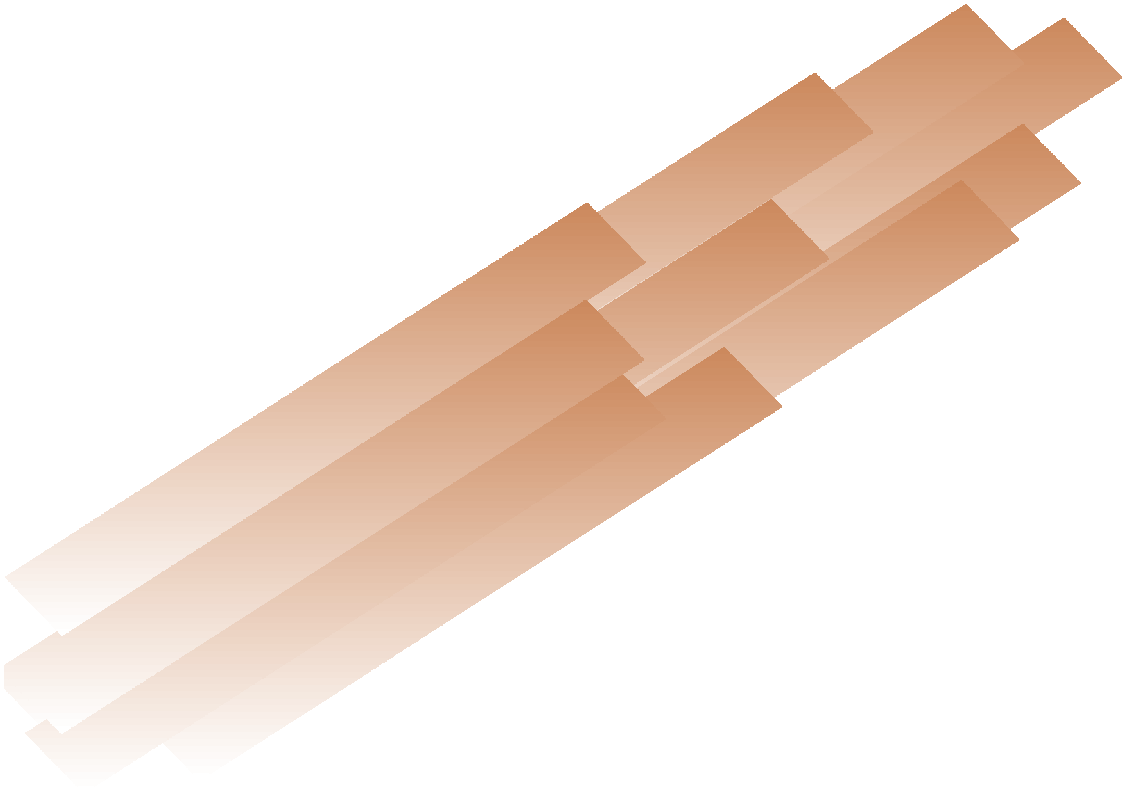
Guidance for Industry

Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro



Department of Health and Human Services
U.S. Food and Drug Administraion
Center for Drug Evaluation and Research
Center For Biologics Evaluation and Research
April 1997

CLIN 3

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5600 Fishers Lane, Rockville, MD 20857
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I. INTRODUCTION

After entering the body, a drug is eliminated either by excretion or by metabolism to one or more active or inactive metabolites. When elimination occurs primarily by metabolism, the routes of metabolism can significantly affect the drug's safety and efficacy and the directions for use. When elimination occurs via a single metabolic pathway, individual differences in metabolic rates can lead to large differences in drug and metabolite concentrations in the blood and tissue. In some instances, differences exhibit a bimodal distribution indicative of a genetic polymorphism (e.g., CYP450 2D6, CYP450 2C19, N-acetyl transferase). When a genetic polymorphism affects an important metabolic route of elimination, large dosing adjustments may be necessary to achieve the safe and effective use of the drug. Pharmacogenetics already has influenced therapeutics. For a drug that is primarily metabolized by CYP450 2D6, approximately 7 percent of Caucasians will not be able to metabolize the drug, but the percentage for other racial populations is generally far lower. Similar information is known for other pathways, prominently, CYP450 2C19 and N-acetyl-transferase. Equally important, if not more so, many enzymatic metabolic routes of elimination, including most of those occurring via the CYP450 enzymes, can be inhibited or induced by concomitant drug treatment. As a result, abrupt changes can occur with a co-administered agent in a single individual. Such interactions can lead to a substantial decrease or increase in the blood and tissue concentrations of a drug or metabolite or cause the accumulation of a toxic substance (e.g., certain antihistamine-antifungal interactions). These types of changes can alter a new drug's safety and efficacy profile in important ways, particularly a drug with a narrow therapeutic range.

