

Washington State University
Institutional Animal Care and Use Committee

Guideline #5: Rationale for Animal Numbers in Protocols

Approval Date: 5.6.2021 (Replacing Version 07.2017)

A. Why Justification for Animal Numbers is Required

Under the Animal Welfare Act and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, the Institutional Animal Care and Use Committee (IACUC) is charged with making certain that every animal protocol has adequate justification for the number of animals requested prior to approving the use of animals in a proposed research or teaching project. The *Guide for the Care and Use of Laboratory Animals* specifies that the requested number of animals must be justified statistically whenever possible.

These rationales and explanations must be written in plain, non-technical English in order to be clear to IACUC reviewers and to informed members of the public. Each protocol must “stand alone” so that a reviewer can understand the justification without need to refer to other active or previously submitted protocols.

Although the *Guide* states that statistical analysis should be employed whenever possible, in some cases statistical analyses are ineffective or inappropriate. Therefore, the information required for IACUC review of projects differs depending on the type of research or other activity being proposed. Animal numbers cannot be justified based on how many experiments laboratory personnel can perform per week or per month, and the cost cannot be used as a determining factor for the use of a particular species, model, or group size.

B. Studies in Which Statistical Justification is Not Possible

If investigators do not provide statistical justification, they must explain why statistical justification is not appropriate. Proposal types for which statistical justification may not be appropriate include:

- a) Pilot studies: Animals may be needed for pilot or proof-of-concept studies. Animal numbers for such studies may be justified based on the probability of observing a desired effect of the experimental procedure or to evaluate a new paradigm. Justification should include any anticipated potential variability and its likely effect on the study. Reference to publications in which similar experiments were conducted may also support a proposed animal number. Pilot studies should generally require only small number of animals enabling collection of data that can then support a statistical sample size calculation.
- b) Breeding protocols may be required to maintain strains of animals available for future research activities. Instead of statistical justification, in such cases the PI should provide information about the number of animals required to maintain the animal strain. Protocols that include both breeding and research components may be required to provide statistical justification for the research objective in addition to justification of the numbers of animals required to maintain the strain.
- c) For teaching protocols, typically the number of animals requested is based on the number of students in the class. Instead of statistical group size justification, the PI should justify the animal numbers in terms of the minimum number of animals required to meet the specific teaching objectives.
- d) Non-intervention field studies (e.g., behavioral observations of wild animals in their native habitat) do not require justification of specific animal numbers.
- e) Protocols for which animals are required to produce tissues, cells, or cellular sequences or similar biological end products (e.g., in cell culture, microarray, etc.). In such instances, the PI should work backward in their justifications: Start by justifying the required number/ amount of products, and then explain how you chose the number of animals required to provide that amount.

C. Studies in Which Statistical Justification is Possible

As mentioned previously, the *Guide* states that statistical analysis should be employed whenever possible to justify the proposed animal numbers represent the smallest number needed to reasonably attain the scientific goals of the project. The information required for a PI to statistically justify the numbers of animals for a project in order to secure IACUC approval depends on the nature of the statistical evaluation to be used. The box below gives an example of the information required for one type of study and analysis: comparing group means using a Student's T-test or related parametric analysis. A statistical power analysis will utilize this information to justify the number of animals needed. Other experimental designs or analysis plans (comparisons of proportions, linear

regressions, analyses or categorical data, time to event data) can also be statistically justified, but the specific methods required will vary.

For example, the information required for IACUC to evaluate experiments to be evaluated by parametric analyses of continuous dependent variable data includes:

- The *P*-value used to detect a statistically significant result. This value is often denoted alpha and indicates the probability of finding a similar or larger difference between groups by chance alone. Typical *P*-values accepted are 0.05 or 0.01; if it is necessary to select an alpha value outside that range, please explain the reason for this to the IACUC.
- The variation expected between animals, frequently expressed as the standard deviation.
- The minimal effect size the PI wants to detect.
- The statistical power desired. Statistical power is only of interest when the experimental results support the null hypothesis. For example, the commonly used statistical power of 0.8 (equivalent to a beta error value of 0.2) means that the researcher can be 80% confident that a larger sample size would still not enable rejection of the null hypothesis. If it is necessary to specify a very high statistical power (>0.9), please explain the reason for this to the IACUC.

Be reasonable: Use of extreme parameters in a statistical power analysis result in unreasonable group size requirements, as with the examples for alpha and beta in the example in the box above. Occasionally extremely low alpha or beta error rates are required for a specific study, but if so the reasons for this will need to be justified to IACUC reviewers.

Replication: Experiments that use group sizes in excess of those supported by the power analysis are inherently unreasonable, as is replication of entire experiments with the justified group sizes. However, it may often be appropriate to request additional animals to account for possible animal losses, experimental failures or other unforeseen needs, but the reasons for these additional animals must be clearly explained to IACUC reviewers.

Previously published results: Pilot studies or previous research involving similar experimental design can be used to justify animal number requirements, but this must be done through the statistical power analysis described above, since successful previously published experiments may have used animal numbers in excess of those necessary for the experiment. However, the data recorded in those experiments will support a statistical sample size justification.

D. How Statistical Justification Can Be Accomplished

Statistical software tools for sample size justification: Numerous tools are available that are specifically designed to assist statistical justification for sample size. These include tools built in to most commercially available statistical analytical software packages and also a wide variety of freely available on-line tools or applications specifically designed for sample size justification for a wide range of analytic approaches. Links to several examples of the latter can be found on the WSU IACUC website, although the list is not comprehensive.

Consult a statistician: For complex cases, or for situations where the PI is unwilling or unable to find appropriate tools to conduct the statistical sample size justification, the WSU IACUC recommends consulting a statistician. Links to statistical resources available within WSU can be found on the WSU IACUC website.

If you have any questions about this guideline, please contact the Animal Welfare Program Office at 509-335-7951 or iacuc@wsu.edu.