

Efforts to reduce poor reproducibility/translatatability: the PREPARE guidelines

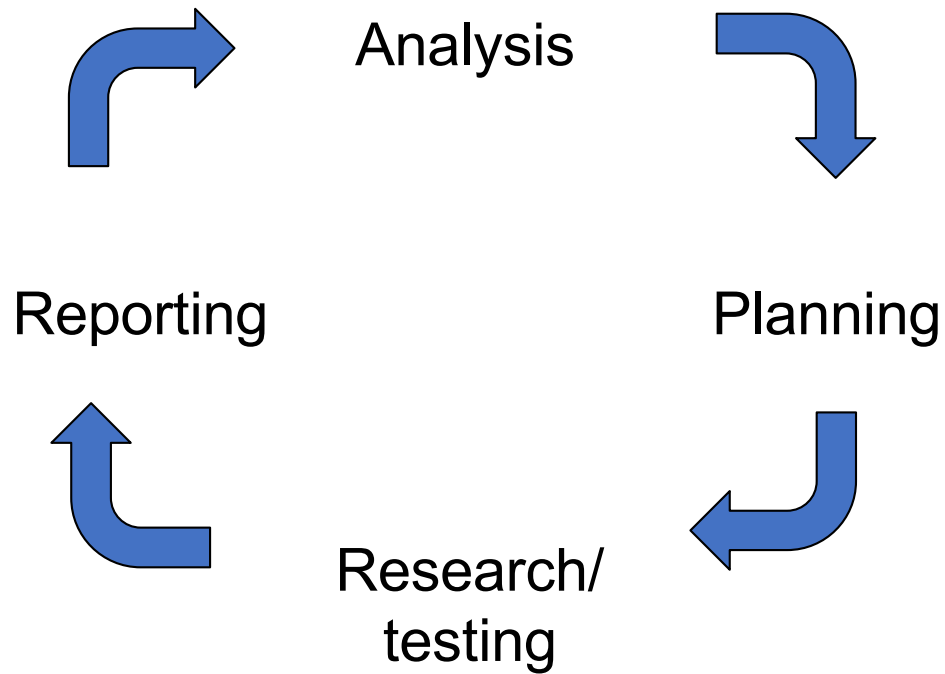
norecopa.no/PREPARE

Adrian Smith, Eddie Clutton, Elliot Lilley, Kristine Hansen & Trond Brattelid

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norecopa.no



Space Shuttle, NASA

Identify and ensure the quality of (at least) the **critical points** in the experiment: critical for animal welfare and scientific value