An understanding of metabolic pathways and potential interactions sometimes allows the use of a drug that would produce an unacceptable level of toxicity if blood levels were not predictable. For these reasons, it is important to learn at an early stage of development whether a drug is eliminated primarily by excretion of unchanged drug or by one or more routes of metabolism. If elimination is primarily by metabolism, the principal metabolizing route(s) should be understood. This information will help identify the implications of

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[1]This guidance has been prepared by the Drug Metabolism/Drug Interactions--In Vitro Studies Working Group of the Clinical Pharmacology Section of the Medical Policy Coordinating Committee in the Center for Drug Evaluation and Research (CDER), with input from the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration. This guidance represents the Agency's current thinking on drug metabolism and drug interaction studies in vitro. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirement of the applicable statute, regulations, or both.

metabolic differences between and within individuals and the importance of certain drug-drug and other interactions. Having such information also will aid in determining whether the pharmacologic properties of certain metabolites should be explored further.

This FDA guidance to industry provides suggestions on current approaches to studies in vitro of drug metabolism and interactions. The guidance is intended to encourage routine, thorough evaluation of metabolism and interactions in vitro whenever feasible and appropriate. As is the case for all FDA guidance documents, suggestions are not requirements, but are offered for consideration by drug development scientists as a means to address potentially important safety concerns. FDA recognizes that the importance of any approach will vary depending on the drug in development and its intended clinical use. The FDA also recognizes that clinical observations can address some of the same issues identified in this document as being susceptible to in vitro study. The suggested approaches delineated in this document, however, are efficient and inexpensive considering the breadth of information they can provide, and often can reduce or eliminate the need for further clinical investigations. This particular guidance is directed toward a broad class of drugs: molecules with a molecular weight below 10 kiloDaltons.

Although the field of in vitro assessment of drug metabolism and drug interactions has progressed sufficiently to allow preparation of this guidance, additional work will be required to allow a comprehensive characterization of drug metabolism in vitro (including induction and inhibition) and its implications for subsequent clinical investigations and product labeling. Because the assessment of drug metabolism in vitro is a rapidly evolving area of drug development and regulation, this guidance may require frequent revision.

Review scientists at the FDA have long been interested in the impact of drug metabolism and drug-drug interactions on drug safety and efficacy. As a result, discussion of this topic also is contained in other FDA guidance documents, including *General Considerations for the Clinical Evaluation of Drugs* (FDA 77-3040), *Guideline for Studying Drugs Likely to be Used in the Elderly* (11/89), and *Guideline for the Study and Evaluation of Gender Differences in the Clinical Evaluation of Drugs* (58 FR 39406, July 22, 1993). To obtain these documents, contact the Drug Information Branch at the Center for Drug Evaluation and Research.

II. OBSERVATIONS AND CONCLUSIONS

The following general observations and conclusions underlie the suggestions found in this guidance:

- The concentrations of the parent drug and/or its active metabolite(s) circulating in the body are the effectors of desirable and/or adverse drug actions.

- A principal regulator of drug concentration in the body is clearance. Metabolism can be a major determinant of clearance.

- Even for drugs that are not substantially metabolized, the potential effect of that drug on the metabolism of concomitant drugs could be important.

- Large differences in blood levels can occur because of individual differences in metabolism. Some drugs, such as tricyclic antidepressants, exhibit order of magnitude differences in blood concentrations depending on the enzyme status of patients. Drug-drug interactions can have similarly large effects when one drug inhibits the metabolism of another. For example, ketoconazole greatly increases concentrations of parent terfenadine, leading to QT prolongation and *torsades de pointes*.

