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PREPARE: guidelines for planning animal research and testing

Adrian J Smith¹, R Eddie Clutton², Elliot Lilley³,
Kristine E Aa Hansen⁴ and Trond Brattelid⁵

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Abstract

There is widespread concern about the quality, reproducibility and translatability of studies involving research animals. Although there are a number of reporting guidelines available, there is very little overarching guidance on how to *plan* animal experiments, despite the fact that this is the logical place to start ensuring quality. In this paper we present the PREPARE guidelines: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence. PREPARE covers the three broad areas which determine the quality of the preparation for animal studies: formulation, dialogue between scientists and the animal facility, and quality control of the various components in the study. Some topics overlap and the PREPARE checklist should be adapted to suit specific needs, for example in field research. Advice on use of the checklist is available on the Norecopa website, with links to guidelines for animal research and testing, at <https://norecopa.no/PREPARE>.

Keywords

guidelines, planning, design, animal experiments, animal research

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Introduction

The quality of animal-based studies is under increasing scrutiny, for good scientific and ethical reasons. Studies of papers reporting animal experiments have revealed alarming deficiencies in the information provided,^{1,2} even after the production and journal endorsement of reporting guidelines.³ There is also widespread concern about the lack of reproducibility and translatability of laboratory animal research.^{4–7} This can, for example, contribute towards the failure of drugs when they enter human trials.⁸ These issues come in addition to other concerns, not unique to animal research, about publication bias, which tends to favour the reporting of positive results and can lead to the acceptance of claims as fact.⁹ This has understandably sparked a demand for reduced waste when planning experiments involving animals.^{10–12} Reporting guidelines alone cannot solve the problem of wasteful experimentation, but thorough planning will increase the likelihood of success and is an important step in the implementation of the 3Rs of Russell & Burch (replacement, reduction, refinement).¹³ The importance of attention to detail at all stages is,

in our experience, often underestimated by scientists. Even small practical details can cause omissions or artefacts that can ruin experiments which in all other respects have been well-designed, and generate health risks for all involved. There is therefore, in our opinion, an urgent need for detailed but overarching guidelines for researchers on how to plan animal experiments which are safe and scientifically sound, address animal

¹Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750, Sentrum, Oslo, Norway

²Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, UK

³Research Animals Department, Science Group, RSPCA, Southwater, Horsham, West Sussex, UK

⁴Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway

⁵Division for Research Management and External Funding, Western Norway University of Applied Sciences, Bergen, Norway

Corresponding author:

Adrian Smith, Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway.

Email: adrian.smith@norecopa.no

welfare and contain links to the best guidance available on more specific topics.

The purpose of this paper is to provide *planning* guidelines, fulfilling a comparable role to *reporting* guidelines such as ARRIVE¹⁴ and others.^{15–19} We have called them PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence). They are designed to be applicable to *all* types of animal research and testing, including field studies, but they also contain topics concerning the management of animal facilities, since in-house experiments are dependent upon their quality. Some elements will be more relevant than others, but experimental bias and inappropriate statistical methodology are frequent causes of poor study design. PREPARE seeks to address the needs of all stakeholders: the animals, their caretakers and animal technologists, technical staff, scientists and designated responsible persons, including named veterinarians, training and competency officers and facility managers. PREPARE should also prove helpful for those evaluating proposals for animal studies, including funding bodies, ethical review boards, national committees and regulatory authorities. A more detailed discussion of these guidelines, with links to global resources is available at <https://norecopa.no/PREPARE>. A comparison between the ARRIVE and PREPARE checklists may also be found there.

The PREPARE guidelines cover 15 main topics as shown in Table 1.

The guidance in this paper should be adapted to the individual research project, animal species and location. The topics in the checklist in Table 1 will not be relevant to all projects, some topics overlap, and they may have to be addressed in a different order to that in the table.

