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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane
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RE: Docket No. 2006D-0344 on Draft Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling

Merck & Co., Inc. is a leading worldwide human health products company. Through a combination of the best science and state-of-the-art medicine, Merck's Research and Development (R&D) pipeline has produced many important pharmaceutical products available today. These products have saved the lives of or improved the quality of life for millions of people globally.

Merck Research Laboratories (MRL), Merck's research division, is one of the leading biomedical research organizations. MRL tests many compounds as potential drug candidates through comprehensive, state-of-the-art R & D programs. Merck supports regulatory oversight of product development that is based on sound scientific principles and good medical judgment.

In the course of bringing Merck drug and biological product candidates through developmental testing, clinical trials and licensure, Merck scientists address several issues related to drug interactions. We have extensive experiences in the development and licensure of drug and biological candidates and the conduct of drug interaction studies; we have utilized that experience to author the comments below.

General Comments

We commend the Food and Drug Administration (the Agency or FDA) for its commitment to foster innovation while serving the public health needs of American citizens. We thank the Agency for the opportunity to comment on the draft Guidance document.

We agree with the Agency's stated goal to consolidate the guidance on drug interaction studies into a single Guidance document (September 12, 2006. Docket No. 2006D-0344). We have identified areas in the draft Guidance document that need additional explanation. Specific comments on the draft Guidance document follow. Of particular importance, we highlight the points raised in Sections 2 and 4 of our comments.

Specific Comments

1. Comments on guidance for metabolism studies

1.1. Metabolism by enzymes other than cytochrome P450
The draft Guidance currently focuses on CYP and transporter-based drug-drug interactions (DDIs). Although other non-CYP enzymes potentially contributing to metabolism are briefly mentioned (P. 25, line 793), we would respectfully ask the FDA to consider including further guidance in this regard. This would be particularly valuable for UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) enzymes, especially since a large body of literature is available on these enzymes. Addition of a table with recommended substrates, inhibitors, and inducers for the major UGTs and SULTs would be valuable for sponsors and would help to standardize the approach used to monitor activity of these enzymes both *in vitro* and *in vivo*.

1.2. Drug interactions studies with compounds not eliminated significantly by metabolism
It is stated in the draft Guidance document that metabolic interactions should be explored, even for an investigational compound that is not eliminated significantly by metabolism (P. 3, lines 94-98). We recommend that the Guidance document provide more discussion and specifics to clarify what the Guidance document means by "metabolic interactions should be explored".

1.3. Interaction studies with more than one CYP inhibitor
The draft Guidance document outlines that in certain cases interaction studies with more than one CYP inhibitor may be appropriate (P. 11, lines 448-466). We recommend that the Guidance document more clearly stipulate what would trigger the requirement for evaluation of the combined drug interaction of three or more drugs. Potentially, sponsors could be required to conduct a very large number of trials to evaluate the many possible permutations of multidrug combinations. We also request that you consider citing published literature to assist in useful scientific and clinically meaningful interpretation. If such published literature is currently limited making it unfeasible to cite such evidence in the Guidance document, we request that the recommendation to do interaction studies with more than one CYP inhibitor be removed from the Guidance document until more information is available to help guide the sponsor. The evaluation of multidrug interactions are clearly very difficult to design into meaningful clinical studies and the results can be difficult to interpret. If the agency still intends to include evaluation of such multidrug interaction studies in the Guidance document, we recommend the agency provide a clearer definition of what will trigger the need for such studies.

1.4. Choice of P450 perpetrator or victim drugs in clinical studies
Clear criteria for classifying a test compound as a strong, moderate or weak inhibitor of CYP3A4 are provided (P. 9, lines 358-373). Similarly, criteria are provided for classifying a test compound as a sensitive CYP3A4 substrate (P. 10, lines 400-427). Although sensitive probe substrates are recommended in the draft Guidance document, it is also indicated that sponsors may use other drugs. We recommend that the Guidance document clearly stipulates that in such cases alternative sensitive substrates or potent inhibitors be used, as otherwise classification of test compounds could be arbitrary.

