

# Responsible Research in Planning for Animal Experiments – A Mini Review

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## ABSTRACT

Animal experiments are routinely carried out worldwide either in educational setups or in research setups. Animal research can be basic or applied, where in the applied research the test compound/drug is tested in animals for its safety and efficacy before going into clinical trials. Planning for conducting *in vivo* animal experiments requires certain preliminary behind-the-scene preparations. These background preparations form the foundation for a valid and reproducible animal experiment. But often these background preparations are overlooked while planning for experiments leading to poor quality, translatability and reproducibility of animal studies. Even though many reporting guidelines are available, there is very little guidance on how to plan for animal experiments. Without better planning, better reporting is not going to help in producing valid research results. Better Planning, careful execution and proper reporting are all essential for good research. Hence, this paper discusses about the steps to follow

while planning an animal experiment to produce a valid, reproducible and translatable results known as responsible research.

**Keywords:** Guidelines, Planning, Animal research, Animal experiments, Prepare.

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## INTRODUCTION

Laboratory animals are commonly used to improve the knowledge about human and animal biological processes, to test the safety and efficacy of new drug products, and to test biological and chemical compounds. Translatability and validity of a study entirely depends upon well planning and careful execution of research keeping in mind all the internal and external variables that could affect the research data or outcome. In animal research, the biggest source of variation will come from the animals themselves. Recently, animal lovers and scientists are criticizing the quality of animal research by using harsh terms like 'research waste', 'false results' etc. Nowadays there is widespread concern about the lack of reproducibility and translatability of laboratory animal research, which is the main reason for the failure of drugs in human trials. It is undeniable that quality of experimental results starts from proper planning and conducting, not on reporting alone. Apart from 3Rs (Replacement, Reduction, and Refinement) of animal experiments, 3S (Good science, Sense, and Sensibility) also needs to be followed for better research outcomes. So far, very few guidelines are available for planning of animal experiments.<sup>1</sup> It is the need of the hour to properly plan animal experiments and maximize the quality and reliability of published research, enabling others to better scrutinize, assess, and reproduce the results.

### What is Responsible Research?

Responsible research is a broad concept, including everything from conflict of interest to reproducibility to data management. It can be described in simple words as the conduct of daily research operations in a reliable way.<sup>2</sup>

### The Need for Responsible Animal Research

Most of the past scientific achievements were made possible with animal research, and majority of scientists agree that the use of animals in research is vital for the progress in biomedical and behavioral research. However, some public members and a few scientists voice out their doubts about the moral justification for the use of animals in research. It is a widely known truth that healthy animals maintained in ideal conditions produce the most reliable data, whereas compromised welfare negatively affects their physiology, immunology, and behavior leading to skewed and misrepresented results.<sup>3,4</sup> In contrast to test-tube ingredients, animals are complex beings, varying in their genetic make-up, microbial composition, and behavioral responses to their environment and procedures to which they are subjected. As the repeatability and reproducibility of experiments are influenced by the variables in animal care and health practices, standardization of such practices will help in reducing variability.<sup>4</sup> Certain experimental variables like housing conditions (temperature, lighting, humidity, noise levels, feed, bedding, etc.), source of animals, genetic background, and their health status (disease status, gut microflora, etc.) can be standardized to some extent.<sup>4</sup> Quality cannot be achieved automatically: it requires detailed planning from the beginning taking into consideration the effects of both the internal and external parameters which affect the animals' response to a procedure. In addition, the animal facility should have many routine procedures, both to maintain a stable environment and to take care of any unexpected emergencies. Most of the scientists do not work on a regular basis within an animal facility and hence they may not be aware of the number and subtlety of many of these factors which could affect their

research results. Thus the input from the facility's veterinarian is crucial to this process. Responsible research covers everything from designing proper animal experiments to better execution, data collection, analysis and reporting. Thus, responsible research has an important role in increasing the validity and credibility of animal research results.

