Formulating a statistical analysis plan as part of the project proposal

To be properly assessed, a project proposal requires not only a research design, but also a general set-up of the statistical analysis plan, as it will provide a reliable estimate of the maximum number of animals needed. This gives a good idea of what efforts you are making to use as few animals as possible – the 'reduction' element of the three R's.

When designing scientific research, it is usual to not only think about the research set-up itself, but also how the final data will be analysed. The statistical analysis plan is also one of the principle ways to determine the minimum number of animals necessary. It is one of the three essential elements of a proposal, along with the research design and the research questions.

The CCD's guide to the forms to use when applying for a project licence ('*Guidelines to the project licence application form*') mentions the statistical analysis strategy, referring to the statistical analyses and to determining the maximum number of animals needed. This indicates that the main emphasis is on your strategy, and that you need not necessarily elaborate every detail.

The statistical analysis plan

The statistical analysis plan contains a rough outline of the analyses, such as whether you plan to use parametric or non-parametric tests. The plan also states whether the analysis consists of comparing the averages of the experimental groups (and with what, if any, post hoc tests), looking at the connection between continuous or categorical variables or another kind of analysis.

Thus, it is important to provide an idea of how the data will ultimately be analysed, in order to be able to verify that the research design will answer the research questions. The minimum sample size is also determined on the basis of statistical analysis. If possible, the minimum sample size is determined using an estimate of the minimum relevant effect size, the desired power and the distribution of the data.

Of course, a plan may deviate from the final analysis. For example, the plan may call for a parametric test, while in the final analysis, the data may show that a non-parametric test is better for the final analysis.

Determining the sample size

An important element of the research design is the minimum number of animals necessary to conduct it. The goal is to find an optimum: no more animals than necessary, but not too few (which gives less predictive value, and too little power). For parametric tests this optimum number can be determined using the power analysis.

The following are needed for the power analysis when you determine the minimum number of animals required (the sample size (n)):

1) the type of statistical analysis to be conducted (in other words, what is the design);
2) one- or two-tailed tests;
3) probability of a type I error (α);
4) desired power (Π);

5) minimum relevant or expected effect (δ) and expected distribution or if applicable, standard deviation (σ).

The main problem is often setting the minimum relevant or expected effect and the expected distribution. When determining the minimum relevant effect, it is useful to ask at what point an effect is no longer interesting or relevant to practice ('clinical relevance'). To get an indication of the expected distribution, you can refer to a pilot study, literature study or general knowledge.

It is sometimes impossible to indicate the minimum relevant effect size or the expected distribution. In that case, the minimum number of animals required cannot yet be definitively determined, and it will suffice to use Cohen's effect size (ratio of effect size to distribution) to realistically estimate the number of animals required. However, it is recommended that you do attempt to arrive at a realistic estimate of the desired effect and distribution.

A power analysis is based on normally distributed continuous data. If you know beforehand that your data will not satisfy this requirement, you will have to estimate the number(s) of animals another way.

It is also wise to keep in mind pilot studies, training employees, validating techniques and/or the potential loss of animals when estimating the maximum number of animals needed. In principle it is not necessary to determine sample size for a pilot study, but it is important that enough observations are made that reliable information can be generated. The Cohen's effect size can also be used but is not standard practice.

The CCD and the statistical analysis plan

The CCD's guide asks about strategy in two places:

1. *In the Non-technical summary* (Niet-technische samenvatting)*, Section 4.2 Reducing:* Indicate how you will ensure that no more animals will be used than are needed to acquire meaningful results. An indication of the general strategy that will be followed (e.g. statistical analysis) is sufficient. Do not elaborate on the details of the analysis itself. (*'*Describe which measures have been taken to ensure that the number of animals used in this project is kept to a minimum. If applicable, relevant information on the general research strategy (conjunction of the individual procedures and the necessity of pilot procedures) may be provided here. It is sufficient to indicate which general strategy will be used for the statistical analysis. Do not describe the statistical analysis in detail.*'*)
2. *In annex on animal experiments, Section A- Experimental approach and primary outcome parameters* (bijlage dierproeven, Paragraaf A- Experimenele aanpak en primaire uitkomstparameters): *'*Both statistical and non- statistical considerations can be used as grounds for the maximum number of animals required. If you cannot use statistical grounds, indicate why not, as well as what other considerations you used for determining the number of animals to be used. Thus the main question is about your strategies and not, for example, power analysis for individual experiments.' (*'*To justify the maximum number of animals required for each type of animal procedure, both statistical and non-statistical considerations may be included. If it is not possible to use statistical calculations, explain why this is not possible and describe which other considerations were taken into account to determine the number of animals. In this section, it is not necessary to provide detailed information on power analysis for individual experiments.')

