JUSTIFICATION OF ANIMAL NUMBERS

Table of Contents

Background

Federal regulations and the *Guide for the Care and Use of Animals* (*Guide*) require that Principal Investigators (PIs) provide justification for the total number of animals proposed for use in research and teaching. The ACUC is charged with evaluating the number of animals to be used and the justification for those numbers. This assessment is essential to determine whether the numbers are sufficient to answer the scientific questions and goals proposed. The rationale provided for the number of animals needed in a study should be based on the nature of the study. Therefore, although statistical analysis is the preferred method for justification, this approach may not be appropriate for certain types of studies.

General Points to include in the Justification (as appropriate)

- Budgetary constraints, time constraints, or the number of experiments that the laboratory personnel can perform in a week, month, etc. are <u>not</u> acceptable for justifying animal numbers.

- The number of animals requested should cover the three-year approval period of the protocol.

- Studies should be designed to provide a statistically significant result with a minimum number of animals.

- The justification should include the:

    o Number of animals per experimental group;

    o Number and types of experimental groups; and,

    o Number of replicates for each study.

- If eggs, fetuses or embryos are used before they reach specific stages based of neural development (described in the [Animal Care and Use Program Policy, III. 3.](#)), they do not require statistical justification. However, justification should include the number of pregnant females required to produce the proposed number of offspring.

- Expected morbidity or mortality that may impact the number of animals needed should be included in the justification.

- For studies where statistical justification is not possible, sequential sampling is often the preferred method of addressing the number of animals that will be required.

Types of Studies and Required Justification

    A.   Pilot Studies

    Because there is often no previously published literature, pilot studies are often performed with small numbers of animals and little or no previous experience to allow statistical justification. In these situations, it is acceptable to reference PI experience, advice from colleagues or literature citations of similar experiments.

    B.   Studies Requiring Inferential Statistical Analyses

        1.   When possible, power analysis should be used to demonstrate the sample size (n) is sufficient. Descriptions should document the alpha, beta, sigma and effect size and the minimum meaningful effect size (e.g., mean differences between groups) and variability (e.g., standard deviation determined prior to doing the power analysis). If power analysis is not possible, inferential statistical analyses following pilot or similar studies (e.g., t-test, regression analyses, analysis of variance (ANOVA), analysis of covariance (ANCOVA), multivariate analyses) may suffice.

        2.   Minimum numbers may be determined based on pertinent literature for comparable studies in which desired effect sizes were shown to be statistically significant (i.e., the authors of the publications have indicated that the group sizes resulted in statistically significant data). In this case, the comparable studies must be sufficiently documented in the justification section and references provided.

    C.   Breeding for Colony Maintenance and Experiments

    The justification of numbers for breeding colonies should be based on the number of breeders and offspring to maintain the colony, including number of offspring used in experiments or culled. Although statistical justification may not be appropriate, PIs should explain how they determine the required number of animals for colony maintenance and use in other studies.

    D.   Studies Involving Tissue/Cell/Fluid Harvest, Antibody Production and Examining Tissues or Cells *In Vitro or In Vivo*

The number requested should be based on that which is necessary to provide adequate cells, tissues, or fluid for *ex vivo* work. When possible, the PI should base the justification on previous experience (i.e., in similar previous studies, X number of animals was shown to be necessary to provide an adequate quantity of cells, tissues, or fluids).

E. Teaching Protocols

1. *Laboratory-based*: Number of animals used should be based on the number of students in the class (e.g., 1 mouse for 4 students). The PI should discuss how the animal-to-student ratio was determined.

2. *Field-based*: Numbers should be based on previous experience of the PI or colleague with similar procedures and/or classes.

F. Field studies

1. Number of animals cannot exceed the numbers on acquired permits. The investigator must consider the effects that sampling will have on a population, especially for threatened or endangered species.

2. Numbers requested for *non-invasive* studies *(e.g., behavioral observations, fecal sample collection)* may be based on literature, PI experience, or advice from colleagues.

3. For *invasive* studies *(e.g., animal capture, blood collection, surgery)*, please refer to the appropriate sections of this guideline, as justification requirements depend on the type of procedure proposed.

References

- Free Statistical Software, Calculators, & Equations
  - [Excel Spreadsheet for Simple, Two-sample Comparison Calculations](#)
  - [Free Statistics](#)
  - [Guide to the Care and Use of Mammals in Neuroscience and Behavioral Research](#) (Appendix A and B)
  - [Statpages.org](#)
  - [Statistical considerations for clinical trials and scientific experiments](#) (Harvard University)

- Statistical Justification
  - Dell, R.B., Hollerman, S., & Ramakrishnan, R. (2002). [Sample size determination](#). *Institute for Laboratory Animal Research*, 43, 4.

  - [Animal Welfare Information Center: Reducing Animal Numbers: Sequential Sampling](#)

- Erb, H.N. (1990). [A statistical approach for calculating the minimum number of animals needed in research.](#) *Institute for Laboratory Animal Research News*, 32 (1), 11-16.

- 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(1), 6-14.

- Muller, K. E., & Benignus, V. A. (1992). Increasing scientific power with statistical power. *Neurotoxicology and Teratology*, 14(3), 211-19.

- Thomas, L., & Juanes, F. (1996). The importance of statistical power analysis: an example from *Animal Behaviour*. *Animal Behaviour*, 52, 856-59.

- Institute for Laboratory Animal Research (ILAR) 43(4)

  - [Shaw, R., Festing, M.F.W., Peers, I., & Furlong, L. (2002). Use of Factorial Designs to Optimize Animal Experiments and Reduce Animal Use](#)

  - Festing, M.F.W., & Altman, D.G. (2002). [Guidelines for the Design and Statistical Analysis of Experiments using Laboratory Animals. Institute for Laboratory Animal Research](#)

- Regulations for the Justification of Numbers
  - Applied Research Ethics National Association (ARENA). Office of Laboratory Animal Welfare (OLAW). *[Institutional Animal Care and Use Committee Guidebook](#)* (2nd edition).

  - Animal Plant Health Inspection Service (APHIS). United States Department of Agriculture (USDA). "Animal and Plant Health Inspection Service, Department of Agriculture". *[Code of Federal Regulations (CFR), Title 9, Chapter 1](#)*. 2020 ed.

  - Institute of Laboratory Animal Research. National Research Council. *[Guide for the Care and Use of Laboratory Animals (8th edition)](#)*. National Academy Press, Washington, D.C., 2011.

  - Office of Laboratory Animal Welfare (National Institutes of Health). 2002. *[Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals](#)*

  - United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). (2000). Animal Care Policy Manual: *[Policy #11: Painful/Distressful Procedures](#)* and *[Policy #12: Consideration of Alternatives to Painful/Distressful Procedures](#)*.

- o Office of Laboratory Animal Welfare. National Institutes of Health. *[U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training](#)*, Section III.
- o National Institute of Standards and Technology. *[6.2.6. What is a Sequential Sampling Plan?](#)*.