

## ANIMAL JUSTIFICATION

### Introduction

According to Federal regulations and the 3Rs principle (1) the number of experimental animals used in a biomedical study should be the minimum necessary to obtain scientifically valid result.

The IACUC recommends that, whenever possible, the calculation of animal numbers be based on statistical methods. When appropriately described prior experience with specific experimental models may also be an acceptable justification.

### Guidelines

These guidelines assume that investigators, or their proxies, have taken the mandatory eIACUC training at <https://era.mssm.edu/training-calendar/> and accessed pre-requisite course videos at <https://peak.mountsinai.org> >Search eIACUC videos.

The following is a guide for completing parts 1-7 of the “Animal Justification” and the “Breeding” sections of the eIACUC Application.

### Animal justification

#### **1. Adjust the number of animals to be used or produced for this protocol as needed:**

The eIACUC software automatically populates the table in part 1 of this eIACUC Section with the total number of animals entered the Experiments section. The totals are calculated for each of the pain categories selected by the PI.

If one or more groups of animals are used in more than one experiment, the totals should be adjusted down to avoid counting animals twice. The “adjusted” totals can be entered directly in the appropriate row the rightmost column of the table.

*Example:* if the same 30 animals (out of a total 150) in category C are used in two separate experiments, the “adjusted” total entered in the table should be 120.

#### **2. If you adjusted the number of animals for this protocol, explain why:**

PIs should provide here a brief explanation for having adjusted animal estimates. If there were no adjustments, PIs may state that this section is not applicable.

#### **3. Provide the rationale for using animals in this protocol:**

Investigators should provide here a concise rationale for the need of using vertebrate animals to achieve the scientific objectives of a study, instead of alternatives such as in vitro methods, or computational models.

#### **4. Justify the number of animals to be involved in this protocol: (the Adjusted Animal Count above)**

The justification of numbers of animal should be based on the stated scientific objectives of a study and on the Experiments needed to achieve such objectives.

PIs must:

- Describe the experimental and control group for each experiment.
- Justify the number of experimental and control animals per group.
- Provide the total number of animals bred for a study. Note that the estimated number must include pre- and post-weaning bred animals that may not have the desirable genotype and may not be used in the study (see “Breeding” section below).
- Justify adding animals to a study to account for potential animal losses due to disease, experimental failure, complications after surgery etc.

*Important Note:* The justification may also be presented in a **table** rather than in narrative form. A table would be the preferred format for applications involving many experiments and large number of animals. A table may be easily uploaded in part 7 (see: Supporting documents”).

#### **5) Justify why each proposed species was chosen for this protocol:**

The scientific rationale for the selection of species should be based upon the relevant biological characteristics that makes the species an ideal model for the study. Please note that any reference to costs, housing space and similar consideration are unacceptable justifications for species selection.

#### **6) Identify each source of animals for this protocol:**

This section is self-explanatory.

#### **7) Supporting documents:**

PIs may upload documents here.

### **Breeding**

The numbers justification for breeding requires a description of:

- 1) the breeding colony that will be used to produce the number of animals needed for experimental use and to maintain one or more lines,
- 2) the total number of animals produced, including those pre- or post-weaning not genotypically useful, and

3) the disposition of animal that do not have the desired genotype.

Regardless of how an animal generated by breeding will be used (i.e. in experiments or future breeding), all animals are to be counted only once when estimating the total number of animals generated by breeding.

As a practical guide:

Estimate the number of animals specifically needed for experimental use.  
 Estimate an approximate number of pups/litter, genotypically appropriate and inappropriate pups/litter, and number of male breeders/female breeders.  
 Add animals in items 1 and 2 to determine the number of animals required to meet the goals of the study and to account for the total number of animals produced.

Other notes:

The total number animals that are requested and counted as "used" includes progeny with useful genotypes that are weaned into the colony, as well as genotypically useless adults and pre-weaning pups, since they undergo the genotyping procedures.

A quick reference table to assist with making these calculations and assigning the appropriate USDA pain category is shown below:

	<b>PAIN CATEGORY GUIDE</b>	<b>NUMBER OF ANIMALS</b>
Estimated number of weaned adults that are used in experiments	Category assigned based on the type of procedure(s) to which the animals are subjected and/or phenotype. Highest pain category is used.	#
Estimated number of pre-weaning pups that are used in experiments	Category assigned is dependent on whether there is a perinatal phenotype that results in pain or distress and/or the type of procedure(s) to which the pups are subjected. Highest pain category is used.	#
Estimated number of mice used only for breeding to maintain the line(s)	Most likely assigned to USDA pain category B, unless there is a phenotype in adults that causes pain or distress.	#
Estimated number of pre-weaning pups that will be genotyped and euthanized at or before weaning and not subjected to experimental manipulation	Most likely assigned to USDA pain category B, but can be different depending on phenotype(s).	#
Estimated number of adult animals that are weaned into the colony but are euthanized shortly thereafter because they do not have the correct genotype.	Most likely assigned to USDA pain category B, but can be different depending on phenotype(s).	#
		<b>Total number of animals requested= X</b>