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PhRMA

November 6, 2006

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

Re: Guideline for Industry on Drug Interaction Studies-Study Design, Data Analysis and Labeling for Dosing and Labeling [Docket No. 2006D-0344]

Dear Sir/Madam:

The attached comments on the above draft guideline are submitted on behalf of the Pharmaceutical Research and Manufacturers of America (PhRMA). PhRMA represents the country's leading research-based pharmaceutical and biotechnology companies, which are devoted to inventing medicines that allow patients to lead longer, healthier and more productive lives.

A PhRMA Joint Committee team has carefully reviewed the draft guideline and would like to take this opportunity to provide comments, which are listed below.

Your consideration of these comments is appreciated. Please contact me if you have any questions.

PhRMA Comments to: FDA draft Guidance Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling. Major comments are ***bolded****.*

General

Our overall impression is that the content is appropriate. However, the document would benefit from greater focus on specific areas that are relatively well-characterized (drug metabolism and clinical drug interactions) and that allow for clear regulatory guidance. **This document summarizes in detail areas that are still not well understood and remain controversial (transporters other than P-gp), and thus cannot be easily regulated. Moreover, the true clinical impact of transporter interactions to date is not well documented. For this reason, the lengthy summarizations regarding**

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transporters may not be ideally covered under a regulatory guidance document and may be better served within another form of publication or manuscript.

The draft guidance addresses conducting in vitro and in vivo drug interaction studies, which can help in dosage adjustments and labeling to avoid undesired consequences due to drug-drug interactions. Many studies described in this document are already being conducted by several pharma companies. However, one of the major issues is that the kinetics of drug metabolism by cytochrome p450 is quite complex and therefore, each substrate-inhibitor interactions are quite unique with variable IC50 and or Ki values. In addition, many times, drugs are not just substrates or inhibitors of CYP isoforms; they also concomitantly induce CYPs and also act as substrates/inhibitors of transporters.

Further, recent research from several academic laboratories indicates that pharmacokinetic interactions due to uptake and efflux transporters results in a more complex interaction profile than originally thought. With such a quite complex mechanisms involved in DDI, using selective probes and inhibitors may not reflect the interaction that may be seen if the interacting co-medications in the market are different from the selective probes/inhibitors used in early clinical DDI studies. We appreciate the effort to address this topic but are very concerned that a better understanding of transporters needs to occur before that section is issued.

The document does not give recommendations as to what stage in drug development DDI studies should be conducted. The guidance charges sponsors to define DDIs during 'early' development and recognizes that a DDI strategy is drug specific. However, can the agency provide any general timings or expectations about when the in vitro and in vivo studies should be considered?
Metabolites are mentioned frequently throughout the guidance, but there is little advice as to criteria for monitoring of metabolites of an investigated drug. For this, a general list of types of triggers or scenarios would be useful. It would be valuable to highlight the importance of bioanalytical capability (i.e., sufficient assay sensitivity to obtain high quality data). A recent reference for consideration of inclusion is the Bjornsson et al PhRMA paper which includes a detailed discussion on analytical considerations for in vitro studies.

Clarify the role of the previous guidance documents (listed below) on similar topics. Does the current DRAFT guidance supersede the previous documents or supplement them?
Drug Metabolism / Drug Interaction Studies in the Drug Development Process: Studies In Vitro, April 1997
In Vivo Drug Metabolism / Drug Interaction Studies – Study Design, Data Analysis, and Recommendations for Dosing and Labeling, November 1999.

Wherever possible, appropriate references supporting recommendations should be included throughout the document

Metabolism by enzymes other than cytochrome P450. The guidance currently focuses on CYP and transporter-based DDIs. Although other non-CYP enzymes potentially contributing to metabolism are briefly mentioned (P. 25, line 793), we would like to encourage the FDA to include further guidance in this regard. This would be particularly valuable for UGT and sulfotransferase enzymes, especially since a large body of literature is available covering these enzymes. Addition of a table with recommended substrates, inhibitors, and inducers for the major UGTs and SULTs would be valuable.

We also suggest the agency provides guidance for investigating interactions at the renal level.

Clinical testing of compounds difficult to evaluate in vitro. The guidance clearly lays out how in vitro tests and clinical studies should be used in concert to evaluate DDI potential and support appropriate labeling. However, in our experience conducting meaningful in vitro studies are sometimes technically not feasible for some drugs showing great promise in the clinic (e.g., due to low solubility, excessive non-specific binding in in vitro systems, or a very low apparent permeability in (polarized) cell lines). The guidance does not address how clinical development of compounds should be conducted for which no robust in vitro data can be generated and what would be a sufficient clinical package for such a compound. Including guidance on this topic is recommended.

Tables in appendices. All tables currently are in appendices. Since the tables are very useful in applying the guidance, placing them more closely to the point of reference in the text would be useful.

Introduction:

Lines 29-31. We request that FDA provide more support of this general statement somewhere in the guidance and cite specific examples that demonstrate clear clinical concerns that would become labeling statements (e.g., >2-fold interaction in AUC or resulting in dosage recommendations) consistent to drug metabolism interactions. If examples of transporter interactions do not meet certain criteria for clinical importance, explanations for when they would still be important would be appropriate

Line 29-32: Unclear whether line 32 relates to previous 2 sentences (transporters and PK/PD) or just the previous sentence (PK/PD).

Line 31: Change sentence to read: "Although less well studied, drug-drug interactions may alter pharmacokinetic/pharmacodynamic (PK/PD) relationships; this important area is not considered in this guidance".

<u>Background:</u>

Line 48: It may be useful to include some background information on transporters in this section, or provide suitable references.

Line 53: We suggest "and/or" instead of "or".

Line 60: Throughout the document in general, the only examples of non-CYP450 enzymes given are transferases (N-acetyl, glucuronosyl etc). Many others such as AO, MAO, FMO, esterases can be important. Does the agency have any recommendations as to their consideration?

Lines 67-69: Does the guidance apply only to active metabolites (e.g., prodrug to active)? Consider to include a decision tree on when phenotyping and inhibition studies need to be conducted.

Line 72: The development of assays for "important" metabolites is mentioned. In the context of drug interactions, we believe that this would refer to those metabolites that contribute substantially to the effects of the drug, rather than metabolites that while possibly representing a considerable concentration in plasma, are inactive. This should be more precisely stated.

Line 87: No definition of the term "narrow therapeutic range" is provided. The development of a working definition of this term would be useful to facilitate communication between groups. Especially for labeling purposes, broad definitions that require interpretation should be avoided.

Line 88: HMG CoA reductase inhibitors may not be "appropriate NTR"

Line 94: It is stated in the guidance that metabolic interactions should be explored, even for an investigational compound that is not eliminated significantly by metabolism. We recommend that the guidance provide more discussion and specifics to clarify what the guidance means by "metabolic interactions should be explored".

Lines 131 to 141: Generally OK, but please consider adding a statement emphasizing the lack of specific probe substrates for the transporters mentioned in this paragraph. Admittedly this is described later in the document, but it could be considered key background information.

Lines 134-136: Please consider adding the ATP-binding cassette and solute carrier nomenclature to this sentence. For example, "...P-glycoprotein (P-gp, ABCB1), ...(OAT, SLC22A), ...(OATP, SLCO1B), ...(OCT, SLC22A), ...(MRP, ABCC), and ...(BCRP, ABCG2)."

Lines 177-188: Please clarify the criteria for "not inhibited or metabolized".

Lines 178 and 183: Please define the criteria for "do not metabolize", or "does not inhibit CYP1A2...".

Lines 188-195: The Draft Guidance fails to mention the cases where in vitro induction of activity of CYP3A or CYP1A2 is found; are we now to evaluate all the other P450 activities, including CYP3A and CYP1A2 again in 3 more hepatocyte preparations? It can not be generalized that if CYP3A is upregulated that CYP2Cs and CYP2B6 are upregulated to the same extent; in many cases they are not and therefore in those cases induction of these enzymes are not likely as clinically relevant as the CYP3A induction.

Line 191: Regarding the statement of co-induction of ABCB1 with CYP3A. The remainder of the paragraph addresses the other CYP enzymes that are co-inducers with CYP3A, but not ABCB1. Please consider adding text confirming whether or not lack of induction with CYP3A also implies lack of induction of ABCB1 or consider dropping the reference to ABCB1 in Line 191.

Line193: Please add ABCB1 to list

Line 195: Please define the criteria for "does not induce ..".

Lines 199-202: Please provide clarification on whether potential drug interactions with CYP2B6 need to be studied for all drugs or provide the criteria used to decide what drugs should be tested.

Line 209: It would be of value to include a perspective on the use the potential application of data from in vivo preclinical models, such the Pgp KO mouse in consideration of clinical studies. (reference to Marathe and Rodrigues, Current Drug Metabolism 7:687-704, 2006).

Lines 223-239: The description of the application of population pharmacokinetics suggests a negative perspective regarding the value of such investigations except to accept that they are informative and may be used to confirm the presence of a drug interaction. There is no indication that population pharmacokinetics can be used to suggest a lack of drug interaction between the NME and other drugs. We suggest that the guidance provide the sponsor the ability to conclude that based on population kinetic data there is no evidence of a clinically important interaction, if no interaction was found with a particular drug.

Lines 230-231: "Simulations can provide valuable insights into optimizing the study design." – It is unclear whether this sentence is a general statement about simulation, whether it is a statement about the use of simulation to design in vivo studies to assess drug-drug interactions, or whether it is a statement about the use of simulation to design large-scale clinical studies in order to detect drug-drug interactions.

Lines 234-236 state "... it is unlikely that population analysis can be used to prove the absence of an interaction that is strongly suggested by information arising from in vivo studies specifically designed to assess a drug-drug interaction." If this is the case, wouldn't the other side also be true, i.e., it is unlikely that population analysis can be used to prove the presence of an interaction that is not suggested by information arising from in vivo studies specifically designed to assess a drug-drug interaction? Any rationale supporting one statement would seem to support the other as well.

Line 241: The in vitro guidance indicates pathways other than the P450s are also of interest, yet the in vivo section of the guidance provides no examples of non-P450 interactions. Are there in vivo substrates, inducers and inhibitors of the UGTs and other non-P450 systems of interest or importance?

