

Animal Study Proposal Guidance for Animal Numbers Justification

Federal regulations and the Guide require that researchers provide to the Animal Care and Use Committee a justification of both the species and the number of animals to be used (Part D of the NCI Animal Study Proposal). Furthermore, whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g. by a power analysis). The obligation is to provide a good-faith estimate that is scientifically adequate, but not likely to be in excess. Minimizing the number of animals used is especially important when there are potential issues of unrelieved pain or distress.

Because many experiments proposed at NCI are pilot studies or novel paradigms, the expected variance is frequently unknown. In many cases it is most reasonable to propose an estimated group size based on previous experience analyzing similar datasets or on publications using a similar model. The basis for the chosen group size should be stated in the text of section D.

The NCI ACUC members request that all experimental proposals clearly identify the various experimental groups. A table that is included with the ASP should reflect the scientific plan and animal number accounting, simplifying the review by the ACUC.

Based on the nature of the science and the goals of the lab, some ASPs require a different approach to the justification of animal numbers, as described below:

- Proposals in which the experimental design dictates the numbers of animals required. Justification should include numbers and types of groups, numbers of animals per group, and number of necessary replications to arrive at a total number of animals necessary to carry on the study over the three year life of the protocol. When possible, it should be demonstrated that the sample size (n) is sufficient to assure reasonable power in significance testing or reasonable precision if the goal is estimation. The sample size can also be determined based on previous laboratory experience or supporting literature. If this information is included in Section E -, a reference to see that section should be made.
- Proposals may also include a breeding program to produce necessary animals for the planned experiments. The NCI LASP has developed a spreadsheet and a web-based animal number calculator (<http://web.ncifcrf.gov/rtp/lasp/intra/calculation.asp>) to assist in determining breeding colony size. The output results may be printed and appended to ASPs when appropriate.
- Proposals wherein the use of animals is primarily for the harvesting of tissues, organs or fluids for *in vitro* use. A short statement citing past usage levels per year (per experiment, etc.) necessary to meet the P.I.'s tissue or fluid requirements for each year of the proposal would be appropriate. If no prior experience is available, a statement relating anticipated amounts of material needed and the number of animals expected to provide that amount would be appropriate, assuming protocols for harvesting tissues have been optimized.
- Proposals wherein animals are used for the production of biologic reagents or cells, i.e. antibodies, bone marrow cells for donation, tumor propagation, etc. Provide an explanation (based on experience or anticipated production capability) of how many animals would be necessary to provide a necessary volume or other measure of material necessary for each year of the proposal.

- Proposals wherein animals are kept for breeding and maintenance purposes and not subject to planned experimental manipulations, other than genotyping. Studies involving genetic analysis are often animal-intensive and may involve culling a large number of excess animals. The ACUC would like the investigator to estimate the number of animals generated to maintain specialized breeding colonies and provide a brief explanation for the numbers if they appear to exceed 100 mice per line per year.

From the Institutional Animal Care and Use Committee Guidebook (2002): *After founder transgenic or knockout mice have been identified, between 80 and 100 mice/year may be needed to maintain and characterize the line. This assumes 5 breeder pairs per line, breeder replacement, and no unusual fertility or maintenance problems.* If the ASP includes justification for maintaining 5 strains for the three year life of the protocol, 500 mice per year, total of 1500 mice, would be allowed without additional mathematical calculations.

Examples:

1) We estimate that we will need to purchase 200 animals for this study because we will be using five (5) animals per group, and examining the effects of five (5) compounds (including vehicle), at four (4) doses of drug per compound, with two (2) replications, (to assure reproducibility), per determination. The compounds are GMC-1, GMC-2, GMC-3, and CMG, and the vehicle is 20% propylene glycol. Past experience and review of referenced publications (specify) have shown that a group size of 5 provides reasonable assurance of statistical power for this type of study, however if our initial experiments justify a larger sample size we will amend our protocol. The total numbers requested will be: $5 \times 5 \times 4 \times 2 = 200$.

2) Animals will be euthanized and the lungs excised for *ex vivo* perfusion and imaging experiments. For experimental metastasis, 10 animals per treatment group are generally required for non-parametric statistical comparisons. We will compare 5 cell lines and three different concentrations of ICAM-inhibitor plus control. $4 \times 5 \times 10 = 200$ mice.

3) In our experience, 10 rats are required to generate enough cells for one experiment. Since we plan to conduct one *in vitro* experiment per week we need 520 rats per year. This number of animals will enable us to optimize experimental conditions and analyze approximately 10 drugs at the desired dosages each year. (For *in vitro* experiments, the ACUC does not need to know the *in vitro* doses and associated statistics

4) Breeding & weaning:

The breeding will be conducted with a 1:1 female:male ratio in accordance with the ACUC guideline.

Justification of animal numbers:

For each subline:

Breeders: 2 male (heterozygotes), 2 female (heterozygotes) (2 breeding cages at one time, change breeders every 6 months). Therefore 4 breeders x 2 times per year = 8 breeders per year

Expected pups/litter: 6

Expected litters/female/year: 4

pups produced/year = 24 pups/breeder female x 4 (2 breeding cages at one time, replaced every 6 months) = 96

total pups/3 years: 288

total transgenic for breeding/3 years: 24

	year 1	year 2	year 3	total
pups	96	96	96	288
breeders	8	8	8	24

total = 312

total for 3 sublines = 936

The mice derived from the breeding will be used for two purposes: 1) experimental studies, 2) breeding subsequent generations.