



Animal Number Justification

It is the responsibility of the investigator to clearly demonstrate that a minimum number of animals are being used to maintain valid results. Careful consideration should be given to the scientific justification of requested animal numbers within a realistic framework including laboratory capabilities, housing accommodations, and the number of experiments that can be reasonably accomplished within the three-year time frame of an IACUC *Proposal*. Requested animal numbers should be clearly explained in Section 13.1 Experimental Groups and justified in Section 13.5. of the *Proposal*.

Reduction of Animal Numbers

During experimental design, Principal Investigators should make every attempt to reduce the number of animals needed. The following table summarizes a variety of methods that may be employed (adapted from *Institutional Animal Care and Use Committee Guidebook*, 2002, ARENA and OLAW, Public Health Service):

Method	Examples
Rational selection of group size	<ul style="list-style-type: none"> ■ Pilot studies to estimate variability and evaluate procedures and effects ■ Power analysis
Careful experimental design	<ul style="list-style-type: none"> ■ Appropriate choice of control groups ■ Standardizing procedures to minimize variability
Maximizing use of animals*	<ul style="list-style-type: none"> ■ Performing several terminal procedures per animal ■ Use of tissues following euthanasia by other investigators
Correct choice of model	<ul style="list-style-type: none"> ■ Use of healthy, genetically similar animals to decrease variability
Minimizing loss of animals	<ul style="list-style-type: none"> ■ Appropriate peri-operative care ■ Avoidance of unintended breeding ■ Planning to ensure that only the appropriate number of animals needed for studies are ordered or bred
Statistical analysis	<ul style="list-style-type: none"> ■ Appropriate use of statistical software to generate maximum information from minimum number of animals

*Reduction of animal numbers should not occur by reusing animals that have already undergone experimental procedures that may have compromised the well-being of the animal.

Adequately Describing Groups

The Proposal must include a complete description of all animals utilized in the protocol, including:

- Experimental animals
 - Number of experimental groups
 - Number of animals per experimental group (including donors and recipients, if applicable)
 - Controls
- Additional animals needed to account for:
 - Expected mortality rate
 - Procedure failure rate
 - Procedure verification or training of new Project Participants
 - Pilot studies
- Breeding colony animals (see separate section below, which also discusses *the use of pre-weanling rodents*)

Note: Show the math! The reviewer must be able to account for every animal that is requested and verify that the numbers requested in the narrative in 13.5 match those requested in the Table in Section 13.1. You must also account for every animal for the entire time it will be housed at UofL.

Justifying Requested Numbers

The number of animals per group (“N”) is best deduced via a power analysis using estimates of error rates and differences between experimental groups. This may require a separate justification for each experiment included if each experiment has different objectives and endpoints and therefore should have different statistical parameters. Other methods that may be acceptable include:

- Sample size required by a funding agent or sponsor
- Extensive experience with the specific model
- Literature citations for similar experimental model

Use of Pre-Weanling Rodents

The IACUC and CMRU track **rodent** (mouse, rat, hamster, or gerbil) animal use only after weaning, *i.e.*, when animals are able to survive independently from their dams. Therefore, even if the *Proposal* involves the use of pre-weanling rodents (*e.g.*, if the study involves neonatal animals or neonatal tissue collection), the total approved and reflected in Table 13.1 should be limited to the total number of weaned animals acquired and/or generated that reach weaning age prior to euthanasia. Note that the written narrative in 13.5 will likely require a description of the number of pups generated and needed, because this provides the justification for the number of adults needed as breeders.

Breeding Colonies

To avoid inadvertent production and euthanasia of unneeded animals, breeding colony planning is an important feature of IACUC review. *Proposals* that include breeding colonies require a description of the breeding colony that will be used to produce the number of animals needed for experimental use **and** to maintain a line once created, including a description of the number of animals produced (even those not genotypically useful) and their disposition.

- Start with a confirmation of the number of animals specifically needed for experimental use.
- Determine an approximate number of pups/litter, genotypically appropriate pups/litter, litters/female, and number of male breeders/female breeders.
- Add to that the number of animals that will be needed to maintain the line.

This allows calculation of the total number of breeding animals necessary for the *Proposal* and the number of animals produced. Rodents that are eventually genetically unusable must be counted as “used” if, and only if, they reach weaning age. *Only include the number of **weaned** rodents in the total animal number requested.* For other species, all animals, including neonates, but be counted, requested, and recorded as “used.”

For rodents, answer the following questions:	Calculation:
How many experimental animals will be needed?	A
Of these, how many will reach weaning age or beyond (expressed as a whole number, not a percentage)?	B
What is the expected litter size?	C
What is the expected number of genotypically-appropriate animals per litter (expressed in a whole number, not a percentage)? If more than one useful genotype is produced in each litter then use the smallest number of appropriate pups in a litter.	D
Number of litters needed:	E = A / D
How many litters will each dam (females) produce?	F
Number of dams needed:	G = E / F
While the CMRU encourages monogamous pairing, how many sires (males) will you need?	H
Estimate number of additional dams and sires needed to continue the line, based on the questions above and expected fecundity for the three-year approval period.	I
<i>Total number of weaned rodents needed:</i>	= B + G + H + I