- Major advances have been made recently in the availability and use of human tissue and recombinant enzymes for studies in vitro of drug metabolism and drug-drug interactions.

- The development of sensitive and specific assays for a drug and its metabolites is critical to the study of drug metabolism and interactions. Development of such assay methods has long been a high priority for drug development programs, and such assays are increasingly available early in development. Once reliable assays are available, the techniques are available for readily assessing drug metabolism and drug-drug interactions in vitro and interpreting the results.

The studies in vitro described in this document are one set of approaches to developing information about drug metabolism and drug-drug interactions. Mechanistic and empirical clinical study approaches are available as well to provide further information. As always, a carefully designed mix of approaches is likely to yield optimal results in the shortest time and at the least cost. Metabolic effects and drug-drug interactions should be considered as early as possible as well as later in the drug development process. Appropriately designed pharmacokinetic/phase 1 studies could provide important information about drug metabolism, relevant metabolites, and actual or potential drug interactions. Blood level data obtained during phase 2 and 3 clinical trials, for example, via a pharmacokinetic screen, also could reveal interactions or marked inter-individual differences. Because clinical trial protocols sometimes limit concomitant drug use, some later studies may not be optimally informative about possible drug interactions. Decreasing exclusions of concomitant drug treatment and measurement of blood levels before and after treatment with a test drug (interaction screen), as well as testing drug blood levels more frequently, could make later phase clinical studies more useful. All of these studies could be more informative if significant metabolites and prodrugs could be identified and their pharmacological properties described.

Identifying metabolic differences in patient groups based on genetic polymorphisms, or on other readily identifiable factors such as age, race, and gender, could help guide the design of dosimetry studies for such populations groups. This kind of information also will provide improved dosing recommendations in product labeling, facilitating the safe and effective use of a drug by allowing prescribers to anticipate necessary dose adjustments. Indeed, in some cases, understanding how to adjust dose to avoid toxicity may allow the marketing of a drug

that would have an unacceptable level of toxicity were its toxicity unpredictable and unpreventable.

III. TECHNIQUES AND APPROACHES FOR STUDIES IN VITRO OF DRUG METABOLISM AND DRUG INTERACTIONS

The goals in evaluating in vitro drug metabolism are: (1) to identify all of the major metabolic pathways that affect the test drug and its metabolites, including the specific enzymes responsible for elimination and the intermediates formed; and (2) to explore and anticipate the effects of the test drug on the metabolism of other drugs and the effects of other drugs on its metabolism. Pharmacologic effects of the test drug and its major metabolites also should be studied, if feasible. Knowledge that a particular drug is not a substrate for certain metabolic pathways is helpful. For example, if it is learned early in drug development that a molecule is not a substrate for CYP450 3A4 or that this pathway represents only a minor contribution to overall metabolism, then concern is lessened or eliminated for possible inhibition of 3A4 metabolism by drugs such as ketoconazole and erythromycin or possible induction of metabolism by drugs such as rifampin and anticonvulsants. Studies in vitro also could indicate whether a drug itself is or is not an inhibitor of common metabolic pathways. The potential for a drug inhibiting the metabolism of other drugs is almost always present for drugs metabolized by the same pathway, but can also be present for entirely separate pathways, including the principal metabolic route for a compound. This potential was first appreciated for quinidine, which is a substrate for metabolism by CYP450 3A4 and is also a very potent inhibitor of CYP450 2D6.

A. Cytochrome P-450, Microsomes, and Related Tools

1. Assessing the metabolism of a test drug

The most mature technology for the study in vitro of drug metabolism (enzymes involved, metabolites formed, and potential inhibitors) is associated with the set of enzymes contained in the cytochrome CYP450 superfamily. These enzymes are responsible for the metabolism of the majority of drugs given to humans. Metabolism usually occurs in the liver, but the enzymes (especially CYP450 3A4) also are important in gut metabolism. Human liver microsomes provide the most convenient way to study CYP450 metabolism. Microsomes are a subcellular fraction of tissue obtained by differential high-speed centrifugation. All of the CYP450 enzymes are collected in the microsomal fraction. The CYP450 enzymes retain their activity for many years in microsomes or whole liver stored at low temperature (e.g., -70^{o} C). Cofactor requirements for CYP450-mediated reactions are well characterized, consisting primarily of a redox sustaining system such as NADPH. Hepatic microsomes can be obtained commercially, with or without prior phenotyping, for most important drug-

metabolizing enzymes.

