parent Drug AUC) as an Interacting Drug of CYP Enzymes.	Please explain/define when kinetic data profile calculated from Concentration vs. Activity curves deviates from basic model. If the basic model is assumed to behave in a Michaelis-Menten fashion, then 'd' should be 1. Please describe how to properly treat and analyze the dataset including transformation of the data (*e.g.*, Eadie-Hofstee Plot) and the criterion for determination of deviation from basic model.
Lines 675-676:	Presentation of basic and mechanistic model equations is confusing.	Employ parentheses in equations to ensure mathematical operations are performed in correct order.
Lines 675-676:	The value of using basic model equations for assessing CYP inhibition or induction is not	Please consider removal of basic model criteria from decision tree given that positive results can be overridden by results from

	clear, given that a negative result from a basic model can also be obtained with mechanistic models, and a positive result from a basic model can be associated with a negative result with mechanistic models. Furthermore, there is low probability for the sponsor to conduct a clinical DDI study on the basis of a positive basic model outcome without first assessing outcome from a mechanistic model.	mechanistic models.
Lines 675-676:	Use of a predefined threshold based on observed induction levels of clinical inducers in different hepatocyte preparations could result in variable interpretation of induction risk among different laboratories. This is potentially the only method for assessing induction risk when it is not possible to evaluate a sufficient concentration range of the investigational drug to determine Emax and EC50 due to solubility limitations or cytotoxicity. It is unclear how predefined thresholds of induction should compare to fold induction values reported for positive control inducers in Table 2. The two recommended methods for *in vitro* induction assessment may be associated with different interpretation of induction risk.	Please clarify establishment of a predefined threshold for assessment of induction potential of the investigational drug. Please provide guidance on interpretation of induction risk when two proposed methods provide opposing results.
Lines 676:	The cut off criteria for CYP inhibition in the Basic models (line 676, Fig.4)) and P-gp	Considerations should be provided over the true value of Ki, additional guidance based on gut lumen concentration, and if

	inhibition (line 1998, Fig.A2)), R=1+[I]/Ki=1.1, are the cut off criteria for reversible inhibition, where [I] is total concentration, but Ki should be unbound concentration. These criteria are much more conservative than those in the last draft guidance, in which Ki could be total concentration. Moreover, in this Draft Guidance, the inhibition in gut should be also considered. In gut, [I] is (molar dose)/250 mL, and the cut off criteria for R is 11. For example, when the compound with M.W of 500 would be administrated in 10 mg/man, [I] will be 80 μmol/L. The compound with Ki value of less than 8 μmol/L should be required in the clinical DDI study. Ki value could be estimated as 1/2 of IC50 value, and unbound concentration in the *in vitro* study is usually 30% to 70% of nominal concentration. This estimation means that the compound with IC50 value of 20 μmol/L or more in a conventional CYP inhibition study should be required in the clinical DDI study. The cut off criteria in basic models should be re-considered. It is not clear whether sponsors should conduct the inhibition study using gut	inhibition studies in gut *in vitro* systems should be used for more appropriate DDI assessments.

	microsomes for the estimation in gut or not. [Total] or [unbound] are not mentioned for EC50 in the induction study and KI in the time-dependent inhibition study.	
Lines 686:	A range of k_{deg} values is presented for various CYP enzymes in the literature.	To ensure consistency between Sponsors in application of mechanistic static and dynamic PB-PK models for time-dependent inhibition, please provide a table or appendix of recommended values of k_{deg} for relevant enzymes. For enzymes with uncertainty in k_{deg} estimates, please provide range rather than single estimate of k_{deg} values along with supporting literature references.
Lines 702:	For industry uniformity, FDA should provide exactly what k_{deg} value one should use. Conflicting messages are presented in the equation at line 702 and 788. Line 702 says that we know k_{deg} for both gut and liver, therefore we can do the calculation, whereas line 788 says that we don't know the k_{deg}, therefore we cannot do simulation.	Please provide the k_{deg} value range one should use.
Lines 702-712 Footnote e:	$I_h=f_{u,b}$ x $([I]_{max,b} + F_a x K_a x Dose/Q_h)$ is liver free concentration calculated from blood. Most of the time, we have plasma conc. available. One should be allowed to use $f_{u,plasma}$ and plasma concentrations for this equation.	Suggest adding "using plasma unbound fraction vs. maximal plasma concentration as an alternative way to calculate I_h. $I_h=f_{u,plasma}$ x $([I]_{max,plasma} + F_a x K_a x Dose/Q_h)$ This is also consistent with OATP inhibition $I_{in,max}$ calculation.

