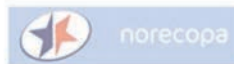


# PREPARE



## The PREPARE Guidelines Checklist

### Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith\*, R. Eddie Clutton†, Elliot Lilley‡, Kristine E. Aa. Hansen§ & Trond Bratteli¶

\*Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; †Royal (Dick) School of Veterinary Studies, Easter Bush

‡Division of Experimental Bioscience, Department of Preclinical Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8144 Dep., 0403 Oslo, Norway; §Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway

¶Division of Experimental Bioscience, Department of Preclinical Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8144 Dep., 0403 Oslo, Norway; †Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway

# Use of PREPARE to identify refinements of severe models and procedures

Adrian Smith, Norecopa

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[@adrian\\_3r](https://twitter.com/adrian_3r)

[norecopa.no/RSPCA-KI](https://norecopa.no/RSPCA-KI)

Topic	Recommendation
<b>(B) Dialogue between scientists and the animal facility</b>	
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.
6. Facility evaluation	<input type="checkbox"/> Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. <input type="checkbox"/> Discuss staffing levels at areas of extra risk.
7. Education and training	<input type="checkbox"/> Assess the current competence of staff members and the need for further education or training prior to the start of the study.
8. Health risks, waste disposal and decontamination	<input type="checkbox"/> Perform a risk assessment, in consultation with the animal facility, for all persons and animals affected directly or indirectly by the study. <input type="checkbox"/> Review, and if necessary produce, specific guidance for all stages of the project. <input type="checkbox"/> Discuss plans for containment, decontamination, and disposal of all items in the study.
<b>(C) Quality control of the components in the study</b>	
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 consultation with expert staff. <input type="checkbox"/> Discuss acclimatisation, 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, immobilisation, marking, and release or rehoming. <input type="checkbox"/> Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and euthanasia.
14. Necropsy	<input type="checkbox"/> Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

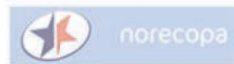
#### References

- Smith AJ, Clutton RE, Lilley E, Hansen KEA & Bratteli T. PREPARE: Guidelines for Planning Animal Research and Testing. *Laboratory Animals*. 2017. DOI: 10.1177/0023677217724623.
- Kilkenny C, Browne WJ, Cuthill IC et al. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biology*. 2010; DOI: 10.1371/journal.pbio.1000412.

#### Further information

<https://norecopa.no/PREPARE> / [post@norecopa.no](mailto:post@norecopa.no) / [@norecopa](https://twitter.com/norecopa)

# PREPARE



## The PREPARE Guidelines Checklist

### Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith<sup>1</sup>, R. Eddie Clutton<sup>2</sup>, Elliot Lilley<sup>3</sup>, Kristine E. Aa. Hansen<sup>4</sup> & Trond Bratteli<sup>5</sup>

<sup>1</sup>Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; <sup>2</sup>Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; <sup>3</sup>Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.;

<sup>4</sup>Section 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; <sup>5</sup>Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE<sup>1</sup> consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE<sup>2</sup>.

PREPARE

- 1.
- 2.
- 3.

The top checklist facilities website The PREPARE and as

Topic

1. Literature search

	<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.

Topic	Recommendation
(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.
6. Facility evaluation	<input type="checkbox"/> Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. <input type="checkbox"/> Discuss staffing levels at times of extra risk.
7. Education and	<input type="checkbox"/> Assess the current competence of staff members and the need for further education or training prior
	and animals affected
	study.
	form them.
	for reporting.
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, immobilisation, 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.

#### References

1. Smith AJ, Clutton RE, Lilley E, Hansen KEA & Bratteli T. PREPARE: Guidelines for Planning Animal Research and Testing. *Laboratory Animals*. 2017. DOI: 10.1177/0023677217724823.
2. Kilkenny C, Browne WJ, Cuthill IC et al. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biology*. 2010; DOI: 10.1371/journal.pbio.1000412.

