



The ACC Connection

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WELCOME to the 17th edition of the **ACC Connection**, a bi-monthly newsletter designed to help researchers with questions regarding animal research at the University of Connecticut Health Center. Our 17th issue is designed to familiarize Principal Investigators with methods to reduce animal numbers.

It is **required** by the Animal Welfare Act and PHS Policy to have, as part of animal user training, instruction on methods that can be used to reduce animal numbers used in research. **Reduction** is one of the “3Rs” (the others being **Refinement** and **Replacement**) that have an impact in laboratory animal use. 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”. 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”. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, principle #3, states “The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered”.

Reduction is desirable in any procedure, however directly humane, which employs large numbers of animals. Reduction is of great importance and, of all modes of progress on the three Rs, it is the one most obviously, immediately, and universally advantageous in terms of efficiency. One general way in which great reduction may occur is by the right choice of strategies in the planning and performance of whole lines of research. Many investigators settle early in their research career on some strategy that appears to suit them and are liable never to change from that strategy.

Methods to Reduce Animal Numbers

Statistics and Power Analysis

The term **Reduction Alternatives** describes methods for obtaining comparable levels of scientific procedures or for obtaining more information from a given number of animals. Proper statistical design is essential. There is evidence that poor experimental design and inappropriate statistical analysis has led to the inefficient use of animals. This may be due to a low level of statistical expertise in the investigators so that they are unaware of the potential value of obtaining statistical advice. Alternatively, they may be unable to do so because of a lack of qualified biometricians with experience in their field of interest.

Statistical significance and biological significance are not the same; however, they 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, determining if 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.

It is beyond the scope of this general introduction to teach statistics to the animal users, but there are methods that are out there that are available. Consultation with a biostatistician can yield benefits to the PI and the experimental animals. For a good general article on selecting appropriate numbers of animals for research, as well as web sites for statistical education that Principal Investigators may find helpful, please refer to the following ACC website: <http://clacc.uchc.edu/ACC/Methods%20to%20reduce%20animal%20numbers/WaystoReduceAnimalNumbers.htm>.

Pilot Studies

Pilot studies are a good way to reduce the number of animal used; the ACC may even require a pilot study when reviewing a protocol. Pilot studies can be used to estimate variability and evaluate procedures and effects.

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) may 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 or recombinant DNA), but it is a method to reduce animal numbers that should be explored by researchers.

Use of 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, there are cases in which there are computer simulations available which can mimic functions of physiology. 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 could be helpful, whenever possible, to design experiments in which animals serve as its own control. For example, if a procedure were to be performed on a limb, instead of doing the procedure on both limbs of an animal- and having separate control animals- do a unilateral procedure with the control being the opposite limb. Though this is not always possible, it is a well-established method for reducing animal numbers.

New Instrumentation and Techniques

Using new instrumentation or innovative techniques that can improve precision can reduce the number of animals needed for a study. This has the added benefit of also being a refinement technique for the protocol.

Finding Reduction Methods- Internet Resources

The USDA requirement for the search for alternatives to painful or distressful procedures and the USDA and PHS Policy requirements to look for alternatives to animal use and non-duplication of animal research work have led to a large number of websites that introduce this topic. Some may be better than others, but all lead you to some valuable information if you have the time.

UHC Alternatives Search Website

Our own website has an extensive listing of websites and search engines to use to fulfill the above regulatory requirements. It can be found at the following link: <http://clacc.uchc.edu/ACC/AlternativeSearchHelp.htm>.

ALTWEB

This site has been described as the global clearinghouse for information on alternatives to animal testing and is a joint effort of the Johns Hopkins Center for Alternatives to Animal Testing and other public and private organizations. It is a good site for news, information, discussion, and resources from the field of alternatives to animal testing. It can be found at the following link: <http://altweb.jhsph.edu/>.

A Guide to Searching for Alternatives to the Use of Laboratory Animals

This is a site from the United Kingdom which provides an overview of how to conduct an alternatives search including search terms, search strategies, and multiple links. This is a good place to start. It can be found at the following link: <http://www.frame-uk.demon.co.uk/guide/index.htm>.

Searching for Alternatives to Animal Research and Testing

This is a site containing guidelines written by the Animal Welfare Information Center (AWIC), a branch of the USDA. It can be found at the following link: <http://www.nal.usda.gov/awic/alternatives/alternat.htm>.

Animal Welfare Institute Enrichment and Refinement Database

Though not extensive, this site can give you some help. It can be found at the following link: http://www.awionline.org/lab_animals/.

National Agriculture Library (AGRICOLA)

Funded by the USDA, this site has citations for books, articles, and conferences as well as publications to help in your search for alternatives. It is a free database to search. It can be found at the following link: <http://agricola.nal.usda.gov/>.

UCHC ACC Methods to Reduce Animal Numbers Webpage

This webpage can direct you to a few websites which can help with statistical analysis. There are some articles on the webpage that PIs may also find helpful regarding statistical and non-statistical methods to determine the optimum number of animal to use. This website may be found at the following link: <http://clacc.uchc.edu/ACC/Methods%20to%20reduce%20animal%20numbers/WaystoReduceAnimalNumbers.htm>.

Frequently Asked Questions

I'm not sure what the best procedure is for a particular activity I need to perform. What should be done?

A pilot study would be the best thing to perform in this case. It ensures that the least amount of animals be used and will show the PI the "best way to go".

Upcoming Training, November 2008 – December 2008

New Animal Users Initial Basic Core Training

Monday, November 24	9:00 am – 12:00 pm	Building 20 conference room
Monday, December 22	9:00 am – 12:00 pm	Building 20 conference room

New Institutional, State, or Federal Regulations

Institutional

None

State

None

Federal

None

Important Reminders to Principal Investigators

⊗ When an individual leaves your laboratory, and is no longer an active animal user, you **must** contact the Animal Care Committee Office (ooacc@uchc.edu) with this information.

⊗ When a new individual enters your laboratory and will use animals, this individual must attend initial animal user training, must enroll in the Occupational Health Surveillance Program, and must be added to your approved protocol(s) by submission of a modification to the protocol(s). Only after these three items are completed will an individual be granted access to CLAC.

⊗ When your old protocol expires, and your renewal protocol comes into effect, you **must** change your cage cards to reflect the new ACC protocol number and expiration date. In addition, please review the [ACC Policy on Cage Identification](#), located on the web, to ensure you are generating cage cards that contain the required information.

⦿ If any unexpected morbidity and/or mortality occurs in any experimental animal, you **must** contact the Animal Care Committee Office (ooacc@uchc.edu) with this information.

⦿ If you have a safety protocol within your approved animal care and use protocol, please remember that veterinary services requires 3 days notice prior to the start of using animals under the safety protocol.

⦿ Always remember to list **ALL** transgenic animals and their origin when filling out the Animal Care and Use application in section 7.

⦿ If there will be transgenic crosses in your animal care and use protocol, you must specifically list all crosses to be performed so that the crosses can be automatically registered by the IBC.

⦿ PIs are reminded that if the scope of their grant work changes significantly, and if you are performing new procedures not listed in your grant, you need to inform the NIH of these changes. For more information as to what constitutes significant changes, please contact your Project Officer in ORSP.

⦿ Laboratory inspections will be performed in December; all laboratories that use USDA regulated species, perform surgical procedures (survival or non-survival) will be inspected. First notifications for scheduled inspections are sent out early in November. Please do all you can to ensure that **SOMEONE** from your laboratory is in the laboratory at the scheduled inspection date(s) and time(s).



Next Issue: Potpourri of Animal Issues

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