	Blood/plasma ratio for conversion of plasma to blood concentrations may be concentration-dependent. Definition of fu in other sections of guidance is based on unbound fraction in plasma.	
Lines 728-730:	Consistent with literature reports, inhibition parameter values can differ between human liver microsomes and cDNA-expressed microsomes. These differences can lead to differences in the interpretation of *in vivo* DDI risk.	Recommend that human liver microsomes are designated as the *in vitro* system of choice for evaluating enzyme inhibition. As in the 2006 Draft Guidance, please include an appendix that discusses advantages and disadvantages of different *in vitro* systems potentially used for assessment of enzyme inhibition.
Lines 737:	The Draft Guidance states: "The use of a cutoff R value of 1.1 where [I] represents maximum total (free and bound) system concentration of the inhibitor is based on an earlier FDA recommendation for reversible inhibition (Huang et al. 2007)."	Please replace "system" with "systemic" so the statement reads: The use of a cutoff R value of 1.1 where [I] represents maximum total (free and bound) ~~system~~ systemic concentration of the inhibitor is based on an earlier FDA recommendation for reversible inhibition (Huang et al. 2007).
Lines 739-742:	Igut ignores solubility limitations.	Please allow simulated Igut based on solubility factor.
Lines 750-753:	This poses a problem when dealing with a compound that is extensively metabolized, and the parent exposure is low compared to overall drug related material in the plasma. The clause needs to be relaxed in such cases to allow use of metabolites as >25% of total drug related exposure, to qualify it for inhibition studies.	Please add: When the parent compounds exposure is low compared to overall drug related exposure in plasma, the exposure to metabolite of ≥25% of the total plasma exposure would trigger inhibition studies by the metabolite.

Lines 788-790:	Given the uncertainty in the k_{deg} parameter describing degradation kinetics of some CYP enzymes, the use of different k_{deg} values in mechanistic static and dynamic PB-PK models could lead to different predictions of the magnitude of TDI-related DDI *in vivo*.	When the basic model equation for TDI suggests potential *in vivo* DDI risk and mechanistic static or dynamic PB-PK models are used as the next step of risk assessment, we recommend indicating that sensitivity analyses should be performed with a range of k_{deg} values to ensure that uncertainty in k_{deg} parameter estimates for some CYP enzymes does not translate to different interpretation of TDI-related DDI risk.
Lines 806-808:	In Figure 4, the basic model approach for CYP induction assessment indicates use of predefined threshold, or calculated R3 value, but the text in Section IV.A.1-b 2 implies that predefined threshold is the same as the R3 value calculated with the basic model approach.	Please clarify the difference between predefined threshold and calculated R3 value in text of Section IV.A.1-b2.
Lines 810-811:	CYP induction can be mediated via transcriptional activation of CYP genes by PXR, CAR, and AhR. When referring to "sufficient number of clinical inducers," it is unclear whether a sufficient number of inducers must be included for each induction mechanism, or irrespective of mechanism. In addition, it is unclear whether a minimum level of induction should be observed with all clinical inducers used to establish a predefined threshold. In addition to use of positive controls,	Please clarify whether a sufficient number of clinical inducers means a sufficient number of clinical inducers for each major nuclear receptor mechanism (PXR, CAR, AhR), or a sufficient number overall. Please provide guidance on what levels of induction should be demonstrated with different clinical inducers. Please specify minimal level of hepatocyte viability (eg, > 75%) that should be demonstrated for each hepatocyte preparation used to assess induction potential of the investigational drug.

