



Institutional Animal Care and Use Committee (IACUC) Directive

1.0: Counting Animals Used in Research & Justification for Animal Numbers

1.0 Directive Purpose

The purpose of this directive is to provide guidance for Principal Investigators (PIs) when determining the numbers of animals needed to achieve projected activities, and to describe the IACUC's position regarding which animals must be counted and when counting must be performed.

2.0 To Whom the directive Applies

This directive applies to all individuals using live vertebrate animals under an IACUC-approved animal use protocol at Brown University.

3.0 Directive Statement

Tracking the numbers of animals used for a research study is essential to ensure that only approved animals are used, to fulfill federal obligations for reporting animal use, and to ensure compliance with IACUC-approved animal use protocols. Public Health Service (PHS) Policy states that "animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results." The IACUC must ensure that every animal protocol has an adequate justification for the number of animals requested before approval is granted. PIs must count and account for all animals used in association with a given IACUC-approved animal use protocol and report those numbers to the Office of Research Integrity (ORI) staff annually or upon request. Additionally, PIs that are approved for breeding must report animal usage to ORI twice a year.

3.1 Justification for Animal Numbers

All animal use protocols must contain a clear hypothesis-driven outline of the experimental design. The animals proposed in all types of studies must include a statement of how many groups of animals will be utilized (both experimental and control) and the number of animals in each group, resulting in the total number requested. The PI may include the number of animals used in previous or published research that is comparable conceptually and technically is germane to the justification of numbers. When appropriate to create the experimental model the PI must include additional animals in the total number requested to account for experimental losses or failures.

The Guide for the Care and Use of Laboratory Animals: Eighth Edition (the *Guide*) states that "whenever possible, the number of animals and experimental group sizes should be Statistically Justified." However, there are certain types of protocols for which statistical evaluation is ineffective. Therefore, the information required from PIs for IACUC review differs depending upon the type of research or use activity that is being proposed.

Studies with Breeding

Breeding is often needed to maintain a certain strain of animal for research activities. For breeding protocols:

- The PI must provide information on how many animals are required to keep the strain of animal available.
- Protocols that include breeding as part of a larger research objective will likely need to provide statistical justification for the research objective in addition to the justification for the number of animals required for maintaining the breeding stock.
- All animals produced must be accounted for with a justification, for example:
 - All adult breeders to be purchased or acquired;
 - animals that will be used for research purposed;
 - animals generated with a percentage that lack needed genotype
 - embryos to be manipulated in-utero.
- PIs that are approved for breeding must report animal usage to ORI twice a year.

Descriptive Studies

Descriptive studies require the following elements in the justification of numbers of animals:

- description of how precise the analysis will be and at what limit the values will be considered significant (e.g., "P" value or alpha),
- the summary measurement utilized (e.g., proportion, mean),
- and the maximum uncertainty (e.g., confidence level or standard deviation) that can be tolerated.

Comparative Studies

Comparative studies require the inclusion of all the information needed for descriptive studies and the following additional information:

- declaration of the type of statistical test utilized to justify the number of animals needed (e.g., paired t-test, Mann-Whitney, ANOVA, chi-square),
- and a notation of the unacceptable beta error rate.

Categorical studies

Categorical studies require a statement that defines the smallest difference or effect that is worth detecting (e.g., change in weight, proportion comparison) for said study.

Continuous Studies

Continuous studies require the PI to state the usual variation found in the measurement or the outcome (e.g., standard deviation or range) of said study and how small a difference is meaningful.

Statistical Power Analysis

Statistical power analysis can be a basis for justifying the number of animals. If this methodology is used, the following key elements must be listed in the justification.

- First, the experimental design must be presented in detail to allow the IACUC to verify that the power analysis is configured in the appropriate manner for the planned analysis.
- Second, the PI must declare and justify estimates of minimum meaningful effect size (e.g., mean differences between groups) and variability (e.g., SD which must be determined previous to the power analysis being performed).

Biostatisticians are trained to perform the kind of calculations used to determine sample size, and it may be helpful for the PI to consult a biostatistician in advance of submission of a protocol to the IACUC.

3.2 Non-Statistical Justification

PIs must provide Statistical Justification for the number of animals they request or explain why a non-statistical justification is instead appropriate. When non-statistical justification for animals is warranted, the PI must describe the justification for the number of animals requested for the proposed activity. Types of proposed activities that may warrant a non-statistical justification include:

- Pilot studies.
- Training (e.g., additional animals that may be used to train new laboratory members).
- Teaching protocols, where the numbers of animals requested are in a predetermined proportion to the number of students in the class.
 - For teaching protocols, the PI must provide information on how the ratio was determined.
- Many non-interventive field studies (e.g., behavioral observations of wild animals in their natural habitat).
- In-depth histological analysis of tissue.
 - For histological analyses, the PI must describe how much material is needed for the relevant analysis and provide information on how the PI arrived at the number of animals needed for the experiment.

