PREPARE before you ARRIVE:
advice on how to plan animal studies from day one

norecopia.no/PREPARE

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Norecopa
National Consensus Platform for the Replacement, Reduction and Refinement of Animal Experiments
European Consensus-Platform for Alternatives

etopa.eu

- Established in 2000
- Recognises National Consensus Platforms (NCPs) with 4 stakeholders equally represented:
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 Romana pentru Stiinta Animalelor de Laborator; Romanian Laboratory Animal Science Association)
ASLAP (American Society of Laboratory Animal Practitioners)

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a lasting resource

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Fish 2005 | Wildlife 2008 | Fish 2009 | Agricultural animals 2012 | Field research 2017 | Past meetings

Meetings Calendar | An informal guide to arranging a scientific meeting
PREPARE: guidelines for planning animal research and testing

Adrian J Smith, R Eddie Clutton-Brock, Elliot Lilley, Kristine E Aa Hansen and Trond Brattli

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

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, even after the production and journal endorsement of reporting guidelines. There is also widespread concern about the lack of reproducibility and translatability of laboratory animal research. This can, for example, contribute towards the failure of drugs when they enter human trials. 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. This has understandably sparked a demand for reduced waste when planning experiments involving animals. 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). 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...
Berti & Cima 1955, quoted in Öbrink and Rehbinder

Fig 7  Influence of room temperature on LD-50 of the drug Chlorpromazine in mouse
Fig 6  The generation of experimental animals from genotype over phenotype to dramatype.
There are many guidelines for reporting animal studies

- GV-SOLAS committee, chaired by AW Ellery (1985)
- Öbrink & Waller, 1996
- Jane Smith et al., 1997
- 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

*More species- and situation- specific guidance is needed*
Guidelines for reporting the results of experiments on fish

Trond Brattelid & Adrian J. Smith
Laboratory Animal Unit, The Norwegian School of Veterinary Science, PO Box 8146 Dep., 0033 Oslo, Norway

Summary
A detailed account of experimental design, including an accurate description of the animals used, is an essential part of good research practice. Without these details, the reader will be unable not only to form an opinion on the significance of the findings but also to repeat the experiment in another laboratory. This paper presents suggested guidelines for reporting experimental studies using fish.

Keywords  Fish; experiment; study; report; refinement

Laboratory Animals, 2000
Guidance on the severity classification of procedures involving fish

Report from a Working Group convened by Norecopa

P Hawkins, N Dennison, G Goodman, S Hetherington, S Llywelyn-Jones, K Ryder and AJ Smith

Laboratory Animals, 45: 219-224, 2011

norecopa.no/categories
Swiss survey highlights potential flaws in animal studies

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

Ramin Skibba
20 December 2016

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

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

Published: 03 October 2015

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.
Why do we need PREPARE when we have ARRIVE?

The ARRIVE guidelines claim that they ‘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.

The elephants in the room...

...the largest of them all is the lack of focus on planning animal experiments
The stress of capture and restraint!

Rory Wilson, Swansea University

ohiobirdsanctuary.com
"Simple" identification methods?

Tags can collect seaweed and shellfish, which dramatically reduce the fish’s ability to swim efficiently

Flipper bands can reduce the penguin’s ability to swim efficiently

Many animals can be identified by non-invasive biometric methods, like photographing the pattern of stripes on the zebra

Contingent suffering

(not just direct suffering caused by the procedure)
e.g. fear, boredom, discomfort

which may caused by
e.g. transport, housing, husbandry, social hierarchy

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

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0111065
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
"Simple" techniques?

- Are you sure that your injection ends up in the same place each time?
- Are the injections painful?
- Are they feasible? e.g. intramuscular injections in small animals
Disposable needles are designed to be used only once!

Lucy Whitfield, RVC and Sally Robinson, AstraZeneca
Photo: AstraZeneca

https://www.nc3rs.org.uk/news/re-use-needles-indicator-culture-care
’the drug was administered by gavage in 3 daily doses’

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

Carefully consider the dose, use of allometric scaling, and the method of administration.
‘Simple’ blood sampling techniques?

At the doctor:
I think I’ll take a blood sample from you tomorrow.
I take my blood samples by sticking a knife into your neck, without anaesthesia.
But don’t worry, I’ll inject 2 litres of liquid into your abdomen first so you don’t die from fluid loss.

medipoint.com/html/for_use_on_mice.html

The best blood sampling techniques are those where you can (1) see the blood vessel, (2) control the amount of blood you remove, (3) stop the bleeding easily and (4) not damage surrounding tissue.
Carol M. Newton (1925-2014)

The three S’s

- Good Science
- Good Sense
- Good Sensibilities


https://norecopa.no/3S
The use of animals in education and training

'We may need the animals, as it were, on the night; but the machines will do very well at rehearsals'
Workshop 11 April in Oslo
norecopia.no/education-training/homemade-educational-materials
Reporting guidelines like ARRIVE describe the experiment. Guidelines like PREPARE are used to plan the experiment (choose the «ingredients» and «baking time»).
PREPARE: Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

PREPARE covers 15 topics:

Formulation of the study
1. Literature searches
2. Legal issues
3. Ethical issues, harm-benefit assessment and humane endpoints
4. Experimental design and statistical analysis

Dialogue between scientists and 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

Methods
9. Test substances and procedures
10. Experimental animals
11. Quarantine and health monitoring
12. Housing and husbandry
13. Experimental procedures
14. Humane killing, release, reuse or rehoming
15. Necropsy

Items in pink are not highlighted in ARRIVE
The PREPARE Guidelines Checklist
Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J Smirke, R Robba-Brustolin, Elliot Lilley, Kristine E As, Hansen & Trond BUstad

PREPARE consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE.
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 specific 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.