	performance of hepatocyte preparations in evaluating enzyme induction by the investigational drug can be assessed by cell viability.	
Lines 812:	Please clarify the "predefined thresholds" for positive induction. Please provide the rationale for adding a negative control on top of the Vehicle control. The mRNA assay produces too many false positives, thus the assay by itself is not reliable and should be used only along with the activity assay.	Please add enzyme activity as an end point, define the thresholds, and remove the negative control.
Lines 821-822:	Some UGT enzymes are co-regulated with CYP3A via PXR or with CYP1A2 via AhR. If the investigational drug induces CYP3A and/or CYP1A2 *in vitro* or *in vivo*, should its UGT induction potential be studied *in vitro* and/or *in vivo*?	Please provide guidance on the evaluation of an investigational drug's UGT induction potential in context of positive *in vitro* or *in vivo* CYP3A and/or CYP1A2 induction results. If UGT induction potential of the investigational drug needs to be investigated *in vivo*, please recommend an acceptable UGT probe substrate.
Lines 835 Table 2:	Table 2 reports fold induction in enzyme activities for *in vitro* positive control CYP inducers. Because the recommendation is to use mRNA as induction endpoint for investigational drug, expected fold induction of mRNA levels should be provided for each positive control. We also note that for some enzymes, a range of enzyme activities is not reported, which is surprising given inter-	If mRNA alone is recommended as induction endpoint, please provide reported fold induction of mRNA levels instead of enzyme activities. Regardless if fold induction of mRNA or enzyme activities is reported, please provide expected range rather than single value.

	donor and inter-laboratory variability in enzyme induction. This implies there is less cumulative experience with these positive controls relative to those reporting a range.	
Lines 859-864:	The Draft Guidance states: "The submission containing the use of such advanced models should include a description of the structural model, source and justifications for both system- and drug-dependent parameters, type of error models, model output, data analysis, and adequate sensitivity analyses."	Please provide additional detail on suggested content and format of PB-PK modeling report(s) that are needed to support the decision to waive an *in vivo* DDI study.
Lines 907-911:	The Draft Guidance states: "For drugs that are highly permeable and highly soluble, the intestinal absorption is not a rate-limiting step, and, therefore, it may be appropriate to exempt such drugs from the in vivo evaluation with a P-gp or BCRP inhibitor. (For further discussion regarding the defining a drug as highly soluble and high permeable (e.g., biopharmaceutical classification class (BCS) 1 drugs), see the Guidance for Industry on *Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System*."	Please exempt BCS Class II compounds as well due to their high permeability.

Lines 919-920 footnote:	How well does preclinical *in vivo* and *in vitro* data correlate to in vivo results in humans? How does one calculate biliary secretion from nonrenal clearance data?	Please provide clarification. Please emphasize the use of human *in vitro* or *in vivo* data in determining relative contribution of biliary excretion to human total clearance.
Lines 920-923:	Renal clearance is dependent on filtration clearance, secretion clearance, and fraction of drug filtered and secreted that is reabsorbed. Secretion clearance is dependent on intrinsic renal secretion clearance with respect to unbound drug and renal blood flow. The equation in footnote 4 does not appear to account for fraction reabsorbed or renal blood flow.	Please provide assumptions that must be met to ensure that the equation in footnote 4 provides accurate assessment of active secretion clearance.
Lines 925-926 Figure 6:	This appears to be inconsistent with Figure 3A. Figure 3A asks to consider the property of molecule before testing *in vitro* whether the compound is a substrate, but Figure 6 asks for *in vitro* testing first.	Please make Figure 6 consistent with Figure A3. From the third box from the top, please remove the words "*in vitro*" from "Determine whether investigational drug is an OATP1B1 and/or 1B3 subs."
Lines 925-926 Figure 6:	Determination of whether hepatic/biliary secretion and renal active secretion is major is based on comparison to total clearance. Figure 2 used the term ”systemic clearance.”	Please use consistent clearance terminology throughout document. Please clarify whether the clearance criterion can be based on apparent oral clearance rather than total or systemic clearance.
Lines 929-931:	Based on evolving research, MATE-1, MRP2, and BSEP should be considered for *in vitro* evaluation. BSEP is also included as transporter of interest in the EMA DDI	Please indicate that evaluation of investigational drugs as substrates for these transporters may be needed on a case-by-case basis.

