

## Justification of Animal Numbers

The Ag Guide and PHS Policy (US Government Principle III) indicates that "the animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results". The minimum number should include animals which cannot be included in analysis due to mortality and expected complications. All control groups should also be described and included in animal numbers justification. Statistical justification by use of a power analysis is the preferred method of justification. The G\*Power statistical power analysis application that is free and downloadable at <http://www.gpower.hhu.de/en.html> is recommended but not required. Other acceptable justifications include citations of peer reviewed research where a similar effect size and error variance is expected.

The statistical tests used should be described in detail, including the following information:

1. The primary outcome, method of comparison, and units (for example, weight difference, comparison of means between two groups, and grams).
2. What kind of variable are you measuring? Examples are:
  - a. Binary – Mortality, diabetes presence, success rate of a procedure
  - b. Continuous – Weight, blood pressure, cd4 counts, glucose, tumor size
  - c. Count – number of pups, number of episodes (e.g. seizures), feeding bouts per unit time
  - d. Ordinal – an order or progression, such as tumor size at time A versus time B
3. The p-value (i.e. the alpha level) you consider significant.
4. The expected variation within a group of animals treated the same way (Assume different variance between study groups unless there is a reason to presume otherwise).
5. The effect size (the smallest difference between groups that you want to find, or you would consider meaningful, e.g. 20% change in the units of the primary outcome).
6. The statistical power you are trying to achieve (one minus the probability of failing to reject the null hypothesis when it is false).
7. When considering the appropriateness of animal numbers, the committee may consider the invasiveness and potential stress caused by the treatments on the animals relative to the rigor required to justify animal numbers.
8. If a power analysis is used all statistics and the values used to calculate the sample size should be presented in the AUP or a screen shot or output from the power analysis included.
9. If multiple variables will be measured power analysis should be done on the most important variable for the study.

Other acceptable justifications include citations of peer reviewed research where similar conditions, variables, effect size and error variance are expected.

### Studies where statistical power analysis is not appropriate:

- *Teaching:* Use of animals in conjunction with a course for the purpose of teaching students. A specified student-to-animal ratio determines numbers. The choice of the specified ratio must be explained in the justification statement.
- Field studies.
- Breeding protocols to maintain a certain strain of animals available for future research activities. The investigator should provide information on how many animals are required to keep the strain of animal available. Protocols that include breeding as part of a larger research objective may need to provide statistical justification for the research objective in addition to the justification for the number of animals required for maintaining the breeding stock.

- *Antibody/tissue production*: Use of animals to produce antibodies or tissue. Numbers are determined by the amount of antibody/tissue required, the ability of an individual animal to provide the needed amount, etc. Provide details on the determining factors in the justification statement.
- *Studies with success/failure as the outcome*: Use of animals to demonstrate success or failure of a desired goal (e.g., production of transgenic animals, to determine whether a poisonous plant induces cleft palate in grazer species). Numbers are determined by the probability of success of the experimental procedure. Provide details in the justification statement.
- *Feasibility or pilot study*: Use of animals in a preliminary study to refine procedures and equipment and to discover problems before the main study begins, in which additional inferential studies will verify any results of interest. When any statistical analysis is done, it is primarily descriptive or exploratory in nature. Numbers are determined by the investigator using experience and judgment; generally, the number is relatively small but should be large enough to provide needed estimates for future sample size analyses.
- *Exploratory studies*: Performed to generate new hypotheses (e.g., carrying out multiple genetic or biochemistry assays without a driving hypothesis). Often many variables are considered. These are often secondary aims in studies. The sample size is usually based on previous experience or, optimally, will make use of the same animals used for the primary aims by collecting additional data.