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Proposed Best Practices for Optimizing the Number of Animals in Toxicology Studies

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ABSTRACT

New drug modalities, accompanied by the limited availability of some of the research animal models, necessitate a continuous evaluation of study designs and technology that will enable a reduction in the number of animals used for toxicology studies, an approach that aligns with the 3Rs of experimental animal welfare.

In rodents, blood microsampling serves as a better option and refinement to the traditional technique of needle and syringe by leveraging the microsampling for accurate collection of low sample volume. For example, by sampling only 10 µL of whole blood, an entire cohort of study animals in the traditional needle and syringe collection was eliminated since serial samples could also be collected from the main study animals. This represented a reduction in the number of study animals by 55% and 100% for mouse and rat studies, respectively. Collection of samples from the same cohort of animals also allows correlations between pharmacodynamic findings and the actual drug exposure profile.

In nonhuman primates (NHP) and dogs, a review was completed of the number of animals in control and recovery cohorts in chronic study designs that had data from prior subacute studies. This enabled a reduction by >25% in the number of control groups with no recovery cohort including in the low-dose group. This NHP study design was reviewed and accepted by a regulatory agency. Each of these approaches will be discussed further to highlight the pros and cons of each in order to allow for a more informed decision when designing toxicity studies.

INTRODUCTION

The 3 R's—Replacement, Reduction, and Refinement - are the tenants of preclinical research. This presentation focuses on the 2nd R—**Reduction**, which refers to methods that minimize the number of animals used per experiment or study consistent with the scientific aims. It is essential for reduction that studies with animals are appropriately designed and analyzed to ensure robust and reproducible findings. The reduction also includes methods that allow the information gathered per animal in an experiment to be maximized to reduce the use of additional animals.

Several methods of reducing the number of animals were utilized by Altasciences in Toxicology studies. This presentation will focus on the methods used and the pros and cons of each method.

MATERIALS AND METHODS

Microsampling strategies in preclinical research allow for the consolidation of satellite toxicokinetic (TK) and main study groups in an effort to reduce animal numbers. Additionally, this method allows for toxicological effects to be correlated with exposure in the same individual. Capillary microsampling techniques also circumvent the hematocrit (HCT) effect often reported for dried blood spot (DBS) analysis; however, the processing is tedious, and drugs exhibiting non-specific binding or requiring matrix stabilization are problematic.

A recent alternative is volumetric absorptive microsampling (VAMS®), wherein an accurate blood volume is absorbed onto a hydrophilic polymeric tip, simplifying sample collection. The use of this technique allows for a dramatic decrease in the number of animals used in a Toxicology study. A toxicokinetic (TK) rat study can successfully be completed with three animals/dose level (serial collection) and a TK mouse study with eight animals/dose level (split into two subsets—sparse). This represents a reduction in population by 50% and 60%, respectively, compared to study designs using a standard sample volume of 0.5 mL/sample.

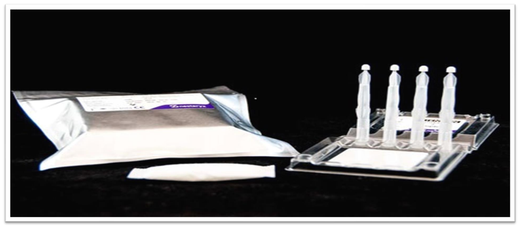

Figure 1. Mitra® Microsampling device

Figure 2. Sample collection

The Mitra® microsampling kit includes the following:

- Four sampling tips allow the collection of 10, 20, or 50 µL samples.
- Clamshell holding case for the tips for storage and shipment—allows for fast and convenient packaging for shipment; no drying time is required when stored in a provided bag with a desiccant.
- Shipping bag with a desiccant—can be labeled with the identity of animals, sample type, etc., and is ready for shipment.
- When the Mitra® tip is fully red, wait two seconds, then remove it. Apply pressure to the collection site to aid in stopping the bleeding.
- Collection is fast and simple with minimal training involved. There is no processing of the samples required, as they are shipped in a holding case and shipping bag provided with the kit.

PROS AND CONS

Study Design for CD-1 Mouse Study

Group	Terminal		Recovery		TK (Mitra)		TK (Terminal; Sparse)	
	Males	Females	Males	Females	Males	Females	Males	Females
1 (control)	10	10	6	6	4	4	6	6
2 (low)	10	10	-	-	8	8	45	45
3 (mid)	10	10	-	-	8	8	45	45
4 (high)	10	10	6	6	8	8	45	45

Fifteen time points were required for a mouse toxicology study for TK profile assessments.