	guidance.	
Lines 938-940:	Are there any suggested transport test systems and inhibitors? Inhibitors, their concentration range, and % inhibition should be listed. If there is no selective control inhibitor available, it would be hard to determine inhibition for NCE for that transporter.	Please provide a table of test systems, Inhibitors, their concentration ranges, and % inhibition at such a concentration.
Lines 939-942:	Different language is used for ATP-binding cassettee (ABC) and OATP transporters (”should be considered”) relative to OCT and OAT transporters (”should be evaluated”), which implies different level of expectation.	Please use consistent language for different transporters when there is a similar level of expectation for evaluation.
Lines 955-956:	It would be useful to provide a list of transporters that are reported to be inducible, their observed levels of induction, and their mechanisms of induction.	Please provide a table indicating whether there is evidence supporting induction of P-gp, BCRP, OATP1B1/3, OAT1/3, OCT2, and other transporters if applicable. If available, please provide observed levels of induction and mechanisms of induction.
Lines 976-978:	It is not clear if metabolite is needed for the transporter substrate or inhibitor test.	Substrate selectivity should be tested only if the metabolite is active or toxic, otherwise just transporter inhibition assessment should be considered sufficient.
Lines 986-987:	Need to specify that qualified assays are appropriate rather than validated assays, unless the metabolite has definitive toxicity or activity concerns.	Please add: Generally, qualified assays are appropriate rather than validated assays, unless the metabolite has definitive substantial toxicity or activity concerns.

Lines 1018-1019:	It is unclear what is considered a combination therapy. Does this refer to a combination therapy which is recommended in the label?	Please clarify what is meant by combination therapy.
Lines 1035:	The decision tree includes reference to *in vitro* studies, but lines 1010 to 1013 indicate that *in vitro* studies have limited value in the qualitative and quantitative projection of clinical interactions because translation of *in vitro* to *in vivo* and animal to human results to date has been inconsistent.	In the decision tree, we suggest considering removing the option of conducting *in vitro* studies to assess the effect of a therapeutic protein on a small molecule drug.
Lines 1035:	Figure 7. Summary of the Types of Studies That Have Been Used During Drug Development to Evaluate Therapeutic Protein (TP)–Small-Molecule Drug (D) Interactions. This includes an evaluation of the effect of TP on D (TP→D) and the effect of D on TP (D→TP). The broken lines suggest the limited use of in vitro studies for informing in vivo study design or labeling. CYP, cytochrome P450. (Modified from Huang et al. 2010)	For cytokine or cytokine modulators (that have known effects on CYPs and transporters): the box on *in vitro* studies should be removed. Such *in vitro* studies are superfluous, as they would only confirm the known effect. On the right side (box: Cases where studies can be considered important…) we suggest removing from the right arrow "or potential for mechanism unknown." This case appears to be identical with/covered by the left arrow at this box, *i.e.* "*No known or suspected mechanisms.*" In the right lower box (*In vitro* or *in vivo* interaction studies), "*in vitro*" should be removed due to the limited use of *in vitro* studies, which will hardly be the sole basis for label information.
Lines 1041:	The current layout of the decision tree has a dotted line from *in vitro* to clinical and to label.	Please clarify.