#### Further information

<https://norecopa.no/PREPARE> | [post@norecopa.no](mailto:post@norecopa.no) | [@norecopa](https://twitter.com/norecopa)

## Disclosures

- Webmaster of [norecopa.no](https://norecopa.no) and the Refinement Wiki
- Lead author of the PREPARE guidelines

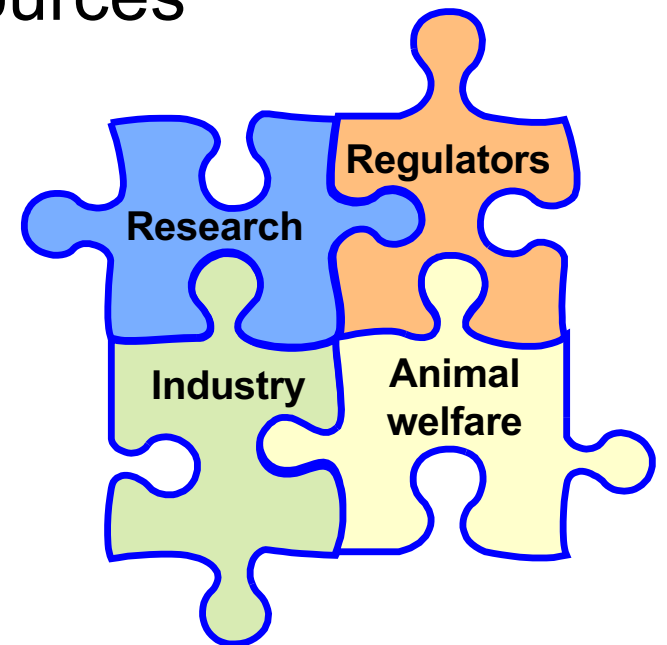
Norway's National Consensus Platform for the  
Three Rs: Replacement, Reduction and Refinement  
and a source of *global* 3R resources



<https://norecopa.no>

*Established in 2007*

Norecopa: PREPARE for better Science



*norecopa.no : an updated overview of global 3R resources*

The image is a screenshot of the norecopa.no website. The header is blue with the norecopa logo (a stylized star) and the text 'norecopa'. To the right of the logo are links for 'NORSK' and 'ENGLISH'. A yellow arrow points from the text 'an updated overview of global 3R resources' to the search bar. Below the header is a navigation bar with links: 'About Norecopa', 'Alternatives', 'Databases & Guidelines', 'Education & training', 'Legislation', 'Meetings', 'More resources', 'News', 'PREPARE', 'Species', and 'Wiki'. Below this is a grid of links: 'Anaesthesia and analgesia', 'Animal facilities', 'Animal welfare organisations', 'Blood sampling', 'Culture of care', 'Email discussion lists', 'Environmental enrichment', 'Ethics', 'Experimental design and reporting', 'Harm-Benefit Assessment', 'Health and safety', 'Health monitoring', 'Humane endpoints', 'Literature searches and systematic reviews', 'Severity classification', and 'Suppliers'. A yellow banner with the text 'we welcome more from you!' is overlaid on the 'Experimental design and reporting' link. Below the grid is a breadcrumb trail: 'norecopa.no / More resources / Experimental design and reporting'. The main content area has the title 'Design and reporting of animal experiments' and a paragraph: 'This page supplements advice given in [Section 4 of the PREPARE guidelines](#). PREPARE covers all aspects of design (including animal and facility related issues)'. A green box contains the text 'approx. 8,900 webpages' and '350,000 hits annually'. Another green box contains the text '7-8 detailed newsletters per year'. On the right side, there is a sidebar with a red border. It contains a 'Search filters' section with 'Order by:' (set to 'Relevance') and 'Typo tolerance:' (set to 'Default'). Below this is a 'Database' section with a list of databases and their counts: '3R Guide database (403)', 'Classic AVs database (118)', 'European Commission Inventory of 3Rs Education & Training Resources (567)', 'European Commission Inventory of 3Rs Knowledge Sources (807)', 'European Commission Inventory of NAMs for Respiratory tract diseases (280)', 'NAL records (1688)', 'NORINA database (3141)', 'TextBase database (1501)', and 'Website (761)'. Below this is a 'Browse the databases' section with a list of categories and counts: 'eBooks (286)', 'Free (199)', 'Held at NMBU Oslo (contact Kristine Hansen, 67 23 21 89) (431)', 'Key products (68)', 'On loan (6)', and 'Reviewed (85)'. At the bottom of the sidebar is a 'Search in the databases' section with a list of search criteria: 'All Text', 'Title', 'Author', 'Publisher', 'Supplier', and 'Record Number'.