1.5. Determining whether an NME is a mechanism-based inhibitor
We note that relatively little guidance is provided on how to determine whether an NME is a mechanism-based inhibitor *in vitro* (P. 34, lines 1067-1074). Since literature suggests that this type of inhibition is a major cause of clinically relevant CYP3A4-mediated drug interactions, we recommend that the Guidance document provide more specific details to further address this topic. We believe that including a table with acceptable positive controls and assay conditions would be helpful. In addition, we suggest that the Guidance document provide illustrative cases to guide the decision on when clinical studies are warranted based on *in vitro* data.

On P. 32 (line 1003), we request to change "at concentrations below its Km" to "concentrations **at or below** its Km". In cases where the metabolic rate of substrates is low, measuring substrate metabolism at Km is appropriate as for competitive and uncompetitive inhibitors $IC_{50} = 2K_i$ under this condition.

We agree that the solvent concentrations in incubations should be as low as feasible (P. 33, line 1021-1023), but we suggest changing the sentence to: "Any solvents should be used at low concentrations (< 1%), but higher concentrations are acceptable as long as the effect on enzyme activity is acceptable." For instance, a final concentration of 1% DMSO would reduce the activity of CYP3A4 to ~40%, whereas activity remaining in the presence of 2% methanol would still be ~70%.

1.6. In vitro identification of enzymes contributing to drug metabolism
On P. 27 (line 860), we suggest changing the sentence "We recommend that at least two of the three…" to "If necessary, we recommend that at least two of the three…", since complete phenotyping may be possible with one method, e.g., antibodies or selective inhibitors.

On P. 28 (Table 2), we request to add *N*-(α-methylbenzyl)-1-aminobenzotriazole to the list of CYP2B6 inhibitors, and (-)-*N*-3-3-Benzyl-phenobarbital (NBPB) and (+)-*N*-3-benzyl-nirvanol as CYP2C19 inhibitors. To footnote 2, we also recommend that you add the following compounds that are known to have the potential to cause mechanism-based inhibition: ticlopidine, diethyldithiocarbamate, troleandomycin, and verapamil.

2. Comments on guidance for drug transport

2.1. Evaluation of an NME as a Pgp substrate in DDI studies

The draft Guidance document recommends using compounds such as ritonavir, cyclosporine A (CsA), or verapamil to study whether transport of an investigational drug is inhibited by a Pgp inhibitor (P. 12, lines 484-486). Unfortunately, no selective Pgp inhibitor appropriate for use in human subjects is commercially available. Due to insufficient specificity among Pgp probes, interpretation of studies is greatly compromised. All three drugs suggested are also associated with safety hazards when given to healthy subjects after multiple doses at exposures required for Pgp inhibition, thus practically limiting their use to single dose administration. All the suggested compounds also inhibit at least CYP3A4 and some other pathways as well as transporters. We recommend that a statement be added to the Guidance indicating "*For a compound that is also a substrate of CYP3A4, no specific test compound is available for use in a clinical study, and that interpretations of results of studies using any of the proposed compounds will be difficult with regard to relative effects on CYP3A4 vs. Pgp.*"

Among Pgp 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 Pgp inhibition. The clinically relevant plasma concentrations of CsA as a Pgp 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 single dose of 600 mg CsA would provide exposure adequate to test the concept of Pgp inhibition. CsA is known to inhibit other enzymes (e.g., CYP3A4) and transporters, like OATP1B1, and as such is not a specific Pgp inhibitor. Nonetheless, CsA (600 mg) currently appears to be the most appropriate probe drug interacting with Pgp and we recommend incorporating in the final Guidance the use of this drug in clinical studies. In cases where no interaction with CsA is observed, the data would suggest that Pgp, 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 Pgp) and/or CYP3A4 play a role in the absorption and disposition of the investigational drug.