	Does this imply that negative results *in vitro* can be concluded as no effect, and that be stated on the label with no *in vivo* assessment?	
Lines 1041:		Please provide more detail around this sentence on the decision tree (page 35): "**Cases where studies can be considered important because of known mechanisms or general concerns other than its possible effect on CYPs or transporters.**"
IV. DESIGN OF IN VIVO DRUG-DRUG INTERACTION STUDIES		
	Several recommendations for conduct of *in vivo* DDI studies (*e.g.*, need to evaluate time course of reversal of TDI or induction DDIs with a third crossover period [lines 1127-1130, 1151-1152]; exclusion of all concomitant medications and numerous foods/ beverages [lines 1159-1170]; and stratification by genotype or powering for genotype status [lines 1194-1197]) are impractical when DDI studies cannot be conducted in healthy volunteers. For example, many oncology agents cannot be evaluated in healthy subjects, requiring conduct of DDI studies in patients with advanced cancers. It is not feasible to conduct 3-period cross-over studies with adequate washout between periods or to require powering cancer patient studies to include multiple genotype groups.	Please remove the recommendations to evaluate reversal of induction/ TDI in clinical DDI studies, and to stratify/ power by genotype, as these are not feasible in DDI studies conducted in patient populations. Time course of reversal of DDIs, where appropriate, can be estimated using PB-PK approaches. Broad-spectrum exclusion of all concomitant medications and the extensive list of foods/beverages is not feasible for cancer patient DDI studies, nor it is practical for healthy volunteer studies, where exclusion criteria should be developed based on the mechanisms of absorption/disposition of the investigational drug.

Lines 1103-1106:	We agree that the highest dose should be used for the interacting drug, but this is not necessary for the substrate. When evaluating a narrow therapeutic range substrate with potentially serious toxicities, such as cytotoxic oncology drugs, a dose lower than the highest dose used clinically may need to be utilized to ensure patient safety in case the magnitude of inhibition by the interacting drug is large. In the oncology setting, it is likely that the highest clinical dose was established in studies where concomitant use of potential inhibitors was prohibited.	Please indicate that the highest dose likely to be used in clinical practice may not be feasible for certain substrate drugs characterized by a narrow therapeutic range. PB-PK-based simulations may be used to predict the extent of interaction with the interacting drug and inform the dose of the substrate drug to avoid toxicity in case of substantial inhibition.
Lines 1103-1106:	When the investigational drug is being evaluated as a substrate and established inhibitors/inducers (*e.g.*, ketoconazole, rifampin) are used as the interacting drugs, there is little value in measuring the plasma concentrations of interacting drugs if their PK has been well-characterized and extensively published in the literature.	Please remove the recommendation to measure plasma levels of interacting drugs that are established probe inhibitors/inducers with well-characterized PK (*e.g.*, half-life, time to steady-state).
Lines 1105-1106:	This is more likely a case when investigational drug is the interacting drug. If the investigational drug is used as substrate, the standard PK of known inhibitor and inducer is already known in the literature, so there is no need to determine the plasma level again. This needs more clarification.	The standard perpetrator (*e.g.*, Ketoconazole or Rifampin) used for testing effects on investigational drug should not be required to assess PK.

Lines 1009-1116:	When the interacting drug effect is delayed, as is the case for inducers and TDIs, we agree that the interacting drug should be dosed for a sufficient period to achieve steady-state inhibition or induction. However, it is unclear why it is necessary for the substrate drug to be evaluated at steady state. If the substrate has time-independent PK, single dose administration of the substrate should be adequate. In addition, it may be problematic to administer a loading dose of an investigational drug characterized by large PK variability or a narrow therapeutic range.	Please remove the recommendation that a substrate drug should be dosed for a sufficient period to achieve steady state levels.
Lines 1118-1124:	When the interacting drug effect is delayed, as is the case for inducers and TDIs, we agree that the interacting drug should be dosed for a sufficient period to achieve steady-state inhibition or induction. However, it is unclear why it is necessary for the substrate drug to be evaluated at steady state. If the substrate has time-independent PK, single dose administration of the substrate should be adequate.	Please remove recommendation that a substrate drug should be dosed for a sufficient period to achieve steady state levels.
Lines 1127-1130:	It is not feasible to conduct 3-period cross-over studies with adequate washouts between periods in clinical DDI studies conducted in select patient populations such as advanced cancer.	Please remove the recommendation to evaluate reversal of induction/ TDI in clinical DDI studies. The time course of reversal of DDIs, where appropriate, can be estimated using PBPK approaches.

