Replacement, Reduction, and Refinement in Animal Research

General Information

Federal laws and Institutional policies require researchers to apply the principles of the 3Rs (replacement, reduction, and refinement) to animal research. Public Health Service (PHS) policy states that the institution must provide "training or instruction in research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress". In addition, the Animal Welfare Regulations state that the institution must include training in "The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress."

The following sections describe the 3R's of animal research and provide guidance on implementing these strategies. This guidance has been adapted from the USDA National Agricultural Library and the University of Connecticut IACUC.

Replacement refers to technologies or approaches that directly replace or avoid the use of animals. Replacement can be broken down into two categories: Full/Absolute and Partial/Relative.

- Full/Absolute Replacement avoids the use of animals completely. Some examples include training manikins, human tissues and cells, computer models, and microphysiological systems.
- Partial/Relative Replacement still requires animal use, but the animals do not experience pain or distress during the study. Some examples include animal-derived tissue/organs for in vitro studies, zebrafish embryos, and transcutaneous electrical resistance test (TER).

Reduction involves methods that help obtain comparable levels of information from the use of fewer animals. Scientists can reduce the number of animals used in research through strategies such as appropriate experimental design, correct statistical analyses, and sharing resources/animals.

- **Power Analysis:** Statistical significance and biological significance are not the same thing; however, statistical and biological significance can be linked through the use of statistical power analysis. The statistical power of a test is the probability of getting a statistically significant result, given that the null hypothesis is false. Power is proportional to the sample size, significance criterion and effect size, and is inversely proportional to the variance in the population. Effect size is a measure of biological significance: it is the difference between the results predicted by the null hypothesis and the actual state of the population being tested. Power analysis can be used to determine whether the experiment has a good chance of producing a statistically significant result if a biologically significant difference exists in the population. Or, in other words, whether the experiment has a high power, given a biologically significant effect size. What constitutes "high power" is best left to the researcher, but conventions of 0.8 and 0.95 have been suggested

in the literature. Power analyses can be performed with most statistical software. Consultation with a biostatistician can yield benefits to the PI and the experimental animals.

- **Pilot Studies:** Pilot studies are a good way to reduce the number of animals used; the IACUC may even require a pilot study when reviewing a protocol. Pilot studies can be used to estimate variability and evaluate procedures and effects. Results from pilot studies can also be used to estimate the parameters that are used in power analyses.

- **Appropriate Use of Endpoints:** the precision of an experiment depends mainly on the sample size and error variance. Careful attention must be given to the type of endpoint used. Qualitative endpoints (e.g., dead/alive) often involve severe pain and distress and generally provide less information than quantitative measurements. More information can generally be found using quantitative endpoints and can, in some instances, lead to a reduction in the number of animals used during an experiment.

- **Sharing Animals:** In some instances, it is possible to share research animals. For instance, animals euthanized by one investigator can provide tissue for use by another investigator. There are instances in which this should never be attempted (e.g., animals have been exposed to biological hazards, chemical hazards, and/or recombinant DNA), but it is a method to reduce animal numbers that should be explored by researchers.

- **Use Quality Animals and Veterinary Care:** When PIs use the correct choice of an animal model- one that uses healthy, genetically similar animals- it generally decreases variability and, hence, animal numbers. You can minimize the loss of animals by providing good post-operative care, avoiding unintended breeding, and planning ahead so that the appropriate number of animals needed for the studies are ordered and/or bred.

- **Computer Simulations:** Though not always possible, computer simulations can mimic biological processes. These are typically most helpful in the case of training protocols.

- **Use of Cell Culture:** When possible, consider the use of cell cultures rather than animals. For example, there are *in vitro* systems which use cell culture to generate monoclonal antibodies rather than using laboratory animals.

- **Auto Controls:** It would be helpful, whenever possible, to design experiments in which animals serve as their own control.

- **New Instrumentation and Techniques:** Using new instrumentation or innovative techniques that can improve precision can reduce the number of animals needed for a study.

- **Appropriate Experimental Design:** Careful experimental design by appropriate choice of control groups and standardizing procedures to minimize variability and increase power.

Refinement refers to modifications of husbandry or experimental procedures that minimize or eliminate animals' pain and distress and improve their welfare. A few examples of refinement are anesthetics and analgesics, humane animal handling, environmental enrichments, and humane endpoints.

Helpful Links

- A Simple, Rapid and Reliable Method for Selecting or Assessing the Number of Replicates for Animal Experiments
- IACUC- non-statistical approach to calculating appropriate animal numbers.pdf
- Isogenic.info
- Rice Virtual Lab in Statistics
- Statistical Considerations for a Parallel Trial where the Outcome is a Measurement
- Web Interface for Statistical Education
- Power and Sample Size