Procedure: IACUC-20
Date: December 2, 2021
Enabled by: PHS/AWA
Supersedes: January 28, 2016

UC Davis
Institutional Animal Care and Use Committee (IACUC)

Title: ***Rationale for Species and Animal Numbers in Animal Care and Use Protocols***

I. **Purpose:**

This document provides guidance to researchers for determining and justifying appropriate species and animal numbers.

II. **Background:**

According to the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Teaching, the animals selected for a procedure should be of an appropriate species and quantity, with the minimum number required to obtain scientifically valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should also be considered.

II. **Policy:**

The Animal Welfare Act and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* charge the IACUC with review of proposed rationale for the species and the number of animals selected. Investigators must demonstrate that they are using the appropriate species and the minimum number of animals necessary to obtain scientifically valid data.

III. **Procedure:**

Justification for the species must include the rationale for why the species selected is most appropriate for the study and why non-animal models will not meet the study objectives.

Investigators are encouraged to consult a statistician and bioinformatics specialist to determine the number of animals necessary for a project. In general, for most experiments, the number of animals can be determined by a power analysis (sample size estimation), which is identified by the variability in the data (e.g. Standard Error from Mean values (SEM)) and the difference the investigator expects between groups.

The power of detecting a given difference from the control group increases by increasing the sample size. Investigators must demonstrate that they are using the fewest number of animals allowed to achieve their scientific objectives. Typically an n=3 is the smallest sample size that can be used to obtain a meaningful approximation of the SEM (so denominator is greater than 1) for a power analysis to determine the minimum number of animals necessary to compare a defined measurable between experimental groups.

Justification of animal numbers begins with a clearly stated summary of the experimental objectives, including experimental animals, donor animals, live-born offspring of pregnant animals, and animals that are produced in breeding colonies but will not be used to test a specific hypothesis. This justification must include information about the proposed experiment such as:

- Purpose of each experiment or set of related experiments
- Number of experimental groups/subgroups
- Number of animals by species/strain per group/subgroup
- Number of animals necessary to provide sufficient tissues/cells
- Typical parameters used to establish statistical significance between groups (e.g. the variable used to evaluate the number of animals in the power calculation)

All animals involved in the study must be addressed in the protocol table as required. List each experimental group separately, **briefly** summarize the procedures, and include the number of animals for each group. This table must account for all animals proposed for use under the submitted protocol. The total number of animals listed in this section must correspond to the total number listed in section 4 “Species” and the justification provided in section 13e “Reduction (Animal numbers justification)”.

Example of table defining groups and procedures:

Group	**Species**	**Number of Animals**	**Procedures/Treatment**
Group 1	Mice	20	Blood and tissue collection
Group 2	Mice	20	Tumor transplants
Control	Mice	20	Sham surgical procedure

Potential animal loss due to morbidity, mortality, or other challenges with the experimental procedures must be described in order to justify the need for additional animals above the minimum required by the power analysis.

The IACUC recognizes that the basis for an appropriate justification of animal numbers depends largely on the nature of the study. The statistical method and reference for which the number of animals was determined must be clearly stated.

Animal numbers **cannot** be justified on the basis of how many experiments the laboratory personnel can perform in a week or month, and the cost cannot be used as a determining factor for the use of a particular species or model.

Examples are listed below, including guidelines for justification of animal numbers.

A. Teaching Protocols: Animal numbers are determined by a specified student-to-animal ratio, which must be fully explained in the justification narrative. Animal numbers should be minimized to the fullest extent possible without compromising the quality of the hands-on teaching experience for students. Please define the student groups and the number of animals per group necessary to achieve the training objective.

B. Tissue Harvest Required for *In vitro* Studies (including antibody production): Animal numbers are typically determined by the amount of tissue/cells required based on experimental conditions, statistical consideration, and the number of individual animals needed to provide the appropriate amount of tissue/cells or antibodies. Please define the parameter(s) to be statistically compared between groups.

C. Exploratory or Pilot Studies: Animals may be needed for a pilot or proof-of-concept study. Animal numbers may be justified based on the probability of observing the desired effect of the experimental procedure or the numbers needed to test a new paradigm. Justification should include potential variability that may be anticipated and how it could impact the study. It is common practice to reference a publication that performed similar experiments and evaluated equivalent parameters to provide an estimate of animal number.

D. S**tudies Requiring Inferential Statistical Analysis:** If possible, animal numbers are determined by statistical power analysis. Alternatively, appropriate numbers of animals may be determined based on comparable studies for which the desired effect sizes were shown to be statistically significant and include references. A power analysis or other relevant statistical tests should be included. It is good practice to refer to the software or website used to compute the power analysis. Consultation with a statistician or use of statistical software during the design phase of the experiment is important to identify the animal numbers required. UC Davis statistical consultation includes the Center for Statistical Consulting for assistance in estimating animal numbers in research proposals 530-752-2361.