Lines 1150-1151:	There is no guidance provided on the optimal delay between dosing of rifampin and the investigational drug in induction DDI studies when the investigational drug is a substrate of OATP.	We suggest: Dosing of rifampin 12 hours prior to the substrate should minimize effects on hepatic uptake during first-pass extraction of the substrate. If the substrate has a long half-life, such that rifampin dosing needs to be continued during the period of its PK assessment in the induced state to evaluate the maximum induction magnitude, it is recommended that rifampin dosing be performed nightly with the substrate dosed in the morning to minimize OATP inhibition while preserving maximum induction of metabolism.
Lines 1151-1152:	It is not feasible to extend the duration of an *in vivo* DDI study in select patient populations (*e.g.*, advanced cancer) to assess reversal of induction/TDI.	Please remove the recommendations to evaluate reversal of induction/ TDI in clinical DDI studies. Time course of reversal of DDIs, where appropriate, can be estimated using PB-PK approaches.
Lines 1159-1170:	This sentence assumes that *in vivo* DDI studies will be conducted in healthy volunteers. Broad-spectrum exclusion of all prior medications and supplements and all of the indicated foods/ beverages is not feasible when DDI studies can only be performed in select patient populations (*e.g.*, advanced cancer patients).	Exclusion criteria should be developed based on the mechanisms of absorption/disposition of the investigational drug and knowledge regarding the effects of specific concomitant medications/foods/ beverages on the relevant mechanisms.
Lines 1194-1197:	It is not feasible to conduct *in vivo* DDI studies in select patient populations (*e.g.*, advanced cancer) with requirement to stratify/power according to genotype.	Please remove the recommendations to stratify/power *in vivo* DDI studies by genotype, as these are not feasible in DDI studies conducted in patient populations. An alternative recommendation is to include EM genotypes, but exclude PM genotypes for polymorphic enzymes (*e.g.*, CYP2C9, 2C19, 2D6) when conducting *in vivo* DDI

		studies.
Lines 1236:	Dronedarone is listed in Table 6 as an *in vivo* P-gp inhibitor and in Table 8 as a dual *in vivo* moderate CYP3A inhibitor + P-gp inhibitor.	For consistency between tables, include dronedarone as moderate in vivo CYP3A inhibitor in Table 3.
Lines 1245-1246:	This appears to be a typographical error.	Please replace "5-fold" with "1.25-fold."
Lines 1435-1438:	If an investigational drug is a substrate for OCT2, OAT1/3, OATP1B1/1B3, or BCRP, it is unclear how to evaluate *in vivo* induction of these transporters. Table 6 indicates there are no known inducers of these transporters at the current time.	Please confirm that *in vivo* evaluation of the effects of OCT2, OAT1/3, OATP1B1/1B3, or BCRP induction on an investigational drug that is a substrate of these transporters is impractical due to unavailability of known inducers.
Lines 1440-1442:	Table 6 (examples of *in vivo* inhibitors and inducers of selected transporters) does not classify transporter inhibitors into strong, moderate, or weak.	As with CYP enzymes, please provide classification of transporter inhibitor strength if recommendation is to initially evaluate strong inhibitor in an *in vivo* DDI study.
Lines 1450-1452:	Table 6 (examples of *in vivo* inhibitors and inducers of selected transporters) does not classify transporter inhibitors according to selectivities or potencies. In addition, several transporter inhibitors in Table 6 may also inhibit CYP enzymes, which may make it challenging to determine the relative contribution of the transporter to the disposition of the investigational drug as substrate, even if a selective transporter inhibitor is used.	It would be useful to classify transporter inhibitors in Table 6 according to potency and selectivity. With regards to selectivity, it would be useful to indicate which transporter inhibitors also inhibit CYP enzymes. It should be indicated that transporter inhibitors may also inhibit CYP enzymes, which further creates challenges in determining the relative contribution of a specific transporter to the disposition of an investigational substrate drug.

