S3A Guidance: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies: Focus on Microsampling

Questions and Answers

Guidance for Industry

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

May 2018
ICH

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Guidance for Industry[1]

The guideline for industry *S3A Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies*[2] (S3A guidance) has been successfully implemented since 1994, and in recent years, analytical method sensitivity has improved, allowing microsampling techniques to be used in toxicokinetic (TK) assessment. This question-and-answer (Q&A) document focuses on points to consider before incorporating the microsampling method in TK studies, acknowledges the benefits (and some limitations) of using microsampling to assess toxicokinetics in main study animals, and acknowledges the overall important contribution of microsampling to the 3Rs benefits (replacement, reduction and refinement) by reducing or eliminating the need for TK satellite animals.

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.

I. INTRODUCTION — SCOPE (1)[3]

1.1: What is the definition of *microsampling*?

[1] This guidance was developed within the Safety Implementation Working Group of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and has been subject to consultation by the regulatory parties, in accordance with the ICH process. The Q&As in this document have been endorsed by the ICH Steering Committee at *Step 4* of the ICH process, November 2017. At *Step 4* of the process, the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan, and North America.

[2] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance web page at https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

[3] Arabic numbers reflect the organizational breakdown of the document endorsed by the ICH Steering Committee at *Step 4* of the ICH process, November 2017.

For the purpose of this document, microsampling is a method to collect a very small amount of blood (typically ≤50 microliters) that is generally used to measure concentrations of a drug and/or its metabolites and subsequently calculate the appropriate TK parameters. The appropriate matrices used for microsampling techniques include blood and its derived plasma or serum, which can be used in liquid or dried form for transportation, storage, and subsequent analysis. Microsampling for toxicokinetics can be used in rodents and nonrodents. Microsampling in non-blood-derived matrices is outside the scope of this Q&A document.

1.2: What are the benefits/advantages of microsampling?

Minimizing the volume of blood collection can reduce pain and distress in animals and improve the animal welfare (refinement) of rodents and nonrodents. It can also eliminate the TK satellite group in which the TK assessment is conducted in main study animals or reduce the number of required animals in a TK satellite group when used in rodent studies (reduction). The benefit is particularly notable for mice, because a significant number of these animals are generally used in satellite groups in TK studies using conventional sample volumes. The main scientific advantage of microsampling is that the relationship between the safety data and drug exposure can be directly evaluated in the same animal.

II. BASIC PRINCIPLE ON APPLICATION OF MICROSAMPLING (2)

2.1: For what types of pharmaceuticals and for what types of safety studies can we use microsampling?

Generally, microsampling is applicable to the majority of pharmaceuticals, including biopharmaceuticals. However, for all types of analytes, consideration should be given on a case-by-case basis as to whether the sensitivity of the measurement method is appropriate for the small sample volumes available.

Microsampling can be used in any type of toxicology study, including single-dose, repeat-dose, carcinogenicity, juvenile, and reproductive. When microsampling is applied, sampling of a representative subgroup is acceptable, as mentioned in the S3A guidance. There are published examples demonstrating no effects on key veterinary clinical pathology or pathological parameters when small volumes of blood are taken from adult animals. However, microsampling is not warranted when the drug concentration is low and the majority or all samples are Below the Limit of Quantification (BLQ) (e.g., exposure after topical or inhaled administration). However, when the bioanalytical method for microsampling has the same Lower Limit of Quantitation (LLOQ) as that for conventional sample volume, microsampling can be used even if the majority or all samples are BLQ.

2.2: What are the points to consider when applying microsampling to TK studies?

As with other approaches to kinetic sampling, to adopt a microsampling technique appropriately, a bioanalytical method should be developed and qualified (or validated for Good Laboratory Practice studies, in accordance with regulatory guideline/guidance in each region) to

ensure the reliability of analytical results. Analytical characteristics, such as LLOQ, accuracy, precision, influence of matrix using dilution prior to storage, and the stability of the analyte(s) in the biological matrix for the entire period of sampling, storage, and processing conditions, should be carefully assessed to establish the microsampling method. When conventional methods have already been used in some studies and microsampling is proposed for others, an assessment of the comparability of the exposure measurement, in the given matrix, between microsampling and conventional methods may be necessary. This comparison is particularly important if the conditions that the TK samples are presented in are substantially different (e.g., dried sample from microsampling vs. liquid sample from conventional sampling). This comparability assessment can be done in a separate pharmacokinetic (PK) study to measure area under the matrix level concentration-time curve (AUC) and/or maximum drug concentration (C_{max}), provided that the appropriate range of concentrations is evaluated and the resulting parameters compared between the two methods. This PK study, if needed, should be completed prior to conducting definitive studies that will include the microsampling procedure. A separate PK study for comparison may be omitted on a case-by-case basis and with appropriate scientific justification, for example, when using similar assay conditions to test blood, plasma, or serum drawn from the same sampling site.

During this comparison process, multiple blood collections at a few time points by microsampling from a few animals and their subsequent measurement of analyte concentrations can be considered to check the variability of sampling. Ideally, the same matrix should be used throughout the TK studies and also in clinical studies for comparison of exposure. When different matrices are used in different studies, the drug concentration relationship among matrices should be defined considering appropriate factors such as hematological parameters, plasma protein binding ratio, and blood/plasma (or serum) ratio of the drug, so that systemic exposure can be evaluated appropriately from each measurement using different matrices.

