Safety Testing of Drug Metabolites

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

November 2016
Pharmacology/Toxicology

Revision 1

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TABLE OF CONTENTS

Contains Nonbinding Recommendations

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I. INTRODUCTION

This guidance provides recommendations to industry on when and how to identify and characterize drug metabolites whose nonclinical toxicity needs to be evaluated. The safety of drug metabolites may need to be determined in nonclinical studies because these metabolites are either identified only in humans or are present at disproportionately higher levels in humans than in any of the animal species used during standard nonclinical toxicology testing.[2]

This guidance applies to small molecule nonbiologic drug products. This guidance does not apply to some cancer therapies where a risk-benefit assessment is considered.[3]

This guidance supersedes the guidance of the same name published in February 2008. The guidance has been revised to be in alignment with the ICH guidance for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals*.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

[1] This guidance has been prepared by the Pharmacokinetic Subcommittee of the Pharmacology and Toxicology Coordinating Committee in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] See the Glossary for the definition of *disproportionate drug metabolite*.

[3] See the ICH guidance for industry *S9 Nonclinical Evaluation for Anticancer Pharmaceuticals* for the safety testing of drug metabolites in cancer therapies. We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

II. BACKGROUND

Nonclinical evaluation of drug safety usually consists of standard animal toxicology studies.[4] These studies usually include assessment of drug exposure, primarily parent drug plasma concentration. Generally, drug plasma concentration and systemic exposure in the nonclinical studies are compared with systemic exposure in humans to assess the potential risks suggested by nonclinical findings and guide monitoring in clinical trials. This testing paradigm usually is sufficient when the metabolic profile in humans is similar to that in at least one of the animal species used in nonclinical studies, but metabolic profiles can vary across species both quantitatively and qualitatively, and there are cases when clinically relevant metabolites have not been identified or adequately evaluated during nonclinical safety studies. This situation can occur if the metabolite is formed only in humans and is absent in the animal test species or if the metabolite is present at disproportionately higher levels in humans than in the animal species used in the standard toxicity testing with the parent drug.

It is not standard practice for drug metabolites to be evaluated separately in a cross-species safety assessment. As a result, their specific contribution to the overall toxicity of the parent drug has often remained unknown. This lack of appreciation of the role of metabolites in drug toxicity may be partly because of the inadequate sensitivity of the analytical methods used to detect and characterize metabolites derived from the parent drug. Technological advances have greatly improved the analytical capabilities to detect, identify, and characterize metabolites and allow for a better understanding of the role metabolites play in drug safety assessment.

Drugs entering the body undergo biotransformation via Phase I and Phase II metabolic pathways. Based on the nature of the chemical reactions involved, metabolites formed from Phase I reactions are more likely to be chemically reactive or pharmacologically active and, therefore, more likely to need safety evaluation. An active metabolite may bind to the therapeutic target receptors or other receptors, interact with other targets (e.g., enzymes, proteins), and cause unintended effects. This is a particularly important problem when such a metabolite is formed in humans and not in animals, but the occurrence of a metabolite only in humans and not in any animal test species is rare. A more common situation is the formation of a metabolite at disproportionately higher levels in humans than in the animal species used in safety testing of the parent drug. This disproportionality stems from the typical qualitative and/or quantitative differences in metabolic profiles between humans and animals. If at least one animal test species forms this drug metabolite at adequate exposure levels (approximately equal to or greater than human exposure), as determined during toxicology testing of the parent drug, it can be assumed that the metabolite's contribution to the overall toxicity assessment has been established.[5]

Metabolites that form chemically reactive intermediates can be difficult to detect and measure because of their short half-lives. However, they can form stable products (e.g., glutathione

[4] See the ICH guidances for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* and *S7A Safety Pharmacology Studies for Human Pharmaceuticals*.

[5] See Appendix A: Decision Tree Flow Diagram. This diagram describes which studies may be needed to determine safety of the drug metabolite.

conjugates) that can be measured. Phase II conjugation reactions generally render a compound more water soluble and pharmacologically inactive, thereby eliminating the need for further evaluation. However, if the conjugate forms a toxic compound such as acylglucuronide, additional safety assessment may be needed.[6]

Demonstration that a metabolite is pharmacologically inactive at the target receptor does not guarantee that it is not toxic. Therefore, it may need to be tested in nonclinical toxicity studies.[7]

III. GENERAL CONCEPTS IN METABOLITE SAFETY TESTING

We encourage the identification of any differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process.[8,9] The discovery of disproportionate drug metabolites late in drug development can potentially cause development and marketing delays.