Division of labour, costs and responsibility

Some elements will be the responsibility of the animal facility itself, rather than the individual research group, since they determine the standard of the facility as a whole. However, a research project often raises questions which are not covered by the facility's normal work routines. These include activities which have potential health and safety risks. Early and open dialogue between the facility and research group, to create a good atmosphere for collaboration, is therefore essential. For example, if a facility cannot safely conduct an experiment without structural changes or investment in new equipment, this should be discussed with the research group at an early stage, however tempting it may be to start collaboration on a prestigious project. Animal welfare and ethics committees can be a useful forum for some of this dialogue.²¹ A set of general planning guidelines such as PREPARE can be used to

help formulate a contract between the research group and the facility. This ensures prior agreement on two significant practical issues: the parameters to be recorded during the study, and the division of labour and costs between the facility and the research group. Failure to do this may result in lost data, making it impossible to publish the research findings, adding to the waste of resources and animal lives. An example of a contract based upon the PREPARE guidelines is given on the website.

The relationship between PREPARE and other guidelines

Over recent years, guidelines have been produced on many subjects related to the use of research animals including harm–benefit assessment, study design, capture, transport, breeding, housing, identification and marking, administration of substances, blood sampling, anaesthesia and analgesia, surgery, humane endpoints and humane killing.

The PREPARE guidelines build upon guidance that was developed at the Norwegian School of Veterinary Science over a 20-year period,²² and they are intended to be an overarching set of recommendations to promote good practice. A comprehensive and curated global list of individual guidelines, databases, information centres and discussion forums can be found on Norecopa's website in the 3R Guide database (<https://norecopa.no/3r-guide-database>), which is linked to the online version of PREPARE.

The European Union (EU) Directive 2010/63 refers to guidelines for education, training and competence, and for the housing, care and use of research animals.²³ Guidance documents from the European Commission, endorsed by the Member States, are a valuable source of information on these topics,²⁴ and may also prove to be useful to non-EU countries. For example, Appendix 1 of the Guidance on Project Evaluation and Retrospective Assessment contains preformulated questions for building a project application template, including harm–benefit assessment.²⁵ These topics have been embedded in PREPARE.

The relationship between PREPARE and ARRIVE

The speaker notes for ARRIVE²⁶ state that they 'provide a logical checklist with all the things that need to be considered when designing an experiment'. There are, in our experience when planning animal research, a number of additional points which need to be addressed at the planning stage, but which are easily overlooked or dismissed as unimportant. This was our motivation for the construction of the PREPARE guidelines.

Table 1. The PREPARE guidelines short checklist.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	<input type="checkbox"/> Form a clear hypothesis, with primary and secondary outcomes. <input type="checkbox"/> Consider the use of systematic reviews. <input type="checkbox"/> Decide upon databases and information specialists to be consulted, and construct search terms. <input type="checkbox"/> Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. <input type="checkbox"/> Assess the reproducibility and translatability of the project.
2. Legal issues	<input type="checkbox"/> Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. <input type="checkbox"/> Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, harm–benefit assessment and humane endpoints	<input type="checkbox"/> Construct a lay summary. <input type="checkbox"/> In dialogue with ethics committees, consider whether statements about this type of research have already been produced. <input type="checkbox"/> Address the 3Rs (replacement, reduction, refinement) and the 3Ss (good science, good sense, good sensibilities ²⁰). <input type="checkbox"/> Consider pre-registration and the publication of negative results. <input type="checkbox"/> Perform a harm–benefit assessment and justify any likely animal harm. <input type="checkbox"/> Discuss the learning objectives, if the animal use is for educational or training purposes. <input type="checkbox"/> Allocate a severity classification to the project. <input type="checkbox"/> Define objective, easily measurable and unequivocal humane endpoints. <input type="checkbox"/> Discuss the justification, if any, for death as an endpoint.
4. Experimental design and statistical analysis	<input type="checkbox"/> Consider pilot studies, statistical power and significance levels. <input type="checkbox"/> Define the experimental unit and decide upon animal numbers. <input type="checkbox"/> Choose methods of randomization, prevent observer bias, and decide upon inclusion and exclusion criteria.
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	<input type="checkbox"/> Arrange meetings with all relevant staff when early plans for the project exist. <input type="checkbox"/> Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. <input type="checkbox"/> Discuss and disclose all expected and potential costs. <input type="checkbox"/> Construct a detailed plan for division of labour and expenses at all stages of the study.

