

 LEHIGH UNIVERSITY	Animal Care and Use Committee (IACUC)		
	Guidance: Justifying the Number of Animals in Research		
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The intent of this Institutional Animal Care and Use Committee (IACUC) guidance is to assist researchers in preparing a response to 7g on the IACUC Protocol Form: “Provide the number of animals requested and the rationale for how the number of animals was determined to be appropriate. Whenever possible, the number of animals requested must be justified statistically”. The guidance is designed to assist researchers in preparing application for review by the IACUC and to help ensure compliance with the institution’s animal welfare Assurance and informed by regulations outlined in the Guide.

Introduction and Background

Investigators are required to use the minimum number of animals necessary to obtain valid results. Question 7g of the IACUC Protocol Form addresses this requirement by providing the rationale for the number of animals requested.

Generally, answering this question requires a power analysis. Power refers to the probability of avoiding a Type II error, or, the ability of a statistical test to detect true differences when they exist. The power of a test generally depends on four variables: sample size, effect size to be detected (medium), the Type I error rate (alpha, usually .05), and the variability of the sample. Power is usually specified at 0.80, that is, 80% likely to be right.

Alternatively, if animal number to be used are based on previous work or publications, detailed citations are acceptable.

Statistical tests provide a way to estimate whether the differences measured between groups of animals treated differently in one experiment are “real” (i.e. will be reproducible nearly all the time), or have just occurred by chance. Most often, this is stated as the p-value. $P < 0.05$ means the “result” could occur by chance less than 5% of the time. From simple t-tests to the most complicated analyses, the mathematical assumptions underlying statistical tests require that the methods of analysis, the p-value, and the minimal difference between groups you want to find, all be decided upon beforehand.

Procedures for Providing Statistical Justification

The IACUC requires the following information about how you a) determine sample sizes, and b) analyze your data:

- A brief description of the experimental design, including the control and experimental **groups** and their **sample sizes**, if applicable. “For example, there will be 4 groups, including one control group that receives vehicle, and three groups, each of which receives 1, 2, and 4 mg of the drug (n = 10 per group, n = 40 total).”
- A description of the statistical methods for determining the sample size, e.g., a Power Analysis, if applicable. Please specify the **variable** used in the power analysis, **and the results of the power analysis**. If these have been determined previously, **cite the publication**. For example, "Using Cohen's d, we determine that a sample size of 10 per group is necessary to detect statistically significant effects of treatment."
- A statement of the **probability value used to detect significant differences** (i.e., the *P*-value, the alpha significance levels). For example “Differences between groups will be considered statistically significant if $P < 0.05$.”
- A statement of the **effect size** that will be considered substantive. For example, “Statistically significant differences will be considered small if the Coefficient of Determination (r^2) is greater than 0.1 but less than 0.3, medium if r^2 is great than .3 but less than 0.5, and large or substantive if r^2 is greater than 0.5.”

The IACUC does not insist on any given alpha or beta levels, and it will evaluate arguments for deviation of either of these from traditionally used values.

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Additional Details

When the animal is not the experimental unit, determine the appropriate sample size for your unit of study (e.g., the number of neurons needed) and extrapolate to determine the maximum number of animals required. Include information regarding the number of experimental units expected to be derived from each individual animal.

When statistical justification is not possible, briefly explain why. Provide a rationale for the proposed number of animals, such as complete citations of previous research or experience. For pilot studies or teaching protocols, the researcher may indicate that statistical justification does not apply because a hypothesis is not being tested.

A firm number (i.e. not a range) must be provided for a three-year period. A maximum number may be listed. It is recommended that a 5-10% overage is considered in order to cover cases where animals must be removed from studies for nonexperimental reasons.

For renewal submissions, provide an updated justification of the number of animals required for the next three years; including all breeding, control, and experimental animals. Total animals requested for a renewal may need to be modified from the previously approved protocol version.

More than one justification may be applicable to the proposed study, depending on the mix of observations and experiments being conducted. Combine non-statistical and statistical justifications as appropriate.

Resources for Completing Power Analyses

There are free web sources available for completing a power analysis for many simple and common experimental designs:

- <http://calculators.stat.ucla.edu/>
- <http://homepage.stat.uiowa.edu/~rlenth/Power/>
- <http://statpages.org/#Power>

References

1. *Guide for the Care and Use of Laboratory Animals*, 8th Ed., National Research Council; National Academy Press: Washington, DC, 2011. <http://www.nap.edu/catalog/12910/guide-for-the-care-and-use-of-laboratory-animals-eighth>
2. Animal Welfare Act and Animal Welfare Regulations. Part 2 – Regulations; Subpart C – Research Facilities; § 2.31, e, 1-2. United States Department of Agriculture: Washington, DC, 2013. http://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Blue%20Book%20-%202013%20-%20FINAL.pdf