**we welcome more from you!**

approx. 8,900 webpages  
350,000 hits annually

7-8 detailed newsletters per year

**Design and reporting of animal experiments**

This page supplements advice given in [Section 4 of the PREPARE guidelines](#). PREPARE covers all aspects of design (including animal and facility related issues).

Norecopa: PREPARE for better Science

**Search filters**

Order by:  
Relevance

Typo tolerance:  
Default

**Database**

- 3R Guide database (403)
- Classic AVs database (118)
- European Commission Inventory of 3Rs Education & Training Resources (567)
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**Search in the databases**

- All Text
- Title
- Author
- Publisher
- Supplier
- Record Number



## 3R-Guide (400 guidelines for animal research and testing)

[norecopa.no/3r-guide](http://norecopa.no/3r-guide)



### Guidance on the severity classification of procedures involving fish

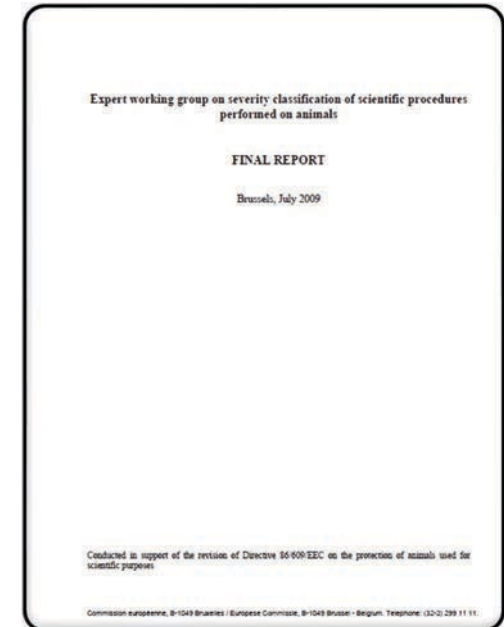
Report from a Working Group convened by Norecopa

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

Laboratory Animals, 45: 219-224, 2011

Norecopa: PREPARE for better Science

[norecopa.no/categories](http://norecopa.no/categories)



[http://ec.europa.eu/environment/chemicals/lab\\_animals/pdf/report\\_ewg.pdf](http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf)

# Webinars and Meetings Calendar

N.B. For information about **courses** in laboratory animal science, [click here](#).

## August 2022

- ▶ [ReThink3R 2.0 Summer School](#), Berlin, 23-26 August 2022
- ▶ [Care and maintenance of lab animal models](#), Envigo webinar, 24 August 2022
- ▶ [Refining severe disease models and procedures](#), Stockholm, 24-25 August 2022
- ▶ [The Future of Science in the European Union and beyond](#), online discussion, 25 August 2022
- ▶ [Ethical dilemmas in veterinary medicine: Advances of alternative methods in toxicology and biomedicine](#), virtual course in Portuguese, 26 August 2022
- ▶ [Function B - Design of scientific procedures and projects involving research animals](#), Stockholm/online, 30 August - 6 October 2022
- ▶ [17th CDC International Symposium on Biosafety](#), Atlanta, 27-31 August 2022
- ▶ [Introduction to two 3Rs Self-Assessment Tools for Research Groups & Institutions to Obtain a Realistic Evaluation of 3Rs Activities](#), webinar, 31 August 2022

## Recorded webinars

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](#)
  - > [Terrestrial animals](#)
  - > [Aquatic animals](#)
13. [Experimental procedures](#)
  - > [Terrestrial animals](#)
  - > [Aquatic animals](#)
  - > [Anaesthesia and analgesia](#)
14. [Humane killing, release, reuse or rehoming](#)
15. [Necropsy](#)

### Other topics:

- A. [Planning animal research in general](#)
- B. [Improving reproducibility](#)
- C. [Sentience](#)
- D. [In vitro methods](#)
- E. [Publishing animal research](#)
- F. [Miscellaneous presentations](#)