During studies to identify metabolic routes of elimination for an investigational new drug, microsomes from several donors should be used, either individually or pooled, to avoid reliance on microsomes that are deficient in one or more metabolic pathways, unless this is a specific objective of the study. With the use of selective chemical inhibitors for each major pathway, the metabolic pathways for a new drug can be readily demonstrated or ruled out. Careful consideration of incubating concentrations of both inhibitor and substrate is essential to maintain a selective approach. For example, quinidine and ketoconazole are relatively selective inhibitors of 2D6 and 3A4, respectively, at concentrations below 1 micromolar, but both will also inhibit other CYP450 enzymes at higher concentrations, an inhibition that is not known to be clinically pertinent. Antibodies to specific CYP450 enzymes also can be used to attempt selective inhibition of metabolic pathways, but at present this approach is limited by lack of wide commercial availability of the antibodies, incomplete characterization of their selectivity, and high laboratory-to-laboratory variation for antibody inhibition results in comparison to chemical inhibitors.

The cDNAs for the common CYP450s have been cloned, and the recombinant human enzymatic proteins have been expressed in a variety of cells. After the apparent metabolic pathway has been determined using microsomes, use of these recombinant enzymes provides an excellent way to confirm results identified in microsomes.

The most complete picture for hepatic metabolism can be obtained with intact liver systems, in which the cofactors are self-sufficient and the natural orientation for linked enzymes is preserved. Isolated hepatocytes and precision-cut slices have these desirable features. Radiolabeled drugs are very helpful at this stage. A major logistic problem with these preparations, however, is that enzymatic activities are not stable for much longer than 24 hours. Overcoming that limitation will be valuable for investigating induction of enzyme activity.

Studies in vitro can identify critical metabolic pathways for a new drug and metabolites that are formed by these pathways. The clinical significance of this information should generally be confirmed via studies in the clinic. Absence of a finding that certain metabolic pathways are important via in vitro studies may obviate the need for further clinical investigations or at least help focus the design of these studies.

2. Assessing effects on other drugs

Human microsomes are also the most useful tool for screening for the effects of a new drug on common CYP450 pathways and for providing rapid initial

information on potential drug-drug interactions. A general assessment of effects on major metabolic pathways can be obtained by simultaneous incubation of the investigational new drug with standard probe compounds, which are available for many CYP450 pathways. The experiments are exceptionally rapid and straightforward, requiring no special equipment. In general, if appropriate concentrations of the test drug are used with established probes, a negative result in vitro (no interaction identified) is reassuring and can generally eliminate the need for further clinical evaluation. Positive results suggest the need for further clinical evaluation.

B. Other Hepatic Enzymes

Although the CYP450 superfamily is the dominant group of metabolizing enzymes, other classes of important enzymes for drug metabolism are present in humans, including enzymes responsible for acetylation, methylation, glucuronidation, sulfation, and de-esterification (esterases). Approaches in vitro are not as widely applied for these enzymes as to the CYP450s, but considerable progress has been made, and further important efforts are underway.

In addition to the CYP450 enzymes, microsomes contain other enzymes, including a variety of transferases. For conjugating reaction pathways, supplementation of microsomal preparations with conjugating moieties as added cofactors has been successful. Cytosolic (soluble) enzymes are not contained in the microsomal fraction, but may be readily investigated using other subcellular fractions (e.g., S9).

C. Gastrointestinal Drug Metabolism

Much emphasis in metabolic research and development has focused on the liver, because this organ has always been regarded as the principal site of drug metabolism. For particular drugs, however, other tissues may predominate (e.g., the kidney or gastrointestinal mucosa). Because most drugs are given orally, interest has been increasing in the effect of gastrointestinal mucosal enzymes on drug entry to the systemic circulation. Drugs susceptible to metabolism via CYP450 3A4 may exhibit low and/or variable bioavailability. Thus, determining the susceptibility of a drug to metabolism by CYP450 3A4 may be important not only in identifying routes of elimination but also in predicting the likelihood of significant first-pass metabolism.

