



## INDIANA UNIVERSITY

Office of the Vice President for Research  
Office of Research Compliance

### Institutional Animal Care and Use Committee (IACUC) Office of Research Compliance (ORC)

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# Allocating, Counting and Justifying Animal Numbers for Animal Use Protocols

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**Responsible University Office:**

*Fred H. Cate  
Vice President for Research*

**Policy Owner:**

*Bloomington Institutional Animal Care and Use  
Committee (BIACUC)*

**Policy Contact:**

*IACUC Administrator*

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## Policy Statement

PHS Policy and the Animal Welfare Act and its regulations require that the IACUC review the Principal Investigator's (PI's) proposal to use laboratory animals. Justification for the type and number of animals must also be reviewed by the IACUC. This document is designed to provide information about the justification of animal species and numbers in Animal Use Protocols. Please contact the IACUC office (ext. 55138) or email [Biacuc@iu.edu](mailto:Biacuc@iu.edu) if you have any questions about this document.

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## Reason for Policy

PHS Policy (III, A) defines an *animal* as "any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes". Applications and proposals (competing and noncompeting) for awards

submitted to PHS that involve the use of animals are required to specify the species and approximate numbers of animals to be used, the rationale for involving animals, and the appropriateness of the species and numbers to be used (PHS Policy IVD1a; IVD1b). The *Guide* states: *The following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC: rationale and purpose of the proposed use of animals...justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis).* (p. 24)

A proper sample size is essential for obtaining valid results and minimizing the number of individuals exposed to the potential risks and harms of research. The IACUC is interested in ensuring that a sufficient but not excessive number of animals are used. A study with too few animals is problematic because the animals may be subjected to potentially painful procedures or loss of life with relatively little benefit to the advancement of knowledge. Likewise, a study using more animals than are truly needed is also problematic, because it unnecessarily exposes extra animals to potentially harmful procedures. The IACUC must be able to determine how the requested animals will be utilized, which research questions will potentially be answered by the number to be used, and why those questions could not be answered with fewer animals.

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## Procedures

- A. Definitions- for the purposes of this BIACUC policy
  1. **Embryonic/Fetal Animal**- (mammals) young from implantation to birth or egg to hatching (e.g., avian, reptile, aquatic species).
  2. **Pre-weanling/fledgling/neonate**- Young animals requiring parental protection or nursing.
  3. **Adult**- Animals which are mature, aged beyond weaning date (for the species), of an age able to reproduce, or offspring of egg-laying vertebrates (zebrafish larvae hatch 3 days post-fertilization).
- B. Policy
  1. Justify the number of animals in the protocol, including:
    - a. The number of animals for breeding and maintaining a colony
    - b. Number of offspring produced (regardless if used for experimental activities or euthanized prior to use)
    - c. Number of animals for experimental procedures by study design, group numbers, etc.
    - d. Number of animals transferred
    - e. Number of animals utilized in field studies
  2. Potential methods that can be used to justify animal numbers include:
    - Report of the numbers of animals needed to achieve statistical significance for the test planned.
    - Citation of previous research that is similar enough in concept and methodology to the present proposal, making it a reasonable model for sample sizes.
    - Derivation of animal numbers from material needs, a clear indication why the specific amount of material is needed, and why the number of animals requested is appropriate to provide that amount of material.

- Regulatory requirement for the particular species of animal and/or number of animals used in a study.
  - Results of a pilot study to estimate the variability in the data before performing a statistical analysis.
3. **Calculation of Animal Numbers for IACUC Protocols:**
    - a. **Purchased from a vendor** (either commercial or research institution); count and list the animals ordered and received upon arrival at the research facility.  
Example: 50 animals arrived from the vendor, only 35 were used in the experiment. All 50 animals are listed in the protocol.
    - b. **In-house breeding colonies:** In a breeding protocol, describe colony management including the number of breeders or replacements, number of young per cage, breeding system (number of females to males), continuous vs. interrupted mating, weaning age, methods for identification of individual animals, method of genotyping, inheritance estimates, etc. Count and list all breeders involved in the breeding of desirable offspring and all offspring produced, even if only a sub-set of the offspring are used for actual experimentation.  
Example: 20 mice are produced from a selected mating of 1 male and 2 females, but genotyping reveals that only 5 of the offspring are the right genotype and these 5 animals are transferred to the researcher for their research project. All 23 animals (1 male, 2 females, 20 offspring) are listed.  
Note: If using a separate breeding protocol and study protocol, animals produced on a breeding protocol that are transferred to a research protocol are listed on **both** the breeding protocol and the research protocol. Thus, the 23 animals from above example are listed on the breeding protocol, and 5 animals would be listed on the research project protocol.
    - c. **Breeding on an experimental protocol:** Count and list all breeders involved in the breeding of desirable offspring, replacement breeders, appropriate sex, mating scheme, genotype, stud males, weaned females to donate embryos for genetic modification, vasectomized males, and sexually mature females with good maternal characteristics for transgenic work and all offspring produced, even if only a sub-set of the offspring were used in actual experimentation. Formulas for calculating animal numbers can be obtained from the veterinarians.
    - d. **USDA Animal Numbers:** The USDA report applies only to "regulated species". This includes all warm-blooded animals **excluding** mice or rats bred for research and birds. The Principal Investigator is responsible for tracking use of animals covered by the USDA (hamsters, voles, rabbits) and then reporting these numbers to the IACUC office annually. Animals are counted as individuals.
  4. Once a protocol is approved, the PI or designee must keep track of the number of animals used on the protocol. A spreadsheet or database can be used to track animal use and counts.
  5. When the PI or designee needs to purchase, breed, capture or transfer animals, he/she must ensure that the activity is approved on the protocol and if not, submit an amendment to the protocol.

6. The PI must check the activity vs. remaining animals available on the approved protocol. If the activity will result in exceeding the number of animals on the approved protocol, then the PI is to submit an amendment to request additional animals.

## Sanctions

Failure to comply with IACUC policies may result in noncompliance reports to the Institutional Official, the Office of Laboratory Animal Welfare (OLAW), the U. S. Department of Agriculture (USDA), and/or the suspension of animal use privileges. In addition, the availability of sponsored research funds may be affected when an Investigator is found to be in violation of these policies.

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## Contacts

<b>Subject</b>	<b>Contact</b>	<b>Phone</b>	<b>Email</b>
Veterinary Concerns	LAR Veterinarians	855-2356	lar@indiana.edu
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## References

1. United States Department of Agriculture, 9 CFR Parts 1, 2 and 3.
2. PHS Policy on the Care and Use of Laboratory Animals, OPRR, 1996.
3. OLAW Website: <http://grants.nih.gov/grants/olaw/>
4. AAALAC Accreditation Guidelines: <http://www.aaalac.org>
5. 8<sup>th</sup> Edition: The Guide for the Care and Use of Laboratory Animals, 2011.
6. Silverman J, Suckow MA, Murthy S. 2007. *The IACUC Handbook 2<sup>nd</sup> Ed.* CRC Press, Boca Raton, FL.
7. [http://vetmed.duhs.duke.edu/PDF/Policies/Animal%20Use%20Policies/policy\\_on\\_counting\\_animals.pdf](http://vetmed.duhs.duke.edu/PDF/Policies/Animal%20Use%20Policies/policy_on_counting_animals.pdf)