3.3 Counting Animals

Identifying and tracking animal numbers are necessary to validate statistical significance and to justify the experimental groups of animals in a study. These activities are also required to fulfill federal obligations for reporting animal use numbers and to assure compliance with IACUC-approved animal use protocols.

What Must be Counted

All animals used in association with each approved protocol must be counted. This holds true for research, testing, teaching, and holding protocols. Adults, Neonate, and Embryos/Fetal animals must be reported. Avian Embryos are **not** considered live animals by U.S. regulatory agencies, and the Brown IACUC does not require full protocol review and approval before use. Rather, when use of avian embryos is proposed, the PI must submit a *Notice of Intent to Use Avian Embryos*.

When Counting Occurs

Animals are counted upon receipt by CARE after purchase or importation, when born as part of a breeding program, and when manipulated as part of a protocol involving in utero procedures.

3.3.1 Animals Purchased from a Vendor or Imported from Outside Institutions

Each animal is counted as used for a particular study upon arrival at the research facility.

Example: Ten female rats with day three litters are received for a study on lactation following parturition. Mammary gland tissue from the Adult females is studied, while the pups are euthanized. All Adult females and their pups must be counted.

3.3.2 Animals Generated via In-house Breeding Colonies

All animals produced (breeders and offspring) as part of a breeding program and counted at birth, even if only a subset of those animals is eventually used for actual experimentation, are counted as used for a particular study.

Example: Eight mice are produced from a selected mating, but genotyping reveals only two possess the correct genotype for the research project. All eight mice must be counted.

3.3.3 Animals Subjected to Embryonic/Fetal Manipulation

Fetal animals and Embryos must be counted as used if they are subject to experimental manipulation prior to birth. Where there is pre-term manipulation, all animals in the litter are counted as used.

Example: Extraction of the uterus revealed eight embryonic pups. Only three were needed for the research. All eight embryonic pups must be counted.

4.0 Definitions

For the purpose of this document, the terms below have the following definitions:

Adults: Animals aged beyond weaning and/or that are able to reproduce.

Embryos/Fetal Animals: Mammals that are in the period from implantation to birth.

Neonates: Young animals not yet weaned and requiring parental protection or nursing.

Statistical Justification: A calculation of sample size by power analysis required to support the need for the number of animals requested in the animal use protocol.

5.0 Responsibilities

All individuals to whom this directive applies are responsible for becoming familiar with and following this it. Animal research program stakeholders (IACUC, CARE, ARC) are responsible for promoting the understanding of this document and for taking appropriate steps to help ensure adherence to it.

6.0 Consequences for Violating this Document

Violation of this document may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violations of this document are a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

7.0 Related Information

Brown University is a community in which employees are encouraged to share workplace concerns with University leadership. Additionally, [Brown's Anonymous Reporting Hotline](#) allows anonymous and confidential reporting on matters of concern online or by phone (877-318-9184).

The following information complements and supplements this document. The information is intended to help explain this document and is not an all-inclusive list of policies, procedures, laws and requirements.

7.1 Related Policies/Directives/SOPs:

- [Avian Embryo Use Policy](#)

7.2 Related Procedures: N/A

7.3 Related Forms:

- Avian Embryo Use Request Form

7.4 Frequently Asked Questions (FAQs): N/A

7.5 Other Related Information: References:

- AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. AVMA: Schaumburg, IL.
- ILAR. 1991. ILAR News 33(4):68-70. Issues for Institutional Animal Care and Use Committee.
- National Research Council. 2011. *Guide for the Care and Use of Laboratory Animals: Eighth Edition*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12910>.
- OLAW FAQ. http://grants.nih.gov/grants/olaw/faqs.htm#App_4 (accessed December 2, 2020).

8.0 Document Owner and Contact

8.1 Owner: IACUC

8.2 Approved by: IACUC

8.3 Subject Matter Contact: Brown University Animal Research Compliance (ARC)

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9.0 Document History

9.1 Effective Date: February 5, 2016

9.2 Last Reviewed: May 6, 2022

9.3 Update/Review Summary: Minor edits to reflect biannual (2x a year) reporting to QA/QI Program Manager February 2, 2024.

- This document is not new; it was pulled out of the University Compliance Policy format and converted to a Directive May 2022.
- IACUC Policy on Counting Animals Used in Research: Date of IACUC Review and Approval: January 8, 2021.