Norecopa is Norway's National Consensus Platform for the advancement of 'the 3 Rs' in animal experiments. In their website, PREPARE guideline is displayed to help the researchers improve the quality of their animal experiments. PREPARE has identified three broad areas which determine the quality of animal studies: Formulation of the study, Dialogue between scientists and the animal facility, and Methods. More details could be accessed from their website at <https://norecopa.no/PREPARE>.

A guideline for reporting animal studies is called ARRIVE guidelines. The purpose of this guideline is to ensure that animal studies are reported in enough detail to add to the existing knowledge base. It guides the researcher throughout the study course – during study planning, during conducting a study, during writing a manuscript and during reviewing the manuscript. More details could be accessed from their website at <https://arriveguidelines.org/about>.

## BASIC STEPS IN PLANNING A RESEARCH EXPERIMENT

The following list enlists some basic steps while planning any research experiment:

1. Formulating a research question
2. Writing your hypothesis
3. Identifying and defining the variables (both dependant and independent) and finding how they are related: An independent variable is the cause, while a dependent variable is the effect. Defining the variables, and deciding how to manipulate and measure them, is an important part of experimental design. Even temperature, barometric pressure, time of day, etc., are variables.
4. Designing the experimental treatments to manipulate your independent variable: Experimental design can be defined as planning a set of procedures to investigate the relationship between variables. Experimental design is essential to the validity of your experiment.
5. Assigning subjects to treatment groups: Randomization. An experimental group or a treatment group receives the treatment, whose effect researchers wish to study, whereas a control group does not. Both groups should be identical in all other ways.
6. Measuring your dependant variables: In experimental research, the internal and external validity of your experiment should be considered.

### Additional Considerations while Planning Animal Experiments

Apart from the steps listed in planning a research experiment, animal experiments need additional considerations as listed below:

1. Literature review – Use search engines (PubMed, Embase, etc) to collect relevant articles, do a systematic review, collect data from your review and do analysis, also collect toxicity data of test compounds to be used in your experiments, so that you will be sure that the test compound is not going to kill your animals. If this data is not available, it is advised to perform *in vitro* toxicity tests to confirm the safety of your test compound before *in vivo* studies.
2. Selecting the appropriate animal model – identify the best animal model for your research problem through literature review. Select a model keeping in mind the 3Rs, practicability, and the resource

availability in your animal facility. While selecting animal models, the primary goal is to use a model system that gives reliable and valid data with minimal confounding variables. The study plan should contain details about the experimental variables being tested, the testing parameters, and testing methods to be used in the study.<sup>5</sup>

3. Interaction with the animal facility veterinarian and support staff – find out about the available resources, instruments availability, and feasibility of the experiment in your research setup. Plan along with the veterinarian about the procedures to be followed in the animals, such as housing, feed, sample collection routes, methods, etc, keeping in mind the welfare of the animals because only well cared and stress-free animals yield better research results.
4. Designing the animal experiment – calculate the sample size (n) needed for the experiment using any of the three approved methods like 'tradition' or 'common sense', the 'resource equation' and the 'power analysis' method.<sup>6,7</sup> The commonly used notion of six animals per group does not have any statistical or scientific basis.<sup>8</sup> If the effect size and standard deviation could be found out from previous studies, then use power analysis method. If both are unknown and it is an exploratory study use resource equation method which uses ANOVA for calculation of sample size.<sup>7</sup> Follow randomization by assigning animals to groups randomly using softwares (e.g., Graph Pad, etc.) or Excel sheet to avoid bias. Depending upon the experimental objective, test groups, control groups and comparator groups (negative or positive) are to be assigned. A pilot study, which is designed to test the feasibility of a procedure, may also not need a control group. 'Experimental Design Assistant (EDA)' is a free online tool from the NC3Rs.<sup>9</sup> It guides a researcher on how to design their experiments – by using minimum number of animals in line with their scientific objectives, by following methods to reduce subjective bias, and by using suitable statistical methods. Animal use can be reduced by good experimental design in two ways:
  - (i) A properly designed experiment will give robust and reproducible data by taking care of the influence of variables and sources of bias, ensuring that data obtained from every animal is used to its full potential.
  - (ii) It is possible to reduce the number of animals required and maximize the information obtained per experiment using proper statistical methods. Researchers can frame complex designs to identify factors which influence the experimental results, thus obtaining more detail about the model they are using.<sup>9</sup>
5. Getting Animal Ethics Committee approval – Fill the Form B by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals, India) answering all the questions including the species used, number of animals to be used, their days of housing, grouping, experimental protocol, surgical procedures to be followed, route and amount of test compound to be administered to each animal, route and amount of blood collection, fate of animals after the experiment, etc. and get IAEC (Institutional Animal Ethics Committee, India) approval. The name given to such committees varies in different countries: Regional Ethics Committees in Sweden; Animal Ethics Committees in Australia and New Zealand; Institutional Animal Care (or Animal Care and Use) Committees in Canada and USA; Animal Welfare and Ethical Review Body (AWERB) in UK; etc.<sup>10</sup> No research on animals is legal without proper approval from animal ethics committee.