Clinical Drug-Drug Interactions Studies:

Weak inhibitors of CYPs are listed in Tables 5 and 6; they are defined as causing 1.25 – up to <2-fold increase in AUC values... (see footnotes). A 25% increase in AUC that is put as a threshold for a relevant inhibition is low. It should be emphasized that the consequences for a compound belonging to this category of weak inhibitors may not be clinically significant.

Many times the design and clinical significance of in vitro interaction may depend on several factors rather than one isolated factor. It should be emphasized that the design of in vivo clinical interaction studies can best be conducted with an integrated assessment of support data (CYP inhibition, Mechanism-based inhibition, CYP induction and transport specificity, induction and inhibition).

Are there any safer alternatives to S-warfarin? Recent data from Tim Tracy's lab using 6 different CYP2C9 substrates (flubiprofen, warfarin, phenytoin, tolbutamide and diclofenac) indicates that inhibition CYP2C9 by several drugs is substrate dependent but most inhibitors were more potent inhibitors of warfarin metabolism than any other compounds. Considering the narrow therapeutic margin of s-warfarin, and potent inhibition of its metabolism, can any other CYP2C9 substrate be employed for in vivo studies?

It will become costly and burdensome to use bioequivalence guidelines for DDI studies? This would require the study of a relatively large number of volunteers to demonstrate this change.

Lines 245-246: We request clarification of the intention of following sentence: "Consultation with FDA regarding study protocols is recommended." As written, the FDA is recommending that all in vivo DDI interactions study protocols conducted as a result of in vitro data should have FDA consultation. This degree of consultation does not seem warranted. There are certain drug-drug interaction

studies that are routine. Consultation with the FDA on these studies would unnecessarily use up FDA resources that would be best served for studies that were not as routine. It may be helpful to be more specific as to what sort of studies or situations would make consultation value added for industry and the FDA.

Line 251: Simplification of study design by not including 'randomised' designs should be considered in order to eliminate blinding, and would not be necessary unless studies involve a PD end point.

Lines 266-268: Dosing with high doses of substrate may not be safe if the magnitude of the inhibitor-substrate interaction is not well predicted. Please separate the recommendation for the inhibiting/inducing drugs from the recommendation for the substrate in this statement. Please consider adding text similar to the text provided in Lines 524 to 528. We would propose that the substrate dose be the lowest dose in the clinically relevant range that will address safety concerns while still resulting in quantifiable concentrations for all treatments.

Line 282: In this bullet point, it is recommended that both the metabolites "of interest" and parent plasma concentrations be measured to determine if they have reached steady-state when the half-life of the metabolite is longer than the parent. We would propose that this is only necessary when the metabolite is also a metabolic inhibitor or inducer. Please consider replacing the sentence, "This is important for both metabolites and the parent drug, particularly when the half-life of the metabolite is longer than the parent, and is especially important if both parent drug and metabolites are metabolic inhibitors or inducers." With the sentence, "This is important for both metabolites of interest and the parent drug, if both the parent drug and metabolites are metabolic inhibitors or inducers and the half-life of the metabolite is longer than the parent."

Lines 294-298: Please consider changing the statement, "..., it may be appropriate to control the variables and confirm the absorption through plasma level measurements of the interacting drug" to "...,it may be appropriate to control the variables or confirm the absorption through plasma level measurements of the interacting drug".

Lines 300-303: At this point it should be mentioned that if a pharmacodynamic endpoint is of interest, then a fourth arm of no drug (or matching placebo for both drugs) should be considered. Without this arm it would be difficult to determine if a pharmacodynamic interaction were synergistic or antagonistic.

Lines 301-303: The statement, "Some design options are randomized ..., and one-sequence crossover" seems out of place in this bullet point and is redundant and less

clear than the statements regarding design options described in Lines 257-258. Consider deleting this sentence.

Lines 309-316: The example statement for exclusion criteria regarding dietary issues is too direct and specific. In particular, the example to exclude selected dietary items for "two weeks" may be quite inappropriate depending on the study being designed and looked upon by sponsors as a direct recommendation from the FDA that may be enforced. If there is insufficient evidence to include such a specific time period in such a general manner, a more flexible option or multiple options as examples should be included. It should also be emphasized that anything included here is definitely only to be used as an example and there is no intention to mandate study design for studies in general. For herbal products one example should be given (e.g., St. John's wort). In the last footnote (P. 22, Table 5), it would be useful to clarify that most grapefruit interactions occur at the level of intestinal metabolism.

Line 311: "....medications, including herbal products, or alcohol within 2 weeks prior to enrollment". We question the need of 2 weeks restriction on alcohol intake. Alcohol is a selective inducer for CYP2E1, not other enzymes, and the induction tends to occur following repeated intake. The sporadic intake of alcohol is unlikely to cause any substantial induction of CYP2E1. In addition, not many drugs are metabolized by CYP2E1. Logistically, it would be difficult to impose such strict restriction. We would like to recommend "alcohol for 72 hours prior to study drug treatment unless the new chemical entity is metabolized by CYP2E1".

Line 312: The list of fruit juices that should not be consumed within two weeks of the start of a drug interaction study seems excessive. While the grapefruit juice interaction is well-established and induction of P450 enzymes by cruciferous vegetables has been known, has it been well-established that 14 days of abstinence from consuming some of the other products listed (e.g. orange juice) is required for a sound interpretation of pharmacokinetic data from a clinical drug interaction study? As these dietary constituents are very commonplace in Western diets, excluding subjects who have consumed them 14 days prior to a study may make it difficult to run studies and, in the absence of data demonstrating a problem, would offer no advantage.

Lines 313-316: Please provide references supporting potential for drug interactions with apple or orange juice and vegetable from mustard green family, etc.

Lines 347-350: Are the substrates identified in the list of example substrates for use in in vivo DDI studies the FDA preferred substrates based on either being a most sensitive substrate (midazolam, repaglinide, omeprazole, desipramine) or the one with a narrow therapeutic range (theophylline, warfarin)? If so, it would be appropriate to call them preferred substrates and more readily encourage their use based on past performance.

Line 348: We recommend to add caffeine for CYP1A2; remove repaglinide as a sensitive/specific probe for CYP2C8 (line 472 supports this); and flubiprofen for CYP2C9 rather than warfarin.

Line 349-354: When would "further studies using other substrates, representing a range of substrates, based on the likelihood of co-administration..." be useful? Product labeling and/or suggestions for dose adjustments? Please clarify.

Lines 358-373: in the document.

Lines 358-373: Clear criteria for classifying a test compound as a strong, moderate or weak inhibitor of CYP3A4 are provided. Do the "fold increases" discussed in this paragraph and in other locations throughout the document refer to the point estimate of the ratio of geometric least squared means based on the inferential statistical analysis? Please clarify. Please provide references supporting the classification of CYP3A inhibitors into strong, moderate and weak based on a particular fold increase in ratio of mean AUC. Similarly, criteria are provided for classifying a test compound as a sensitive CYP3A4 substrate (lines 400-427). Although sensitive probe substrates are recommended in the guidance, it is also indicated that sponsors may use other drugs. It should be added that in these cases alternative <u>sensitive</u> substrates or <u>potent</u> inhibitors should be used as otherwise classification of test compounds could be inadequate.

Lines 360-368: An example is given of labeling CYP3A inhibitors as strong, moderate or weak inhibitors. It is not clear whether the definitions are based on the point estimate or the confidence bounds. If based on point estimates, can no interaction also be claimed based on point estimate rather than 90% confidence interval?

Line 373-383: Provided that the NME is a sensitive substrate for the enzyme induced, one can draw conclusions from single vs multiple dose data.

Line 382: If a compound is proven not to be an inducer of 3A (via a sensitive probe such as midazolam), does this remove the need to complete an OC DDI study?

Lines 387-398: The simultaneous administration of a mixture of substrates of CYP enzymes in one study ("cocktail" approach) is discussed. The information on the "cocktail" approach to assess drug interactions is relatively superficial. For greater utility and applicability to the sponsor, we suggest the guidance provide more detailed information, particularly as the literature offers more than what is suggested in this section.

Lines 402-427: In the guidance, overall elimination of a drug by one pathway by > 25% is defined as substantial. Our experience is that testing for CYP3A4 inhibition in molecules with 25% CYP3A4 elimination through this pathway would rarely be warranted. In theory, even complete inhibition of such an enzyme would result in

only a 1.33-fold increase in exposure. Possibly consider > 50% as a better alternative.

Line 408: Please clarify what is considered a "substantial" contribution refers to a particular metabolic pathway, or does it refer to the overall CYP related metabolism?

Lines 429-433: For labeling purposes, this statement should specifically address sensitive 3A4 substrates or 3A4 substrates with narrow therapeutic range.

Line 429: As there are food stuffs other than grapefruit juice that could affect the systemic exposure, we recommend to make a more general statement -- " food stuffs (e.g. grapefruit juice) may have ....."

Lines 435-439: For labeling purposes, this statement should specifically address sensitive 3A4 or P-gp substrates or 3A4 or P-gp substrates with narrow therapeutic range.

Lines 441-445: Is there a preference of studying poor metabolizers versus doing a formal drug-drug interaction trial. Poor metabolizers are typically difficult to recruit; thus without a preference, industry will probably most often default to a drug-drug interaction trial.

Line 441: Unlike CYP2C19 or 2D6, study of CYP2C9 poor metabolizers is complicated. CYP2C9 mutants excepting CYP2C9*6 have enzyme activities with different affinities for substrates.

Line 443: Please include "..poor metabolizers (naturally or chemically induced) versus ...

Lines 443-444: "When the above study shows significant interaction, further evaluation with weaker inhibitors may be necessary." When would these be necessary, as you already know what the maximal interaction would be using PMs particularly for CYP2D6 and CYP2C19. Would this be for product labeling and/or suggestions for dose adjustments? Please clarify.

Line 445: We recommend changing "When the above study shows significant interaction, further evaluation with weaker inhibitors may be necessary." To "When the above study shows that significant interaction will be expected, further evaluation with weaker inhibitors may be necessary."

Line 448-453: We question the practical value of assessing the effect of multiple CYP inhibitors in humans, when the new chemical entity is metabolized by more than one CYP isoenzyme. It is understandable that inhibitions of multiple isoenzymes would have a greater magnitude of interaction than that of single inhibitor alone. If significant interaction was observed with one inhibitor, consequently this information will be reflected on the label with some caution or

restriction of one inhibitor. It would be difficult to provide clear instruction for dosing adjustment in the presence of 2nd or 3rd inhibitors. It would be equally challenging to provide useful guidance to physicians based on the available information. To minimize likelihood of drug interaction, it is desirable to have a drug to be metabolized by more than one isoenzyme. In our opinion, this kind of approach provides little practical, clinical useful information.

