IACUC Standard Operating Procedure

Subject: Justification of Numbers of Animals for Protocols

Background: 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”. Additional guidance from PHS policy (IV.D.a. IV.D.1.b) adds some additional clarification and states that the identification of species, approximate number of animals and rationale for the appropriateness of both should also be provided in the animal protocol. Likewise, the USDA regulations state similar principles for protocols using animals. In reviewing protocols, they specify that “identification of the species and the approximate number of animals to be used must be included” as well as “a rationale for involving animals and for the appropriateness of the species and numbers of animals to be used”.

The most specific comments are found in The Guide for the Care and Use of Laboratory Animals (Guide) which specifies that the requested number of animals must be justified statistically whenever possible. The Institutional Animal Care and Use Committee (IACUC) is charged with making certain that every animal protocol has an adequate justification for the number of animals requested before they approve the use of animals in a proposed research or use project.

Inadequate justification of the number of animals requested on a protocol may result in the protocol either being tabled or denied approval by the IACUC. Either will result in a delay in the ability to begin work on the proposed project. Examples of well-structured justifications of animal numbers are available on the IACUC website.

Procedures:

Although the Guide states that statistical analysis should be employed whenever possible, there are certain types of protocols for which statistical evaluation is ineffective. Therefore, the information required from investigators and the IACUC review of proposed projects differs depending upon the type of research or use activity that is being proposed.

I. Studies in which statistical justification for animals is inappropriate.

Investigators shall provide statistical justification for the number of animals requested or provide justification for why statistical justification is inappropriate. When investigators believe that statistical justification for animals is inappropriate, they must provide information on how they arrived at the number of animals requested for the proposed activity. Inappropriate types of proposed activity may include

1. Pilot Studies
2. Breeding protocols to maintain a certain strain of animals available for future research activities. Instead of statistical justification, the PI 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.

3. Teaching protocols where the numbers of animals requested are in a predetermined proportion to the number of students in the class (e.g. 1 mouse per 3 students). Instead of statistical justification, the PI should provide information on how the ratio was determined.

4. Many non-interventive field studies (e.g. behavioral observations of wild animals in their natural habitat).

5. Exclusive use protocols such as polo, riding classes, etc.

6. In-depth histological analysis of tissue. Instead of statistical justification, this type of protocol requires the Principal Investigator (PI) to describe how much material is needed for the analysis and provide information on how s/he arrived at the number of animals needed for the experiment.

II. Studies in which statistical justification is appropriate.

Justification of animal numbers is a complex issue contingent on complicated variables including, but not limited to, the type of study being proposed. PHS policy, USDA regulations, and ethical principles allow for no species-related variance in criteria for justification of the number of animals requested for a proposed research or use activity.

Outlined below is the minimum information required by the Principal Investigators for statistical justification of the number of animals needed in order to secure approval of a protocol by the IACUC.

1. The protocol must contain a clear hypothesis-driven outline of the experimental design. The number of animals proposed in all types of studies must include a statement of how many groups will be utilized (both experimental and control) and the number of animals in each group resulting in the total number requested. Inclusion of a statement that correlates the proposed research to any previous or published research that is comparable conceptually and technically is germane to the justification of numbers.

2. As appropriate, consideration should be given to the necessity to include additional animals in the total number requested to account for experimental loses or failure to create the experimental model.
Brief Statistical Guide:

1. Descriptive studies are those used to reveal patterns or connections within a group but have no analytic comparisons between any of the groups utilized. This type of study is often useful in the formulation of hypotheses (e.g., certain pilot studies). Pilot studies by virtue of their nature require the use of smaller numbers of animals.

The following elements need to be present in the justification of numbers of animals for these types of studies. A description of how precise the analysis will be and at what limit will the values be considered significant is required. Precision of the analysis is often expressed as the “P” value or alpha. The P value indicates the likelihood that the PI is incorrect when they conclude that there is a difference, effect, association, correlation or dependence in the data set. Commonly accepted values for significance are 0.10, 0.05 or 0.01 although other values may be justified. A statement that the P value is either 1-sided (values either greater than or less than the critical value are important) or 2-sided (values greater than and less than the critical value are important) should also be included. The summary measurement utilized (e.g., proportion, mean) has to be included as well as the maximum uncertainty (e.g., confidence level or standard deviation) that can be tolerated.

2. Comparative studies are used to decide whether or not two or more values are equivalent. Justification of animal numbers for these types of studies requires the inclusion of all the information needed for descriptive studies along with the additional information delineated below. Declaration of the type of statistical test utilized (e.g., paired t-test, Mann-Whitney, ANOVA, chi-square) is necessary. A notation of the unacceptable beta error rate, (the risk the PI is willing to take of missing a true effect or difference), needs to be included. The beta error is commonly expressed as a value of 0.20, 0.10, 0.05 or 0.01 although other beta errors may be justified. Comparative studies can then be further broken down into two sub-categories, categorical (count-type) or continuous (measurement-type). Categorical studies require a statement that defines the smallest difference or effect that is worth detecting (e.g., change in weight, proportion comparison). Continuous studies require the PI to state the usual variation found in the measurement or the outcome (e.g., standard deviation [SD] or range) and how small a difference is meaningful.

3. A statistical power analysis can be an excellent basis for justification of animal numbers. If this methodology is used, several key elements must be listed in the justification. It is crucial that the experimental design is presented in detail to allow the IACUC the ability to verify that the power analysis is configured in the appropriate manner for the planned analysis. Use of a power analysis requires the PI to declare and justify estimates of minimum meaningful effect size (e.g., mean differences between groups) and variability (e.g., SD which must be determined
previous to the power analysis being performed).

4. Biostatisticians are trained to perform the kind of calculations used to determine sample size, and it may be helpful to consult them in advance of submission of a protocol to the IACUC.

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