6. Procuring the needed chemicals, drugs, instruments and other items before starting animal experiments. Standardize any techniques or chemicals to be used beforehand and do the necessary calculations prior to starting the experiments.
7. Procuring animals from CPCSEA approved breeders (in India) with proper supporting documents.
8. Provide proper quarantine and acclimation time for the animals before beginning the experiments. Check their microbiological and health status before issue to experiments. Some facilities may follow deworming procedures during quarantine.
9. Perform the experiment within the stipulated time as mentioned in the approved protocol. Record any minor deviations during the study and include as amendment in the approved protocol. Major deviations should be included in the IAEC protocol (Form B) and approved by IAEC before beginning the experiment. Clearly know about the humane endpoints (animals that are moribund or obviously in pain and showing signs of severe and enduring distress) and terminate the suffering of animals by euthanasia. More details about humane endpoints can be referred in OECD guidance document No.19.<sup>11</sup> Pain assessment methods like facial grimace scoring,<sup>12</sup> general appearance and behavior of the animal should be followed when the experimental protocol involved painful procedures. No animal should be allowed to unnecessarily suffer in the name of research. Humane endpoints should be clearly mentioned in the research protocols involving painful procedures and it is the responsibility of researcher and facility veterinarian to ensure them.
10. Data collection and proper reporting – collect all the necessary data from animal studies and preserve the raw data intact even after publishing.
11. Publish with all the details including animal details (age, sex, strain, body weight), husbandry details (temperature, humidity, photoperiod, feed type, water, housing etc.), inclusion and exclusion criteria followed, IAEC approval number, power of the experiment, sample size justification, randomization and grouping details, details of drugs, chemicals or plant extract used, sample collection methods followed, statistical tests used, etc. It is better to publish negative results too as they will guide the other researchers to focus their research in the right direction.

## CONCLUSION

During the past few decades, a fierce debate is going on between the scientists who do animal experimentation and patient organizations hoping for new treatments on one side and animal welfare groups on the other regarding the use of animals in basic research and in testing of potential new therapeutics. Even though most people accept the benefits of animal experimentation, some ardent and militant groups demand a complete ban on animal use. The question of how to protect animals from being used for unnecessary experiments and of how to alleviate their suffering remains a valid, sensitive and controversial issue for science, society and politics. It is critical to achieve a uniformly high standard for the care and use of animals in experimental research. All experiments

should be designed to avoid distress and unnecessary pain and suffering to the experimental animals. Due to the growing concern of society in general regarding the treatment of animals not only in research, but also in farming and agriculture, it is becoming increasingly important to address the ethical issues involved and to find a solution that will both benefit society and address the needs of the scientific community. In animal research, responsibility is linked to providing reassurances about how animals are used and cared for during the research process.<sup>13</sup> Doing responsible research lies in the hands of every researcher who has to feel the need for producing better research results following the ethical guidelines for use of animals. Every animal researcher should understand that only well cared, happy and stress-free animals produce better research results that can be translated to human subjects.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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