Line 450:...interaction study with more ~~that~~ ***than*** one inhibitor if all of the following.....

Line 451: We have concerns about the conditions "drug exhibits blood concentration-dependent safety concerns". The majority of drugs will have concentration dependent safety concerns if a wide range of dose is explored. We would like to recommend instead "serious safety concern or drug with narrow therapeutic index".

Line 464: Some sponsors are using software such as SimCYP to provide predictions around drug interactions and, where appropriate, would recommend either conducting or not conducting in vivo studies based on these predictions. Please consider commenting on the acceptability of computer simulated predictions to support decisions regarding conduct of in vivo drug interaction studies.

Line 455: Theoretically, the magnitude of change should not be predicted based on the product of AUC fold changes. It must be predicted based on clearance changes associated with the sum of each individual inhibitor. (e.g., if one inhibitor has 10% inhibition and a second has 20% inhibition, then the combined inhibition would be 30%).

Lines 479-480: Please elaborate on this comment such that useful guidance is provided. Considering the importance placed on P-gp-mediated DDIs throughout the guidance document, it seems that limited statements provide minimal guidance. What specific in vitro data drives the conduct of these in vivo studies?

Line 482-486: These studies are only definitive if the investigational drug is not a substrate for CYP3A, as (depending on the concentration) cyclosporine, ritonavir and verapamil are all inhibitors of CYP3A and as well, rifampin is an inducer of CYP3A. This should be made clear. It is very difficult to differentiate P-gp efflux effects from metabolism, especially in the presence of induction and suggest that erythromycin, cyclosporine or ketoconazole be considered for clinical studies, particularly for drugs with limited metabolism (experience with fexofenadine, Ximelagatran). If there is no interaction, then transporter (and metabolism) based DDIs can be considered low. Adding a split to the transporter decision tree based on metabolism of the drug may help.

Lines 484-488: To date there is no validated substrate probe for P-gp. Digoxin is not specific for P-gp.

Lines 484-486. The guidance recommends using compounds such as ritonavir, cyclosporine A (CsA), or verapamil to study whether transport of an investigational drug is inhibited by a P-gp inhibitor. Unfortunately, no selective P-gp inhibitor appropriate for use in human subjects is commercially available. Due to insufficient specificity among P-gp probes interpretation of studies is greatly compromised. All three drugs suggested are also associated with safety hazards when given to healthy subjects long term at exposures required for P-gp inhibition, thus practically limiting their use to single dose administration. The compounds suggested all also inhibit at least CYP3A4 and some other pathways and transporters as well. We recommend that a statement be added to the guidance indicating that for a compound that is also a substrate of CYP3A4, no specific test compound is available for use in a clinical study and interpretations of results from any of the proposed compounds will be difficult with regard to relative effects on CYP3A4 *vs.* P-gp. Among P-gp inhibitors, the ones which could be considered include CsA, quinidine, ketoconazole, clarithromycin and atorvastatin, but quinidine, ketoconazole, clarithromycin and atorvastatin all produce human plasma concentrations at therapeutic doses that are an order of magnitude lower than the IC_{50} or K_i required for P-gp inhibition. The clinically relevant plasma concentrations of CsA as a P-gp inhibitor range from approximately 1000 – 5000 ng/mL. A single oral dose of 300 mg CsA yields mean peak plasma concentrations of approximately 1000 ng/mL with an elimination half-life of approximately 8 hours. It is therefore expected that a dose of 600 mg CsA would provide exposure adequate to test the concept of P-gp inhibition. CsA is known to inhibit other enzymes (e.g., CYP3A4) and transporters, like OATP1B1, and as such is not a specific P-gp inhibitor. Nonetheless, CsA (600 mg) currently appears to be the most appropriate inhibitor drug interacting with P-gp and we recommend the use of this drug in clinical studies. In cases where no interaction with CsA is observed, the data would suggest that P-gp, and several other transporters, like OATP1B1, do not play a significant role in the absorption or excretion of the investigational drug. In cases where an interaction is observed, the data would suggest that transporters (potentially including P-gp) and/or CYP3A4 play a role in the absorption and disposition of the investigational drug. Because of ritonavir's safety profile, clinical trials in healthy subjects may be more difficult to execute. An alternative sentence would end with "...should be studied by using a strong inhibitor of both P-gp and CYP3A, such as ritonavir or ketoconazole." Exposing healthy subjects to multiple doses of rifampin in an interaction study may not be warranted if substantial induction is predicted based on a strong inhibitor interaction, unless the sponsor intends to make dose adjustment recommendations based on the findings of such a study. This is, however, likely to be rare. Once an NME is considered a sensitive substrate, clinical interaction studies with inducers should be considered optional as long as appropriate labeling is agreed upon.

Line 490: Recommend to include other transporter/substrate pairs such as rosuvastatin/pravastatin for OATP, or fexofenadine for multiple transporters, and to add rifampin single dose as a probe inhibitor for OATP. Celiprolol may be an alternative in vivo probe.

Line 516: Can the agency clarify what is meant by shortest dosing interval? Ketoconazole has been dosed previously at 200 mg, BID. Is this a better design than 400 mg, QD? Can there be a comment regarding duration of inhibitor dosing prior to addition of the test article (eg 5 half-lives)?

Lines 518-528: Comments on application of dose selection for probes and substrates are useful and should be expanded to cover the important, well-established probes and substrates much more effectively. This information should be effectively consolidated in a single area of the guidance regarding the dose and application of the probe/substrate. This includes ketoconazole, rifampin, midazolam, clarithromycin, diltiazem and other well known and frequently used drugs. Expansion and consolidation, especially in a tabular form, regarding dose and application would be very useful.

Line 520: suggest rewording to ..."testing should maximize the possibility of finding an interaction within acceptable safety limits".

Line 523: "multiple days" – please provide guidance on minimal timeframe

Line 523: It is important to recognize that rifampin may display significant inhibition of OATP1B1 and this may produce altered hepatic uptake for OATP1B1 substrates (Vavricka, et al., 2002). Therefore, the response to the first dose of rifampin when co-administered with an OATP1B1 substrate, may produce altered pharmacokinetics when compared to chronic response of rifampin induction of CYP3A.

Lines 542-561: On the PK endpoints, we note that, since it is impossible to separate out effects on clearance or volume from those on bioavailability, CL and volume of distribution are rarely appropriate parameters for evaluation of DDIs of orally administered products. Therefore, we respectfully disagree with the inclusion of these endpoints. The focus on AUC and C_{max} as primary PK endpoints needs to be qualified as appropriate only for evaluation of effects following single dose (SD) administration. Following multiple dose (MD) administration, C_{trough} is an important parameter to evaluate. In addition, this parameter is also important for single dose administration of drugs where efficacy is highly dependent on trough concentrations. Therefore, C_{trough} needs to be added to this list and some discussion of the difference between SD and MD studies added.

Line 559: This line implies that metabolites should be measured for the substrate. Was the intention for this to be done with all substrates, that is, for the investigational drug and the probe substrates?

Line 560: "For the substrate, whether the investigational drug or the approved drug, determination of the pharmacokinetics of **important active metabolites** is important". Does the agency have a recommendation as to a definition of "important active metabolite"?

Line 577: Systems with high variability such as drugs metabolized by 3A4 require either a large number of subjects or relaxed goal posts. The guidance should indicate this.

Line 579: should read: whether there is any **significant** increase or...

Line 579: Recommend to delete this paragraph and replace with the paragraph on lines 627-630.

Lines 583-589: It is suggested to use a 90% confidence interval to estimate and not to use hypothesis testing approach to test the interaction. It is stated (605-626, page 14) that if the sponsor wants to make specific claim of no interaction, no-effect boundaries should be pre-specified. However, reasonable boundaries sometimes are very difficult to specify.
For example, if the investigational drug is a substrate and rifampin is used as inducer for CYP3A, the induction could be 4 to 31-fold (Appendix C-3, Table 5, page 35). With such a big range, no-effect boundaries are very difficult to specify. Also, Approach 2 (620-625, page 15) is definitely not applicable since it allows only 20% decrease and 25% increase. One option in this case is to:

1. Design a study with reasonably acceptable sample size based on the standard deviations of the endpoints.
2. Calculate the 90% confidence interval for the ratio and claim no interaction within the observed 90% confidence interval (this approach is called the delta-boundary approach in equivalence analysis).

If the observed lower bound is not too small and the upper bound is not too large, the no interaction claim should be accepted. Otherwise, the potential impact of the interaction should be evaluated through the PK/PD modeling (613-614, page 15) or further investigation should be conducted (412-417, page 10). Even when reasonable no-effect boundaries can be obtained through Approach 1 (613-618, page 15), delta-boundary approach is still applicable. For example, if the no-effect boundaries from Approach 1 are (0.25, 4.0) and the observed 90% confidence interval is (0.5, 2.0), we should claim no interaction within (0.5, 2.0) rather than (0.25, 4.0).

Line 584: The suggestion of using a ratio of geometric means of all PK parameters may not be statistically appropriate since all PK parameters may not have a log-normal

distribution. Some language around the appropriateness of transformation should be included for parameters other than Cmax and AUC.

Lines 585-587 state "Confidence intervals provide an estimate of the distribution of the observed systemic exposure ratio of (S+I) versus (S alone) ..." The first part of the sentence actually refers to what prediction intervals or tolerance intervals would provide, not confidence intervals. Related to this, the guidance does not directly mention prediction or tolerance intervals. Sponsors have sometimes been asked by a regulatory agency to provide a 95% prediction interval for the distribution of individual subject ratios. There have been some recommendations in the industry that prediction intervals (worst case individual subject effect) are sometimes more relevant than the average effect across all subjects. It would be useful for the guidance to address this.

