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Strategies for Reducing the Number of Animals in Toxicity Testing: A Comparative Approach for Rodent and Large Animal Studies

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ABSTRACT

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, an approach that aligns with the 3Rs of experimental animal welfare (Replacement, Reduction, and Refinement).

In rodents, we adopted blood microsampling as a refinement to the more common needle and syringe technique by leveraging the Mitra™ Volumetric Absorptive MicroSampling (VAMS®) device for accurate and precise collection of low sample volume. For example, by microsampling only 10 µL of whole blood, an entire cohort of study animals in the traditional needle and syringe collection was eliminated from the project 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. Collecting samples from the same cohort of animals allows correlations between PD findings and the actual drug exposure profile.

In nonhuman primates (NHP) and dogs, we carefully reviewed the number of animals in control and recovery cohorts in chronic studies with data from prior subacute studies. This enabled reduction by >25% in the number of the control group with no recovery cohort, including in the low-dose group. The NHP study design was reviewed and accepted for conduct by a regulatory agency. Each of these approaches will be discussed further to highlight the pros and cons of each 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 pharmacokinetic (PK) rat study can successfully be completed with three animals/dose level (serial collection) and a PK 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.

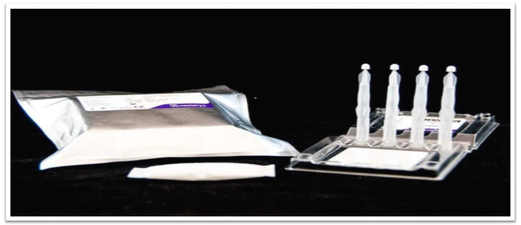

Figure 1. Mitra® Microsampling Device

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.

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.

BENEFITS

Figure 1. Mitra® Microsampling Device

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.
Place the Mitra® tip back into the clamshell box, and when all four samples are collected, place them into the transfer bag with the desiccant for shipment.

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 use in the dry blood collection technique

Study Design for Cynomolgus Monkeys

Group	Terminal		Recovery	
	Males	Females	Males	Females
1 (saline control)	3	3	-	-
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 8 animals (4 males and 4 females).

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.

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).

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.

CONCLUSIONS

The use of Mitra® tips can significantly decrease the number of animals required in a routine 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.

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).