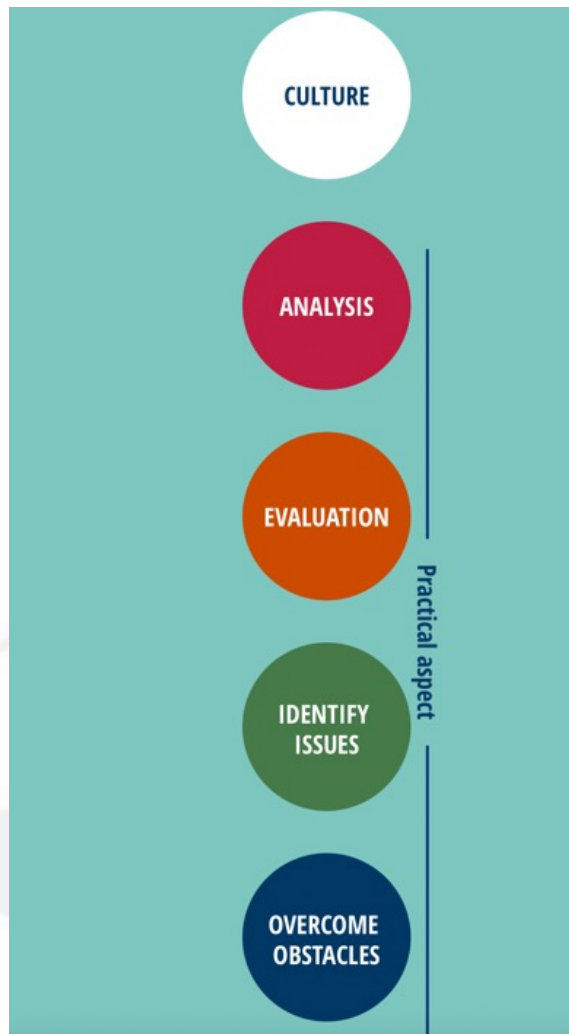
KAROLINSKA INSTITUTET LAS WEBINAR SERIES, 1 APRIL 2022

## IS THIS A HARMFUL PHENOTYPE? HOW TO RESPONSIBLY ASSESS GENETICALLY INDUCED PHENOTYPES IN RODENTS.

DR. ANNE ZINTZSCH  
ANIMAL WELFARE OFFICER



copyright 2016 by Russ Hodge  
<https://goodsciencewriting.wordpress.com>



Culture of Care Network

PREPARE guidelines

*CIRS-LAS website*

The Refinement Wiki





The International Culture of Care Network  
[norecopa.no/coc](http://norecopa.no/coc)

A demonstrable commitment, throughout the establishment, to improving:

- animal welfare
- scientific quality
- care of staff
- transparency for all stakeholders, including the public

*It goes beyond simply complying with the law!*

Norecopa: PREPARE for better Science



## Communication and the Culture of Care

Penny Hawkins, RSPCA Research Animals Department  
 on behalf of the International Culture of Care Network\*

Effective two-way communication between scientists and animal technologists is essential for a good Culture of Care  
 The European Commission suggests the 'development of formal and informal communication channels, for mutual benefit with respect to science and animal welfare'  
 Here are some examples from International Culture of Care network members

### Regular meetings

Scheduled meetings for scientists, animal technologists, vets, unit managers and AWERB members



Regular refresher/update meetings for all organised by NTCO



### Special events

Duo-talks: researcher talks about their science, and animal technologists talk about techniques and animal care within the project



ELH organises an informal meeting for all, in which anyone can raise welfare issues



### Building communication into existing processes

Each study has a pre-start and wash-up meeting involving everybody



Three Rs improvements reported to AWERB & shared at external user meetings



### Other ideas

A 'boxless' event: anyone can submit 'out of the box' ideas to improve practice



A staff survey for all e.g. how much do you agree with statements such as 'in our group we listen to each others' ideas about animal welfare'



\*norecopa.no/culture-of-care





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## Centres

- ☒ [Replacement](#) i
- ☒ [Reduction](#) i
- ☒ [Refinement](#) i
- ☒ [ecopa](#) i

## Associations

- ☒ [ACURET](#) i
- ☒ [AFLAS \(includes South Korea\)](#) i
- ☒ [Culture of Care Network](#) i
- ☒ [ecopa](#) i
- ☒ [EU-NETVAL](#) i
- ☒ [EU3Rnet](#) i
- ☒ [FELASA](#) i
- ☒ [FESSACAL](#) i
- ☒ [Scand-LAS](#) i
- ☒ [Concordat on Openness](#) i



## *Culture of Care facilitates honest discussion*



"because we've always done it that way"

"as often as necessary"

"there are no alternatives"

*Closely related to a culture of care is*

a **Culture of Challenge** (Louhimies, 2015).