1) Columbia

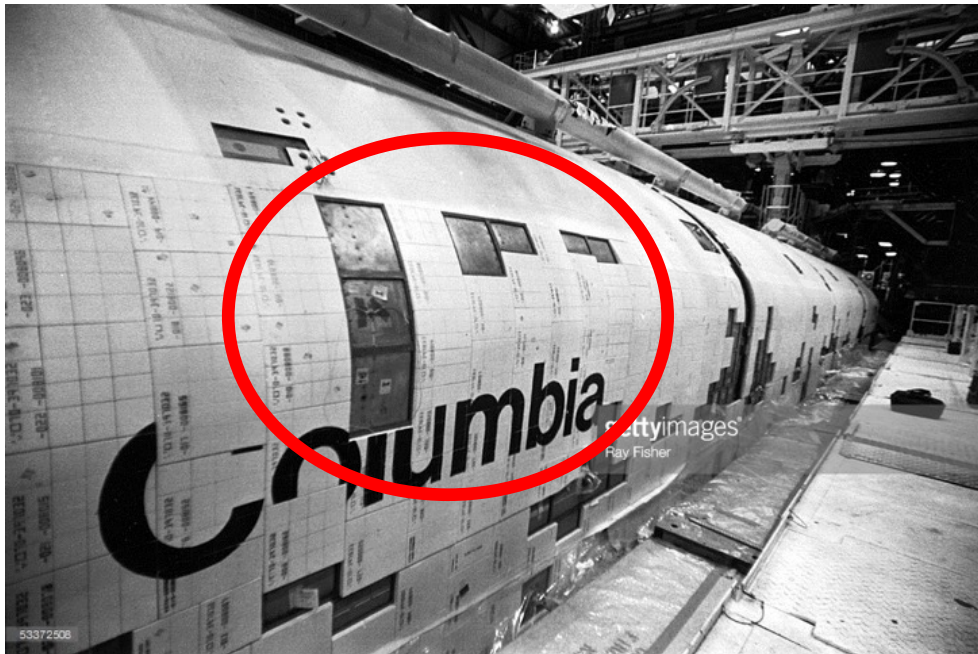
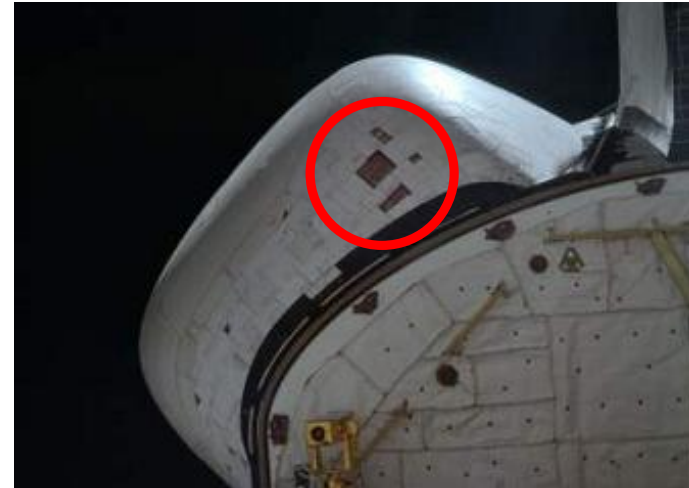


Photo: gettyimages.no

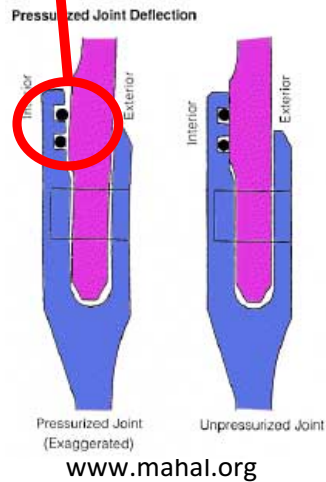
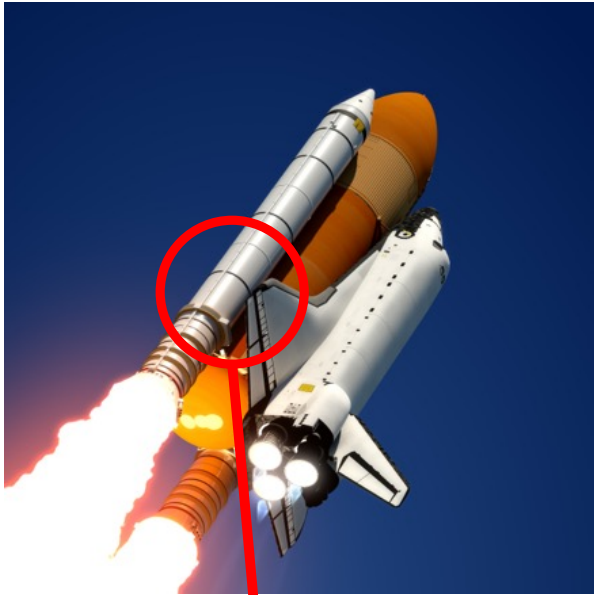


First shuttle flight, Columbia, in April 1981
Photo: nasaspaceflight.com



Columbia burnt up in 2003, killing all 7 crew members
Photo: cbsnews.com

2) Challenger



Challenger disintegrated in January 1986
killing all 7 crew members

Photo: no.wikipedia.org

Details are important!!

Good planning is critical!