<table>
<thead>
<tr>
<th>Topic</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>(A) Formulation of the study</td>
<td></td>
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<tr>
<td>1. Literature searchers</td>
<td>Form a clear hypothesis, with primary and secondary outcomes.</td>
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<td>Consider the use of systematic reviews.</td>
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<td></td>
<td>Decide upon databases and information specialists to be consulted, and construct search terms.</td>
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<td></td>
<td>Access the relevance of the species to be used, its biology and its suitability to answer the experimental questions with the least suffering, and welfare needs.</td>
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<td></td>
<td>Assess the reproducibility and transplantability of the project.</td>
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<td>2. Legal issues</td>
<td>Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety.</td>
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<td></td>
<td>Locate relevant guidance documents (e.g. EU guidance on project evaluation).</td>
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<tr>
<td>3. Ethical issues, harm-benefit assessment and humane endpoints</td>
<td>Construct a key summary.</td>
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<td>In dialogue with ethics committees, consider whether statements about the type of research have already been produced.</td>
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<td></td>
<td>Address the 3Rs (replacement, reduction, refinement) and the 35% (good science, good sense, good animal welfare).</td>
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<td>Consider pre-registration and the publication of negative results.</td>
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<td>Perform a harm-benefit assessment and justify any animal harm.</td>
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<td>Discuss the framing objectives, if the animal use is for educational or training purposes.</td>
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<td>Allocate a severity classification to the project.</td>
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<td>Define objectives, easily measurable and unequivocal humane endpoints.</td>
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<td></td>
<td>Discuss the justification, if any, for death as an end-point.</td>
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<tr>
<td>4. Experimental design and statistical analysis</td>
<td>Consider pilot studies, statistical power and significance levels.</td>
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<td>Define the experimental unit and decide upon animal numbers.</td>
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<td>Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.</td>
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References

Further information
https://norecopa.no/prepare
https://norecopa.no/preparedocumentation
https://norecopa.no/

Two pages, translated into 13 languages so far
In addition to the checklist, much more information is available on:

norecopa.no/PREPARE
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 unalleviated 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.
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.
Are we prepared for equipment failure?

*Anything that can go wrong, will go wrong* (Murphy’s Law)
Are the animals ready for the experiment?
We strongly recommend the PREPARE checklist and its associated webpages with more detailed recommendations. Some, but by no means all, of the challenges include:

- health status, acquisition, transport and acclimation to new buildings
- quarantine and adaptation to new feeding regimes
- establishment of new social groups
- provision of sufficient space for exercise, sampling, anaesthesia and necropsy
- ventilation issues
- the differences in practices between traditional farm work and those used in controlled studies in a laboratory environment
- health, safety and general hygiene
- waste disposal (e.g. contaminated carcases)
- containment of pathogens
- identification of sufficient numbers of staff who are familiar with, and competent to handle, farm animal species

Many of these issues are exacerbated by the sheer size of the animals.
Contract between the animal facility and the research group

The division of labour, responsibilities and costs between the two parties, with the aim of clarifying all stages of the experiment and ensuring that all necessary parameters are recorded.
Quality assurance and a culture of care at all levels of the animal facility

- **SOPs** describing good techniques, carried out by competent operators
- **Checklist** (“contract”) between researcher and the facility
- The AAALAC *Program Description template* as an overall performance checklist
  - Institutional policies on animal care and use
  - Animal environment, housing and management
  - Veterinary care
  - Physical plant
- A **Master Plan** as a weekly checklist for the whole facility during the year

*https://www.aaalac.org/programdesc/index.cfm*
A simple but effective Master Plan
Think ”3R-Alternatives” at all stages

- Breeding
- Transport
- Acclimation
- Procedures, e.g. choice of
  - dose
  - method of administration
  - methods of data collection (blood sampling, body temperature, heart rate, blood pressure etc.)
- Pilot studies

*Consult the technicians from Day 1:*
- they have a right to know and will be more motivated
- they know the possibilities (and limitations) in the animal facility
- they often possess a large range of practical skills and are good at lateral thinking
- they know the animals best
- the animals know them best
Even experienced pilots use checklists as an aide memoire...
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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• Stiansen Foundation
• Universities Federation for Animal Welfare (UFAW)
• US Department of Agriculture, Animal Welfare Information Center (AWIC)

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