

WHY, WHEN, AND HOW TO CONDUCT ^{14}C HUMAN STUDIES

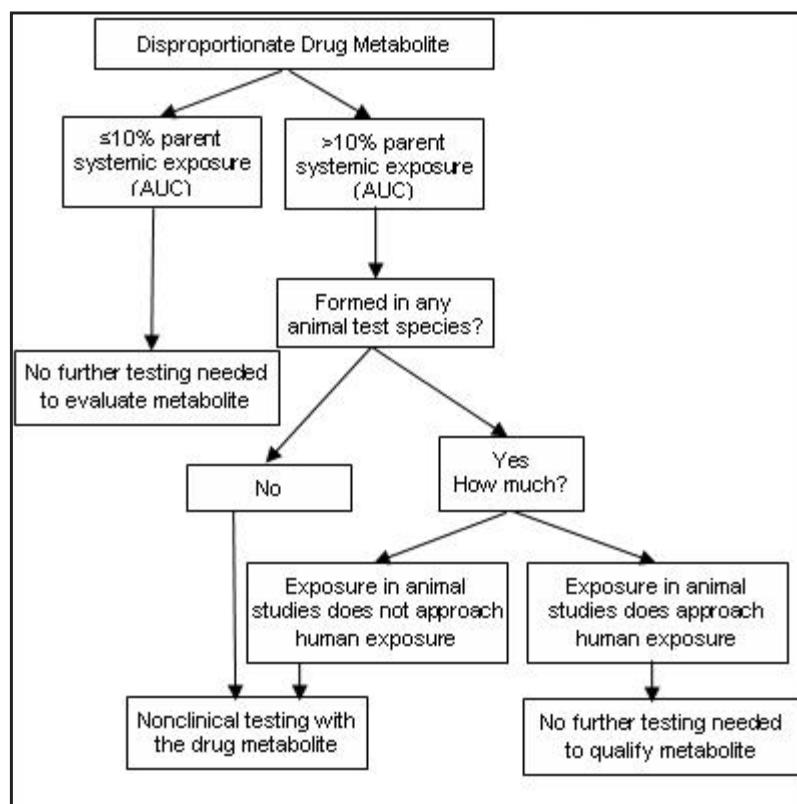
**AUTHOR:****ALAIN MIGNOT, PHARM. PH.D., SR CONSULTANT EARLY CLINICAL DEVELOPMENT, SGS LIFE SCIENCE SERVICES**

INTRODUCTION

The knowledge of the disposition and metabolism of drugs is an important part of the drug development process to appropriately understand the safety and efficacy of drug candidates. As recently summarized by the last FDA Guidance on safety testing of drug metabolites¹, there is a high concern when “drug metabolites are either identified only in humans (but this is rare) or are present at disproportionately higher levels in humans than in any of the animal species used during standard nonclinical toxicology (a more common situation)”.

Overall, the FDA “encourages the identification of differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible during the drug development process. The discovery of disproportionate drug metabolites late in drug development can cause marked delays in drug development. It is underlined that “human metabolites that can raise a safety concern are those formed at greater than 10 percent of parent drug systemic exposure at steady state.” The Guidance also depicts a decision tree that describes the circumstances where non-clinical studies using direct administration of the disproportionate human metabolite may be requested (Figure 1).

As a consequence, the knowledge of the metabolic fate of a drug candidate needs to be established during the drug development process and mainly before large population of patients are enrolled in clinical trials.

**FIGURE 1: FDA DECISION TREE FLOW DIAGRAM**

This article aims at presenting diverse strategies supporting the management of the risk related to drug metabolites,

and how the final ¹⁴C-human ADME (Absorption, Distribution, Metabolism, Excretion) studies should be conducted.

WHY CONDUCT ¹⁴C HUMAN STUDIES?

Early and comprehensive knowledge of pharmacokinetics and key metabolites is critical in drug discovery and development. It has now been more than two decades since SGS Life Science Services began conducting ¹⁴C ADME studies in humans. These studies have several objectives including the:

- a)** Determination of the mass balance of total drug-related material
- b)** Determination of the routes of excretion of total drug-related material
- c)** Determination of the pharmacokinetics of the parent drug and total drug-related material
- d)** Identification of the metabolites
- e)** Quantification of the relative abundances of the metabolites in excreta and circulation

In vitro models based on liver tissue, e.g. pooled human liver microsomes, liver S-9 fraction, and hepatocytes are generally

used to study the metabolism of drugs. These *in vitro* systems are deemed to provide appropriate information on *in vivo* circulating metabolites and metabolic pathways. In a recent study² using these three systems, the prediction of human excretory and circulating metabolite profiles of forty-eight compounds of different structures and routes of excretion for which the human ADME data were available was good for primary metabolites and less reliable for secondary metabolites.

However, even in these conditions it is generally recognized that identifying the human excretory and circulating metabolite profiles from these *in vitro* systems is not sufficient to mitigate the risk of disproportionate metabolites in humans.

Except for drugs that are entirely eliminated via the kidney, it is generally difficult to conclude that all the drug

metabolite have been identified and all drug related materials eliminated before having conducted human studies (metabolic profiling of plasma samples from first-in-human studies) and radiolabeled ADME studies in human. There are even suggestions that the starting point for quantification of circulating metabolites should be based on the results from the human metabolism study³.

Although there is no debate on the necessity to conduct a radiolabeled human ADME study to address all the issues related to the safety of drug metabolites, it is difficult to provide detailed requirements on what is sufficient to avoid additional toxicity studies. Overall, this uncertainty reflects the complexity of the drug development process and therefore, one cannot be surprised that different risk-based strategies are followed to fulfil the requirements of the guidance on the safety of testing of drug metabolites.

