

October 25, 2006

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

RE: Docket 2006D-0344

To Whom It May Concern:

Please find enclosed our comments on the recently released docket entitled "Guidance for Industry Drug Interaction Studies-Study Design, Data Analysis, and Implications for Dosing and Labeling". The docket notice was published in the Federal Register on September 12, 2006 (Volume 71, Number 176, page 53696). We have been working in the fields of *in vitro* drug safety assessment and drug metabolism (in particular human P450 gene regulation) for a combined total of over 35 years, and currently work with a majority of top-tier pharmaceutical companies and a number of mid-sized and smaller companies. Because of this experience and our relationships with the pharmaceutical industry, we feel we have first-hand knowledge of certain industry practices. With regards to the draft document, our area of expertise falls under "In Vitro Studies" and in particular, those related to Appendix C-3 "In Vitro Evaluation of CYP Induction", we will confine our comments and concerns to this section of the draft document. A point-by-point critique and suggestions for improvement follow.

Sections I and II.

The introduction of this guideline addresses an important issue, drug-drug interactions, a very serious health concern to the American public and the subject of other FDA forum. However, it does not address new drugs as a source of adverse drug interactions (ADRs) nor suggest that ADRs are caused by mechanisms other than ADME related issues (i.e., disruption of endogenous homeostasis pathways). In this response we specify how ADRs resulting from ADME and non-ADME systems can be addressed with a single technology.

Section III, General Strategies, In Vitro Studies

1. The statement that in vitro studies can "serve as a screening mechanism to rule out the importance of a metabolic pathway and the drug-drug interactions that occur through this pathway so that subsequent in vivo testing is unnecessary" holds

merit. This is well documented in the literature and should be considered during drug development by all pharmaceutical companies. Furthermore, in vitro studies can be used to predict the ability of new chemical entities (NCEs) to produce drug-drug interactions early in the drug development process, thereby eliminating those compounds that are most likely to produce adverse drug reactions. This would save time and reduce costs associated with drug development and is in alignment with the FDA's *Critical Path Initiative*. However, several techniques fall short in effectiveness of those currently in use in basic science and hence the guidelines could better serve its constituents by addressing the more advanced methodologies.

2. In paragraph 190-197, we are concerned about the statement that "the initial in vitro induction evaluation may include only CYP1A2 and CYP3A" as a means for predicting co-induction of CYP2Cs, CYP2B and P-gp. In contrast to CYP3A4, the expression of the human CYP2C enzymes may be mediated by more than one mechanism and those mechanisms have not been completely elucidated. In other words, there may be instances where one or more CYP2C enzymes are induced by a NCE without affecting CYP3A4 expression. Along these same lines, studies demonstrate that regulation and expression of P-gp exhibits tissue specificity [1-3]. As CYP3A induction is primarily assessed in hepatic products, results generated may not predict the effects of agents on intestinal expression of P-gp. Taken together, enthusiasm for this paragraph is low.

Appendix C-3 "In Vitro Evaluation of CYP Induction"

General Comments:

There is concern that the methods suggested for use in the draft guidelines are antiquated. The rationale and scientific methods described within were modern several years ago, but more reliable techniques are currently available. These lower cost techniques are also more predictive of the potentially adverse effects of the NCE upon the biological system. Hence, the new guidelines fail to incorporate more modern techniques. Indeed, as written this draft will hamper the rapid development of safer drugs and will increase the costs of drug discovery rather than lower them. Because FDA guidelines are essentially regulations in practice that industry strictly follows, more reliable, less costly techniques will only be utilized by some of the more advanced scientists in the industry.

The new guideline appears to be at odds with other FDA documents. For example, these FDA mandates were designed to improve the efficiency of drug development and the safety of drugs in the market. The *Critical Path Initiative* calls for a rapid need to improve the drug development process. Its narrative clearly points out many of the problems facing the pharmaceutical industry and the speed in which new science is used in the drug safety assessment process. The FDA whitepaper, *Innovation Stagnation* (page 3), clearly states "that applied science has not kept pace with advances in basic sciences", and the drug development process "is inefficient, due in large part to the current reliance on cumbersome assessment methods".

Specific Comments:

1. On page 35, the authors of the document present a table (Table 5) of preferred and acceptable chemical inducers to use as positive controls for in vitro experiments. With the exception of CYP2E1, this table is comprehensive and should provide a set of guides that will produce easily interpretable results. However, it is unclear why an inducer for CYP2E1 was not included in the list. It is well documented that this P450 enzyme is induced by drugs such as halothane and ethanol. In addition, medium chain fatty acids also induce this enzyme at the transcriptional level [4]. While there may not be a plethora of drugs metabolized by this P450, CYP2E1 accounts for one of the most well documented drug-drug interactions, i.e. acetaminophen and alcohol [5]. Thus, we feel it is an oversight of this guideline to disregard the significance of CYP2E1 in drug-drug interactions and neglect to include inducers of this enzyme in Table 5.

