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Pfizer Global Research & Development

November 7, 2006

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

Dear Dockets Management:

Re: **Draft guidance for Industry on Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling**
[Docket No. 2006D-0344, 71 *Federal Register*, 53696, September 12, 2006]

Pfizer welcomes the opportunity to provide comments to the Draft Guidance on Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling. Overall, we agree with many of the points made and applaud the agency for the creation of a well thought out, progressive, and science-driven draft guidance.

Our response represents the compiled comments of many of our researchers who possess expertise in the areas of clinical pharmacology, drug metabolism, and the emerging field of drug transport.

Again, we appreciate the opportunity to provide comments and commend the Agency for pursuing guidance on this topic. Additionally, we would invite direct dialog with the Agency if you would consider the opportunity valuable.

Sincerely,

A handwritten signature in cursive script that reads "William R. Murphy".

William R. Murphy
Director
Pfizer Global Research & Development

Background:

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.

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)."

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.

Clinical Drug-Drug Interactions Studies:

Lines 245-246. It is recommended that the sponsor consult with the FDA regarding study protocols. The general nature of this statement implies that sponsors should consult with the FDA on all protocols. Many drug interaction protocols can be straightforward in design and should not require special consultation with the agency, in particular if the sponsor closely follows the guidance in this document. It seems more likely that a sponsor would desire consultation on the overall project plan with respect to drug interactions for a drug with a complex metabolic profile or possibly feedback on a protocol when the protocol design is unusual does not follow one of the 3 designs described in the guidance document. Please consider elaborating on this statement to give guidance on when it might be appropriate to consult with the agency.

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 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 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. If study variables are well controlled, then it may not be necessary to confirm the absorption of the interacting drug through plasma level measurements. 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 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.

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 358-373. It is assumed that 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. This is not directly stated in the document. Please clarify in the document.

Line 408. We disagree that a metabolic enzyme contributing >25% to clearance represents a "substantial" contribution that merits a clinical drug interaction study. In theory, even complete inhibition of such an enzyme would result in only a 1.33-fold increase in exposure. We propose that a value of 50% be the cutoff as complete inhibition of such an enzyme would result in a 2-fold increase in exposure.

Lines 448-466. In general, provide clarification on the practical application of the guidance in this paragraph. It may not be clinically relevant to perform interactions with multiple inhibitors. There may be instances in some diseases, such as HIV, where it is more relevant because multiple drugs are commonly combined to treat a specific indication. In other indications, this may be less important.

Line 488. Please consider either substituting ritonavir with ketoconazole as a suggested inhibitor of both P-gp and CYP3A or add ketoconazole in addition to ritonavir. 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."

Line 523. It is important to recognize that rifampicin 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 rifampicin when co-administered with an OATP1B1 substrate, may produce altered pharmacokinetics when compared to chronic response of rifampicin induction of CYP3A.

Line 546. It is recommended that clearance, volume of distribution and half-life be obtained in every study. However, these parameters are not used for decision making as described in Section IV. G. Please consider revising the sentence on Line 546 such that it ends after "...and others as appropriate." Change the remainder of the sentence to a new sentence that reads, "Pharmacokinetic parameters such as clearance, volume of distribution and half-life may be considered as supportive data when interpreting the results of the trial." The revised wording should indicate that these parameters may be interesting, but are secondary and supportive.

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.

Line 678. Table 1. 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).

Line 688. Table 2. We have some suggestions regarding the list of in vivo substrates, inhibitors, and inducers. For CYP1A2, tizanidine is a sensitive substrate and should be included (Granfors, et.al., 2003; Granfors, et.al., 2004). Clopidogrel should be listed as an inhibitor for 2B6 and bupropion (with measurement of the hydroxy-bupropion/bupropion ratio) should be listed as an additional substrate for 2B6.

Line 716. Table 4. While tizanidine is a substrate with narrow therapeutic range, it should also be listed as sensitive CYP1A2 substrate. Please consider including this drug in both columns of the table.

Line 726. Table 5. Would the authors consider the feasibility of adding a table similar to Table 5 that classifies strong, moderate, and weak inducers of CYP3A4? We would be interested in discussing our thoughts on such a table or figure based on our recent experiences.

Line 760. Appendix B. Figure 1. Please add CYP 2B6 to the first box that describes In Vitro Metabolism Information.

In Vitro Drug Metabolism Studies:

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

Lines 787 and 848. In line with our comment to line 408, we challenge the idea 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 classic equation of Rowland and Martin, 1973). We propose that this value should be 50%.

Line 889. Table 2. We have some suggestions regarding the list of inhibitors. 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. For CYP2C19, N-benzylrivanol can serve as a selective inhibitor (Suzuki, et al., 2002) and should be added.

Line 900. 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) are also mechanism-based inactivators and should be preincubated when used as chemical inhibitors.

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).

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 use of up to 1% final concentration is acceptable.

Line 1044. The approach listed of using $[I]/K_i$ ratios (where $[I]$ = systemic C_{max}) 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; Venkatakrisnan 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 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.

Line 1071. Consider adding methylenedioxy as a substituent known to cause mechanism-based inactivation.

Line 1073. We recommend a more specific criterion for mechanism-based inactivation kinetics to warrant requiring an in vivo study. In our experience, we have found compounds that show inactivation in vitro, but because the K_i is high or the dose is low, these do not cause an interaction.

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). We propose that mRNA be an endpoint in induction studies to avoid false negatives.

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.

Line 1199. We would contend that the statement "It is generally accepted that co-administration of drugs that interact with this transporter can result in drug-drug interactions...." is not generally accepted. There remains considerable controversy as to whether significant or clinically meaningful drug interactions are commonplace for this transporter.

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

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

Line 1224. While it is true that the bi-directional transport system can be viewed as a reliable system, it certainly has some caveats. 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.

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 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 μM 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 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 comment. 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 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.

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.

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 1341. There is not a requirement that there be a 30 min preincubation.

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 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 would 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. 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 1356. Recovery determination should be required, not recommended. The data from an experiment in which recovery is low (<75%) is suspect, and efforts should be made to understand what happened to the substrate (e.g. metabolism, chemical instability, sequestration in the cells, etc).

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

Line 1415. There is no need to specify experimental time periods as those should be based on the where flux is linear for the compound being studied.

Appendix D. Section 2(g). 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.

Lines 1438 and 1449 (and Figure 1, line 1465). As systems vary from lab to lab, a cutoff value of 2 is inappropriate for all experiments. 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 1453. The alteration of flux in the respective direction should be the metric to use rather than alteration in efflux ratio.

Line 1457-8. More specificity is needed here. What type of in vivo data is being referred to here?

Line 1463. We 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.

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

Line 1511-1555. We have the same comment as we did for Appendix D. Section 2(g) regarding the value for in vivo [I] that should be compared to in vitro IC_{50} . 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. (Estimates of drug concentration in the intestine can be derived either from clinical dose/250 mL or if solubility is low, then the limit of solubility can be used. These values should represent the worst case scenario.) And as before, we maintain that the IC_{50} values should be calculated from the effect of the inhibitor on unidirectional flux and not from efflux ratios.

Comments Applicable to the Subject in General:

The use of computer simulation to estimate the combined effect of inhibitors is mentioned briefly on Line 464. We 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.

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