Thus, the CCD is indicating that although you do need a plan for your statistical analysis, it may be a general one. The statistical analysis plan must be clear enough that there can be no longer be any argument about the type of analysis itself when the research plan is further worked out. There can still be discussion, however, about things like exactly which groups are being defined, or, when further working out the plan, about whether the test will be parametric or non-parametric.

A reliable estimate of the final maximum number of animals needed can be made based on the preliminary estimate of the minimum number of animals required, the number of animals required for a pilot study (if applicable), the expected percentage of potential loss of animals and other factors. After approval by the CCD, the research proposal in the work protocol can be further elaborated in consultation with the Animal Welfare Body Utrecht (*Instantie voor Dierenwelzijn Utrecht*).

Elements necessary for assessing your application

The following elements of your application are important for the proper assessment of your statistical analysis plan:

1. What are the research questions? Make a distinction between primary and secondary research questions.
2. What is the design of the animal experiment? Think about what you are going to measure, how you are going to measure it, what animals you need, whether it will be a paired or unpaired design, how you are organising the randomisation and blinding, how you will analyse the data afterwards, what practical requirements there are, etc.
3. How are the data going to be analysed? In other words, what is the statistical analysis plan? Think of what results you will want to describe, test or model; what hypotheses you intend to test; what statistical analysis you intend to use; whether you intend to do one- or two-tailed tests, etc.
4. What is the minimum number of animals you need? Determine this using the design and the statistical analysis plan. Think about whether the determined sample size may result in any practical problems. If necessary, modify the design, statistical analysis plan or the power analysis.

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Websites

The following website has a great deal of information on the above subjects: www.3Rs-reduction.co.uk. You can also test your own knowledge with its self-test.

Literature

Van Zutphen, L.F.M. et al., (2012) Handboek proefdierkunde, proefdieren, dierproeven, alternatieven en ethiek, Springer Media B.V.
This book contains basic information (in Dutch) about designing an animal experiment.

Bate, S.T., Clarke, R.A., (2014) The design and statistical analysis of animal experiments
This book contains basic information about designing an animal experiment and information about the statistics you can use for the analyses.

Lara-Pezzi, E et al., (2015) Guidelines for Translational Research in Heart Failure. J. of Cardiovasc. Trans. Res. 8(1)
An article in which the main focus is on the translation of models for heart failure in animals and effective suggestions for designing similar studies. Although the focus is on heart failure, the information is also suitable for other fields.

Steward, O., Balice-Gordon, R. (2014) Rigor or mortis: best practices for preclinical research in neuroscience. Neuron. 84(3)
This article discusses best practices in experimental design and statistics in preclinical studies in the field of neurological and psychiatric disorders. It also addresses data management. Although the focus of the article is on neurological and psychiatric disorders, the information is also suitable for other fields.

Festing, M. F. W., Altman, D.G., (2002) Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals. ILAR 43(4)
This article helps you answer your research questions step by step using different kinds of experiments. It shows you how to prevent errors and gather meaningful data. Written with the use of animals in research in mind, it emphasises the 3 R's and good statistical analysis.

Aban, I.B., George, B., (2015) Statistical considerations for preclinical studies, Exp. Neurol. 270
This article discusses statistical concepts, with improving the quality of animal studies as its goal. This article was specially written for people using animals in preclinical studies, so that the data can be appropriate as preparation for the clinical phase of research.

Tweel, I. van der (2006) Sample size determination. Intern Report nr 4
(http://portal.juliuscentrum.nl/Portals/2/Disciplines/Biostatistics/SAMPLE%20SIZE%20DETERMINATION_electronic%20version.pdf)
This report explains the simplest way to determine sample size.