- Complex machines (animals) create *known or unknown unknowns* (interactions between parts that are impossible to foresee until you “fly”)
- **Possible design weaknesses must be discussed** (damage from foam, and susceptibility to low temperature, *which the engineers knew about!*)
- **Avoid “pressure to launch”** (political, media). = Publish or perish.
- **Don’t make bad management decisions** (pushing the safety envelope):
 - “We’ve got away with it before”
 - = “We’ve managed to publish the experiments before”
- Often **a combination of many factors, each of which may be harmless until they occur simultaneously**
 - Don’t ignore “insignificant” issues!
 - Pay Attention to Detail

NATURE | NEWS



Swiss survey highlights potential flaws in animal studies

Poor experimental design and statistical analysis could contribute to widespread problems in producing preclinical animal experiments.

Ramin Skibba

20 December 2016

Pain management in pigs undergoing experimental surgery; a literature review (2012–4) FREE

A. G. Bradbury, M. Eddleston, R. E. Clutton

Br J Anaesth (2016) 116 (1): 37-45. DOI: <https://doi.org/10.1093/bja/aev301>

Published: 03 October 2015

with analgesic properties, but only 87/233 (37%) described postoperative analgesia. No article provided justification for the analgesic chosen, despite the lack of guidelines for analgesia in porcine surgical models and the lack of formal studies on this subject. Postoperative pain assessment was reported in only 23/233 (10%) articles. It was found that the reporting of postoperative pain management in the studies was remarkably low, reflecting either under-reporting or under-use. Analgesic description, when given, was frequently too limited to enable reproducibility. Development of a

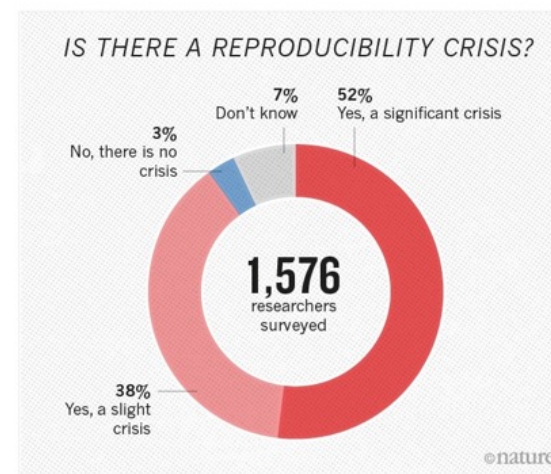
NATURE | NEWS FEATURE

1,500 scientists lift the lid on reproducibility

Survey sheds light on the 'crisis' rocking research.

Monya Baker

25 May 2016 | Corrected: 28 July 2016



More than 70% of researchers have tried and failed to reproduce another scientist's experiments, and more than half have failed to reproduce their own experiments. Those are some of the telling figures that emerged from Nature's survey of 1,576 researchers who took a brief online questionnaire on reproducibility in research.



Missing mice: gaps in data plague animal research

Reports of hundreds of biomedical experiments lack essential information.

Monya Baker

05 January 2016

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Two studies have unveiled widespread flaws in the reporting of animal experiments — the latest in a series of papers to criticize [shoddy biomedical research](#).

Whereas reports of clinical trials in major medical journals routinely state how many patients die or drop out of analysis during the course of a study, animal studies generally fail to report this figure — or drop animals without saying why, according to a team led by Ulrich Dirnagl at the Charité Medical University in Berlin. That lapse could significantly bias results, the team reports in the journal *PLoS Biology*¹.

In a second study in the same journal², a team led by John Ioannidis, an epidemiologist at Stanford University in California who has repeatedly called for more reproducible and transparent research, criticizes the lack of data availability and detailed protocols in biomedical papers.



News › Science

Scores of scientific studies based on mice thrown into doubt because they were picked up by the tail

Mice picked up by the tail – standard practice in labs – are stressed and anxious so don't act naturally in some experiments, new study finds

Ian Johnston Science Correspondent | [@montaukian](#) | Tuesday 21 March 2017 10:58 GMT | [3 comments](#)



Single-housed male mice show symptoms of what in humans would be characterised as depression

Increased hypothermia in response to treatment with a serotonergic agonist

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0111065>

Identification methods are not always 'simple'



Photo: T. Poppe, NMBU



http://blogs.discovermagazine.com/notrocketscience/2011/01/12/flipper-bands-impair-penguin-survival-and-breeding-success/#.VLU6_8Y7_wo



photo: Svalbardposten



SMERTER: Denne villreinen måtte avlives i Nordfjella. En isklump på GPS halsbåndet hadde vokst seg større enn hodet på reinen.