Lines 585-589 state that estimation is the appropriate approach for DDI evaluation and hypothesis testing is not appropriate. However, lines 605-625 says that in order to claim 'no drug-drug interaction' in the label, the sponsor has to define a no effects boundary and the 90% CI should fall within the no effect boundary; the no effect boundary has to be pre-defined based on dose/concentration - response relationships or can be 80-125%. This 'no effect boundary' approach (regardless of the limits of the boundary) is the same as equivalence approach, which is actually a hypothesis testing situation. Thus, there is a contradiction in the statements (between lines 585-589 and lines 605-625) given in the guidance. In one place, it says hypothesis testing is not appropriate, but in the other, requires a hypothesis testing approach for a label claim.

This section addresses the key issue of determining sample size in order to formally (by statistical test) conclude no interaction between the new drug S and the interacting substance I. This issue is very similar to determining the sample size in a bioequivalence study, where S would be compared to S+I. Designing such a study is known to require defining the no-effect boundaries. [Note the existing FDA guidance for bioequivalence, Guidance for Industry. Statistical Approaches to Establishing Equivalence, FDA/CDER, Jan 2001, concentrated on data analysis, not computing the sample size.] Specifying no-effect boundaries are recommended in this guidance (lines 605-609, page 14) when the sponsor wishes "to make specific claims in the package insert that no drug-drug interaction of clinical significance occurs." Generally the sponsor is prepared to include drug use restrictions in the package insert when there is clear S-I interaction. Thus the question: is it acceptable to compute the sample size based on a targeted precision (no power consideration), and then, as suggested in lines 583-589, page 14, to assess exposure changes from the 90% confidence interval of S vs S+I effect, without any formal testing? If it is concluded there is an 0 interaction, does this approach entail restriction in the package insert, or should an additional formal positive "equivalence" study be undertaken to avoid PI restrictions?

Line 587: We agree that 'tests of significance are not appropriate'. Recommend using 'clinically relevant' changes in place of 'significant' changes.

Line 592: ....indicate ***two-fold*** (or lower for certain NTR drugs)……

Line 597: Regarding the statement "For inhibition or induction by the investigational drug, the main consequence of a finding will be to add the drug to the list of inhibitors or inducers likely already present in labeling of the older drug" is there a mechanism for implementation and/or communication?

Lines 605-630: Since inhibitors are now classified according to the magnitude of the change of substrate exposure, it is appropriate to give guidance regarding the sample size for studies where one expects a clinically relevant change. One way to accomplish this is to consider the desired precision and power accordingly when using a substrate that has a well-established variance. For instance, one could power a CYP3A4 study based on the known variance of the commonly recommended substrate midazolam, assuming a 3.5 fold change in AUC (the midpoint of a moderate inhibitor) such that there is an 80% probability that the resulting 90% CI will lie entirely within the 2-fold to 5-fold range for a moderate inhibitor. Please consider adding guidance for sample size if a clinically relevant change is expected.

Lines 611-625: There are two things that should be pointed out here. 1) If the sponsor is to define its own no effect boundaries (Approach 1), then they should consult with the FDA in order to have agreement that these indeed would be considered no effect boundaries by the FDA. 2) In a study in which the goal is to rule out an entire class by using the most sensitive substrate, 80-125% is the only acceptable no effect boundaries.

Lines 620-625: If the classification of the inhibitors is based on a ratio of AUC of marker substance with and without the inhibitor present, shouldn't this approach be based on the ratios rather than confidence limits for equivalence?

Regarding Approach 1 for setting no effect boundaries, many interaction studies are conducted before there is sufficient information about drug safety / efficacy to specify appropriate no effect boundaries. In such cases, it would be useful to know if it is permissible to:

1. Choose N using the Approach 2 default 80-125 boundaries in the protocol,
2. If the 90% CI fails to be within the 80-125 boundaries, retain the possibility to later use (with appropriate justification from improved understanding of the therapeutic index) some wider Approach 1 boundaries for submission purposes/ labeling. This is a common practice by some sponsors.

When a formal no-interaction study is felt to be needed, why is approach 2 (page 15) not considered the standard method, as it is with equivalence studies? Of note, there are cases where the standard 80-125% boundaries lead to infeasible studies for testing equivalence, because of large variability (as alluded to in lines 624-625, page 15).

Approach 1 (page 15) is generally more difficult to implement because the relationship between exposure and PD response is not known with enough precision, nor is the exposure/clinical response relationship known.

Will the guidance related to equivalence be modified in the line of this guidance?

Non-primary endpoints – In drug-drug interaction trials, pharmacokinetic variables will be the primary endpoints; however, safety and other end points will be measured. Care must be taken in order not to over-interpret results from the non-primary endpoints. Some discussion of this is warranted. For instance, suppose the investigational product is a substrate. Typically, the trial would produce information on S and S+I. Suppose, a lab value is shown to be higher for S+I than for S. Suppose I causes the concentration of S to be higher. At this point confounding exists, since it cannot be discerned whether I or the increased concentrations of S caused the increase. The only way that this could have been discerned is if a third treatment arm, I, were included in the trial. Thus, it would be useful to have guidance when this sort of situation is conceivable.

Line 678, Appendix A, Table 1, Major Human Transporters:

- We feel that it is important that this table should be split into two. Table 1 should contain only the clinically relevant transporter, i.e. ABCB1. The title should be changed accordingly. A second table should contain the rest of the transporters with unknown clinical relevance clearly labeled as "for information only".
- Most of the substrates listed are also substrates of different transporters and/or CYP isoenzymes. Similarly, the listed inhibitors are not selective and also inhibit CYP isoenzymes. The overlap of substrate specificity between different transporters and CYP isoenzymes would impose a challenge to define the relative role of these transporters in drug disposition for a given drug in humans.
- For ABCB1, hyperforin should replace St John's wort. For ABCG2, daunorubicin and doxorubicin transport has been demonstrated to occur in BCRP (R482T) and MXR (R482G) variants found in human cancer cell lines that have been selected with cytotoxic agents. These variants display a gain of function phenotype and have no in-vivo ADME relevance with respect to the known reference gene sequence reported for this transporter in humans (Allikmets et al., 1996).
- Indinavir is a substrate for CYP3A4 and multiple transporters and is too broad to be useful in vivo..
- ABCB1 inhibitors are listed, but not all of these are commercially available. For example, GF120918, LY335979, and PSC833 are proprietary compounds and not commercially available. It would be helpful if the agency recommends other drugs/compounds that are commercially available.

Line 688, Table 2, substrate, inhibitor, inducer examples of in vivo CYP :

- For CYP1A2, tizanidine is a sensitive substrate and should be included (Granfors, et.al., 2003; Granfors, et.al., 2004). In addition, bupropoin for 2B6, flurbiprofen for 2C9,and clopidogrel for 2B6 should be included. Omeprazole should be added as a CYP1A2 inducer. Please note in the footnotes that retonavir is not specific for CYP3A4 and should possibly be removed from this list.
- Please cross reference the tables to assure everything in T2 is in T4. For example tolbutamide.
- Note that there is a concern about repaglinide and tolbutamide as substrates. Since most drug interaction studies are performed under fasted condition, the hypoglycemic effect of these two drugs can be dangerous. Alternatively, a safe dose should be provided.
- Please note in the text that although dextromethorphan is an in vivo substrate for CYP2D6, there is high variability in the metabolic ratio confounding clinical pharmacokinetic interpretations.
- For CYP3A4/3A5, it would be beneficial to rank the substrate according to robustness of scientific data.
- Recommend to clarify table titles with "clinical" probe substrate or inhibitors, as both in vitro and in vivo studies are addressed in the guidance.

Line 704, Table 3: The table lists sensitive substrates based on interactions with inhibitors. A footnote needs to be added to highlight that these sensitive substrates can be used as probes for induction studies as well

Line 718, Table 4: The guidance states that repaglinide is a sensitive CYP2C8 substrate based on a >5X effect of gemfibrozil on its exposure. Rosiglitazone, another 2C8 substrate that is also affected by gemfibrozil is on the list of 2C8 substrates but is not considered as sensitive (since interaction is <5X). However, from a mechanistic standpoint, the reason for a >5X interaction between repaglinide and gemfibrozil is due to inhibition of both metabolism by 2C8 and transport by OATP1B1, since repaglinide is a dual substrate of both CYP2C8 and OATP1B1 and the contribution of this transporter to its disposition is supported by clinical pharmacogenetic data. Therefore, it is not currently established that repaglinide will be more sensitive than rosiglitazone to CYP2C8 inhibition by a NME that is a "pure" CYP2C8 inhibitor. Should either of these substrates be considered for clinical DDI studies evaluating NMEs as CYP2C8 inhibitors?

Line 726, Table 5: Please add a table that classifies strong, moderate, and weak inducers of CYP3A4.

Line 742, Table 6:
Nefazodone has been withdrawn from many markets including US. We suggest either delete or indicate restrictions as in Table 3, for example. Gemfibrozil is not a strong 2C8 inhibitor; it should be removed.

Line 757 Appendix B Figure 1, CYP based DDI:

- Appendix B has only one figure, yet the title says Appendix B- Figures...
- Please add CYP 2B6 to the first box that describes In Vitro Metabolism Information
- Top box on the left, please define what is meant by a "major" contribution of the pathway
- There should not be a mandatory requirement to study other inhibitors and inducers after a positive finding with the most potent inhibitor/inducers. The decision tree should allow us to look at safety window or therapeutic window, or, allow us to accept the most strict labeling from the most potent inducers/inhibitors.
- Please clarify, if in vitro studies are negative (left box), does this mean no clinical studies are required? Please expand on the comment on line 1059 refering to the rank order approach"NME not a substrate or NME a substrate but contribution of pathway **not major**" and "NME is a substrate and contribution of **pathway to elimination major** or unclear" boxes. Does the "major" refer to > 25% of the clearance pathway as it is defined in line 408 above? Recommend to add a footnote to the fig 1 to define "major pathway".

- Line 767: to 'negative results from a' add; appropriately powered

Guidance suggests that *"experiments designed to identify and quantitate the relative importance of individual CYP enzymes mediating a drug's metabolism should use drug concentrations ≤Km"*. This is not clear whether guidance indicates the Km determined for the parent compound disappearance is sufficient or Km for each metabolite formed. This means that each metabolite needs to be synthesized and quantitated. That should not be required due to limited value and high costs

Line 784: For clarity, are in vivo or in vitro metabolite profiles being referred to here?

Lines 787 and 848: We do not feel that a 25% contribution of CYP enzymes to total clearance is enough of a contribution to require reaction phenotyping, since complete inhibition of such a pathway in vivo would only yield, at most, a 33% increase in exposure (using the equation of Rowland and Matin, 1973). We propose that this value should be 50%.