(continued)

Table 1. Continued

Topic	Recommendation
6. Facility evaluation	<input type="checkbox"/> Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs.
7. Education and training	<input type="checkbox"/> Discuss staffing levels at times of extra risk. <input type="checkbox"/> Assess the current competence of staff members and the need for further education or training prior to the study.
8. Health risks, waste disposal and decontamination	<input type="checkbox"/> Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study. <input type="checkbox"/> Assess, and if necessary produce, specific guidance for all stages of the project. <input type="checkbox"/> Discuss means for containment, decontamination, and disposal of all items in the study.
(C) Quality control of the components in the study	
9. Test substances and procedures	<input type="checkbox"/> Provide as much information as possible about test substances. <input type="checkbox"/> Consider the feasibility and validity of test procedures and the skills needed to perform them.
10. Experimental animals	<input type="checkbox"/> Decide upon the characteristics of the animals that are essential for the study and for reporting. <input type="checkbox"/> Avoid generation of surplus animals.
11. Quarantine and health monitoring	<input type="checkbox"/> Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.
12. Housing and husbandry	<input type="checkbox"/> Attend to the animals' specific instincts and needs, in collaboration with expert staff. <input type="checkbox"/> Discuss acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing).
13. Experimental procedures	<input type="checkbox"/> Develop refined procedures for capture, immobilization, marking, and release or rehoming. <input type="checkbox"/> Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, reuse or rehoming	<input type="checkbox"/> Consult relevant legislation and guidelines well in advance of the study. <input type="checkbox"/> Define primary and emergency methods for humane killing. <input type="checkbox"/> Assess the competence of those who may have to perform these tasks.
15. Necropsy	<input type="checkbox"/> Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

A more detailed discussion, with references and links, is available at <https://norecopa.no/PREPARE>, together with a downloadable pdf version of this checklist in several languages.

Attention to detail not only helps promote excellent study quality and optimal animal welfare, but also the safety of humans and animals affected directly or indirectly by the work. Particular considerations highlighted in the PREPARE guidelines, which are not so prominent in

the ARRIVE guidelines, include a harm–benefit assessment; health risks, waste disposal and decontamination; quarantine and health monitoring; the use of humane endpoints; the fate of the animals (humane killing, release, re-use or re-homing); and necropsy.

A Swiss study indicates that journal endorsement alone does not ensure guideline use: half of the researchers who had last published in a journal endorsing ARRIVE had never heard of the guidelines.²⁷ Emphasis on reporting guidelines in the EU Commission's Guidance on a common education and training framework²⁸ and in recommendations produced by other authorities will hopefully improve this situation.

Concluding remarks

It is our hope that the PREPARE guidelines will draw scientists' attention to the wide range of factors which require consideration at the planning stage. This should lead to an increase in scientific validity, reproducibility and animal welfare. Improving the quality of publications will also facilitate systematic reviews, thereby generating new knowledge through the synthesis of evidence, without the use of animals.^{29,30}

Planning guidelines have greater potential than reporting guidelines for assisting funders, regulators and ethical review committees in the assessment of applications for new projects. We therefore propose that funders make adoption of the principles in PREPARE or similar guidelines a condition of funding.

As with the ARRIVE (reporting) guidelines, the PREPARE (planning) guidelines are neither meant to be mandatory, absolutely prescriptive, nor a standard formula. Biomedical subspecialties may find it useful to produce their own supplementary guidelines, such as Australian scientists have done for osteoarthritis research³¹ and the STAIR conferences³² for stroke models. The Strategic Planning Poster from FRAME (Fund for the Replacement of Animals in Medical Experiments) provides a flowchart with good general advice on planning animal research.³³ PREPARE is designed to provide a detailed, universally relevant checklist which reduces the risk of problems, artefacts or misunderstandings arising once studies have begun. Furthermore, it can serve as the basis for a contract for the distribution of labour between the animal facility and research group. This will also reduce the risk of the researchers being unable to respond to journals' requests for more observations in an experiment, which can lead to manuscript rejection, wasting both animal lives and human resources.

