

The USDA has indicated that we have not been providing adequate justification for the number of animals used in experimental protocols (i.e., item C3 in the protocol application form). Typically, PIs have listed the number of animals used per group or treatment, but have not explained why these numbers are justified. Generally, the USDA does not consider it adequate to assert that the number of animals chosen is necessary to assure statistical significance. To address item C3 appropriately, please consider the following suggestions:

1. A survey of pertinent literature will usually identify studies that contain similar variables for which sample sizes were determined scientifically (i.e., using a statistical power analysis). A sample size at least as large as those used in these comparable studies may then be justified.
2. A power analysis may be used to justify group sizes. Three easily understood articles regarding determination of sample size when doing animal experimentation are cited below and are attached as PDF files.

Crouse, D.A., Mann, M.D., and E.D. Prentice (1995). **The logical determination of "n" in animal experimentation.** In *"Current Issues and New Frontiers in Animal Research*, K.A.L. Bayne, M. Greene, and E.D. Prentice, eds., Greenbelt, Maryland: Scientists Center for Animal Welfare, pp. 19-23.

Erb, H. (1996). **A non-statistical approach for calculating the optimum number of animals needed in research.** *Lab Animal* 25(3): 45-49.

Khamis, H.J. (1997). **Statistics and the issue of animal numbers in research.** *Contemporary Topic in Laboratory Animal Science* 36(2): 54-59.

3. A power analysis to determine sample size is not possible if you do not have an idea of the standard deviation of the experimental variables. In this case, under C3, you should identify the experiment as a pilot study and provide further explanation for the number of animals you require. For pilot studies, experience and judgment of the investigator are used to determine the number of animals. Generally, it is appropriate to use 4-8 animals per experimental group in a pilot study.