Line 793: Suggest to re-word as described "...is encouraged, ***particularly since the role of non-CYP enzymes in drug metabolism is an emerging scientific area***."

Line 799: Expectation of non-P450 pathways should be discussed

Line 808: Instead of determining whether the metabolic pathways are parallel or sequential, it may be just as informative to determine the extent of metabolism via individual pathways. It is often not feasible, nor important, to obtain information on the parallel or sequential nature of metabolic pathways during the early part of the drug development process.

Line 827: Please change to (1) specific chemical *inhibitors* or antibodies (delete "as specific enzyme inhibitors").

Lines 827-828: We suggest replacing this sentence with "Further analysis of the radioactive peaks by MS/MS, UV, fluorescence, etc., may provide structural elucidation capabilities."

Line 833: Suggest to re-word "Expected steady state in vivo plasma concentrations ***or predicted clinical Cmax*** may be helpful ..."
Line 842: Table 1: add isovanillin as an aldehyde oxidase inhibitor

Line 848: Can the agency clarify as to what in vivo study or data would be used as basis of the 25% drug clearance as a trigger for in vitro CYP identification studies? (e.g. HRS, cold urine analysis, etc)

Line 859: Please add (3) *correlation analysis based on* a bank of human liver ....).

Lines 865-866: Please delete the sentence "For correlation analysis ..." since it is redundant with item #3 in line 859.

Line 876: Would the agency comment further on when studies of individual isomers vs. racemic drugs be completed? For instance: If drug was not significantly cleared or metabolized by P450, or if there was rapid metabolic conversion to one, or both, of the isomers, we would not evaluate isomers.

Line 889, Table 2: We have some suggestions regarding the list of inhibitors for in vitro experiments.

- For CYP2C8, quercetin is not selective enough to be used to identify metabolism by this enzyme. Quercetin also inhibits CYP1A2 and CYP3A (Walsky, et al., 2005).
- Interpretations made using ticlopidine as an inhibitor need to be made with caution as this compound potently inactivates both CYP2B6 and CYP2C19, and inhibits CYP2D6 (Turpeinen, et al., 2004).
- Fluconazole, fluvoxamine, and fluoxetine are non-specific for CYP 2C9 at relevant concentrations and should be removed from the table as acceptable inhibitors.
- Include N-benzylnirvanol (Suzuki, et al., 2002), omeprazole as the CYP2C19 inhibitors.
- Include azamulin as the <u>preferred</u> CYP3A inhibitor as it is more selective than ketoconazole or itraconazole;
- Include bufuralol as a CYP2D6 inhibitor
- Include diclofenac as a CYP2C9 inhibitor.
- methoxsalen not selective 2A6 inhibitor (also inhibits 1A2, 2D6, etc)
- orphenadrine as a 2B6 inhibitor
- tranylcypromine also inhibits 2C19 as well as 2A6.
- Although gemfiborzil is a potent inhibitor for CYP2C8 in vivo, it is not a potent selective inhibitor in human liver microsomes. We recommend that it is excluded from the list of acceptable in vitro inhibitors for CYP2C8.

Table 2: Inhibitors/mechanism based inhibitors:

- We recommend adding that other mechanism-based or time-dependent inhibitors ticlopidine and clopidogrel (Richter, et al., 2004), phencyclidine (Jushchyshyn, et al., 2006), thioTEPA (Richter, et al., 2005), diethyldithiocarbamate (Guengerich, et al., 1991), troleandomycin (Zhao, et al., 2005), and verapamil (Wang, et al., 2005). These should all be preincubated when used as chemical inhibitors. Please note in footnote 2.

Line 892: Please change statement to "Cited studies were performed using following substrates"

Line 897: hydroxylase is misspelled

Line 912: If the clinical concentration exceeds Km, a clinical concentration should be studied, not an artificial concentration with no clinical relavance

Line 916: It is stated that "Noncompetitive and mechanism-based inhibitors are not dependent on the drug (substrate) concentration." While this statement is true for noncompetitive inhibitors, it is not true for mechanism-based inhibitors. We recommend that the original statement is replaced with the following statement: Mechanism-based inhibitors are time- and concentration-dependent.

Line 918: This maybe too much detail 'Preincubate for 15-30 minutes'. Suggested re-wording ***"...for 15 to 30 min in presence of cofactors and investigative drug to generate inhibitory metabolites (prior to addition of probe substrate"***. Line 923-925: The enzyme activity measured in recombinant P450s does not provide information on the relative importance of the individual pathways in human liver microsomes, *unless it is scaled to the relative abundance of that enzyme in the liver.* It is a common practice in industry to do this and the addition of this point to this line in the guidance would make the point of it's validity more clear.

Line 964: In the section on Correlation Analyses the authors should address the use of multivariate correlation analyses to demonstrate the role of multiple enzymes.

Line 993, Table 3: We question the usefulness of S-mephenytoin N-demethylase as a selective probe activity for CYP2B6, since this activity also has a low K_M component that is catalyzed by CYP2C9 (Ko, et al., 1998). Testosterone 6β-hydroxylation is listed as one of the two preferred substrates for CYP3A4/5. We recognize that testosterone 6β-hydroxylation has been widely used as an in vitro research assay for CYP3A4/5 activity, and testosterone represents a distinctive binding site from other CYP3A4/5 substrates including midazolam, nifedipine and erythromycin. However, there is no relevant in vivo probes that can be used in human to test testosterone 6β-hydroxylase activity. We recommend that nifedipine oxidation which represents not only a distinctive binding site, but also a class of clinical relevant substrates of CYP3A4/5 be added to your preferred substrate list.

Line 1014: Shouldn't this be >30% substrate depletion?

Line 1021: For many new molecular entities, final delivery solvent concentrations will need to be above 0.1% (v/v), which is listed as the preferred upper limit solvent concentration. We agree that if the solvent used is DMSO, then 0.1% is an appropriate upper limit since this solvent can have profound effects on P450 activities (Chauret, et al., 1998). But for other solvents such as acetonitrile or methanol, the percentage could be higher as long as the method is validated at that amount of solvent.

Line 1027: Section 3 is valid for non-CYP targets (transporters, other enzymes etc). Therefore, we recommend that this section come at the end of the guidance, not at the end of the CYP section. Recommend to note that IC50 is equally acceptable as the use of Ki when substrate concentrations are set to its Km.

Line 1035: To this paragraph a discussion of the role of the fraction of the inhibited drug metabolized by the enzyme inhibited should be added (see for example: Ito et al Drug Metab Dispos 33:837-844, 2005).

Line 1044: The approach listed of using $[I]/K_i$ ratios (where [I] = systemic C_{mac}) is too simplistic an approach to predicting the likelihood of drug interaction. Data in the scientific literature suggest that consideration of the fraction of the victim drug metabolized by the inhibited enzyme is very important (Brown, et al., 2005; Ito, et al., 2005; Venkatakrishnan and Obach, 2005; Obach, et al., 2006). Furthermore, it was stated in the document that systemic C_{max} is the concentration that should be used in comparing [I] to K_i. However, it is highly unlikely that this is the appropriate concentration available to the enzyme. Protein binding of the inhibitor is an important consideration. This is exemplified by the recent example of montelukast, a very potent inhibitor of CYP2C8 in vitro. Systemic total C_{max} concentrations would suggest a high likelihood of a drug interaction with a CYP2C8 cleared drug (e.g. repaglinide), however no such interaction was observed. This is due to the high plasma protein binding of montelukast, and when this is also considered, it would be predicted that there would be no interaction in vivo (Kajosaari, et al., 2006). Such an approach is also consistent with the well-established principle of free drug concentrations being those that can exhibit pharmacological effect (i.e. the "free-drug hypothesis"), in this case considering inhibition of P450 enzymes as the pharmacological effect.

Lines 1048, 49 and Table 4: It is unclear that if the ratio of [I]/Ki <0.1, then no additional studies would be necessary.

Line 1050, Table 4 [I]/Ki >0.1 recommends clinical study. Does MDI always require follow up irrespective of [I]/Ki? We would suggest that IC_{50} under certain conditions ([S] = Km) can substitute for a Ki value, as noted above. The last entry in table 4 is a confusing. Suggest replacing "0.1 > [I]/Ki" with "[I]/Ki < 0.1"

Lines 1067-1074, Mechanism-based inhibition:

- Relatively little guidance is provided on how to determine whether an NME is a mechanism-based inhibitor in vitro or in vivo. Since literature suggests that this type of inhibition is a major cause of CYP3A4-mediated drug interactions, addressing this topic more extensively is recommended.
- Including a table with acceptable positive controls and assay conditions would be helpful. Providing guidance in which cases clinical studies are warranted is also recommended.

- The use of 30 min is usually excessive and not useful for detailed kinetic analyses.
- There needs to be more guidance for the magnitude of MBI to substantiate a clinical DDI study in humans.
- It is stated that "Any time-dependent and concentration-dependent loss of initial product formation rate indicates mechanism-based inhibition." The time- and concentration-dependency can only suggest metabolism-based inhibition. Other criteria for irreversible or quasi-irreversible inhibition are required to determine mechanism-based inhibition.
- Please add methylenedioxy as a compound known to cause mechanism-based inactivation.
- It is stated that "Detection of time-dependent inhibition kinetics in vitro indicates follow-up with in vivo studies in humans." It is unclear what type (or to what extent) of time-dependent inhibition kinetics in vitro such as K_I and k_{inac} indicates that in vivo studies in humans should follow. More clarification is needed for this statement.
- The terms of "mechanism-based inhibition" and "time-dependent inhibition" are used inter-changeably. Some time-dependent inhibitors are not mechanism-based inhibitors. There are more strict criteria for mechanism-based inhibitor than time-dependent inhibitor. If the guidance is only for mechanism-based inhibitor, more clarification should be made in this section.

Line 1078, APPENDIX C-3. There is no recommendation for categorization (strong-moderate-weak) for enzyme induction-related interactions. The categorization of strong, moderate, and weak for enzyme inhibition (based on AUC changes, page 9) is helpful, and a similar ranking of AUC changes due to enzyme induction with possible outcomes would be equally helpful to standardize the language across this field.

