**Justification of Animal Numbers for Teaching, Research, and Testing Purposes:**

Studies should be designed to provide a statistically significant result with the minimum number of animals and the method of determination.

**Common types of research studies include:**

**Teaching Studies**: Sample size specified by student-to-animal ratio. Consideration of minimal animal numbers without impacting hands-on-teaching experience.

**Exploratory Study Requiring No Statistical Analysis**: (e.g. murine production colony): Animal sample size are justified based on the probability of success of the experimental procedure.

**Epidemiological Studies (intervention and observational):** Animal sample size calculations are used to estimate a population, test a hypothesis (intervention and observational), or obtain results that support the detection of an event.

**Behavioral Studies:**Animal sample size calculations depend on the type of animal behavioral study employed (e.g., field work vs. human-animal bond)

**Pilot Studies**: Animal numbers are determined by researchers experience and personal judgment. These animal numbers are generally small. Data obtained in pilot studies can be used to determine statistically relevant sample size calculations for future experiments.

**Note: Some studies may not fall into these categories**

**A key principle regarding ethical use of animals in research, teaching or testing include the 3Rs (reduction, replacement, & refinement).**

Animal number justification begins with a clearly stated, hypothesis-driven outline of the experimental design. **Principal investigator should consider the following**:

* Experiment’s purpose
* Number of experimental groups/subgroups by species/strain per group/subgroup
* Total number of control and experimental animals from the experimental design (Consider including a grid or flowchart)
* If tissue harvest is required, ensure that the relationship between the amount of tissue needed for the experiments is directly correlated to the number of animals required to produce that tissue
* Anticipated animal losses or removal due to morbidity, mortality or other expected difficulties with the experimental procedures. (Report to IACUC if Adverse Event)

**Statistical techniques for sample size calculation:**

* Power analysis: Most popular and researcher should have information and knowledge on the effect size, standard deviation, type 1 error, power, direction of effect, the appropriate statistical tests, and attrition or death of animal.
* Resource equation method: if statistical justification such as effect size, standard deviation is unable to be determined or if there are multiple factors involved.

**Studies Requiring Inferential Statistical Analysis**:

* Justification statement must include the values of alpha, beta, sigma, and effect size used in the power analysis to determine sample size.
* Alternatively, minimum numbers of animals may be determined based on pertinent literature for comparable studies in which the desired effect sizes were shown to be statistically significant.

**Consultation with a statistician or use of statistical software during the design phase of the experiment may be warranted**

**Websites helpful in determining appropriate sample size:**

<https://stats.oarc.ucla.edu/other/mult-pkg/seminars/intro-power/>

<https://epitools.ausvet.com.au>

<https://epitools.fp7-risksur.eu/tools/index?toolId=46>

<https://epidemiology.sruc.ac.uk/shiny/apps/samplesize/>

<http://statpages.org>

<https://arxiv.org/abs/1707.00222>

**References**

1. Public Health Service. (1996) U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and training. PHS Policy on Humane Care and Use of Laboratory Animals. Washington, D.C.
2. National Research Council. (2011) Guide for the Care and Use of Laboratory Animals, Eighth Edition. National Academy Press, Washington, D.C.
3. CFR (Code of Federal Regulations) (1985) Title 9 (Animals and Animal Products), Subchapter A (Animal Welfare). Washington, D.C.: Office of the Federal Register.
4. ILAR Journal Volume 55, Issue 3, 2014 - Experimental Design and Statistics <http://ilarjournal.oxfordjournals.org>
5. Erb, H.N. (1996) A non-statistical approach for calculating the optimum number of animals needed in research. Lab Animal, 45-49.
6. Festing, M. F. W. 2002. Introduction: the design and statistical analysis of animal experiments. *ILAR Journal* 43(4):191-193.
7. Mann, M.D., Crouse, D.A., Prentice, E.D. (1991) Appropriate animal numbers in biomedical research in light of animal welfare considerations. Laboratory Animal Science 41:6-14.
8. Mohan S, Foley PL. Everything You Need to Know About Satisfying IACUC Protocol Requirements. ILAR J. 2019 Dec 31;60(1):50-57. doi: 10.1093/ilar/ilz010. PMID: 31361817; PMCID: PMC7304469.

<https://pubmed.ncbi.nlm.nih.gov/31361817/>

1. <https://stats.oarc.ucla.edu/other/mult-pkg/seminars/intro-power/>
2. <https://www.frontiersin.org/articles/10.3389/fvets.2015.00016/full>
3. <https://www.frontiersin.org/articles/10.3389/fvets.2020.539573/full>
4. <https://www.uwo.ca/animal-research/doc/sample-size-in-animal-studies.pdf>