



INDIANA UNIVERSITY

RESEARCH

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)  
BLOOMINGTON**

**Justifying Animal Numbers & Species in Animal Use  
Protocols**

Statement	Effective: 9-25-2017
Reason	Last Updated: 3-27-2023
Procedures	Responsible University Office:
Contacts	Vice President for Research
References	Policy Owner: <i>Bloomington Institutional Animal Care and Use Committee (BIACUC)</i>
	Policy Contact: <i>IACUC Manager</i>

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**GUIDANCE STATEMENT**

PHS Policy and the Animal Welfare Act and its regulations required that the IACUC review the Principal Investigator’s (PI’s) proposal to use laboratory animals. This document is designed to provide information about justifying animal species and numbers in Animal Use Protocols. Please contact the IACUC office if you have any questions about this document.

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**REASON FOR GUIDANCE**

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 groups sizes should be statistically justified (e.g., provision of a power analysis).* (p. 25)

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 IACUC guidance
  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 – (mammals) 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. Guidance
  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 (including those not 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. Justify the species to be used with research activities as follows:
    - a. Describe the characteristics that make this species appropriate for this study or class.
    - b. Explain your rationale for animal use, including why non-animal models are not suitable.
  3. 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.
4. 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 colony, include the number of breeders or replacements, number of offspring per cage breeding scheme (continuous vs interrupted mating), weaning age range, methods for identification methods of genotyping, etc. Count and list all breeders and offspring, even if only a sub-set of the offspring are used for 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.
5. 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.
6. When only adding less than 10% of the total number of animals approved, this request may be submitted as an administrative amendment.

## CONTACTS

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

1. *PHS Policy on the Care and Use of Laboratory Animals*, OPRR, 1996.  
<https://grants.nih.gov/grants/olaw/references/phspolicylabanimals.pdf>

2. OLAW: <https://www.nih.gov/grants-funding>
3. 8<sup>th</sup> Edition: *The Guide for the Care and Use of Laboratory Animals*, 2011.  
<https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>
4. Silverman, J., Suckow, M.A., Murthy, S. 2007. *The IACUC Handbook 2<sup>nd</sup> Ed.* CRC Press.