Av **TOR-HARTVIG BONDØ** og **LINN K. YTTERVIK**
(VG) 22.01.2016 20:27

49

photo: vg.no

Are we prepared for equipment failure?

Are there potential human hazards?



Health risks: there are many people to think about

People engaged in animal capture, transport and breeding

Animal carers and technologists

Security personnel

Administrative personnel with occasional access to the animal facility

Students

Sales representatives and those delivering supplies or equipment

Craftsmen carrying out facility repairs

Other visitors, including inspectors, journalists and students

Cleaning staff

Waste disposal personnel

Those who re-home research animals

Many of these people often possess a number of features which increase their health risks

They may:

- enter the facility **outside normal working hours**, when advice on hazards may not be readily available
- **not understand** messages left in the facility, especially if scientific jargon is used. Special consideration should be paid to employees with other native languages.
- **have little knowledge** of animal research, scientific method and the need for controlled experiments
- **have no intrinsic concern** of potential health hazards unless these are pointed out to them. Ironically, the cleaner and tidier an animal facility appears to be, the less likely they are to be fearful of such hazards.
- **have not been health-screened** before entering the facility. Those predisposed for allergy or asthma are particularly at risk when working with animals.
- **be planning a family**. Early embryonic development and spermatogenesis are known to be at risk upon exposure to ionising radiation and chemicals, including volatile anaesthetics.

'the drug was administered by gavage in 3 daily doses'



photo: NMBU

"How much ethanol do I need to give a mouse to be the equivalent of 2 glasses of red wine in the evening?"

Dose, allometric scaling, method of administration etc.

Effects of microflora and microbial hazards



wikipedia.org



colourbox.com

- *Effects of microflora on the experiment*
- *Effects of subclinical and clinical infections*
- *Challenges with containment of micro-organisms*
- *Waste disposal (more complicated the larger the animal)*

There are many guidelines for *reporting* animal studies

- Öbrink & Waller, 1996
- Jane Smith *et al.*, 1997
- Adrian Smith & Trond Brattelid, 2000 (fish)
- Öbrink & Rehbinder: Animal definition: a necessity for the validity of animal experiments? *Laboratory Animals*, 2000
- **ARRIVE Guidelines**, 2010 (Kilkenny *et al.*, NC3Rs)
- Gold Standard Publication Checklist, 2010 (SYRCLE)
- Institute for Laboratory Animal Research, NRC, 2011
- Instructions to authors, in many journals
e.g. Nature's Reporting Checklist

Why do we need PREPARE when we have ARRIVE?

The ARRIVE guidelines 'provide a logical checklist with **all the things that need to be considered when designing an experiment**'.

In our experience when planning animal research, a number of additional points need to be addressed at the planning stage.

These items not only improve study quality and animal welfare (and therefore reproducibility), but also the safety of humans and animals affected directly or indirectly by the work.

* <http://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/ARRIVE%20Guidelines%20Speaker%20Notes.pdf>

Some examples...



PREPARE: guidelines for planning animal research and testing

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

Laboratory Animals
0101 1-7
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nav
DOI: 10.1177/0023677217724823
journals.sagepub.com/home/lan
SAGE

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

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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

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The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

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PREPARE¹ consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE². PREPARE covers the three broad areas which determine the quality of the preparation for animal studies:

1. **Formulation of the study**
2. **Dialogue between scientists and the animal facility**
3. **Quality control of the components in the study**

The topics will not always be addressed in the order in which they are presented here, and some topics overlap. The PREPARE checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidance on the management of animal facilities, since in-house experiments are dependent upon their quality. The full version of the guidelines is available on the Norecopa website, with links to global resources, at <https://norecopa.no/PREPARE>.