D. Interspecies Metabolic Comparisons and Other Uses of Animal Data

Animal toxicology studies are an important component of assessing safety for subsequent human exposure. Although comparative metabolism has long been of interest, this emphasis has grown in recent years, and many drug development

programs now produce extensive characterization of metabolites in animals. This work has not regularly been linked to parallel findings in humans, but the availability of tools for the study of human metabolism in vitro provides an opportunity to refocus and enhance the goals of pharmacokinetic and metabolic studies in animals.

Animal studies provide the means to determine whether new chemical species generated by human metabolic studies in vitro are active pharmacologically (toxicologically) and how they compare to the parent compound, often a critical determinant of the effect of drug-drug interactions or genetic diversity. Early identification of human metabolic routes of elimination and metabolites by studies in vitro can provide clear direction for preclinical studies in animals.

An especially valid application of in vitro and appropriate clinical follow-up studies is to compare drug and metabolite exposure in humans and animals. Reasonably similar exposure supports the relevance of a particular animal species to the assessment of a potential human risk, and knowledge of differences (e.g., a toxic metabolite in animals, but not in humans) could aid in interpretation of clinical data. The earlier this is done, the easier it will be to use the information in planning and interpreting clinical studies. Although the use of in vitro techniques to determine the most metabolically relevant species for nonclinical testing may enhance the value of these studies, selecting appropriate species or strains is not a simple matter. The need for historical control data and prior experience in toxicology studies for a particular species and strain could limit the ability to select species and strains based on similarities of metabolic pathways to humans. Nonetheless, major metabolic dissimilarities between the test species and humans reduce the confidence in these studies as predictors of safety in humans.

IV. CORRELATION BETWEEN STUDIES IN VITRO AND IN VIVO

A complete understanding of the relationship between metabolism in vitro and in vivo is still emerging. Strong correlations have been documented between well-conducted studies in vitro and in vivo, but considerable effort is necessary before complete validation of these correlations is obtained, including an appreciation for whatever limits may exist for the correlations. When a difference arises between findings in vitro and in vivo, the results in vivo should always take precedence over studies in vitro. In many cases, however, studies in vitro, which are inexpensive and readily carried out, will serve as an adequate screening mechanism that can rule out the importance of a metabolic pathway and make in vivo testing unnecessary. If investigations in vitro suggest that the answer to the question "Does CYP450 2D6 metabolize this drug?" is "no," clinical studies to identify the impact of the slow metabolizer phenotype or to study the effect of CYP450 2D6 inhibitors will not be needed. Because studies in vitro, however, cannot adequately define the importance of a metabolic pathway, if the in vitro study answer is “yes,” additional clinical studies will be important to answer whether CYP450 2D6 is clinically important to the elimination of the drug.

Additional information also may be necessary to identify which inhibitors, if any, affect metabolism in vivo significantly. For example, although a drug may be extensively metabolized in vitro, a mass balance study in vivo may demonstrate that metabolism is less important than urinary or biliary excretion. In addition, inhibition studies often will not be definitive in vitro unless only a relatively low degree of inhibition is present, with intermediate to high degrees of inhibition needing subsequent clinical confirmation. In this setting, inhibition of metabolic pathways will not have a clinical impact except for patients with severe impairment of excretory function, and the effect of induction on elimination will be limited. In general, if there is some, but not a large, effect in vitro, predicting the effect in vivo will be difficult. Experiments in vitro should be conducted at concentrations similar to the relevant concentration in vivo. As previously noted for chemical inhibition studies, different pathways may be affected at various concentrations. This may be difficult to determine, however, when the interaction occurs in the gut.