Lines 1461 Table 6:	Indinavir and telaprevir are indicated as strong *in vivo* CYP3A inhibitors in Table 3 and as strong *in vivo* CYP3A inhibitors + P-gp inhibitors in Table 8. Therefore, these drugs should be included in Table 6 as examples of *in vivo* inhibitors of P-gp.	For consistency between tables, please include indinavir and telaprevir in Table 6 as examples of *in vivo* inhibitors of P-gp.
Lines 1500 Table 7:	There is a typo.	Please correct "Irrinnotecan" to read "Irinotecan."
Lines 1520-1534:	No guidance is provided on interpretation of cocktail DDI studies.	Please provide criteria for classifying results from a cocktail DDI study as negative or positive.
Lines 1570-1577:	Table 8 does not identify any moderate CYP3A inhibitors that are non-P-gp inhibitors. Although it would be possible to assess the effect of a moderate CYP3A and P-gp dual inhibitor on a substrate investigational drug, Table 8 suggests it would be difficult to assess the effect of a moderate CYP3A inhibitor alone on the disposition of a dual CYP3A and P-gp substrate investigational drug.	Please revise last sentence to read: or inhibitors for one particular pathway only may be recommended, if such inhibitors are available.
Lines 1616:	There is a typo.	Please correct "Intraconazole" to "Itraconazole."
Lines 1688-1692:	We agree that the highest dose should be used for the interacting drug, but this is not necessary for the substrate. When evaluating a narrow therapeutic range substrate with potentially serious toxicities, such as cytotoxic oncology drugs, a dose lower than	Please add the caveat that limitations of using lower dose of substrate investigational drug is relevant only when investigational drug exhibits nonlinear PK.

	the highest dose used clinically may need to be utilized to ensure patient safety in case the magnitude of inhibition by the interacting drug is large. In the oncology setting, it is likely that the highest clinical dose was established in studies where concomitant use of potential inhibitors was prohibited.	
Lines 1760-1763:	While we agree it is important to provide clinical recommendations for the substrate investigational drug affected by other drugs, it may be impractical to provide specific recommendations for marketed substrate drugs affected by the investigational drug. In addition, it is not possible to extrapolate the effect of an investigational drug on one substrate drug to other substrate drugs.	Please clarify that clinical recommendations should be provided for substrate investigational drug, not for marketed probe substrate drug, particularly if the latter drug is unlikely to be co-administered with the investigational drug.
V. LABELING RECOMMENDATIONS		
Lines 1820-1825:	This recommendation somewhat conflicts with the recommendation provided in Figure 2. If the investigational drug affects the disposition of the most sensitive substrate, Figure 2 indicates that additional *in vivo* studies or mechanistic modeling should be conducted with other substrates based on likely co-administration and/or narrow therapeutic range.	Please clarify whether it is acceptable in the label to extend results from one sensitive substrate to all other sensitive and NTR substrates of the affected enzyme, without having to conduct additional dedicated studies or mechanistic modeling.

Lines 1827-1831:	This recommendation somewhat conflicts with the recommendation provided in Figure 2. If the substrate investigational drug is affected by a strong inhibitor/inducer, Figure 2 indicates that additional *in vivo* studies or mechanistic modeling should be conducted with other less strong inhibitors/inducers.	Please clarify whether it is acceptable in the label to extend results from one strong CYP3A inhibitor/inducer to less strong CYP3A inhibitors/inducers, without having to conduct additional dedicated studies or mechanistic modeling.
Lines 1976-1996:	Since there is no BCRP specific inhibitor, it may be useful to have the *in vivo* human comparative PK study based on genotype to understand more of BCRP contribution to DDI and PK variability.	Please add a footnote under the Figure A1 (appendix): "*In vivo* human comparative PK study based on genotype to understand more of BCRP contribution to DDI and PK variability is encouraged."
Lines 1979:	If the P-gp substrate is evaluated by MDR1-transfected cells, this step should be skipped.	Please change the language in the box to reflect this.
Lines 1987-1988:	Please consider effect on absorption as well.	Please edit text to read: In particular, the relative contribution of the transporter-mediated pathway to the overall absorption and clearance of the drug is the primary determinant of whether an inhibitor will have a major effect on the disposition of the investigational new drug.
Lines 1998:	The mean unbound steady-state Cmax value should be used as $[I_1]$, because it is typically recognized as the pharmacologically active concentration. Besides, the criteria $[Cmax,total/IC50] \geq 0.1$ is too conservative to judge the potential risk	Please provide rationale for the use of total concentration as $[I]_1$. Please allow use of $[I]_1/Ki$ only, where Ki =IC50/2. Need a uniformly applicable guidance.