2. On page 36, first paragraph the authors state that “the most reliable method to study a drug’s induction potential is to quantify enzyme activity of primary hepatocyte cultures following treatments including the potential inducer drug, a positive control inducer drug and vehicle-treated hepatocytes (negative control), respectively.” There are several concerns regarding this statement. First, human hepatocyte cultures have been utilized by many researchers in the drug metabolism arena for over 20 years largely because better alternatives, including animal hepatocytes, were not available or in the case of animal tissues the results could not be extrapolated to humans. Thus, using primary human cells has become reluctantly accepted as a means for identifying inducers of P450 enzymes. However, there are many inherent problems associated with the use of human hepatocytes. In addition to requiring seven days to complete experiments (low-throughput, high cost), the primary problem stems from variable results which are often difficult to interpret. This variation is not entirely due to individual differences among donors, but rather due to culture conditions. Indeed, cultured human hepatocytes are so variable in their induction response that drug sponsors often cannot interpret study data even if they follow the draft guideline and use three preparations from three different human livers. Moreover, Madan *et al.*, [6] demonstrates variation on positive controls from zero fold induction to 145 fold (CYP 3A4 activity after rifampicin treatment, n=61). This variability is such that moderate and mild inducing agents cannot be accurately classified. Also, sponsors have submitted data where results are too variable for accurate interpretation (personal communications). Furthermore, the large variability witnessed in human hepatocyte studies is not indicative of the *in vivo* situation where the greatest variability for CYP3A4 induction does not exceed 10 fold [7;8]. Therefore, the variability in induction exhibited from lab-to-lab and from hepatocyte culture-to culture does not lend itself to being “the most reliable method” for identifying P450 inducers.

Over the past 8 years, mechanisms involved in the induction of CYP3A4 and CYP1A2 have been elucidated. Enhanced expression of these enzymes by chemical entities occurs at the transcriptional level and involves the receptors, pregnane X receptor/steroid xenobiotic receptor (PXR/SXR) constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR). To date, there have not been any other mechanisms of induction identified for these two enzymes. Because these

receptors are involved, techniques such as functional receptor activation (reporter gene) assays can be utilized in an easy and rapid manner to identify inducers of CYP3A4 and CYP1A2. There are several reports (not all listed here) documenting the reliability of this method to predict induction of unknown chemicals in hepatocytes [9], liver slices [10], and *in vivo* [11]. Thus, it is unclear why this methodology is only briefly mentioned on P. 37, part 4 (c) of the draft document. It is also unclear why only primary cultures of human hepatocytes are the “preferred method”. The use of the formula shown on p. 36 under “Endpoints for subsequent prediction of enzyme induction” can also be applied to receptor activation studies. Indeed, because of the robustness and lack of variability, EC₅₀ values are much easier to determine with receptor activation technology.

3. An additional concern with the draft document is the use of enzyme activities to assess induction of P450 enzymes. This method alone should not be accepted as many inducers may also be inhibitors, producing false negative results. Determining enzyme activities should be coupled with mRNA or protein quantification. Enzyme activities should never be the sole measurement for P450 induction. In fact, assessing enzyme activity is cumbersome and reports suggest that measuring CYP3A4 or CYP1A2 mRNA levels are sufficient to identify P450 inducers [12].

4. There is also concern regarding the terminology used to describe receptor activation studies on page 37, part 4 (c). In this section, these studies are incorrectly termed “receptor gene assays” which suggests that both receptor binding and reporter gene assays exhibit similar predictive powers. On the contrary, each assay produces different results. For instance, binding assays and cell-based assays (functional) differ in their ability to predict an *in vivo* event. The latter utilizes a living cell (hepatic in most cases) that with a complement of transporters, enzymes, and functional cascades of gene-mediated processes can better predict an *in vivo* event. These receptor activation assays with intact cells are also very accurate at predicting the ability of a NCE to bind to a nuclear receptor and produce a response. On the other hand, ligand binding assays only determine the affinity of an NCE to bind to the receptor, but do not assess the effect of that binding on gene activation (transcription) or repression. Moreover, there is low correlation between receptor activation and receptor binding assays [13]. Therefore, the terms are too loose and fail to indicate to the reader the vast differences in the described technologies.

Appendix D:

The new material on P-glycoprotein (P-gp) in this draft guideline is welcome and will benefit the industry as P-gp drug interactions can be a source of adverse drug reactions. The guideline addresses methods to examine if the NCE is a substrate or inhibitor; pointing out the importance of “modulating” this protein and the adverse effects that can occur. However, it does not address methods to determine if a NCE would act as an inducer of P-gp, or modulate this transporter through induction. Increased expression of P-gp can be a source of adverse drug reactions, including multidrug resistance, in much the same manner as induction of a major cytochrome P-450. Reliable *in vitro* tools are in place for prediction of such

an event. Collectively, this guideline should address methods for assessing P-gp induction and not rely upon results generated in human hepatocytes that determine CYP3A4 induction.

Recommendations for the authors:

We feel that a more comprehensive list of “preferred methods” should be incorporated into this document. One such technique should be reporter gene/receptor activation assays. Proper tools and methods for measuring receptor activation are in wide-spread use and have been commonplace for at least 8 years [9-11;14-16]. Thus, these tools are reliable, have endpoints that permit clear decision-making and exhibit much less variance than the “reliable” “preferred” method of using human hepatocytes and measuring enzyme activity. The guideline does little to encourage readers to use more advanced technologies.

Finally, there is a distinct advantage in determining receptor activation, particularly PXR/SXR and CAR as these receptors are also involved in the regulation of key enzymes involved in endogenous activities including cholesterol synthesis and degradation, bile acid metabolism, vitamin metabolism and steroid hormone metabolism [17-21]. Recent reports also describe a direct link between PXR and drug-mediated antagonism of NF- κ B, a key regulator of inflammation and the immune response [22]. Elevated expression of PXR can also disrupt glucocorticoid and mineralocorticoid homeostasis [23] in addition to its role in cholesterol detoxification [24] [25] and in protecting against liver toxicity from lithocholic acid[26]. Activation of PXR can also result in increased metabolism of 1,25(OH) vitamin D(3) diminishing intestinal effects of this hormone and contributing to osteomalacia [21]. Thus, determining receptor activation can be beneficial in two ways. First, by predicting adverse drug reactions that occur when NCEs activate these receptors *in vivo* and second, identifying potential drug-drug interactions.

We are hopeful that our suggestions will be incorporated into the draft document and appreciate this opportunity to present our critique of the current guidelines. We anxiously await your response.

Sincerely,

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