The Justification of Animal Numbers;
The Role of Sample Size, Precision and Power Analysis in Assigning and Justifying Animal Numbers

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Introduction

To assure alignment with the provisions of The *US Animal Welfare Act (1966,1985)* all Janssen R&D animal model based research and development is accountable to its local *Institutional Animal Care and Use Committee* (IACUC). In particular, this support must account for animal welfare considerations in addition to those of the scientific conduct of the study.

To meet potential regulatory and animal bioethics concerns, as well as those related to basic study design and sample size, many Research Investigators have turned to biostatisticians for statistical support and justification of the animal numbers used in their studies during the IACUC Protocol development and review process.

A New Challenge

Recently, the US Department of Agriculture (*Inspector General, 2005*) has begun to look more closely at the *justification and assignment of animal numbers* and more generally, at identifying reduction alternatives to the related study practices.

A Solution from the Statistical Sciences

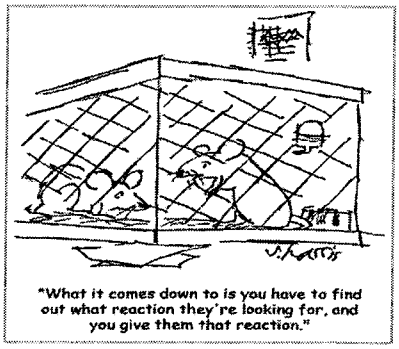
From the perspective of biostatistics, the animal sample size to detect a study treatment effect with specified power and precision, a controlled error rate, and sensitivity is related mathematically to the experimental design and the test statistic used for the data analysis; in short they are all interconnected aspects of the same statistical structure. Thus, for conducting studies comparing individual or multiple treatments to a control, the *optimal animal numbers* are those based on the sample size required for the intended statistical test.

An Extended Solution from Statistical Computing

To continue facilitation of study planning, user-friendly web based sample size and power analysis software tools have been created to assist the Research Investigators while designing their experiments and writing the Statistics Section of the IACUC Protocol. These software tools use programs previously employed by the biostatistician for sample size support. They allow the Research Investigator menu options to estimate sample size and power values assuming (1) prior summary study information, (2) and the anticipated method of data analysis - whether it be a t-test, the analysis of variance, or *multiple treatment comparisons*. Additionally, the software generates a table and customized text reporting sample size for a range of treatment effects with at least 60% power in addition to an interpretative explanation in basic English. Both can be downloaded and inserted into a standard document for report or presentation purposes. Note that this software is not commercially available.

Implications and Summary

An immediate consequence has been to define *an empirical statistical standard for the support of animal numbers.* In particular, we can now control the risk of using *too few* animals, where variation can hide the potential activity of the research drug, thus using animals unnecessarily without meeting our research goal. Similarly, we can control the risk of using *too many* animals, thus avoiding the unnecessary pain and distress of extra animals. Either case supports real or potential animal reduction.



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Fifteen Ways of Reducing Animal Sample Size Without Severe Impact to Power *

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1. **Increase effect size.** If the minimum acceptable treatment effect can be reduced, then so will the sample size needed to detect it, e.g., fasted animals may preclude large sample for detecting a change in glucose.

2. **Reduce variability** Sample size is reduced if the sources of component variability are reduced - a key point of experimental design. Increase measurement and/or method accuracy. Refine the bioassay or chemical assay. Look to inbred strains, transgenics, or litter mates.

3. **Use robust statistics.** Sample size is reduced if the sources of component variability are reduced; trimmed mean in place of the mean. The median and median absolute deviation (MAD) in place of the mean and std dev, respectively.

4. **Use an outlier-resistant method to downweigh extreme values**, such as Huber's robust functions. Then use your standard least squares software.

5. **Revaluate control group use** Use animal as their own control. Revaluate control/vehicle group precision need. Revaluate need for multiple controls. Combine control groups creatively. "borrow strength" by using historical controls.

6. **Design a study better** *Experimental Design* premise: there is a better way to use resources to reduce variability and improve efficiency. *Example:* Is it better to us a 4-point study to evaluate a response profile and ED50 with n = 8 mice per group OR a 5-point study with n = 6 mice per group? Use cross-over designs; use fractional factorial designs.

7. **Use Dunnett's test** to compare treatment to control means. Controls the false positive rate with more power than Tukey's test; in fact a ***planned linear contrast*** in often better than a multiple comparison test used naïvely.

8. **Repeat samples from same animals** Obtain larger arrays of parameters from same animal; better techniques. Design for *repeated measures*, and analyze the data accordingly (use a mixed effects model).

9. **Use the *Statistics Section* of papers from your literature search** Most well written papers from the literature define their statistics clearly and provide clear summaries of the data and the analysis results.

10. **Use a one-sided test statistic** Using a one-tailed test to detect either an increase or decrease, but not both, (what is a two-sided test?) will reduce sample size. Requires some anticipation of response outcome direction May also preclude unexpected opposite outcome.

11. **Use a Trend Analysis** Designing a study to detect a trend will reduce sample size and provide a more precise estimate of the *dose response profile.* Assumes response monotonicity and use of an appropriate proper trend statistic.

12. **Use noninvasive methods** Imaging or thermal method may improve accuracy of response detection, thus impacting routine sacrifice. Use in vitro embryonic or cell cultures. Sub-cellular assays; DNA array; *simulations !*

13. **Do a Pilot Study** If successful, base the sample size for the large study on the variability of the response variables of interest from the pilot.

14. **Curtail artificial inflation of sample** The practice of artificially inflating animal number orders is usually a function of not bothering to calculate actual need. Typically for an order of, say, "1,000 rats" - when really it's 975 rats. Then annual totals will not add up. Need extras ? then document that number based on prior loss rates.

15. **Report the number animals in a table** Detail the breakdown by the number of animals per treatment group (including controls), the number of groups, the number of studies. These should all sum clearly to the total number anticipated for the annual project and be reported accordingly. Less room for arithmetic errors or ambiguity here.

Sample Size	Groups	Treatments	Studies		Total
5	4	10	4	=	800

* *Power* is the probability of not making a false negative; of detecting a significant effect, with the corresponding sample size, when it exists.