Line 1080: Use of Immortalized cells: On one hand the guidance states that PXR reporter assays, an immortalized cell model designed to respond to PXR activators, can *"provide supportive evidence for a compound's induction potential"* but *"do not necessarily reflect the enzyme activities" (pg 35)* and on the other hand state that the use of other *"immortalized liver cells are acceptable if it can be demonstrated with positive controls" (pg 36)*. This may be a problem since immortalized cells are not yet fully characterized and therefore to conclude they represent primary hepatocytes with regards to response to CYP inducers may be misleading; for example we know these cells contain PXR and AhR but do they also express CAR? What about drug transport function, how do they compare to a primary cell? Even though these may be excellent models to use early in Discovery they may not be adequate stand alone assays for in vivo prediction.

CYP2B induction: On page 5, the guidance suggests that compounds that induce CYP3A also induce CYP's 2C and 2B and therefore may be a good indicator for the

potential induction of all these CYP's. However, on pg 37 they indicate that CYP3A4 induction is indicative of CYP2C8, 9 and 19 induction but do not mention CYP2B6. Literature on CYP2B6 induction has concluded that induction can occur through other mechanisms separate from PXR activation (mostly via CAR) and so potential for CYP 2B induction may not be predicted by CYP3A induction.

Line 1084: Consider re-wording to "... could lead to increased formation of an active ***and/or toxic*** compound, resulting in an adverse event."

Lines 1091-1093. Chemical Inducers as a Positive Control. It is stated that "The positive controls should be potent inducers (>2-fold increase in enzyme activity of probe substrates at inducer concentrations <500 μM)". This is contradictory to the information contained in Table 5 that refers to phenobarbital and pyrazole at concentrations greater than 500 μM.

Line 1095, Table 5:Please add rifampin as a preferred or acceptable inducer of CYP2B6.

Lines 1105-1135. In the section about design of in vitro drug induction studies, there is lack of criteria about acceptable cell viability. To strengthen the validity of cell-based enzyme induction models, appropriate measures of biochemical cellular toxicity would be beneficial. We suggest a minimum cell viability of >70% when initially plating cells. In addition, it is highly recommended that cell viability be measured at the end of the experiment on control and drug treated cells. Significant cytotoxicity caused by test articles can result in false negative interpretations. We suggest that any test article (and its associated concentration) that causes >50% cytotoxicity in a biochemical toxicity assay should not be used in the final interpretation of results.

Lines 1112-1113: Design of In Vitro Drug Induction Studies. Immortalized liver cells for induction. This is a significant departure from accepted norms. In the statement "... ...immortalized liver cells are acceptable if it can be demonstrated with positive controls that CYP3A4 and CYP1A2 are inducible " , it is not clear whether this refers to enzyme activity or other measurements such as mRNA and western blot. In addition immortalized cell lines do not appear to retain all of the fully functional mechanisms of enzyme induction as do primary hepatocytes. For example, Fa2N-4 cell line responds appropriately to PXR- and AhR-mediated enzyme induction, but does not appropriately respond to CAR-mediated enzyme induction. In addition, some inducers may involve multiple mechanisms such as PXR- and CAR activation. To clarify those concerns, we recommend the following modifications to this statement: "... ...immortalized liver cells are acceptable if it can be demonstrated with positive controls that CYP3A4, CYP1A2 and CYP2B6 enzyme activities are inducible" The Bjornsson et al PhRMA perspective paper (Drug Metabol Dispos 31:815-832, 2003) does not endorse the use of these cells However, these immortalized could be very useful as a screening tool .

Line 1115: It is stated that "Test drug concentrations should be based on the expected human plasma drug concentration (to) be used" The word "to" is missing. More importantly, it would be helpful to clarify whether the expected human plasma drug concentration refers to free or total drug concentration. To be consistent with the inhibition section, this should be total concentration.

Lines 1116-1120: It is important when measuring CYP activities in cell monolayers after an induction period with an investigational drug that the CYP inhibition properties and metabolic stability in hepatocytes of the investigational drug is known. If not, measurements of mRNA are important when the investigational drug is also an inhibitor to eliminate false negatives. The importance of measuring mRNA is mention in Line 1172, but may be of great enough importance to mention it earlier.

Lines 1121-1125. There should be some mention that enzyme inhibition data (of the test article) is necessary when interpreting enzyme activity results from probe substrates as inhibition can confound such results.

Lines 1127-1128. It is stated that "When conducting experiments to determine enzyme activity, the experimental conditions listed in section Appendix C-2 are relevant". In Appendix C-2, there are discussions about how probe substrates at K_m concentrations should be employed for enzyme inhibition study, however, this is not true for induction studies where concentrations approching V_{max} are employed. We recommend that appropriate language such as "probe substrate concentration at 3-5 x K_m, or maximum solubility of the probe" should be added in this section.

Lines 1142-1143: The guidance recommends that in cases where the induction of enzyme activity is equal or greater than 40% of the positive control, the compound can be considered as an enzyme inducer. Interpretation of this number in isolation is difficult. It is not stated whether it is 40% at the maximum achievable concentration, response, or at therapeutic concentrations and does not mention which positive controls are recommended. The following points should be considered: (i) The percentage induction measured at a particular drug concentration should be evaluated in the context of clinically relevant drug concentrations. (ii) Induction of activity will be low or absent if the test compound is an inhibitor of the enzyme evaluated. In these cases quantitative mRNA expression data should be taken into consideration in evaluating the propensity of a test compound to cause induction in humans. (iii) Whether a compound will be qualified as an inducer will depend on the positive control used. For induction of PXR target genes such as CYP3A4, we think the use of rifampicin should be recommended as the most appropriate positive control as it elicits a response near maximum induction. (iv) Interpretation of the percentage of the positive control is only possible if the window for this control is reasonably high (~5-10-fold).

Line 1152: It is important to note that the measurement of CYP3A activity as the only endpoint could be misleading regarding conclusions of in vitro induction studies, and that measurement of mRNA is also warranted. This is proposed because some agents can

be simultaneous PXR activators (which results in the induction of many enzymes/proteins as stated in the document; e.g. CYP2C9, P-gp, etc) and CYP3A inactivators. Using a CYP3A activity endpoint could misclassify such a compound as a non-inducer, and induction effects on substrates of CYP2C9, P-glycoprotein, etc could be overlooked. An example of this was described in the scientific literature (Luo, et al., 2003).

Line 1156: Recommend adding the following text: "(d) The clinical dose and dosing regimen (single dose vs repeat dose, acute vs. chronic) are key considerations when for an in vivo DDI study based on in vitro hepatocyte induction data."

Lines 1157-1187: Please include comments on the use of computer simulation as an additional method to identify and predict drug interactions.

Line 1175: Suggest replacing line 1175 with: "RT-PCR can quantify mRNA expression for a specific CYP enzyme. Although a one-to-one quantitative relationship between mRNA expression and enzyme activity is not expected, with carefully defined criteria, changes in mRNA levels, in most cases, are indicative of changes in enzyme activity."

Drug Transport Studies:
Overall, we agree with the agency that the area of drug transport merits investigation with regard to drug disposition and drug interactions. This is an emerging area of science, and while our knowledge of P-glycoprotein has advanced to the degree to which we can reliably interpret the data from in vitro and in vivo studies, there are many other drug transporters that may gain in importance in the future. As this guidance regarding P-gp potentially sets the stage for future guidance around other drug transporters, we want to ensure that the principles underlying the guidance are scientifically sound and not excessive. We agree with the principles outlined, but we have many comments regarding the specifics around the conduct of assays, selection of systems, and selection of approaches used to interpret the data. At this early point, we believe that the recommended experimental details described in this document may be detrimental to the advancement of the science around drug transporters and would ask that the guidance remain flexible with regard to experimental details.

Flux ratios of >2 occur very routinely and should not be alarming. This should be raised to at least 3-4 but should actually be considered along with other properties of the drug when designing follow-up programs

APPENDEX D. The terms of "P-glycoprotein" and "efflux" are used inter-changeably though the section. Since there is lack of specific substrate and inhibitor for P-gp, it is difficult to assess the contribution of other efflux transporters such as BCRP and MRP-2 to the net flux ratio of the cell culture systems. Therefore, cautions should be taken when concluding whether or not the test article is a P-gp substrate or inhibitor.

Line 1192: Add ABCB1 to list of names. Technically the protein should be referred to as ABCB1 and not P-gp or MDR1 throughout the document.

Line 1192, P-gp: There is significant concern regarding the detail of experimental design outlined in this section. The details are too specific with respect to membrane, seeding density, TEER, days in culture, etc. and recommend stating that the test system be well characterized by the laboratory with positive controls should demonstrate the functional activity of the test system as described in D2.a and b. This section could be qualified by stating "General Tissue Culture Considerations to Ensure Functionally Polarized Cells" (Line 1321) In addition, it is not consistent with the other parts of the guidance where broad concepts are outlined. This is of concern because the maturity of the field is just the opposite as that of the P450s; that is, there is not yet a consensus of the most appropriate methods to do transporter experiments. Thus the authors of the guidance should significantly reduce the specific methodology and focus on concepts. Specific examples of clinical consequences of interactions with P-gp would be of benefit.

Line 1199: "It is generally accepted that co-administration of drugs that interact with this transporter .... can result in drug-drug interactions...." is not truly generally accepted. The language is extremely strong considering that there is no state of the art in the field of transporter DDI's. To date there is very little clinical understanding of the implications and the probes are not even close to ideal. There remains considerable controversy as to whether significant or clinically meaningful drug interactions are commonplace for this transporter. Therefore we should be cautious of regulatory creep adding studies which sound good but have unknown clinical relevance.

Line 1206: The language around modulation of P-gp and resultant outcomes is vague – since this is a relatively new area, we would suggest that some specific P-gp DDI cases in which this has been observed be referenced.

Line 1221, Table 1: The table is confusing. The statement: "Tends to fail to identify substrate and/or inhibitor with low permeability." Should be also added to the assay type of Bi-Directional Transport. (i.e. Fexofenadine is not identified in MDCK assay's as a P-gp substrate since it's uptake transporter is not present.

Line 1221, Table 1: A fourth assay type should be added: In-side out vesicles The Tissues: Cell line over-expressing ABCB1 Parameters: Uptake into the vesicles Comments: 1. Requires a radiolabel or highly sensitive analytical assay 2. Direct measure.

Line 1221, Table 1: Consider adding the phrase "permeability ratio" to the terms describing the parameters for bi-directional transport assays.