V. TIMING OF METABOLISM STUDIES

A question frequently raised is when during drug development should studies in vitro be conducted. Sponsors are reluctant to allocate resources during the investigation of a drug that has not yet demonstrated a suggestion of clinical activity. This is a reasonable concern, and where possible, it is reasonable to identify some useful activity in short-term clinical studies before embarking on a major metabolic evaluation. Nevertheless, an early understanding of how a compound is metabolized could influence selection among several pharmacologically similar agents and could lead to dose regimens that would be more likely to detect a positive clinical effect. When attempting to determine the most appropriate time to conduct metabolism studies in vitro, it is helpful to reconsider the reasons for conducting such studies. Two of the major clinical reasons, as previously mentioned, are (1) to identify all of the major metabolic pathways that affect the drug and its metabolites and (2) to anticipate the effects of the drug on the metabolism of other drugs. With these objectives in mind, an understanding of the metabolic profile of a drug in vitro would be useful prior to the initiation of phase 2 studies and is especially important before phase 3 trials, when a broader population will be studied. This knowledge would permit the efficient design of clinical dose/response, interaction, and special population studies and also would enable adequate attention to be given to patient variability and potential interactions in phase 2 and 3 studies. Of course, drugs have been developed successfully even when the evaluation of metabolic routes of elimination occurred during the later phases of drug development or were not explored at all. Today, however, it is difficult to justify marketing a drug without knowing how it is metabolized or how it could influence, or be influenced by, the drugs being taken with it. Therefore, sponsors are encouraged to conduct appropriate metabolic studies prior to commencement of phase 3 trials.

VI. LABELING

Each year, large numbers of new drug-drug interactions are discovered, precluding the possibility that any prescriber could memorize them all. Based on the increasing amount of valuable information that is available, it is now possible to label for class effects for various enzymes, and the ability to extrapolate from partial data is growing. Standardized approaches to labeling are likely to emerge and be helpful, in a manner analogous to the class labeling used for certain categories of drugs. For example, certain powerful inhibitors (quinidine for CYP450 2D6, ketoconazole for CYP450 3A4) are likely to affect all drugs metabolized by these pathways. For this reason, if a new drug is found to be a substrate for certain CYP450 enzymes, then certain interactions may be anticipated, even though specific data are lacking. This understanding relies on knowledge about the activity of the drug and its metabolites. Similarly, it would be helpful to know what metabolic pathways are not involved in the elimination of a drug. When generalizations are made from studies in vitro, the conditions of extrapolation should be explicitly stated. Thus, conclusions based on data gained from in vitro studies that are extrapolated to the clinical situation should be identified and distinguished from conclusions based on clinical observations in vivo. Under these circumstances, the best advice available at any given time may be provided, and class effects may be updated as new information is obtained. The following text is an example of class labeling based on studies in vitro:

> *Although clinical studies have not been conducted, on the basis of this drug's metabolism by CYP450 3A4, ketoconazole, itraconazole, erythromycin, and grapefruit juice are likely to inhibit its metabolism. Furthermore, rifampin, dexamethasone, and certain anticonvulsants (phenytoin, phenobarbital, carbamazepine) may induce this drug's metabolism. Thus, if a patient has been titrated to a stable dosage on this drug, and then begins a course of treatment with one of these inducers or inhibitors, it's reasonable to expect that a dose adjustment may be necessary to prevent toxicity or therapeutic failure.*

The example below demonstrates where the class effects would be inserted and also where information on the drug's inhibitory effects would be stated:

> *This drug is metabolized by CYP450 3A4 < insert current statement > . At clinical doses, the drug itself does not inhibit the metabolism of other 3A4 substrates, but does inhibit the metabolism of substrates metabolized via the CYP450 2D6 pathway.*

Given the tendency to include many potential interactions, it is sometimes unclear if anything is noninteracting. In such a circumstance, labeling statements that denote both positive and negative expectations may be helpful. For example:

> *This drug is a substrate for CYP450 1A2. Although inhibition of its metabolism by ciprofloxacin is observed, quinidine, erythromycin, ketoconazole, and itraconazole are not inhibitors.*

VII. RELATED APPLICATIONS AND CONSIDERATIONS

The same techniques for evaluating potential drug-drug interactions can also provide information related to the influence of social (smoking, alcohol), environmental (diet, e.g., grapefruit juice), and genetic factors upon therapeutics. For example, several studies have demonstrated that tobacco smoking is a strong inducer of the CYP450 1A2 enzymes. As a result, larger doses of theophylline are recommended for patients who smoke and receive this drug. In the future, evidence of induction as well as inhibition may also be developed via in vitro studies. Metabolic characterization of racemic drugs should be conducted in accordance with previously expressed guidances and guidelines on the development of stereoisomers. In particular, if the development of a single enantiomer is to be pursued, the preclinical metabolism studies in vitro should be conducted with the relevant enantiomer, rather than the racemic mixture.