Generally, metabolites identified only in human plasma or metabolites present at disproportionately higher levels in humans than in any of the animal test species should be considered for safety assessment. Human metabolites that can raise a safety concern are those formed at greater than 10 percent of total drug-related exposure at steady state.[10] The choice of a level of greater than 10 percent for characterization of drug metabolites reflects consistency with FDA and Environmental Protection Agency guidances.[11,12]

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[6] Faed, EM, 1984, Properties of Acyl Glucuronides. Implications for Studies of the Pharmacokinetics and Metabolism of Acidic Drugs, Drug Metab Rev, 15, 1213–1249.

[7] See Appendix B: Case Examples of Drug Metabolites.

[8] Baillie, TA, MN Cayen, H Fouda, RJ Gerson, JD Green et al., 2002, Drug Metabolites in Safety Testing, Toxicol Appl Pharmacol, 182, 188–196.

[9] Hastings, KL, J El-Hage, A Jacobs, J Leighton, D Morse, and R Osterberg, 2003, Drug Metabolites in Safety Testing, Toxicol Appl Pharmacol, 190(1), 91–92.

[10] Exposure should be at steady state unless there is some justification for a different measure of exposure. Comparison between human and animal exposure generally is based on area under the curve, but sometimes it may be more appropriate to use C_{max}. See ICH M3(R2).

[11] U.S. Environmental Protection Agency, 1998, Health Effects Test Guidelines, OPPTS 870.7485, Metabolism and Pharmacokinetics (http://www.epa.gov/epahome/research.htm).

[12] See the Veterinary International Conference on Harmonization guidances *GL46 Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-producing Animals: Metabolism Study to Determine the Quantity and Identify the Nature of Residues (MRK)* and *GL47 Studies to Evaluate the Metabolism and Residue Kinetics of Veterinary Drugs in Food-Producing Animals: Comparative Metabolism Studies in Laboratory Animals* available on the Veterinary International Conference on Harmonization (VICH) Guidance Documents Web page at http://www.fda.gov/RegulatoryInformation/Guidances/ucm122050.htm.

A. General Approaches for Assessing Metabolite Safety

A metabolite identified in animals that is not present in humans can mean that a toxicity observed in that animal species, attributed to the metabolite, may not be relevant to humans. Conversely, a drug metabolite identified during clinical development that is not present in animal test species or is present at much lower levels in animals than in humans can suggest the need for further studies in animals to determine the potential toxicity of the metabolite. In such cases, two approaches can be considered to assess the drug metabolite. The first approach is to identify an animal species routinely used in toxicity studies that forms the metabolite at adequate exposure levels (equivalent to or greater than the human exposure), and then investigate the drug's toxicity in that species. The second approach, if a relevant animal species that forms the metabolite cannot be identified, is to synthesize the drug metabolite and directly administer it to the animal for further safety evaluation. In this approach, analytical methods that are capable of identifying and measuring the metabolite in nonclinical toxicity studies should be developed.

We acknowledge the difficulties associated with synthesizing a specific metabolite as well as the inherent complexities that accompany its direct administration. Direct dosing of a metabolite to animals may lead to subsequent metabolism that may not reflect the clinical situation and thus may complicate the toxicity evaluation. Moreover, new and different toxicities may arise from administration of the metabolite that were not observed with the parent drug. However, notwithstanding these possible complications, identification and evaluation of the potential toxicity of the drug metabolite is considered important to ensure clinical safety, and the decision to conduct direct safety testing of a metabolite should be based on a comprehensive evaluation of the data on the parent drug and any information available for the metabolite. Appendix B provides three case examples when drug metabolites were formed at disproportionately higher levels in humans than in test animals used in the nonclinical studies and how the safety evaluation was approached. In Case 1, testing of the drug metabolite was not needed because the metabolite was adequately characterized in nonclinical toxicity studies with the parent drug. However, in Cases 2 and 3, the drug metabolites had to be tested in toxicity studies by direct administration to the animal. In Case 3, the drug metabolite was pharmacologically inactive at the therapeutic target receptor but showed a unique toxicity not observed with the parent molecule.