Line 1221, Page 39: Nomenclature (table 1):
MDCK-MDR1 or MDR1-MDCK (as on pg 40)
LLC-PK1 MDR1 or MDR1-LLCPK (as on pg 40)

LLC-PK or LL-CPK1 or LLCPK or LLC-PK1 (as on pg 39, 40, 42, 43)

Line 1221, Table 1: Change "tissues" to "cell systems"
Bi-directional transport Comment: Please clarify or delete how a bi-directional study helps identify the localization/identification of the transporters within the apical or basolateral membrane.

Line 1222, Table 1: In the "Tissues" column, "oocytes injected with cRNA for transporters" is listed. This heterologous expression system has the additional disadvantage of expression of transporters on a membrane type (frog egg) that is markedly different from epithelial and endothelial cells that typically express transporters endogenously. Thus, it would be a good idea to list this in the Comments section.

Line 1223-1231:

The use of cells which over-express specific transporters (eg. Pgp or BCRP) have been useful in determining if investigational drugs are inhibitors of these transporters using specific fluorescent probe substrates and measurements of the inhibition by flow cytometry. Potency of the inhibition is described by calculations of IC50s using the Hill equation, as well as by magnitude of the inhibition.(ref).

Line 1224: While it is true that the bi-directional transport system can be viewed as a reliable system, it certainly has some caveats. First, drug efflux is not directly measured. In this system, permeability is measured and comparison of conditions (A to B and B to A, +/- inhibitors) infers the involvement of P-gp. Some language around the caveat of using this system, namely that permeability rather than efflux itself, is being measured to gather information about P-gp should be included. For example, a P-gp substrate that has very low intrinsic membrane permeability can be missed using this system.
Please change "definitive" (line 1224) to "preferred"

Lines 1232: Should read; " Although the bi-directional transport assay may fail to identify highly **and poorly** permeable compounds as P-gp substrates,"

Lines 1235: "the transcellular transport **or vesicles assays** should be used..."

General Comment on Appendix D Section 2. In cell-based bi-directional transport assays, pH can have a large effect on the data. Should pH values be better defined because of this?

Line 1246: There is not a known probe that is selective of P-gp. Therefore this should not be stated so firmly.

Line 1246: Is a selective probe "preferred" even for recombinant cell lines, such as MDCK-MDR1 or LLC-PK1 MDR1? This may be needed for Caco-2 cells, however, there

may be some flexibility with individually transfected cell lines. Suggestion to item (1) is to state "ideally" the probe is selective for Pgp.

Line 1247: The range of permeability is model/plate-format dependent. Actual numbers should not be listed for these ranges.

Line 1259, Table 2: We challenge the need for such detail in the description of the probes used at certain concentrations and efflux, particularly in light of the statement of ambiguity made on line 1252. Some of these conditions are excessively challenging; for example running quinidine at 0.05 uM in Caco-2 is much lower than needed and creates overly burdensome analytical challenges. We also challenge the absolute ability of the ratio of ratios to be able to correctly assign P-gp substrates in the MDR1-MDCK system. A significant caveat of using the background correction approach with the MDR1-MDCK and MDCK cell lines is the presence of functionally active P-gp in the MDCK cells; this can lead to false negatives. In some cases there is enough P-gp efflux in MDCK via endogenous canine P-gp to maximize the impact of P-gp. In other words, the effect of P-gp is not linearly related to protein expression and dog and human transporters are homologous enough to mediate the same effect. As an example, macrolide antibiotics, which are some of the most well-established P-gp substrates, would not be classified as P-gp substrates using a ratio of ratio approach.

Line 1259, Table 2: Add HIVPIs (e.g., saquinavir, amprenavir) and paclitaxel (Taxol) as Pgp substrates.

Line 1285: "Most P-gp substrates with high affinity are also potent competitive inhibitors." This is not true for digoxin or vinblastine. Suggest that this sentence be deleted.

Appendix D lines 1292-1294, 1361-1364, 1514-1518, Table 3 p.44: It will be helpful to list the transporters that are inhibited by the indicated agents in Table 3, so that the most appropriate inhibitors may be chosen to address the transporters in question. Since the inhibition of uptake (e.g. OATP) versus efflux (e.g. P-gp) transporters will have very different effects on the efflux (BA/AB) ratios in a monolayer system, and dependent on the system used, the recommended usage of at least 2-3 P-gp inhibitors may not be always necessary.

Line 1292 (and Table 3): It is stated that the use of multiple inhibitors is recommended to determine whether the efflux activity observed in vitro is related to P-gp and this is proposed in reference to the overlapping inhibitory potency of some P-gp inhibitors. While it is true that many overlap with other transporters, not all cell lines express all of these transporters. We recommend that language be included to add the need to characterize some of the more relevant transporters in the cell system to be used to gauge inhibition and then select the most appropriate inhibitor based on that system. Using fewer inhibitors in a system that is well characterized with regard to P-gp and other

transporter expression would be an approach that would provide greater confidence in the conclusion vs. running several inhibitors in an uncharacterized system.

Appendix D. Section 2(c). General comments. The level of detail is excessively prescriptive and restrictive. The key to doing sound transwell bi-directional transport studies resides in the use of positive and negative controls. These address the integrity of the monolayer and expression of transporters. For example, the use of TEER (line 1326) is not necessary if data for positive and negative controls are acceptable. In fact, in the case of TEER, there are other perfectly adequate approaches to ensure monolayer integrity (e.g. Lucifer yellow). One experimental parameter that requires specific attention is the pH of the medium on apical and basolateral sides. Use of the same pH (7.4) on both sides of the monolayer reduces artifacts due to sequestration caused by substrate ionization differences. Additional detail should be supplied about 'preferred cell lines'. In particular, what is the reference gene sequence for ABCB1 (MDR1). What is the method of stable transfection? Do we know which variant of ABCB1 is expressed in Caco-2 cells? Should there be guidelines from CDER with respect to cell line best practices?

Appendix D, section (c): Tissue culture considerations to ensure functionally polarized cells. No guidance is provided regarding to the appropriate pH value for bi-directional studies. We recommend that pH 7.4 for both sides should be considered as appropriate.

Lines 1293: Due to lack of inhibitor specificity, it is suggested that "the use of multiple inhibitors" be employed. It may be a good idea to clarify that this type of study would be most successful if the various inhibitors were used either it separate experiments, or separately and then also in a "cocktail" format in order to get the most useful information.

Lines 1316-1359: Detailed recommendations are made regarding conducting transport experiments with polarized cell monolayers. Although such information will be of value to scientists not familiar with these types of studies, the guidance provided is very broad in several cases (e.g., P. 43, line 1323, it is recommended to seed 0.05-5.0 x 10^6 cells/cm^2 on membrane filters). Since providing guidance on experimental detail is difficult, **we propose that only general recommendations should be provided.** CaCo-2 are not a preferred cell line for many reasons. The cells are highly variable from lab to lab and even over time in the same laboratory. They also express a vast array of transporters and it is difficult to interpret the data. They are a secondary choice if the lab does not have an over expressing cell line available.

Lines 1316-1317: The use of wild type cells as a control is not necessary and may confound data interpretation. Upon over expression of ABCB1 other transporters are also up and down regulated. The over expression of ABCB1 is also know to alter membrane composition and may alter the permeability of certain compounds. It is better to use the over-expressing cell line with inhibitor present as the control.

Lines 1319-1329: This section is over prescriptive (as is section d and section f). If the model is working for control substrates it is not necessary to follow the details outlined. Specifically, TEER is not a particularly useful measure and is highly variable. The use of a paracellular marker is also unnecessary if a control compound is used and within historical values.

Line 1328. Other paracellular markers with other means of detection (e.g. nadolol, with mass spectrometric detection) would also be adequate so radiolabelled mannitol should not be a preferred marker.

Line 1335-36: ... "using polycarbonate filter inserts" – : Bidirectional assays are not typically done with side by side diffusion chambers. Add, or polyethylene terephthalate (PET) membrane inserts <u>or</u> side-side diffusion chambers...." Also add "transwells" as alternative. Currently, with most cell based permeability assay performed using transwell system and results are no different from the side-by-side chambers.

Lines 1338-1339: A concentration range is not necessary. If a compound is not a substrate at low concentration then the work is unnecessary at higher concentrations.

Lines 1338 and 1417: We disagree with the requirement to run the transport experiment at three concentrations. If the data for a compound suggest that it is a transport substrate at the low concentration, there should not be a requirement that it be run at higher concentrations.

Line 1338: Early in development, a single low concentration (<10 uM) is sufficient to demonstrate a compound is a substrate. This is true also for a single time point. Final registration studies may use more than one concentration and time points. Recommend to ease this wording/detail in section D.

Lines 1340: Why preincubate for 30 min...could be 15. Should read for "approximately" or for the "appropriate amount of time".

Line 1347: Concerning the selected times (1, 2, 3, 4 hrs), since there is already a half-hour pre-incubation, with another 4-hr experiment, the membrane integrity could be affected. So it is suggested "using at least four sampling time points for permeability measurement" instead of the current selected time points.

Line 1347. The use of four sampling times is not necessary and specific times should not be listed, but rather left to the experimental design for individual compounds.

Lines 1347-1350: The timepoints should not be listed. Timepoints used are normally much shorter that those listed. It should read that the permeability should be measured under initial rate kinetics. Also many labs do not use aliquots, but actually remove the total volume from the receiver chamber to maintain sink conditions. The key is the maintenance of sink condition.

Lines 1349-1350: Extensive repeat should only be requested for negative results if used for labeling.

Lines 1352 and 1409: We would contend that using a transfected cell line (e.g., MDR1-MDCK and MDR1-LLC-PK1) to identify substrates or gauge inhibitory potential requires duplicate experiments be performed in the untransfected cells. Our contention is primarily due to reasons which are related to accuracy of substrate identification. Especially in the case of MDR-MDCK, the MDCK cell line has functionally active canine P-gp that mediates an identical effect on the candidate as the transfected human P-gp, and therefore wild-type MDCK cannot be a true negative control. Furthermore, it is not entirely possible to accurately distinguish the action of canine P-gp from human P-gp via comparison of efflux ratios generated in transfected and wild-type cells (see comments regarding Table 2). This phenomenon has been observed because P-gp-mediated efflux activity is not linearly related to expression. As presented as comments for Table 2, in some cases there is enough P-gp efflux in MDCK via endogenous canine P-gp to maximize the impact of P-gp thus leading to a misleading conclusion using a ratio of ratios approach (Troutman and Thakker, 2003). Several studies have shown that use of the transfected cell line alone provides an excellent system for which to identify P-gp substrates (Polli, et al. 2001, Tang et al. 2002). We would suggest that performing studies in the wild-type lines remain optional as this experimental approach does not provide further ability to accurately identify substrates above that achieved using the transfected cells alone.