2.2. In Vivo interaction studies for compounds with net flux ratio of $\geq$ 2 measured in vitro

In the draft Guidance document, several polarized cell lines are presented suitable for measuring Pgp-mediated transport. It is indicated that an *in vivo* Pgp interaction study is warranted for compounds with a flux ratio ≥ 2 measured in these cell lines (P. 47, Figure 1). We draw your attention to published values for digoxin transport ratios and other Pgp substrates that have varied as much as ten-fold. As illustrated in Table I, differences are observed between different cell lines or in the same cell line if used in different laboratories. Normalizing the transport ratios for test compounds to the ratio obtained for vinblastine compensates for differences in Pgp expression yielding more consistent

results as illustrated by the data presented in Table I. Similarly, differences in the Pgp expression level in Caco-2 cell monolayers resulted in a transport ratio obtained for Compound A to be above or below 2 (Table II). Normalizing the ratio to that for vinblastine resulted in consistent results between different experiments. We therefore respectfully disagree with using a net flux ratio of ≥ 2 and rather recommend including in the guidance the suggestion to compensate for variability in Pgp expression by presenting the transport ratios for test compounds relative to one or more marker substrates (e.g., vinblastine, ritonavir, and digoxin).

Normalizing the transport ratio of the test compound to a marker substrate could also help defining a new cut-off for the level of Pgp flux that would warrant potential clinical evaluation of the test compound. Although digoxin is the only clear example of a drug sensitive to pharmacologically meaningful Pgp-mediated DDIs, studies by Wang et al. (2005) and Doan et al. (2002) demonstrated that over half of the marketed non-CNS drugs show transport ratios > 2. This suggests that for some transport models a set B-A/A-B ratio of 2 might be too conservative. Drugs for which Pgp has been implicated to be contributing to their pharmacokinetic behavior (i.e. saquinavir) tend to show high Pgp transport ratios comparable to digoxin. Since digoxin is the most accepted example of Pgp inhibition, we recommend to incorporate in the guidance that clinical studies to evaluate compounds as a victim of Pgp inhibition should be *limited to investigational drugs with transport ratios of $\geq$ 50% of the value for digoxin (or another comparable marker substrate)*. As indicated below (*Section* 2.3), this ratio should be considered with other drug properties in assessing the need for clinical DDI studies.

2.3. Consideration of drug properties in determining the need for clinical evaluation of investigative drugs as a victim of Pgp inhibition

The need for clinical drug interaction studies should not only depend on the B-A/A-B ratio of the test compound but drug properties like therapeutic index, extent of absorption and mechanisms of elimination should also be considered. In particular, if a compound is absorbed extensively (> 80%) and cleared primarily by metabolism, Pgp inhibition is unlikely to significantly increase oral exposure beyond the cut-off for detecting a meaningful drug interaction. It is our believe that such considerations should exclude Biopharmaceutics Classification System (BCS) class I compounds from the need for clinical studies to address their liability as victims of Pgp inhibition, since by definition these compounds have permeability and solubility characteristics consistent with oral absorption greater than 80%. We request the following text be added to the guidance: *"For instance, BCS Class I compounds may not require clinical studies to address Pgp inhibition, since, by definition, these compounds have permeability and solubility characteristics consistent with oral absorption greater than 80%.*

Table I

Transport ratios observed for prototypical Pgp substrates in different Pgp transport models: Comparison of observed transport ratios to transport ratios normalized to vinblastine transport

(This Table is intended to be used to support the comments in this document)

	MDR1-MDCK		LLC-PK1 MDR1		Caco-2	
Drug	GSK[1]	Abs Systems[1]	MRL[1]	Takeuchi et al.[1]	BMS[1]	Troutman et al.[1]
Digoxin	52	35	10	10	14	20.2
Loperamide	31	237	20			
Labetalol	9	61				
Vinblastine	56	27	13	12.5	12	14.1
Amitriptyline	1.34	2.8				
carbamazepine	0.98	1.4				
chlorpromazine	1.27	2.6				
Fluoxetine	1.18	2.7				
Indomethacin	0.97	3.9				
Indinavir	25		9.9		10	
Ritonavir	54		16	10		7.3
Saquinavir	165				15	17
Transport ratios normalized to vinblastine[2]						
Digoxin	0.93	1.31	0.75	0.78	1.18	1.47
Loperamide	0.55	9.08	1.58			
Labetalol	0.15	2.31				
Vinblastine	1.00	1.00	1.00	1.00	1.00	1.00
Amitriptyline	0.01	0.07				
Carbamazepine	0.00	0.02				
Chlorpromazine	0.00	0.06				
Fluoxetine	0.00	0.07				
Indomethacine	0.00	0.11				
Indinavir	0.44		0.74			
Ritonavir	0.96		1.25	0.78		0.48
Saquinavir	2.98				1.27	1.22

[1]Data published from BMS (Bristol Meyer Squibb), GSK (Glaxo Smith-Kline), Abs Systems (Absorption Systems) MRL (Merck Research Labs) and academic labs was compiled from Wang et al., 2005; Polli et al., 2001; Mahar et al., 2002; Yamazaki et al., 2001; Takeuchi, 2006; Balimane et al., 2004; Troutman et al., 2003.