WHEN TO CONDUCT ¹⁴C HUMAN STUDIES?

While it is recognized that the human radiolabeled ADME study is the necessary step to definitely fix the strategy to tackle the issue of a disproportionate metabolite, different approaches to fulfil the requirements in the Guidance can be envisaged. Indeed, examples of different approaches are described here⁴:

Approach 1 is resource intensive with low risk for delay consists of:

- Extensive preclinical ADME
- Toxicokinetics (TK) of parent and possible disproportionate metabolites
- Pharmacokinetics (PK) of parent and possible disproportionate metabolites during Phase I studies
- Phase I human ADME

Approach 2 is resource minimized with high risk for delay consists of:

- Preclinical ADME
- TK of parent
- PK of parent during Phase I studies
- Human ADME after Proof of Concept
- Synthesis of formal reference standards
- Assessment of disproportionate metabolites from toxicology and human samples

- GLP analyses of human and toxicology samples.
- Evaluation of disproportionate metabolites and decisions concerning additional toxicology.
- Human ADME performed in Phase II to verify disproportionate metabolites decisions.

Another approach is also possible as described in Figure 2, where the continuous building of knowledge along with the drug development program is depicted⁵.

Approach 3 is a compromise between approach 1 and 2 and consists of:

- Establish metabolite profiles in preclinical studies; isolation of metabolites
- Metabolite profiling in toxicology species
- Metabolite profiling in Phase I studies to estimate presence of disproportionate metabolites.

Overall, there are several strategies regarding metabolite profiling during drug development, each of which includes the possibility to conduct the human radiolabeled ADME study at different step of the program. However, it is generally recognized that this human study should be done prior to conducting large patient trials.

PRECLINICAL***IN VITRO* METABOLITE SCREENING, *IN VIVO* ANIMAL METABOLITES**

Is there a human unique metabolite?

Is the metabolite likely to be pharmacologically active?

PHASE 1**EARLY CLINICAL METABOLITE INVESTIGATION:**

metabolite identification during FIH (SAD/MAD) studies

Are there human metabolites not present in animal?

Has potent target pharmacology or HERG activity?

PHASE 2

↓ YES

Case by case consideration:
Do animal studies support
safety assessment for
metabolites?

↓ NO

YES →

Clinical development to POC

↓ NO

YES ↗

Conduct appropriate safety
studies:
Is appropriate safety of
metabolite demonstrated?

↓ NO

NO ↘

Resolve safety concern or
discontinue**POC DEMONSTRATED**

↓ YES

**DEFINITIVE RADIOLABELED
ADME** studies in animal and
human**PHASE 3****FIGURE 2: DECISION TREE FOR METABOLITE SAFETY RISK ASSESSMENT (ADAPTED FROM DON WALKER ET AL, 2009).**

FIH: First-in-Human; SAD: Single Administration Dose; MAD: Multiple Administration Dose; POC: Proof of Concept

HOW TO CONDUCT HUMAN ^{14}C -ADME STUDIES

A typical ^{14}C human ADME study consists of administering male healthy subjects with a single dose of the drug generally labelled with Carbon-14. The total radiolabeled dose used in these studies is in the range of 50-100 μCi depending on predictions of exposures of specific tissues from tissue distribution studies conducted in animals (mainly rats). The total dose (radiolabeled + cold drug) is generally in the range of pharmacologically active doses.

After administration, the study subjects are maintained in the clinical pharmacology unit (CPU) until the radioactivity related

to the radiolabeled drug is quantitatively recovered in the excreta (thresholds pre-specified in the study protocol, generally in the range of 95% total and blood $< 1\text{Bq}/\text{mL}$). Blood samples are also collected for determination of pharmacokinetics of parent drug and total radioactivity. The samples obtained from this study are used in metabolite profiling of the excreta and circulation.

Several aspects of the quality of the radiolabeled drug need to be managed closely by the clinical site where the study drug is administered to the subject. SGS laboratories in the near vicinity of the

Antwerp CPU perform chemical analysis on raw materials and finished products, microbiology assays, stability testing, as well as radiopurity assay. For intra-venous dosage forms additional controls including sterility tests and assay for endotoxins are also performed.

Due to the safety importance of results obtained from these samples, full Good Manufacturing and Clinical Practices apply at SGS Life Science Research for both the manufacturing of the study drugs (radiolabeled and cold) and the bioanalytical methods developed for these laboratory assessments.

CONCLUDING REMARKS

Several risk-based strategies to assess metabolites exist. Whatever the strategy chosen, the conduct of a radiolabeled human ADME study is recognized to be necessary in order to fulfil the requirements of the last Guidance* on safety testing of drug metabolites.

It is worthy to note that while there is

some flexibility to perform these human ADME studies at different steps during the drug development process, they should definitely be completed before starting the large patient trials in order to avoid major delays in the registration of new candidate drugs.

With innovative study designs, optimal facilities and strong regulatory intelligence, SGS can favorably impact client's drug development timelines and decision-making process.

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CONTACT INFORMATION

EUROPE

+ 33 1 53 78 18 79
clinicalresearch@sgs.com

WWW.SGS.COM/CRO

NORTH AMERICA

+ 1 877 677 2667
clinicalresearch@sgs.com

ASIA

+ 65 98 26 25 98
clinicalresearch@sgs.com

WHEN YOU NEED TO BE SURE