B. Identification of Metabolites

Metabolite concentrations cannot be inferred by measurement of parent drug concentrations. The metabolic profile of the drug should be identified during the drug development process. This identification can be accomplished at different stages of development using in vitro and in vivo methods. In vitro studies can use liver microsomes, liver slices, or hepatocytes from animals and humans and generally should be conducted before initiation of clinical trials. In vivo metabolism study results in nonclinical test species generally should be available early in drug development, and their results will either confirm the results obtained from the in vitro studies or reveal quantitative and/or qualitative differences in metabolism across species. It is the latter situation that may pose a safety concern. Human in vivo metabolism studies usually have been conducted relatively later in drug development, but we strongly recommend in vivo metabolic evaluation in humans be conducted as early as feasible.

Adequacy of exposure to drug metabolites that are present at disproportionately lower levels in animals used in nonclinical studies should be considered on a case-by-case basis. Generally, systemic exposure is assessed by measuring the concentration of the parent drug at steady state, in serum or plasma. However, when measurements cannot be made in plasma of the test species for any reason, verification of adequate exposure can be made in other biological matrices such as urine, feces, or bile.[13] We encourage contacting the FDA early in drug development to discuss these issues.

C. General Considerations for Nonclinical Study Design

When designing a nonclinical study for a disproportionate drug metabolite, it is important to consider the following factors:

- Similarity of the metabolite to the parent molecule
- Pharmacological or chemical class
- Solubility
- Stability in stomach pH
- Phase I versus Phase II metabolite
- Relative amounts detected in humans versus the amounts detected in animals

Additional factors to consider include the proposed drug indication and patient population (e.g., nonclinical studies can be abbreviated for serious indications such as amyotrophic lateral sclerosis (ALS)). The proposed duration of use (short term, intermittent use, chronic) and levels of exposure at the therapeutic dose also should be considered when designing the nonclinical studies for the drug metabolite.

IV. RECOMMENDED STUDIES FOR ASSESSING THE SAFETY OF METABOLITES

Good laboratory practice guidelines apply to the nonclinical studies with the drug metabolite designed to evaluate safety (21 CFR part 58). The following studies may need to be conducted to assess the safety of the disproportionate drug metabolite.

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[13] See the ICH draft guidance for industry *S3A Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies — Questions and Answers*. When final, this guidance will represent the FDA's current thinking on this topic.

Although the drug metabolite of toxicological concern usually is one circulating in plasma at greater than 10 percent of total drug-related exposure, other metabolites also can elicit safety concern. For example, a drug metabolite representing greater than 10 percent of urinary excretion relative to the bioavailable dose, or a human fecal metabolite in cases where biliary elimination is the predominant route of excretion in humans, may reflect potential localized renal or bile duct toxicity, respectively. Further characterization in these instances should be addressed on a case-by-case basis with the review division.

A. General Toxicity Studies

The potential toxicity of a disproportionate drug metabolite should be evaluated to enable comparisons between the metabolite and its parent drug. The duration of the general toxicity study with direct dosing of the metabolite should follow the recommendations listed in ICH M3(R2). The toxicity of the drug metabolite should be investigated at multiples of the human exposure or at least at levels comparable to those measured in humans. We also recommend using the parent drug's intended clinical route of administration. However, with justification, other routes can be used to achieve sufficient exposure to the disproportionate metabolite. If the clinical route is oral, it is important to verify the stability of the metabolite in the stomach environment. It is crucial to gather toxicokinetic data from this type of study to ensure adequate exposure.

B. Genotoxicity Studies

The potential genotoxicity of the drug metabolite should be assessed in an in vitro assay that detects point mutations and in another assay that detects chromosomal aberrations. It is important that these assays be conducted according to the recommendations in the ICH guidance for industry *S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use*. If one or both of the in vitro tests are equivocal and/or positive, results from a complete standard battery of genotoxicity studies may be warranted.

C. Embryo-Fetal Development Toxicity Studies

When a drug is intended for use in a population that includes women of childbearing potential, embryo-fetal development toxicity studies should be conducted with the drug metabolite. We may ask for other reproductive toxicity studies on a case-by-case basis, depending on results of the general toxicity and embryo-fetal development studies. Reproductive toxicity studies should be conducted in accordance with the ICH guidance for industry *S5(R2) Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility*. Sometimes the conduct of an embryo-fetal development toxicity study in only one species that forms the drug metabolite can be justified.