	of P-gp mediated drug-drug interaction. For IC50 determination, the unified method should be employed in order to avoid misunderstandings of the potential P-gp inhibitory effect of compounds. Net flux ratio is the appropriate parameter to estimate IC50 values of P-gp inhibitors according to our experimental data and the model analysis (Sugimoto et al., J. Pharm. Sci., 100: 4013-4023 (2011)).	
Lines 1994-1996:	Consideration is needed for the lack of clinically usable BCRP inhibitor.	Please edit text to read: A similar decision model may be used for a BCRP substrate; however, clinical studies would differ. Due to the lack of specific clinical BCRP inhibitor, the comparative human PK based on the BCRP genotype can help identify the importance of BCRP-mediated drug disposition.
Lines 2001:	Need to add MDR1-overexpressing membrane vesicle, as also suggested by ITC paper.	Please edit text to read: Bi-directional transport assay with a probe P-gp substrate (e.g. in Caco-2, MDR1-overexpressing polarized epithelial cell lines or appropriate in vitro models such as MDR1-overexpressing membrane vesicles)
Lines 2005:	If the investigational drug has low solubility, the simulated Igut should be allowed to be considered.	If the inhibitor has low solubility (much less than dose/250mL), simulated Igut value can be used.

Lines 2013:	Need to clarify: should the NME having all the listed physiological properties or only one property be tested as OATP substrate?	Please clarify.
Lines 2013:	Is hepatocyte model (as ITC paper suggested) acceptable?	Please edit text to read: Investigate uptake in OATP1B1- or OATP1B3-overexpressing cell lines compared to that in empty vector cells or in hepatocytes with appropriate one or more OATP inhibitor to confirm.
Lines 2018:	If hepatocytes are accepted as the OATP substrate and inhibitor testing model, we suggest to add the criteria for OATP substrate in hepatocyte as well.	Please add to the footnote 'b' hepatocytes as well along with transfected cells for testing.
Lines 2027:	The rationale for initially using total inhibitor concentration to assess the risk of OATP1B1/1B3 inhibition versus using unbound inhibitor concentration to assess the risk of OCT2 and OAT1/3 inhibition is not clear. If there is a specific publication supporting this difference, it should be cited.	Please provide rationale for differences in risk assessment strategies for OATP1B1/1B3 versus OCT2 and OAT1/3, as well as rationale for 2-step approach for OATP1B1/1B3.
Lines 2027:	The rationale of criteria for judgment of OATP inhibition study in clinical is not clear and may be too conservative. There is no evidence as to whether adverse effects (*e.g.*, rhabdomyolysis) would be caused in the case of plasma concentration increase of statins by	Please consider adding DDI study based on safety margin.

	a total Cmax/IC50 of >0.1 or a R value of 1.25. DDI risk should be considered based on the safety margin.	
Lines 2049:	Other appropriate and well characterized *in vitro* models should be accepted as well.	Please edit text to read: Investigate uptake in OCT2, OAT1 or OAT3-overexpressing cell lines compared to that in empty vector cells or other appropriate in vitro models.
Lines 2063:	There are no selective probe substrate drugs available for OAT and OCT inhibition testing in the clinic. Although it was described that metformin could be a probe substrate of OCT2 in the clinical studies, it was reported that it was actually MATE that was involved in the DDI between metformin and cationic compounds (Ito S et al., J Pharmacol Exp Ther 340: 393-403 (2012)). Therefore, it is a critical issue that probe substrates for OAT and OCT be identified.	Please provide specific substrate drugs for testing as probes in the clinic.