When justifying animal numbers based on a statistical test the IACUC will expect a description of the test used and the following information in the IACUC protocol:

- Include the *p*-value you set for a statistically significant analysis of an experimental measure. This value is also often denoted the alpha significance level (or probability of a difference between two mean values).
- The variation between animals treated the same way. The variance is often expressed as sigma, the sample standard deviation (statistical way of expressing the precision of the observed measures within a group of animals treated the same way).
- The minimal effect size (difference in the measures between groups) you are looking for, the size of difference that you would consider to be biologically meaningful and worth interpreting.
- The statistical power you are aiming to achieve. Power is one minus beta (the probability of failing to reject the null hypothesis when it is actually false), and most researchers accept powers of 0.8.
- The minimal number of animals per group needed to find the minimal effect size from a power analysis appropriate to your statistical test using the above information.

Replication: As part of the protocol review process the IACUC is charged with ensuring there is not unnecessary duplication of experiments and that the minimum number of animals needed to obtain valid data is being requested. If you will be requesting that group numbers exceed the minimum number statistically determined from a power analysis you will need to provide a rationale for this increase or for the complete replication of a study. It should be noted what variables will be altered that could warrant repeated study. These may include; change in technician, seasonal variation, differences in reagents or other consumables used in the subsequent experiments. If there is inherent variability in an experiment, it is good practice to refer to predicted sources. If justification is based on journal requirements or similar rationale provided by a funding agency, please provide those references in the IACUC protocol. See below for examples of acceptable number justifications involving replicates.

1. "To obtain statistically significant results, 15 animals will be needed per treatment and control group. This number is based on data from our previous studies, in which the standard deviations of the fold-change **protein expression** levels measured by western analysis were around 0.8. Based on a two-sided t-test, 7 animals would provide 90% power to detect a 2-fold change in protein expression signal at a significance of *P*=0.05. All experiments will be duplicated to control for day-of-testing variability, as we have experienced variation that may be due to differences in the preparation of the injected protein label, animal state on a given day, animal handling on a given day, or work being done by other investigators in the same housing area. Allowing for the loss of even one animal in each group would bring the total to 15 animals per group. This number of animals

is also consistent with similar studies [XX et al., (2007) Nature Neuroscience, 10:XXX]."

2. "Experimental replication is a basic principle of science and serves to verify that any statistically significant data from a single data set are not due to an uncontrolled experimental variable. It is fairly common to observe experimental variability in mouse experiments that could be a consequence of batch changes in reagents, intermittent animal vendor issues, or infectious agents or microbiota alterations in the animal housing room. Given the intermittent nature of these variables and the potential for them to affect interpretation of an entire experiment, these issues are best controlled for by performing replicate experiments. Adjusting the alpha to a more conservative level in a power analysis or simply increasing the "n" of every experiment does not deal effectively with these issues and will lead to unnecessary animal use. Additionally, most data publication requires replicate experiments, either as journal policy or due to data analysis by reviewers who appreciate the issues discussed above.

3. "In our experiments, we have assumed that every experiment will be repeated twice for a total of 3 experiments. Thus, three independent experiments are anticipated and animal numbers adjusted accordingly. In reality, not all experiments provide interesting results that will require replicates. However, it is impossible to predict ahead of time which of these experiments will show effects that require replication and which do not. As a rough estimate, we would anticipate that approximately 50% of our experiments require a direct replicate and 25% require a second replicate. A second replicate is often important, either because of conflicting data from two experiments, an experimental failure (tissues inadvertently destroyed in processing for example), or a particularly surprising result that challenges current dogma and therefore requires additional verification before publication. It should be appreciated that these are very rough estimates. However, if the IACUC permits an estimate for 50% of animals in a first replicate and an additional 25% for second replicates, this should be sufficient. We will revisit the issue with the IACUC if additional animals are needed."

E. Breeding Colony: Animal numbers are determined by the number of breeders and the TOTAL estimate of the number of pups to be generated. If only a percentage of the pups can be used for the project (e.g., only male pups or a specific genotype), that estimate should be included in the calculation of the final number required for the study and should be represented by a breeding group referred to in the Protocol Section 13f "Study Groups and Numbers Table".

IV. Resources:

1. Animal Welfare Act and Regulations
   https://www.nal.usda.gov/awic/animal-welfare-act
2. ILAR, Guide for the Care and Use of Laboratory Animals
   http://nap.edu/12910
3. PHS Policy
   https://olaw.nih.gov/policies-laws/phs-policy.htm
4. Stat Lab Statistical Consultation
   https://statistics.ucdavis.edu/stat-lab