D. Carcinogenicity Studies

Carcinogenicity studies should be conducted on metabolites of drugs that are administered continuously for at least 6 months, or that are used intermittently in the treatment of chronic or recurrent conditions when the carcinogenic potential of the metabolite cannot be adequately evaluated from carcinogenicity studies conducted with the parent drug. A single carcinogenicity study or an alternative bioassay should be conducted and the studies should be conducted in accordance with the ICH guidances for industry *S1A The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals*, *S1B Testing for Carcinogenicity of Pharmaceuticals*, and *S1C(R2) Dose Selection for Carcinogenicity Studies*.

V. TIMING OF SAFETY ASSESSMENTS

Early identification of disproportionate drug metabolites can provide clear justification for nonclinical testing in animals, assist in interpreting and planning clinical studies, and prevent delays in drug development. If toxicity studies of a drug metabolite are warranted, studies should be completed and study reports provided to the FDA before beginning large-scale clinical trials.

To optimize and expedite drug development for serious or life-threatening diseases other than cancer (e.g., ALS, stroke, human immunodeficiency virus), the number and type of nonclinical studies for the drug metabolites can be modified on a case-by-case basis for those drugs with major beneficial therapeutic advances, and for drugs for illnesses that lack an approved effective therapy. Sponsors should contact the appropriate review division to discuss such situations.

