EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

13 May 2016
EMA/CHMP/ICH/320985/2016
Committee for Human Medicinal Products

ICH Guideline S3A: Note for guidance on toxicokinetics: the assessment of systemic exposure in toxicity studies - questions and answers

Step 3

Adopted by CHMP for release for consultation	26 May
Start of public consultation	31 May
End of consultation (deadline for comments)	31 August

Comments should be provided using this template. The completed comments form should be sent to ich@ema.europa.eu

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

12 Document History

Code	History	Date
S3A Q&As	Approval by the ICH Assembly under *Step 2* and release for public consultation.	February 2016

13 ICH S3A - Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity
14 Studies - October 1994

15

ICH Guideline S3A- questions and answers

Table of contents

Preface

The S3A Guideline has been successfully implemented in 1994 and, in recent years, analytical method sensitivity has improved, allowing microsampling techniques to be widely used in Toxicokinetic (TK) assessment. This Q&A document focuses on points to consider before incorporating the microsampling method in TK studies, acknowledges its benefits (and some limitations) for assessment of TKs in main study animals and its overall important contribution to the 3Rs benefits (Replacement, Reduction and Refinement) by reducing or eliminating the need for TK satellite animals.

1. Introduction – scope

#	Date of Approval	Questions	Answers
1.1	January 2016	What is the definition of microsampling?	Microsampling is a method to collect a very small amount of blood (typically ≤50 µL) to measure TK parameters of the drug and/or its metabolites. The appropriate matrices for microsampling bioanalytical samples 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 TKs can be used in rodents and non-rodents. Microsampling methods in other matrices (e.g. lung lavage and lymph) are not yet validated and therefore, are outside the scope of this Q&A.
1.2	January 2016	What are the benefits/advantages of microsampling?	Minimising the volume of blood collection can minimize pain and distress in animals and improve the animal welfare (refinement) of rodents and non-rodents. It can also reduce or eliminate the number of required animals in a TK satellite group for rodents (reduction). The benefit is notable particularly for mice since a significant number of the animals is generally required in TK studies (with conventional sample volumes) as satellite groups. Because microsampling is performed on the main study group, its main advantage is that the relationship between the safety data and drug exposure can be directly evaluated in the same animals.

2. Basic principal on application of microsampling

#	Date of Approval	Questions	Answers
2.1	January 2016	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 and biopharmaceuticals. However, for all types of molecules, consideration should be given on a case-by-case basis as to whether the sensitivity of the measurement method is appropriate with the small sample volumes available. Microsampling can be used in any type of safety study, such as single-dose or repeated-dose safety studies, and other safety studies (e.g. juvenile and reproductive studies).

#	Date of Approval	Questions	Answers
			There are published examples demonstrating no impact 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 Lower Limit Of Quantification (LLOQ) of the bioanalytical method is insufficient for the planned sample volume due to low drug exposure levels (e.g., exposure after topical or inhaled administration).
2.2	January 2016	What are the points to consider when applying microsampling to TK studies?	As with other approaches to kinetic sampling, in order to adopt a microsampling technique appropriately, a bioanalytical method should be developed and qualified (or validated for GLP studies, in accordance with regulatory guideline/guidance in each region) to ensure the reliability of analytical results. Analytical characteristics, such as LLOQ, matrix effects 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 in order to establish the microsampling method. When conventional methods have been already used in some studies and microsampling is proposed for others (for example if a method is improved to allow a lower LLOQ), the comparability of the exposure measurement between microsampling and conventional methods should be confirmed in a given matrix in each animal species before selecting the microsampling procedure. This comparison can be done in a separate Pharmacokinetic (PK) study provided that the appropriate range of concentrations is evaluated. This separate PK study for comparison may be omitted on a case-by-case basis and with appropriate scientific justification, for example, when using the same assay conditions in the same matrix to test blood samples drawn from the same site. During this comparison process, multiple analyses at each time point of blood collection by microsampling can be considered in order to check variability of measurement. Furthermore, it is advisable to evaluate the method of adding internal or external standard substances, and the sample dilution. 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 for some reasons, the drug concentration relationship among matrices should be defined considering various factors such as

#	Date of Approval	Questions	Answers
			hematological parameters, plasma protein binding rate 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	January 2016	What types of blood collection and what types of pretreatment methods 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 can be treated as the measurement sample either in a liquid or dried form. In the case of liquid samples, the isolated plasma or serum can also be used when blood samples are centrifuged after collection. In some cases, the sample is diluted with the appropriate solvents or blank matrices prior to storage, shipment and subsequent analysis. Dried sample methods are also available, wherein the sample is usually spotted directly onto cellulose-based or other types of materials and then dried. A fixed diameter sub-punch or the whole quantity of the spot on the card/device can be extracted and measured/analyzed. Recent and on-going advancements in microsampling devices 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.

35 ## 3. Effect on safety evaluation

#	Date of Approval	Questions	Answers
3.1	January 2016	How to evaluate the effect of blood sampling on the toxicity data and wellbeing of the animal in 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 the volume and the number of samples taken in a given period, the properties of the test drug (e.g. effects on red blood cells or anticoagulant properties), the test system (e.g. species, age, body weight, total blood volume), site of collection, and the study duration. Therefore, even with microsampling, sampling protocols should be appropriately established, as frequent and repeated blood collection

#	Date of Approval	Questions	Answers
			may affect physiological data, such as hematological parameters, unless total volumes drawn are carefully considered. It is considered 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 in establishing whether any suspected effects are related to test drug or to procedures, within the context of the specific study conditions. If there is evidence in previous studies of test drug-related changes to hematological parameters that could be exacerbated by frequent blood sampling for kinetics, or it is suspected that the pharmacological action of the test drug may induce such effects, then the use of satellite groups of animals for TK purposes might still warrant consideration, even if microsampling techniques are to be used. Alternatively, sparse sampling might also be considered in conjunction with microsampling, if scientifically justified.

36 ## 4. Issues regarding the bioanalytical method

#	Date of Approval	Questions	Answers
4.1	January 2016	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) confirmation of the sample homogeneity, 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, and 4) impact of addition of anticoagulants to small containers/capillaries, resulting in dilution of the

#	Date of Approval	Questions	Answers
			sample. For dried sampling techniques (spot on cellulosic/non-cellulosic card, polymer matrix, etc.), the method with best recovery and lowest matrix interference on the drugs should be selected. If the sub-punch of the dried spot approach is used, the effect of different hematocrit values should be evaluated, such as the reproducibility of the quantified value using blood with different hematocrit values spiked with test drugs of known concentrations. Also, it is important to confirm the uniformity of the spots by means of an evaluation using multiple samples punched out from one spot or by visual evaluation using radiolabels. Both of these issues can be minimized if an accurate volume of blood is collected on the device and the whole sample is subsequently analysed, rather than a sub-aliquot. 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 for ISR.

5. Annex: Q&As linked to the respective sections of ICH S3A guideline

Sections of ICH S3A Guideline	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 Guidance)	Other ICH Guidelines
1. Introduction – Scope							
1	1		3.10		Note 1		
2	1		3.5		Note 1		
2. Basic principal on application of microsampling							
1		2		4			
2			3.1 3.10				
3			3.10				
3. Effect on safety evaluation							
1			3.3 3.5				
4. Issues regarding the bioanalytical method							
1			3.10		Note 1		