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How many animals?

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states that proposals to Animal Ethics Committees (AECs) must justify the number of animals required, and that this should be "the appropriate number of animals (neither too few nor too many)" (NHMRC, 1997). No specific guidance is given as to how this number should be determined. The aim of this article is to bring to the attention of AECs and investigators objective statistical methods for determining required animal numbers. While these methods may incorporate information from the experience and judgement of the investigators, this is done in a defined and transparent manner, so that all aspects of the process may be subjected to the scrutiny of the AEC.

The use of sequential sampling techniques has been discussed in a previous issue of *ANZCCART News* (Chamove, 1996). One of the problems that AECs must grapple with when dealing with sequential designs is that the number of animals to be used cannot be specified in advance. The methods described here allow the required number of animals to be calculated before the experiment is commenced.

Different methods of determining animal numbers are required for different experimental designs. In complex situations, such as experiments with multiple treatment groups or multiple, possibly interacting, treatments, the advice of a statistician should be sought during the planning stages. In simple cases, for example involving the comparison of two groups of equal size, approximate determination of the required numbers is relatively straightforward and may often be undertaken by the investigator. Two cases will be described here. The first is for continuous outcomes, in which the response of each animal can take one of many possible values, and it is desired to determine whether the outcome is affected by an experimental treatment. Blood pressure, serum enzyme concentrations and body weight are examples of continuous outcomes. In the second case, the outcome is dichotomous, i.e. some characteristic is either present or absent, and it is desired to determine whether the proportion of animals with the characteristic is affected by the treatment. Examples of dichotomous outcomes are cured vs not cured, infected vs not infected. Sometimes it is useful to convert a continuous outcome to a dichotomous outcome, e.g. blood pressure elevated vs not elevated.

1. Continuous outcomes: Student's 2-sample t-test

A common experimental design involving a continuous outcome compares the mean response of a control group of animals to the mean response of a treated group. If the data are to be analysed by Student's 2-sample t-test, the approximate required sample size can easily be determined. The investigator must specify the desired Type I error, the desired Type II error, the minimum treatment effect to be detected, and the variability of the data.

Type I error

The Type I error, or α, is the highest significance level which will be accepted as demonstrating a difference between treatments. This is commonly set at 0.05. A lower level, such as 0.01, might be preferred if the hypothesis to be tested is especially novel. A smaller Type 1 error will require more animals.

From a table of the standard normal distribution, a value Z_α is determined as the value which includes $(1 - \alpha)$ of the area under the normal curve. For a 2-tailed test with Type I error of 0.05, Z_α = 1.960, as 0.025 of the area falls above 1.960 and 0.025 falls below -1.960. For a 1-tailed test with a Type I error of 0.05, Z_α = 1.645, as 95% of the area falls below 1.645.

Type II error

The Type II error, or β, is the probability that the experiment will fail to detect a significant difference when there is, in fact, a true difference of the magnitude specified. The

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This issue contains the Facts Sheet on the Importance of non-statistical design in refining animal experimentation. The Facts Sheet is also available as an offprint.