[2]Normalized transport ratios = (test compound B-A/A-B ratio - 1)/(vinblastine B-A/A-B ratio - 1) where 1 is subtracted from the transport ratios since a ratio of 1 reflects no Pgp transport.

2.4 Clinical interaction studies for Pgp inhibitors

The draft Guidance document identifies a compound as a Pgp inhibitor warranting clinical DDI studies when the $[I]/IC_{50}$ value is > 0.1 (P. 49, Table 1). The calculation used to determine the IC_{50} for evaluating Pgp inhibition in Table 1 is based on 50% inhibition of the Pgp-mediated B-A/A-B transport ratio. Since the IC_{50} is a measure of the inhibitor concentration at which Pgp transport activity is reduced to half the value for the substrate in the absence of test compound, we propose that the IC_{50} should be defined as the concentration at which the net flux (transport from B-A minus the transport from A-B) is reduced to half the flux in the absence of inhibitor using the following equation:

$\Delta Papp_i/\Delta Papp = (I)/(I + IC50)$, where $\Delta Papp_i$ and $\Delta Papp$ are the net flux of a marker substrate in the presence or absence of the Pgp inhibitor, and I is the extracellular concentration of the Pgp inhibitor.

Determining the IC_{50} value based on the transport ratio would introduce both systematic and, potentially, technical errors. Systematic errors would be introduced especially for substrates with a high B-A/A-B ratio since small changes in the flux could double the transport from A-B, resulting in a 50% decrease in the B-A/A-B ratio. Consequently, the IC_{50} value would be highly influenced by the marker substrate used and the level of Pgp expression in the transport model. For similar reasons, an IC_{50} calculated from transport ratios would also 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).

Table II

Impact of culture conditions on Pgp expression in Caco-2 cells: Normalizing transport of Compound A to vinblastine compensates for differences in Pgp expression

(This Table is intended to be used to support the comments in this document)

Culture conditions	Transport ratio		Normalized transport ratio
	Compound A	Vinblastine	
Condition A	1.30	3.60	0.12
Condition B	2.20	11.20	0.12
Condition C	2.80	16.50	0.12
Condition D	1.34	3.74	0.12
Normalized transport ratios = (compound A B-A/A-B ratio -1)/(vinblastine B-A/ A-B ratio -1), Unpublished results.			

2.5. Consideration of IC_{50} and dose in assessing the need for clinical DDI studies
In the draft Guidance document, IC_{50} values for inhibition of Pgp are related to systemic exposure (P. 49, Fig. 1). Although this takes into account the importance of exposure for Pgp inhibition, it does not account for inhibition of Pgp during intestinal absorption. Rautio et al. (2006) have proposed an alternative approach based on empirical observations that compounds sensitive to Pgp-mediated DDIs tend to show IC_{50} values < 15 μM and are administered at relatively high dose (> 100 mg). Since the approach described by Rautio et. al. is based on the dosage size as opposed to systemic exposure, inhibition during oral absorption would be taken into account. In addition, the criteria for the identification of compounds as potential inhibitors of Pgp *in vivo* are supported by empirical pharmacokinetic DDI data. Comparison of reported affinities of potential perpetrators of Pgp-mediated DDIs listed in the University of Washington Drug Interaction Database with their impact on digoxin exposure suggests that DDIs associated with inhibition of Pgp are primarily associated with high affinity compounds (Km or IC_{50} values ≤ 10 μM (Table III)). We recommend incorporating these considerations in the Guidance document.

