

Iowa State University Institutional Animal Care and Use Committee	
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Institutional Animal Care and Use Committee (IACUC) Policy

ANIMAL NUMBERS ON IACUC PROTOCOLS

1. Background

The Animal Welfare Regulations (9 CFR 2.31)¹ require justification of the number of animals to be used, as well as justification of the rationale and appropriateness of the species. Additionally, the *Guide for the Care and Use of Laboratory Animals* (Eighth Edition)² outlines that species justification along with the proposed number of animals to be used is to be reviewed by the IACUC, and that “whenever possible, the number of animals and experimental group sizes should be statistically justified.”

The US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training³, Principle III states, in part, “The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results.”

Therefore, accurately evaluating animal numbers is central to the mission of the IACUC as animal reduction is one of the committee’s responsibilities. The IACUC must also ensure that studies requiring live animals are conducted in a responsible manner that is consistent with *The Guide*. One of the more challenging roles of the IACUC is to review animal protocols and ensure that each includes an estimate of the minimum number of animals needed to accomplish research, teaching or testing objectives. Current IACUC standards are to count all animals ordered and received as though they are being used for the project.

2. Policy

Justifying Animal Numbers

The proposed number of animals to be used must be clearly stated in the justification section of the protocol along with the method of determining those numbers, such as

The IACUC has added text (see below) to the protocol (“Animal Number” tab) to provide further guidance on what the IACUC seeks for responses to this question.

The IACUC protocol numbers justification requires:

1. The number of animals should be the minimum number required to obtain statistically valid results, accounts for expected mortality, attrition, infection rate, and phenotype expression during the experiment(s).
2. The experimental unit(s) for each experiment will be defined.

3. The trait(s) to be measured for N determination are clearly stated in each experiment.

The animal numbers (N) for each experiment can be justified by providing either a power calculation, some other statistical analysis, or a publication reference with its complete citation. Investigators are also encouraged to reference other specific published studies, include data from previous research, or include a detailed formal explanation specific to their subject area to strengthen the justification.

The Guide recommends that the number of animals should be the minimum number required to obtain statistically valid results. A power analysis or other statistical analysis to predict a minimum sample size is strongly encouraged to justify group sizes when appropriate.

Examples of acceptable justifications are below, or consult a statistician for advice (Iowa State University Statistical Consulting; see <http://stat.iastate.edu/statistical-consulting>). Justifications that are based solely on “past experience”, funding, space limitations, or other non-statistical approaches are generally unacceptable. Investigators are encouraged to reference specific published studies, include data from previous research, or include a detailed explanation specific to their subject area to strengthen the justification.

Here are some resources to help investigators adequately justify a sample size of live animals for their particular study.

1. Most protocols involve a Manipulative Experiment (treatment and control groups), a Survival Study, a Comparison of Groups (single group to a specified value, or paired groups), or similar design. For these types of studies the best justification comes from a power analysis or minimum sample size calculation. The OpenEpi Web site (http://www.openepi.com/Menu/OE_Menu.htm) is an excellent resource. From the main page, in the menu at left, choose the most appropriate method under either the “Sample Size” or “Power” option. Another good option is StatPages (<http://statpages.org>); select the link “Power, sample size and experimental design” from the bottom of the table of contents and you will find a list of options specific to diverse study designs. It is acceptable to use published work from a similar previous study as long as the justification includes citations for all relevant work(s).
2. If you are conducting a Pilot Study, please make this clear and justify how the requested number of animals will be used to guide future studies. There are formal sample size calculators for a pilot study (<http://137.120.14.46/epid/n1.htm>) <http://www.crutzen.net/n.htm> if you want to use one.
3. If this is a Teaching Protocol please justify the number of animals with respect to the stated student learning outcomes for the course.
4. If required by the FDA or other federal agency, the federal code, regulation or standard, etc., must be cited.
5. If required by international testing requirements, the code, regulation, standards, etc., must be cited.

Ordering Extra Animals

In some situations, animal suppliers send an overage to ensure that enough animals survive the shipping conditions. In some cases, an investigator may need to select animals from within a larger group to reduce variability and reject others. These extras create a problem for PIs since, in many cases, PIs have not planned for spares or extras. To help avoid this pitfall and similar unexpected events, the IACUC recommends requesting approval for 5 to 10% more animals than the minimum required and including them in the count for the protocol. Then, depending on the animals' condition when received, extra animals can be assigned to the protocol, assigned to a different protocol, or euthanized. However, euthanized animals will still count as used on the protocol.

Since changing animal numbers is a major amendment, inclusion of a few extra animals in the original application may eliminate the need for full committee or designated member review (FCR/DMR) of an amendment to increase animal numbers. In these cases, the investigator should list the number of animals needed for the experimental design and also specify the number that are designated as extras. Extra animals can be assigned to treatments as replacements, but it must be clearly specified what will ultimately happen to all animals requested.

In special circumstances under which all of the animals ordered are not appropriate for the study (i.e., animals have an unacceptable health status) and new animals are needed to conduct the study, the IACUC will work with the PI to expedite the review of your protocol to add animals to your protocol. Please be sure to notify the IACUC office when you have special circumstances.

Animals used continuously in non-surgical, non-invasive, and/or non-infectious protocols or procedures over multiple years should only be counted once on the protocol. An example would be a dairy cow that is on a project over the course of multiple lactations. If a project is not continuous, such as distinct grazing research projects over several years, each animal should be counted as a new animal each time it is used, regardless of whether or not it was on the study in a previous year. The reason for this difference is that the PI may not have oversight and control of animals while they are off-study.

Counting Animals When Projects Extend Past Three Years

Because the IACUC approves projects for a three-year period, PIs must justify the total number of animals to be used during that same period. If additional animals are needed during that three-year period, an amendment must be submitted or a request made during the required annual review process.

If the project extends past the first three-year approval period,

- a new application must be submitted, and the PI must again justify the animal numbers needed;
- individual animals counted or used on the study during the first three-year approval period may be carried over; however, that total number approved for the next three-year approval period will include any carry-over animals plus new ones to be

acquired. This policy applies regardless of the species of animals or the anticipated length of life of the animal.

Breeding Colonies

If pre-weaned or weaned animals will be manipulated in any way, such as thymectomy, toe clip or ear notch for identification, or tail tip excision for genotyping, the number of manipulated animals must be included in the estimated number of animals used. If animals will be euthanized at, or prior to, weaning without any data collection or manipulation, their numbers are counted.

To assist the IACUC in reviewing breeding colony forms, the following information should be included:

- Maximum estimated number of breeders needed to produce desired numbers
- Maximum estimated number of replacement breeders held (i.e., for rodents, this is usually 2 to 3 x number of breeders used at one time)
- Maximum estimated number of animals (pre-weaned, weaned, or adult) to be subject to manipulations
- Euthanized

PIs with concerns about animal numbers should contact the Office of Research Ethics or the IACUC chair.

3. References and Regulations

1. Animal Welfare Regulations, 9 CFR 2.31
2. *Guide for the Care and Use of Laboratory Animals*, Eighth Edition. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 2011.
3. Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, 1985.

Additional resources:

4. *Guide for the Care and Use of Agricultural Animals in Research and Teaching*, Fourth Edition, Federation of Animal Science Societies, Champaign, IL, 2020.
5. U.S. Department of Health and Human Services, Office of Laboratory Animal Welfare, "PHS Policy on Humane Care and Use of Laboratory Animals Frequently Asked Questions," F2 <https://olaw.nih.gov/guidance/faqs#F> accessed January 13, 2022.

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