Line 1354: This requirement seems excessive particularly if a well-characterized system is used with controls. We recommend that some language be added around the need to characterize P-gp functional activity (using positive control) as a function of key parameters for the system such as days in culture and passage number jor use of Pgp inhibitors (such as GF120918) could be sufficient instead of running control cell lines.. This would be done to establish the range of P-gp activity in the system and to provide confidence in reproducibility of the results.

Line 1354: If the test system is well characterized with inclusion of a positive control, there is no need for study conduct on different days, so that this text should be deleted. Agree that triplicate wells on a single day are sufficient.

Line 1363: There needs to be a better rationale for suggesting the use of 2-3 inhibitors. The use of a single potent and specific inhibitor in a well-characterized system is adequate.

Lines 1365-1367: Using MDR1-overexpressed cells study should be optional. The efflux ratio differences between wild-type and overexpressed-MDR1 cells may not directly

relate to in vivo interactions so that the extent of P-gp contribution to efflux activity will not be important.

Line 1375: Recommend to add the exact permeability (Pexact) equation as an alternative, as this equation is more robust in dealing with mass balance issues. See Tran TT et al., (2004) Kinetic Analysis of Passive Transport across a Polarized Confluent MDCK Cell Monolayer expressing Human Multi-Drug Resistance Protein. Biochem. J Pharm Sci 93:2108-2123.

Lines 1393-1396: It should not be required that one has to do a ratio of ratios for transfected cell lines, rather than just generating direct efflux ratios for these models. There is no rationale or evidence to show that this experimental approach is more valid than directly generating the efflux ratio for the system alone.

Line 1393: Do not agree that (R) = (RT)/(RW) is necessary for individually transfected cell lines. Suggest to remove.

Lines 1398 - 1419: Section F- There is too much detail in this section and we do not feel an overly prescriptive outline should be mandated. Please soften or reduce this section. Some specific comments follow: State that substrate concentration should be at or below its Km.
,PI
Line 1403: Inhibition assays are not typically or solely done with side by side diffusion chambers (literature is typically Transwell configuration). This wording should be deleted or PET inserts and Transwells shouldbe added.

Line 1406: The cells should be pre-incubated with inhibitor for ≥30 min prior to adding probe compound.

Line 1409 Note that a control cell line is not necessary if the test system has been previously characterized and is well understood.

Lines 1409-1411: It should be noted somewhere that MDCK-WT cells express canine P-gp, and this must be considered carefully when comparing transport data using MDCK-MDR1 and MDCK-WT cell lines. Studies have shown that canine P-gp demonstrates some substrate overlap with human P-gp (example: vinblastine).

Line 1411-1412: The use of 3 different filters is excessive . Measurement of non specific binding may provide an alternate quality control.

Lines 1412-1414. It is said that "After incubation of the cells for 0.5-1 hour at 37°C, the medium is removed from the apical or basolateral side of the monolayer and replaced with the appropriate concentration of the selected probe P-gp substrate (see Table 2). As good practice, the test article as inhibitor should be kept on both sides for the entire duration of incubation to avoid false negatives.

Lines 1415-1416: The time course will vary and is going to be dependent on the system and the probes. Experimental time periods will vary and should be based on where flux is linear for the compound being studied.

Appendix D. Section 2(g) and line 1511-1555. We disagree with calculating IC_{50} values from efflux ratios, but rather the effect on unidirectional flux should be used. This is common in the literature from several leading investigators and the use of efflux ratio for this calculation is disputed (Gao et al. 2001, Rautio et al. 2006, Keogh and Kunta 2006). The value used for [I] should be the systemic unbound C_{max} when considering drug interactions at the kidney, brain, or liver, and should be an estimate of GI fluid concentration when considering drug interactions at the intestine. The latter could be estimated by dividing the dose by a standard fluid volume (e.g. ~240 mL) or by using the upper limit of solubility of the test compound at intestinal pH.

Line 1425: Suggest that the flux of probe substrate across a cell monolayer be normalized to the percent of control as an option for IC50 calculations (our preferred approach). Further, the option of completing this study in one direction should be given.

Lines 1427-1486: The text (as it is) implies that we would have to do an interaction study for every compound that we find to be a P-gp substrate, irregardless of whether it is cleared via P-gp or a different mechanism like metabolism. This is not necessary and should be clarified
To observe clinically significant changes in systemic exposure of an orally dosed Pgp substrate, the substrate must be dosed at a low dose as to not saturate the transporters and to be mainly cleared systemically via the specific transporter. We do not recommend dosing at artifiacally low concentrations just to demonstrate a non clinically relevant effect.
More information on the fraction of the drug cleared by the specific transporter should be described before recommending a DDI study
Better Pgp inhibitors need to be deliniated (specific for Pgp and not CYP3A).

Line 1428: It is stated that "where (REi/REa) represents the efflux ratio of the probe P-gp substrate in the presence of inhibitor concentration (I) relative to that for the control without inhibitor." There is another approach which has been widely used to calculate the inhibition potency (Kim et al, Pharm Res 1999, 16, 408-414). We recommend that this approach is included as an alternative.

Line 1431: "effect" should be deleted.

Lines 1438 and 1449 -1479 (and Figure 1, line 1465): A minimum flux ratio of 2 is very common for many compounds in development and is too low to set as a point for in vivo interactions studies.. Variability of in vitro test results occur within and between labs for many reasons including the use of acceptable but different cell lines and level of expression of P-gp in the different cell lines. Perhaps most importantly, a lack of availability of in correlations between the in vitro and in vivo clinical data does not support such tight standards. If FDA requires a number we recommend that the following criteria is used in the decision tree (Figure 1): Net flux ratio ≥ 3 as positive result, net flux ratio < 3 as negative result.

We advocate the use of positive and negative control compounds to define the efflux ratio cutoff for any given system.

Line 1441: For consistency throughout the document, the term 'net flux ratio' should be replaced with 'efflux ratio.'

Line 1457: Consider adding: ...evaluation of in vivo data ***(dose linearity, DDI observed, BDDCS classification)*** can help....". It should be noted that the efflux ratio may or may not reflect the importance of Pgp affecting a compound's in vivo disposition, as other properties such as permeability, dissolution, solubility, metabolism and PPB will also influence disposition and drug interaction potential. Please add examples of the types of in vivo data you are talking about.

Line 1463: We strongly disagree that in vitro findings in which a directional efflux is observed, but which is unaffected by P-gp inhibitors should be further investigated. The science is not as well developed in these cases, so we would not know what further 'warranted' studies could be done. Furthermore, we would not know how such information could be used in the clinic.

Line 1465To compensate for the variability in expression, and for a more integrated perspective on potential clinical significance, we propose to analyze the test compounds in parallel with one or more marker substrates (e.g., verapamil, ritonavir, and digoxin)..

Line 1465 and 1527: Please clarify what is meant by an in vivo study (clinical or preclinical?).

Appendix D. Section 3. General comment. The unidirectional flux should be used instead of a ratio of ratio approach (e.g. line 1486).

Lines 1491-1492: There should be clarification on the % value reduction. Is there any clinical justification of this value?

Lines 1500-1501: Net flux ratios vary from lab to lab and from day to day. This is why appropriate controls should be run in all experiments.

Line 1502: What if a Km cannot be determined (e.g., digoxin)? Will simply showing that efflux is not saturated over a broad concentration range a sufficient demonstration that substrate concentration is below the Km?

Lines 1520-1525: Need to clarify [I].Is it the steady state Cmax or drug concentration in the GI tract?. What value should be used for the inhibitor concentration? This is a challenge for intestinal transporters, as [I] will often be very high (> 1000 uM) as it would translate for clinical studies needed with investigational drugs having IC50 values = 100 uM. We believe that this cannot be so prescriptive, and the decision needs to be considered with other factors such as dosing schedule, therapeutic area and co-medications.

Lines 1520-1525. I/IC50 (or Ki) ratio should not be the sole determinant for conducting a clinical P-gp interaction studies, as, unlike CYP inhbition, this has not been validated clinically. Addionally, if a drug has high permeability coefficient, then the decision tree presented on page 49 is likely not clinically relevant. Permeability has to be considered in evaluating overall impact, or misleading labeling and useless studies will occur. Please add a footnote to the table or another box capturing that all other factors should be considered in assessing the need for a Clinical P-gp study.

Line 1520: Is there a recommended approach for exploring Pgp inhibition at CNS?

Line 1521: Since fexofenadine has been demonstrated and used as an appropriate P-gp substrate for clinical study, we recommend that fexofenadine is included as an alternative to digoxin.

Line 1527: "Figure 1" should be "Figure 2"?

Line 1542: The calculation used to determine the IC_{50} for evaluating P-gp inhibition in Table 1 is based on 50% inhibition of the P-gp-mediated B-A/A-B transport ratio. We do not think this is an appropriate method to calculate IC_{50} values as the number would be highly influenced by small technical variations (e.g., a compound with a low A-B transport would be highly sensitive to small absolute changes in A-B transport). We propose that

the correct approach to determine IC_{50} values is to calculate the net flux (transport from B-A minus transport from A-B).

. A clinical induction study specific for Pgp will be difficult, especially with metabolism and transport inter-dependency. Could the agency provide further guidance on how such a study should be done?

Lines 1573-1582. The draft guidance has provided a reasonable approach to assess P-gp induction potential based on results of CYP3A4 induction. However, in lines 1573-1574, it is said that "Thus, the P-gp induction potential of an investigational drug can only be evaluated in vivo". It would be helpful to make the statements consistent throughout the document.

Lines 1581-1582: Since the the clinical significance of P-gp induction is not fully known at this time, s not yet appropriate to request a P-gp induction study for all 3A inducers

PhRMA trusts these comments are useful to FDA.

Sincerely,

Michael Garvin

Michael Garvin, Pharm.D.

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