'It is perfectly true, as philosophers say, that life must be understood backwards. But they forget the other proposition, that it must be lived forwards.' (Søren Kirkegaard 1813–1855)³⁴

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Early versions of these guidelines were inspired by the pioneering work of Öbrink and coworkers.^{35,36} An earlier version of the contract between researchers and the animal facility

was published in compendia in *Laboratory Animal Science* produced by the Norwegian School of Veterinary Science.²² We thank Anton Krag, Norwegian Animal Protection Alliance, Oslo; Dr Ute Weyer, Animal and Plant Health Agency, UK; Professor Malcolm Macleod, Centre for Clinical Brain Sciences, University of Edinburgh, and the referees for their valuable advice during the preparation and review of this paper.

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Résumé

Il existe de nombreuses inquiétudes au sujet de la qualité, de la reproductibilité et de la traduisibilité des études impliquant des animaux de laboratoire. Bien que de nombreuses orientations en matière de reporting soient disponibles, il existe très peu de principes directeurs sur la manière de *planifier* les expérimentations animales, malgré le fait qu'il semble logique d'étudier cette question pour pouvoir assurer la qualité des expériences. Dans cet article, nous présentons le document intitulé « PRÉPARATION : Lignes directrices pour la planification de la recherche et des procédures d'expérimentation animale : Recommandations en matière d'excellence ». Le document « PRÉPARATION » couvre les trois principaux domaines qui déterminent la qualité de la préparation des études menées sur les animaux : l'élaboration des études, le dialogue entre les scientifiques et le laboratoire animal, et le contrôle de la qualité des différentes composantes de ces études. Certains sujets peuvent se recouper et la check-list du document « PRÉPARATION » doit donc être

adaptée en fonction des besoins spécifiques, par exemple pour les travaux de recherche sur le terrain. Des conseils sur l'utilisation de la check-list sont disponibles sur le site Internet de Norecopa <https://norecopa.no/PREPARE>, qui inclut notamment des liens vers les lignes directrices relatives à la recherche et à l'expérimentation animales.

Abstract

Bedenken zu Qualität, Reproduzierbarkeit und Übertragbarkeit von Studien mit Versuchstieren sind weit verbreitet. Es existieren zwar verschiedene Berichtsleitlinien, doch allgemeingültige Richtlinien bezüglich der *Planung* von Tierexperimenten gibt es kaum – trotz der Tatsache, dass dies der logische Ausgangspunkt für die Gewährleistung von Qualität ist. In diesem Dokument präsentieren wir die PREPARE-Richtlinien: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (Planung von Forschung und Versuchen mit Tieren: Empfehlungen für Excellence). PREPARE berücksichtigt die drei umfassenden Bereiche, die die Qualität der Vorbereitung von Tierstudien bestimmen: Erarbeitung, Dialog zwischen Wissenschaftlern und Tiereinrichtung sowie Qualitätskontrolle der einzelnen Komponenten der Studie. Dabei überlappen sich einige Themen, und die PREPARE-Checkliste sollte an die konkreten Erfordernisse angepasst werden, zum Beispiel in der Feldforschung. Hinweise zur Nutzung der Checkliste sind auf der Norecopa Website zu finden, darunter Links zu Richtlinien für Tierforschung und Tierversuche: <https://norecopa.no/PREPARE>.

Resumen

Existe una preocupación generalizada sobre la calidad, reproducibilidad y aplicación de los estudios con animales de investigación. A pesar de que existe una serie de directrices disponibles, no hay muchas normas globales sobre cómo *planificar* los experimentos con animales, a pesar del hecho de que ese es el punto más lógico para empezar a garantizar la calidad. En este estudio presentamos las directrices de PREPARACIÓN: Planificación de procedimientos experimentales y de investigación con animales: recomendaciones para conseguir la excelencia. Este estudio cubre las tres áreas generales que determinan la calidad de la preparación de estudios con animales: formulación, diálogo entre científicos y las instalaciones para animales, y el control de calidad de los distintos componentes del estudio. Algunos temas se solapan y la lista de comprobación del estudio de preparación debería adaptarse a las necesidades específicas, por ejemplo en la investigación de campo. Para asesoramiento sobre el uso de la lista de comprobación visite la página web de Norecopa, con enlaces a directrices para la realización de pruebas e investigación con animales, en <https://norecopa.no/PREPARE>.