2.3: What types of blood collection are used for microsampling?

Blood can be collected from the tail vein, saphenous vein, etc., using capillary tubes or any appropriate miniaturized collection devices. The collected blood, and its derived plasma or serum, can be used to measure drug concentrations in either liquid or dried form. In some cases, liquid samples can be diluted with the appropriate vehicles or blank matrices before storage, shipment, and subsequent analysis. Dried sample methods are also available, wherein the sample is usually applied directly onto cellulose-based or other types of materials and then dried. A fixed-diameter subpunch or the whole quantity of the sample on the card/device can be extracted and analyzed. Recent and ongoing advancements in microsampling methods have demonstrated the ability to collect precise volumes of blood, such that the entire sample can be used for analysis without additional volumetric measurements. In addition, newly developed techniques could also be considered with adequate validation.

III. EFFECT ON SAFETY EVALUATION (3)

3.1: How to evaluate the effect of blood sampling on the toxicity data and well-being of animals in the main study group?

When blood sampling is performed on the main study animals, it is important to consider the effect of blood collection on the physiological condition of the animals. The main factors to consider include: (1) the volume and the number of samples taken in a given period; (2) the properties of the test drug (e.g., its effects on red blood cells, anticoagulant effect, or hemodynamic properties); (3) the test system (e.g., species, age, body weight, total blood volume); and (4) the site of collection. Because frequent and repeated blood collection may affect physiological data, such as hematological parameters, sampling protocols should be appropriately established, even with microsampling. It is prudent to record the relevant animal data, such as changes in body weight, food consumption, hematological parameters (e.g., red blood cell count, hemoglobin level, hematocrit value, mean corpuscular volume, electrolytes, total proteins), and any effect on the blood collection site (e.g., tissue damage, inflammation). Evaluation of these parameters compared to matching control animals, which have had the same number and volume of samples drawn as the test drug groups, will be important to establish whether any suspected effects are related to the test drug or to procedures, within the context of the specific study conditions. If previous studies show that test-drug-related changes to hematological parameters could be exacerbated by frequent blood sampling or if it is suspected that the pharmacological action of the test drug may induce such effects, the use of satellite groups of animals for TK assessment would be warranted, even if microsampling techniques are used. Alternatively, sparse sampling[4] can be used in conjunction with microsampling, if scientifically justified.

IV. ISSUES REGARDING THE BIOANALYTICAL METHOD (4)

4.1: What are important points to consider in bioanalytical method development and validation of treatment of liquid or dried samples?

In addition to the analytical method validation stipulated in the bioanalytical guideline/guidance in each regulatory region, the following points should be considered when analyzing samples derived from microsampling:

- For liquid sampling, the following issues should be considered: (1) ensuring sample homogeneity, for example by pipetting; (2) small volume handling issues (e.g., potential freezing/drying effects during the storage and subsequent freeze/thaw process, as applicable); (3) potential increase in the LLOQ due to the limited sample volume; (4) impact of addition of anticoagulants to small containers/capillaries, resulting in dilution of the sample; (5) potential for increased adsorption of the analyte to the collection container (i.e., increased surface area to volume ratio); (6) maintenance of the appropriate preservation conditions of the sample; (7) risk of contamination and difficulty of repeated sampling using some methods.

[4] Sparse sampling in TK studies usually involves the collection of a few blood samples at specified time points from each animal in a treatment group. The samples are allocated at different time points for different animals, often allowing some replicates, and statistical inferences are then made about the concentration-time behavior of the test compound. By using proper study designs, investigators can limit the number of samples and the amount of blood drawn, so as not to affect the animals' health status, yet still achieve the customary TK objectives in a study.

- For dried sampling techniques (e.g., spotting onto cellulosic or noncellulosic card, polymer matrix, etc.), it is important to select the method with adequate and reproducible recovery and minimal matrix interference with detection of the drugs. If the subpunch of the dried spot approach is used, it is important to ensure that detection of the analyte is not affected by different hematocrit values, especially for small molecule drugs. The effect of hematocrit on analyte detection can be measured by using blood with different hematocrit values and spiking with test drugs of known concentrations. It is also important to confirm the uniformity of the spots by evaluating analyte levels from multiple samples punched out from one spot or by evaluation, for example, using radiolabels. Both of these issues can be minimized if an accurate volume of blood is collected on the device and the entire sample is subsequently analyzed.

Incurred sample reanalysis (ISR) should be conducted according to each regional guidance/guideline, if described. When doing ISR, care should be taken to ensure sufficient sample volumes or numbers of replicates (e.g., spots, containers, or tips) are retained for ISR.

V. ANNEX: Q&As LINKED TO THE RESPECTIVE SECTIONS OF S3A GUIDANCE

Sections of S3A Guidance	1: Introduction	2: The objective of toxicokinetics and the parameters which may determined	3: General principles to be considered	4: Toxicokinetics in the various areas of toxicity testing – specific aspects	5: Supplementary notes	6: References (other ICH guideline)
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