2.6. Normalization of Pgp transport ratios to control cells
In the decision trees for identifying Pgp substrates and inhibitors, the B-A/A-B ratios for the transfected cells are normalized to the parental cells (P. 47 and 49, Figs 1). We respectfully disagree with this approach as it may not be scientifically justified and may amplify the effects of random small experimental errors. Since the endogenous transport is likely to be mediated by efflux transporters acting in parallel with the transfected Pgp, the impact would be additive, not multiplicative (the effects would be multiplicative if the transporters function in series). Therefore, the transport ratio measured in the parental cell monolayers should be subtracted from the ratio in the transfected cell monolayers, assuming that the expression of the endogenous transporter(s) is the same in the parental and the transfected cell monolayers. Since the Pgp expressing cells are clonally selected, differences in the level of endogenous transporters likely will exist between the various cell lines. Given these issues, we recommend that the transport ratios for the control and transfected cells be presented as separate values without applying corrections.

Table III

Comparison of Pgp inhibitor affinities with their impact on digoxin systemic exposure following oral administration

(This Table is intended to be used to support the comments in this document)

Drug (Pgp inhibitor)	Pgp IC_{50} or Km* (μM)	AUC_{inh} / AUC_{cntrl}	$Cmax_{inh}$ / $Cmax_{cntrl}$	Css_{inh} / Css_{cntrl}
Atorvastatin	> 50 uM	1.04	1.1	
Carvedilol	0.16	1.6	1.4	
Clarithromycin	4	1.7	1.8	
Dipyridamole	1.5	1.12	1.23	
Itraconazole	0.9			1.8
Ritonavir	10	1.23	1.25	
Talinolol	100*	1.17	1.44	
Telithromycin	2			1.55
Trospium	>100	1.08	1.07	
Valspodar	0.1	1.7-3.04	1.7-2.5	

Compounds reported to produce clinical drug interactions with digoxin were identified in the University of Washington Drug Interaction Data Base.
AUC_{inh} / AUC_{cntrl}, $Cmax_{inh}$ / $Cmax_{cntr}$, and Css_{inh} / Css_{cntrl} refer to the ratio of the plasma digoxin AUC, Cmax, and steady state concentrations when coadministered with the Pgp inhibitor.
Values for affinity for Pgp were compiled from internal unpublished MRL data and Hochman et al., 2004; Rautio et al., 2006; Verstuyft et al., 2003; Pachot et al., 2003; Collett et al., 2005; Barchmakov et al., 2006; Sandage et al., 2006; Eberl et al., 2006.
Clinical effects on digoxin exposure were compiled from: Verstuyft et al., 2003 ;Sandage et al., 2006; Kovarik et al., 1999; Nenciu et al., 2006; Westphal et al., 2000; Rengelshausen et al., 2003; Penzak et al., 2004; Partanen et al., 1996; Ding, 2004; Boyd, 2000; Barris et al., 2006.

2.7. Evaluation of monolayer integrity

Transepithelial electrical resistance (TEER) measurements and [^{14}C]-mannitol flux are recommended in the draft Guidance document as tests for cell monolayer integrity in transporter studies (P. 43, lines 1326-1329). In our experience, the use of a fluorescent marker (e.g., Texas Red Dextran or Lucifer Yellow) has proven valuable since these can be included at low concentrations with the investigational drug allowing monitoring of the monolayer integrity in each individual well over the course of the transport experiment. This provides both a measure of monolayer integrity in individual wells and a means to assess the effects of an NCE on monolayer integrity (i.e. cytotoxicity and disruption of tight junctions). We recommend that the Guidance document be amended to add monitoring of the para-cellular flux of fluorescent compounds as a method to measure cell monolayer integrity.

2.8. Guidance in performing in vitro transport experiments
The draft Guidance document makes detailed recommendations regarding conducting transport experiments with polarized cell monolayers (P. 43, lines 1316-1359). Although such information may be of value to scientists not familiar with these types of studies, the draft Guidance document 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 the Guidance document provide only general recommendations along with key references that sponsors could review for more detailed information on specific Pgp transport models.