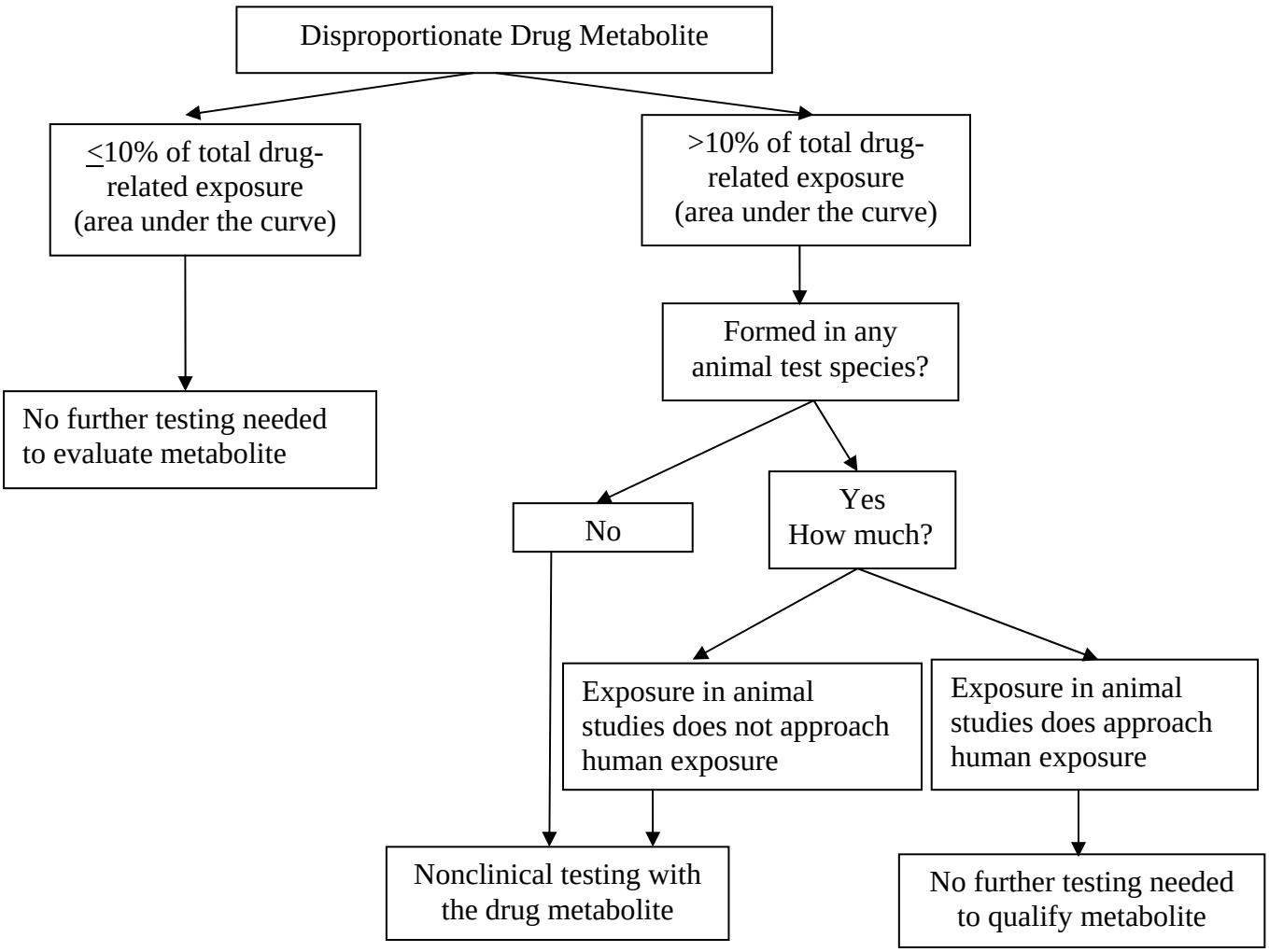
GLOSSARY

Disproportionate drug metabolite — A metabolite present only in humans or present at higher plasma concentrations in humans than in the animals used in nonclinical studies. In general, these metabolites are of interest if they account for plasma levels greater than 10 percent of total drug-related exposure, measured as area under the curve at steady state.

Metabolite — A compound derived from the parent drug through Phase I and/or Phase II metabolic pathways.

Pharmacologically active metabolite — A metabolite that has pharmacological activity at the target receptor. The activity may be greater than, equal to, or less than that of the parent drug.

APPENDIX A:
DECISION TREE FLOW DIAGRAM



APPENDIX B:
CASE EXAMPLES OF DRUG METABOLITES

Case 1.
From an initial mass balance study, a metabolite represented 1 to 2 percent of total radioactive dose in rat plasma, 5 percent in dogs, and 20 percent in humans (radioactivity of this metabolite in urine and/or feces was minimal). Based on the up-to-20 fold greater exposure in humans than in animals, nonclinical safety testing was recommended. However, the data generated in the general toxicology studies with the parent drug in the rat and dog suggested that the maximum doses tested produced metabolite exposures that represented at least the therapeutic exposure at the maximum recommended human dose. Also, the plasma concentrations of this metabolite measured in the in vivo genetic toxicity study, embryo-fetal development toxicity study, and carcinogenicity studies conducted with the parent drug provided adequate exposure and characterization of the metabolite. Therefore, no additional testing with the metabolite was needed.

Case 2.
Two primary hydroxylated metabolites, M1 and M2, were shown to undergo further oxidation to form secondary metabolites M3 and M4 using hepatic microsomes and hepatocytes from human, monkey, rat, dog, rabbit, and mouse. This metabolic profile was later confirmed by in vivo data. The results showed the following:

- M1 and M4 were the predominant metabolites in human, monkey, and dog microsomes, whereas rat, mouse, and rabbit formed M2 and M3

- M4 was formed in humans at 4 fold higher levels than total drug-related exposure, but M4 was formed at very low levels in rodents and only represented one-third of the total drug-related exposure in monkey (see Table 1)

Table 1: $AUC_{0\text{-}24hr}$ at the Maximum Dose*

	Human (MRHD)*	Monkey	Rat
Total drug-related exposure	1,800	15,000	12,500
M4	7,700	5,000	135

* AUC = area under the curve; MRHD = maximum recommended human dose

- Severe drug-related and novel target organ toxicities were observed with the parent drug in monkeys but not in rats

- M4 was pharmacologically inactive at the drug target receptors

The following additional studies were done with M4:

- Subchronic toxicity study: 3 months in the rat
- Embryo-fetal development study in the rat
- In vitro genotoxicity testing: M4 was positive for point mutation and chromosomal aberration; the parent drug was negative
- Because of the positive genotoxicity, a carcinogenicity study that included M4 was recommended

Case 3.
M2 is a Phase I oxidative metabolite that formed up to 50 percent of total drug-related exposure in humans, 10 percent of total drug-related exposure in mice, 15 percent of total drug-related exposure in dogs, and only trace amounts in rats. In vitro metabolism studies in these species supported the in vivo findings. Based on structure activity relationship analyses, there was no reason to anticipate any difference or exaggeration in toxicity of the metabolite compared to parent molecule. The parent drug showed no significant toxicity or identifiable target organ of toxicity in any of the animal species tested in safety assessment studies. Because disproportionate human exposure was identified, further safety testing was needed. When M2 was tested in a short-term tolerance study in the dog, it produced unexpected and significant cardiotoxicity at all doses and in all of the dogs. M2 was pharmacologically inactive at the therapeutic target receptor.