Allocating and Counting Animal Numbers for Protocols

Justifying Animal Numbers

The Animal Welfare Regulations require justification of the number of animals to be used (9 CFR 2.32), as well as justification of the rationale and appropriateness of the species. The proposed number of animals to be used must be clearly stated in the justification along with the method of determining those numbers, such as:

1. required for statistically significant results (tests used or statisticians consulted should be included);
2. based on scientific literature or past experience (references should be cited);
3. based on results of a pilot study;
4. required by FDA or other federal agency (federal code, regulation or standard, etc., must be cited);
5. required by international testing requirements (code, regulation, standards, etc., must be cited);
6. based on the number of students/animal and procedures needed to learn.

Counting Animals

Accurately evaluating animal numbers is central to the mission of the IACUC since animal reduction is one of the committee’s responsibilities. Current IACUC standards are to count all animals ordered and received as though they are being used for the project.

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 modification, inclusion of a few extra animals in the original application may eliminate the need for full committee review of a modification 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.

As a reminder, if the extra animals are included in the treatment groups, the final animal numbers must be communicated to the IACUC as a matter of record. This may be done during the annual continuing review or via an email to the IACUC administrator. 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 administrator 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, a modification must be submitted or a request made during the required annual continuing 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**

Determining the answer to Question 8 of the Animal Breeding Colony form can be challenging. The question asks “How many breeding animals will be maintained in the colony?” Federal guidance indicates that if pre-wean or weaned animals will be manipulated in any way, such as thymectomy, toe clip or ear notch for identification, tail tip excision for genotyping, or behavior tests, 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 not counted.

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

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

PIs with concerns about animal numbers should contact the Office for Responsible Research or the IACUC chair.