The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

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). <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 end-point.
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 randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Carol Kilkenny¹, William J Browne², Innes C Cuthill³, Michael Emerson⁴ and Douglas G Altman⁵

¹The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK, ²School of Veterinary Science, University of Bristol, Bristol, UK, ³School of Biological Sciences, University of Bristol, Bristol, UK, ⁴National Heart and Lung Institute, Imperial College London, UK, ⁵Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	
Study design	6	For each experiment, give brief details of the study design including: <ol style="list-style-type: none"> a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. 	
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: <ol style="list-style-type: none"> a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used). 	
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	

The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010¹

PREPARE:

Planning Research Involving Experimental Procedures on Animals: Recommendations for Excellence

PREPARE recommends attention to the following:

Preparation

1. Literature searches
2. Legal issues
3. Ethical issues, Harm-Benefit Assessment and humane endpoints
4. Experimental design and statistical analysis

Dialogue with the animal facility

5. Objectives and timescale, funding and division of labour
6. Facility evaluation
7. Education and training
8. Health risks, waste disposal and decontamination

Not highlighted in the ARRIVE checklist

Methods

9. Test substances and test procedures
10. Experimental animals
11. Quarantine and health monitoring
12. Housing and husbandry
13. Experimental procedures
14. Humane killing, release, re-use or re-homing
15. Necropsy

The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith¹, R. Eddie Clutton², Elliot Lilley³, Kristine E. Aa. Hansen⁴ & Trond Bratteli⁵

¹Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0108 Oslo, Norway; ²Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; ³Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; ⁴Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; ⁵Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

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The PREPARE checklist

<https://norecopa.no/PREPARE/prepare-checklist>
is available so far in:

English
Brazilian Portuguese
Danish
French
German
Italian
Japanese

Korean
Mandarin Chinese
Norwegian
Portuguese
Russian
Spanish
Swedish

Presentation of PREPARE on
<https://norecopa.no/PREPARE>
for free downloading:

The PREPARE Guidelines for Planning Animal Research and Testing

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norecopa.no

In addition to the checklist, much more information is available on:

norecopa.no/PREPARE



[PREPARE Checklist](#) | [1-Literature searches](#) | [2-Legal issues](#) |
[3-Ethical issues, Harm-Benefit Assessment and humane endpoints](#) | [4-Experimental design and statistical analysis](#) |
[5-Objectives and timescale, funding and division of labour](#) | [6-Facility evaluation](#) | [7-Education and training](#) |
[8-Health risks, waste disposal and decontamination](#) | [9-Test substances and procedures](#) | [10-Experimental animals](#) |
[11-Quarantine and health monitoring](#) | [12-Housing and husbandry](#) | [13-Experimental procedures](#) |
[14-Humane killing, release, re-use or re-homing](#) | [15-Necropsy](#) | [Comparison with ARRIVE](#)

PREPARE utilises the resources on norecopa.no



The screenshot shows the top navigation bar of the norecopa.no website. It features the norecopa logo (a blue and red star in a white circle) and the text "norecopa" in white on a blue background. In the top right corner, there are language options for "NORSK" and "ENGLISH". A search bar with the text "Search:" and a magnifying glass icon is highlighted with a red circle. Below the navigation bar, there is a horizontal menu with various categories: "About Norecopa", "Alternatives", "Databases & Guidelines", "Education & training", "Legislation", "Meetings", "News", "Other resources", "Species", and "Feedback". Below this menu, there is a list of specific topics: "Anaesthesia and analgesia", "Animal facilities", "Animal welfare organisations", "Blood sampling", "Email discussion lists", "Environmental enrichment", "Ethics", "Experimental design and statistical analysis", "Harm-Benefit Assessment", "Health monitoring", "Journals", "Organisations", "Severity classification", "Suppliers", and "Systematic reviews". Below the list, there is a search bar with the text "Search all Norecopa's databases and webpages simultaneously:" and a magnifying glass icon. The search bar is highlighted with a red box. To the right of the search bar, there are social media icons for Facebook, Twitter, Email, and a plus sign.

