Help with Outline of Animal Use and Justification of numbers.

According to federal regulations the UCAR needs assurance that:

1) a sufficient number of animals is used to answer satisfactorily the scientific questions posed, and
2) an excessive number of animals is not used.

Provide the justification for all animals mentioned in the protocol for all 3 years, including all animals that will be bred.

- Fetuses are not included in the number of animals requested, but should be discussed here since the number of fetuses needed justifies the number of pregnant females needed.
- Do not break the justification down by year. The justification is for the total number of animals requested.
- **Do** include all animals that you will have, including all those that are bred, not just those used for experiments. This is particularly important if you must breed many more animals than you can use, either because you require a particular genotype for your experiments, or because you must have a specific sex and cannot use all the pups in a litter.
- For each group of animals, or set of experiments, indicate whether it is category (stress level) C, D or E.

The type of justification required depends on the animal work being done. Several examples are given below. In general, summary information and tables are the best way to convey this information. Please do not provide the entire Experimental Design section of your grant. Take into account that your response will be read by both scientific and non-scientific members of the committee. It is important to make it clear how you determined the number of animals you have requested. Please keep it as simple as possible and do not provide rationales for the studies, procedural details, or descriptions of assays to be done on tissue samples. All procedural details will be addressed in other parts of the protocol. Remember, if you would not want to read it, neither would the reviewers.

Parametric studies: In order for reviewers to evaluate animal numbers for these kinds of studies, you must provide them with the following:

An outline of the proposed experiments involving animals. Generally the information should include a listing of experimental and control groups, treatments, time points, etc. – in other words, a description of the experimental design (not procedural details).

A statement of how group size was determined. Power analysis is the gold standard here, and should be described if possible. Consultation with the Department of Biostatistics (275-0343) may be helpful in addressing this section, especially in consideration of the estimated sample size (by power analysis) required for a given level of statistical significance in a study. If a power analysis has not been

done, the basis for your decision about group size should be discussed. This might include previous experience with this type of measurement, published literature, limits on the number of samples that can be handled at a time, etc. The procedural details (e.g. the doses, routes of administration, etc.) should be provided in the procedure description, not here.

Example:

The proposed studies investigate the effects of CNS Bozoamine receptor blockers on the executive behavior (measured by the Enron behavior battery, AIG subset) of old and young male and female mice. The study will be done in duplicate so that tissue can be taken for chemical assays and histology. In all cases, group size will be 8 (estimating the variability of behavioral measures and similar behavioral studies in the literature (Felten et al. Effects of bozoamine of the brains of certain species of administrators, *Deanus Americanus and Chairmanus Horribilis*, (J MUS, 1:1-12, 1983:). These studies are category C.

n	Male	Female	8 weeks	18 months	Doses (0.1 – 100 mg/Kg)
8	+		+		6
8		+		+	6
8	+		+		6
8		+		+	6
					= 384

Alternately:

8n X 2 sexes X 2 ages X 6 doses X 2 assays = 384 mice

If you have a number of different experiments, a summary or table for each experiment may be the clearest way to provide the information. If you have a number of experiments, all with the same design, you might describe the design, then discuss how many different experiments will be done. Using the above example, you might state that the same design would be repeated for each of 8 different receptor blockers: 384 mice/ blocker X 8 blockers = 3072 mice.

Transgenic mice and studies done in a single sex: Studies done with transgenic mice often require the investigator to breed many more animals than are needed for the study in order to get the required number of mice with the correct genotype. This information should be included in your justification. Breeding pairs must also be included.

Example:

The proposed studies examine the effects of Bozoamine receptor knockout on the executive behavior in male mice. Only males will be used because previous studies have

determined that executive behavior is dependent on Bozoamine binding in males but not females.

Genotype	n	Assays	Total	Breeding Ratio
BoRKO	8	2	16	1:4
Wild type	8	2	16	
Total			32	

A total of 32 experimental animals will be required, of which 16 will be BoRKO males. The ratio of BoRKO:wt is 1:4, and males 1:2 therefore we expect approximately 1 of every 8 mice to have the appropriate genotype and sex. We must breed

16 (# transgenics needed) X 8 (4x2) = 128 mice

to obtain the required experimental animals. This breeding will also produce the required number of controls. Estimating 8 mice/litter, we will require 16 litters for a total of 128 pups + 16 breeding pairs (32 mice) = 160 mice.

Numbers determined by amount of tissue needed:

Often the determining factor for the number of animals needed is the amount of tissue or the number of cells that can be obtained from an animal. Such a justification would include;
       Estimate of the number of experiments/assays
       Estimate of how many animals would be required to produce tissue for each assay

Example:

We propose to isolate and culture Bozoamine expressing cells from the brains of wild type male mice for studies on transcription factors that regulate the expression of Bozoamine synthase. These dose response assays are run in triplicate and require $1x10^6$ cells/measurement. One brain yields $5x10^5$ cells or 2 brains/measurement.

2 brains/measurement x 6 transcription factors x 10 doses/factor x 3 repeats = 360 male mice needed for assays. Note that if mice are being purchased, the justification is complete, and the number requested would be 360. If animals are being bred, you would have to estimate the litter size, number/litter and add the breeders:

We estimate that each litter will produce 10 mice, of which 5 will be male, so to produce 360 male mice we would require 360/5=72 litters (720 mice). We anticipate that each breeding pair will produce 6 litters, so require 72/6=12 breeding pairs for a total of 744 mice (720 mice bred + 12 pairs).

An alternative approach might be

The proposed studies use mice as a source of cells for a large series of in vitro experiments associated with the expression and regulation of the *onno* gene. We anticipate doing 2 assays per week over the course of 3 years for a total of approximately 300 assays. Each assay requires cells from 1 neonatal (P01) mouse brain (2 per week). Because cells will last in culture only a few days, we will require a new litter each week. We will begin with 8 breeding pairs, then use mice not used for experiments to replace the retired breeders. 8 pairs + (150 litters x 8 mice/litter) = 1216 mice.

General principles:

When you are providing your justification keep in mind that it must be clear enough so that reviewers can follow your thinking. Tables and summaries help. Leave out experimental and procedural details. This is an outline that should give the reviewers a brief overview of the kinds of procedures the animals will experience and how many animals will experience them. You should make it clear how you determined the number of animals (show your work!). In addition, it is important to state how the size of each group was determined.