Pros:

- In a routine study with terminal blood collection, a total of 102 mice would be required for a sparse collection with three animals/sex/timepoint.
- With the use of Mitra® tips, the number of animals could be reduced to 56 animals (sparse collections with four animals/sex/time point).

Con:

- A new bioanalytical method will need to be validated for the use of the dry blood collection technique.

Study Design for Sprague Dawley Rat Study

Group	Terminal		Recovery		TK (Mitra)		TK (Terminal; Sparse)	
	Males	Females	Males	Females	Males	Females	Males	Females
1 (control)	15	15	5	5	-	-	3	3
2 (low)	15	15	-	-	-	-	9	9
3 (mid)	15	15	-	-	-	-	9	9
4 (high)	15	15	5	5	-	-	9	9

Fifteen time points were required for a rat toxicology study for TK profile assessments.

Pros:

- In a routine study with serial blood collection, a total of 90 rats would be required for a serial collection with three animals/sex/timepoint.
- With the use of Mitra® tips, the TK subset of animals can be removed completely, and blood samples can be collected from the terminal Tox animals using a less invasive method, thus eliminating any potential complications associated with jugular blood collections (routine for TK sample collection).

Con:

- A new bioanalytical method will need to be validated for the use of the dry blood collection technique.

Study Design for Cynomolgus Monkeys

Group	Terminal		Recovery	
	Males	Females	Males	Females
1 (saline control)	2	2	-	-
2 (low)	3	3	-	-
3 (mid)	3	3	2	2
4 (high)	3	3	2	2

- Typical study design would require 2 recovery animals per sex per group.
- The control group was administered Saline, therefore eliminating the need for a recovery subset.
- There were no TA-related changes anticipated at the low dose, based on previous studies, therefore, eliminating the need for a recovery subset.

Pros:

- Reduction of a total of 10 animals (5 males and 5 females).
- Design accepted by the regulatory agency.

Cons:

- Resolution of any potential unanticipated TA-related changes in the low-dose group cannot be tracked.
- Control article needs to be well characterized and established to allow for the elimination of the recovery subset and, thus, comparator group for recovery animals.
- A robust historical dataset for clinical and anatomic pathology changes in age-matched Cynomolgus monkeys may be required.
- The lack of sufficient animals in the control group (3/sex/group) precluded any comparative statistical analysis from being conducted during the terminal phase. Suggestion is to increase the number of animals to 3/sex/group.

Study Design for Beagle Dogs

Group	Terminal		Recovery	
	Males	Females	Males	Females
1 (control)	3	3	2	2
2 (low)	3	3	-	-
3 (mid)	3	3	2	2
4 (high)	3	3	2	2

Typical study design would require 2 recovery animals per sex per group.

- There were no TA-related changes anticipated at the low dose, based on previous studies, therefore, eliminating the need for a recovery subset.

Pros:

- Reduction of a total of 4 animals (2 males and 2 females).
- Depending on the nature of the control article, potential removal of the recovery subset from the control group will lead to further reduction of animals.

Cons:

- Resolution of any potential unanticipated TA-related changes in the low-dose group cannot be tracked.
- A robust historical dataset for clinical and anatomic pathology changes in age-matched Cynomolgus monkeys may be required.

CONCLUSION

The use of Mitra® tips can significantly decrease the number of animals required in a routine small-animal toxicology study, thus addressing the critical 3Rs of preclinical research. The procedure, which is much less invasive than a traditional approach (jugular draw), has the added benefit of eliminating potential complications (death, histological changes, etc.). Collection of TK samples from Main Toxicology animals allows for correlation between possible test article-related effects with the exposure levels, much like what is typically done in non-human primate studies.

With careful consideration, large animal studies can be designed to provide robust and statistically significant data sets while still reducing the overall number of animals.

Altasciences is actively working on creating a robust background dataset for potential use as virtual controls in the future.

It is essential that the scientific community continually re-evaluates study designs and technology that will enable a reduction in the number of subjects used for toxicology studies. This approach aligns with the 3Rs of experimental animal welfare (Replacement, Reduction, and Refinement).