2.9. Model substrates and inhibitors listed in Table 1
In Table 1 (P. 17), many of the transporter substrates and inhibitors listed interact with multiple transporters and drug metabolism enzymes. For instance, indinavir is listed as a model substrate for ABCB1, ABCC1 and ABCC2. Although this drug is a substrate for these pathways in vitro, clinical drug interactions with indinavir are dominated by CYP3A4 effects and therefore it is unclear what if any signal can be explained by these transporters. To avoid misinterpretation, we recommend that a footnote be added to the table clarifying that the listing of compounds in Table 1 does not imply selectivity for the individual transporters and that pharmacokinetic properties of the compounds may be dominated by other transporters or metabolic pathways.

3. Comments on guidance for enzyme induction

3.1. Application of receptor gene assays to measure the potential of test compounds to cause enzyme induction
The draft Guidance document briefly discusses the use of nuclear receptor activation or binding assays to identify Pregnane X Receptor (PXR), Constitutive Androstane Receptor (CAR) or Aryl Hydrocarbone Receptor (A*h*R) activators (P. 37, lines 1179-1187). Although we agree that these assays are valuable for screening compounds in drug discovery, we think that, in addition, understanding the molecular mechanism causing enzyme induction is extremely valuable during the drug development process. For instance, knowing whether a compound is a PXR activator provides guidance regarding which other off-target pathways could be activated by the test compound in cases where a clinically significant induction of CYP3A4 was found. We recommend that the final Guidance document provide opportunity for the application of knowledge of the molecular mechanism of enzyme induction in the drug development process.

3.2. In vitro hepatocyte induction studies
The draft Guidance document recommends that in cases where the induction of enzyme activity is equal or greater than 40% of the positive control, the compound could be considered as an enzyme inducer (P. 36, lines 1142-1143). It is our belief that interpretation of this number in isolation could be 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. Given the above concern, we recommend that the final Guidance document include the following points:

(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 instance, for induction of PXR target genes such as CYP3A4, we recommend the use of rifampicin as the most appropriate positive control as it elicits a response near maximum induction. To limit the potential for variation between sponsors in qualifying the propensity of test compounds to cause induction, we recommend limiting the number of compounds suggested as positive controls. We propose incorporating the positive controls listed in Table IV in the guidance.

On P. 36 (line 1123), the words "whole cell monolayer" should be replaced with "plated hepatocytes" as the cells do not necessarily form a monolayer under the conditions typically employed in induction studies.

We suggest the Guidance document (P. 36, lines 1115-1119) recognize that the amount of compound in solution can be limited for NMEs with a low solubility. In addition, cytotoxicity may be observed at high concentrations of the NME. In designing *in vitro* induction experiment, these two factors should be taken into account.

The draft Guidance document makes recommendations for substrates and concentrations to be used in activity assays (P. 36, lines 1127-1128). With enzyme activity assays performed with plated hepatocytes, the concentration within the cell can be much different from the concentration in the incubation. Often concentrations within the incubation of 2-5-fold the Km are necessary in whole cell assays. Optimal concentrations should be determined experimentally.

On P. 37 (line 1164), we recommend to delete the word "polyclonal" as both polyclonal and monoclonal antibodies can be used in Western blot and immunoprecipitation experiments.

Table IV

Suggested chemical inducers to be used as positive controls in *in vitro* induction experiments

(This Table is intended to be used to support the comments in this document)

CYP	Inducer – Preferred	Inducer Concentrations (μM)	Inducer – Acceptable	Inducer Concentrations (μM)
CYP1A	Omeprazole β-naphthoflavone	25-50 30-50	3-methylcholanthrene	1-2
CYP3A	Rifampicin	10-30	Phenobarbital	500-2000

3.3 Induction of CYP2B6

In the draft Guidance document, it is stated that DDIs based on CYP2B6 are emerging as important interactions, yet no sensitive CYP substrates or inhibitors to measure this enzyme are given (P. 21 and 23, Tables 4 and 6). We recommend including a list in the Guidance document with suitable substrates or inhibitors that can be studied.

The draft Guidance document states several times that CYP3A induction is a sensitive predictor for induction of CYP2B6, -2C8, -2C9, and -2C19. We therefore suggest adding CYP2B6 to P.5 line 193 to read: "Therefore, to evaluate whether an investigational drug induces CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP3A, the initial *in vitro* induction evaluation may include only CYP1A2 and CYP3A." In addition, we suggest adding CYP2B6 to P. 37, line 1154.

