Version No. 1 **Policy No. 109**

Determination and Justification of Animal Numbers

I. Purpose:

A key principle governing the ethical use of animals in research, testing and teaching is that no animal life is wasted; the number of animals used in each project must be the minimum necessary to obtain valid and meaningful results.(1)

By Federal regulation, the IACUC must review the number of animals requested in each protocol and agree that the number is appropriately justified in terms of the stated goals of the project.(2), (3)

II. Experimental Design Outline:

Justification of animal numbers begins with a clearly-stated, hypothesis-driven **outline** of the experimental design. All experiments involving live animals or animal tissue must be outlined in the protocol, and the number of animals required must be directly relevant to the experimental design in order to achieve the goals of the project. All animals must be justified, including experimental animals, donor animals and live-born offspring of pregnant animals and animals that are produced in breeding colonies but cannot be used in research.

> NOTE: Animals used to establish and maintain a breeding colony (breeding pairs, offspring that cannot be utilized because of genotype, sex, etc.) should not be included in the research application, but should be listed separately in the IACUC Breeding Colony Supplemental Application.

In order to assess the number of animals requested, the Principle Investigator must provide the IACUC with an overall outline of the proposed experiments in the protocol application. This outline must include (at minimum):

- The purpose of each experiment or set of related experiments,
- The number of experimental groups/subgroups;
- The number of animals by species/strain per group/subgroup,

The total number of control and experimental animals should be drawn from the experimental design and summarized in the overall outline. For many studies, this information can be effectively presented using a table or grid design.

If the project involves euthanizing animals for tissue harvest, the relationship between the amounts of tissue/cells needed for the experiments must be directly correlated to the number of animals required to produce that tissue (e.g., each animal produces X amount of tissue, which is adequate to perform Y experiments.)

Anticipated animal losses due to morbidity, mortality or other expected difficulties with the experimental procedures must be carefully described in order to justify the need for additional animals.

III. Justification of Animal Numbers:

Research and testing studies should be designed to provide a statistically significant result with a minimum number of animals, and the method by which the number of animals was determined must be clearly stated. Statistical techniques and/or power analysis are appropriate in most cases to maximize the analysis of the data generated from each animal. However, the IACUC acknowledges that the basis for an appropriate justification of animal numbers depends largely on the nature of the study itself. Prior experience and expertise with the model in question may be taken into account as well, but must be carefully documented in the protocol.

Consultation with a statistician or use of statistical software during the design phase of the experiment may be useful. The UC Merced IACUC can be contacted for assistance in estimating animal numbers in research proposals.

Websites that may be helpful in performing a power analysis include:

- https://archive.org/details/CAT10401495021
- http://statpages.org

Five basic types of studies are listed below, along with guidelines for justification of animal numbers appropriate for each type of study. These guidelines are intended to provide direction – any given study may not fall neatly into one of these five categories:

Teaching Protocols**:** Animal numbers are determined by a specified student-to-animal ratio, which must be explained in the justification narrative. Animal numbers should be minimized to the fullest extent possible without sacrificing the quality of the hands-on teaching experience for students.

Tissue Harvest Required for In-vitro Work (including antibody production): Animal numbers are determined by the amount of tissue required and the number of individual animals needed to provide the appropriate amount of tissue, antibodies, etc. A detailed explanation of how the required number of animals was determined must be included in the justification narrative.

Exploratory Study Requiring No Statistical Analysis (use of live animals to demonstrate success or failure of a desired goal, such as the production of transgenic mice): Animal numbers are justified based on the probability of success of the experimental procedure; a detailed explanation of how that probability was determined must be included in the narrative.

Pilot Studies**:** Animal numbers are determined by the investigator's experience and personal judgment, and are typically small. Data collected in pilot studies are generally used to determine statistically relevant sample size calculations for future experiments.

Studies Requiring Inferential Statistical Analysis: If possible, animal numbers are determined by statistical power analysis; the justification statement must include the values of alpha, beta, sigma, and effect size used in the power analysis to determine sample size. Alternatively, minimum numbers of animals may be determined based on pertinent literature for comparable studies in which the desired effect sizes were shown to be statistically significant.

Animal numbers cannot be justified on the basis of how many experiments the lab personnel can perform in a week, month, etc. The cost of the animal should not be considered as the primary justification for the use of a particular species or model.

IV. References:

1. PHS Policy on Humane Care and Use of Laboratory Animals.
2. *Guide for the Care and Use of Laboratory Animals.* (8th Edition)
3. Code of Federal Regulations 9 CFR Chapter 1, Subchapter A Erb, H.N. (1996) A non-statistical approach for calculating the optimum number of animals needed in research. *Lab Animal*, 45-49.
4. Mann, M.D., Crouse, D.A., Prentice, E.D. (1991) Appropriate animal numbers in biomedical research in light of animal welfare considerations. *Laboratory Animal Science* 41:6-14.