Organisations of relevance to animal research

Organisations within Laboratory Animal Science

[AAALAC International](#) (Association for Assessment and Accreditation of Laboratory Animal Care International)

[AALAS](#) (American Association for Laboratory Animal Science)

[ACLAM](#) (American College of Laboratory Animal Medicine)

[AniMatch](#) (an online sharing platform for the exchange of organs and tissues)

[ARSAL](#) (Asociatia Româna pentru Stiinta Animalelor de Laborator; Romanian Laboratory Animal Science Association)

[ASLAP](#) (American Society of Laboratory Animal Practitioners)

6,000 webpages
80,000 links
22,000 unique links
<3.5% dead links



- [PREPARE Checklist](#) | [1-Literature searches](#) | [2-Legal issues](#) | [3-Ethical issues, Harm-Benefit Assessment and humane endpoints](#) | [4-Experimental design and statistical analysis](#) | [5-Objectives and timescale, funding and division of labour](#) | [6-Facility evaluation](#) | [7-Education and training](#) | [8-Health risks, waste disposal and decontamination](#) | [9-Test substances and procedures](#) | [10-Experimental animals](#) | [11-Quarantine and health monitoring](#) | [12-Housing and husbandry](#) | [13-Experimental procedures](#) | [14-Humane killing, release, re-use or re-homing](#) | [15-Necropsy](#) | [Comparison with ARRIVE](#)

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Harm-Benefit Assessment

[Harm-Benefit assessment](#), an evaluation of the likely sources and level of suffering of a planned procedure, followed by an assessment of the potential benefits of the research weighed against these harms, lies at the heart of [legislation in the EU](#) and elsewhere. [A framework for severity assessment and severity classification](#) must be established and justified. The likely adverse effects of each procedure should be described, along with their likely incidence and methods of recognising them, with indications of how these effects can be mitigated by implementing refinement. This necessitates the involvement of personnel with the relevant expertise to recognise, assess and reduce animal suffering, especially severe suffering. [Guidance on this is available on the RSPCA website](#). Specific justification of all unanticipated animal suffering must be provided. An estimate must be made of the maximum amount of pain, distress or lasting harm to which an individual can be exposed.

Links to quality guidelines worldwide on e.g. blood sampling, injection volumes, housing and husbandry, analgesia, humane endpoints, experimental design

Contract between the animal facility and the research group

The division of labour and responsibilities between the two parties, with the aim of clarifying all stages of the experiment and ensuring that all necessary parameters are recorded.

	Animal facility	Researcher	Not applicable
Animal:			
Arrival date			
Species			
Strain/stock and substrain			
Supplier (full name and address) or bred on the premises			
Number and sex			
Age, weight, stage of life cycle on arrival			
Pre-treatment (surgical or medical) from supplier			
Quality (e.g. SPF, germ-free, gnotobiotic, conventional)			
Acclimation time before the start of the experiment			
Time and duration of fasting (with/without water and bedding)			
Environment:			
Type of housing: barrier/conventional			
Temperature (mean \pm variation)			
Light schedule			
Relative humidity (mean \pm variation)			
Number of air changes in the animal room/cabinet per hour			
Environmental enrichment			
Housing:			
Free-range, shelf, cabinet, isolator			
Cage type and size			
Number and method of distribution of animals per cage			



ARRIVE

PREPARE



<https://www.bls.gov/ooh/images/3077.jpg>

PREPARE

ARRIVE



<https://www.dreamstime.com>

PREPARE is **not just a checklist**, published once and for all.

The PREPARE website will form a dynamic set of recommendations which will evolve and contain more links as more species- and situation-specific guidelines are produced, and as best practice within Research Animal Science progresses.

PREPARE is not prescriptive and is not meant to suffocate creativity, it are designed to help eliminate the artefacts caused by factors which have nothing to do with the treatment itself.

Something for wildlife researchers as well?



wikipedia.org

Søren Kirkegaard (1813-1855)

*It is perfectly true, as philosophers say, that life must be understood backwards. **Reporting!***

*But they forget the other proposition, that it must be lived forwards. **PREPARE!***

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