4. Comments on General Strategies

4.1. Population pharmacokinetic screens

In describing the application of population pharmacokinetics (Pop PK) (P. 6, lines 223-239), the draft Guidance document implies limited value of such investigations beyond their potential utility to confirm the presence of a drug interaction. Indeed, the draft Guidance document explicitly states that there is no indication that Pop PK can be used to suggest a lack of drug interaction between the NME and other drugs. We suggest the Guidance document provide the sponsor the ability to conclude that based on Pop PK data there is no evidence of a clinically important interaction if no interaction was found with a particular drug. In addition, we suggest the Guidance document explicitly describe the design and statistical power requirements for the use of Pop PK to screen for potential DDIs and provide specific examples when such a result could be incorporated in the label. We believe that appropriately performed Pop PK screens for DDIs can be used to provide informed guidance to physicians since such an analysis can provide corroborative evidence of clinically meaningful DDIs.

5. Comments on design of *in vivo* drug-drug interactions studies

5.1. Study design

The example statement for exclusion criteria regarding dietary issues (P. 8, lines 309-316) 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. 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, an 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.

5.2. Choice of substrate and interacting drugs
The simultaneous administration of a mixture of substrates of CYP enzymes in one study ("cocktail" approach) is discussed (P. 9, lines 387-398). 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 document provide more detailed information, particularly as the literature offers more than what is suggested in this section.

Table 2 (P. 19) identifies "Examples of *in vivo* substrate…" and Table 4 (P. 21) identifies "Examples of sensitive CYP substrates …" We recommend addressing this perspective further in the Guidance document as confusion might occur when a sponsor selects a substrate to assess a potential drug interaction. As an example of the application of the two tables as they appear in the present draft Guidance document, Table 2 identifies repaglinide and rosiglitazone as substrates to assess effects on CYP2C8 activity, and Table 4 specifically identifies repaglinide as a sensitive CYP substrate. A sponsor could consider this in different ways: One possibility is that an option for the selection of two acceptable substrates is offered in Table 2, while a warning is given in Table 4. Alternatively, it is also possible for a sponsor to assume that the FDA is differentiating substrates between the two tables because sensitive substrates are appropriate for testing and are acceptable to the FDA as studies intended to be used to make claims in the label. The application of Table 2 versus Table 4 is critical to help clarify to the sponsor what options are available when selecting substrates, inhibitors and inducers.

5.3. Investigational drug as a substrate of CYP enzymes
In the draft Guidance document, overall elimination of a drug by one pathway by > 25% is defined as substantial (P. 10, line 402-427). Our experience is that testing for CYP3A4 inhibition in molecules with 25% CYP3A4 elimination through this pathway would rarely be warranted. We consider > 50% a more appropriate alternative cut-off.

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. We, therefore, suggest that 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.

5.4. Dose selection
Comments on application of dose selection for probes and substrates are useful (P. 12, lines 518-528) 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 document 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.

6. Comments on endpoints

6.1 Pharmacodynamic endpoints
On the PK endpoints (P. 13, lines 542-561), 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.

7. General points

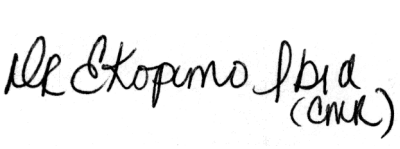
7.1 Clinical testing of compounds difficult to evaluate in vitro
The draft Guidance document clearly lays out how *in vitro* tests and clinical studies should be used in concert to evaluate DDI potential and support appropriate labeling. However, in our experience conducting meaningful *in vitro* studies 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 draft Guidance document does not address how clinical development of compounds should be conducted for which no robust *in vitro* data can be generated. We recommend the Guidance document recognizes the existence of such compounds and specifies what would be a sufficient clinical package for such a compound.

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

7.3. Definition of narrow therapeutic range
No definition of the term "narrow therapeutic range" (P. 3, line 87) 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.

We appreciate the opportunity to provide our comments on the Agency's draft guidance: "Guidance for Industry: Drug Interaction Studies–Study Design, Data Analysis, and Implications for Dosing and Labeling."

Sincerely,



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Director
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