**Look for the acceptable, rather than choosing the accepted.**



# PREPARE: On the pathway to better research



Norecopa: PREPARE for better Science

[norecopa.no/PREPARE](https://norecopa.no/PREPARE) and  
[ivd-utrecht.nl/en/news/better-animal-research-through-open-science-1](https://ivd-utrecht.nl/en/news/better-animal-research-through-open-science-1)



# Prepare



Original Article

## PREPARE: guidelines for planning animal research and testing

Adrian J Smith<sup>1</sup>, R Eddie Clutton<sup>2</sup>, Elliot Lilley<sup>3</sup>,  
Kristine E Aa Hansen<sup>4</sup> and Trond Brattelid<sup>5</sup>

### 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

Date received: 5 April 2017; accepted: 27 June 2017

### 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,<sup>1,2</sup> even after the production and journal endorsement of reporting guidelines.<sup>3</sup> There is also widespread concern about the lack of reproducibility and translatability of laboratory animal research.<sup>4-7</sup> This can, for example, contribute towards the failure of drugs when they enter human trials.<sup>8</sup> 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.<sup>9</sup> This has understandably sparked a demand for reduced waste when planning experiments involving animals.<sup>10-12</sup> 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).<sup>13</sup> 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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<sup>2</sup>Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, UK  
<sup>3</sup>Research Animals Department, Science Group, RSPCA, Southwater, Hershaw, West Sussex, UK  
<sup>4</sup>Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway  
<sup>5</sup>Division 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](mailto:adrian.smith@norecopa.no)

Pre-published under Open Access on 3 August 2017,  
sponsored by the Universities Federation for Animal  
Welfare (UFAW), UK

<https://doi.org/10.1177/0023677217724823>



Over 25,000 downloads from the  
journal website so far

[norecopa.no/prepare/endorsements](https://norecopa.no/prepare/endorsements)

Norecopa: PREPARE for better Science



# PREPARE



## The PREPARE Guidelines Checklist Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith<sup>1</sup>, R. Eddie Clutton<sup>2</sup>, Elliot Lilley<sup>3</sup>, Kristine E. Aa. Hansen<sup>4</sup> & Trond Brattli<sup>5</sup>  
<sup>1</sup>Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; <sup>2</sup>Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; <sup>3</sup>Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwate, Harsham, West Sussex, RH13 9RS, U.K.; <sup>4</sup>Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8140 Dep., 0033 Oslo, Norway; <sup>5</sup>Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE<sup>2</sup>. 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, as a checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidelines, since in-house experiments are dependent upon their quality. The full version of the 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.

Three Rs!

Topic	Recommendation
<b>(A) Formulation of the study</b>	
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 to 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 sensibility). <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"/> Associate 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.

Topic	Recommendation
<b>(B) Dialogue between scientists and the animal facility</b>	
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.
6. Facility evaluation	<input type="checkbox"/> Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. <input type="checkbox"/> Discuss staffing levels at times of extra risk.
7. Education and training	<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.
<b>(C) Quality control of the components in the study</b>	
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, immobilisation, 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.

### References

1. Smith AJ, Clutton RE, Lilley E, Hansen KEA & Brattli T. PREPARE: Guidelines for Planning Animal Research and Testing. *Laboratory Animals*, 2017, DOI: 10.1177/0023677217724923.
2. Kilkenny C, Browne WJ, Cuthill IC et al. Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biology*, 2010, DOI: 10.1371/journal.pbio.1000412.

### Further information

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3-Ethical issues, harm-benefit assessment and humane endpoints	
3a	Construct a lay summary.
3b	In dialogue with ethics committees, consider whether statements about this type of research have already been produced.
3c	Address the 3Rs (Replacement, Reduction, Refinement) and the 3Ss (Good Science, Good Sense, Good Sensibilities).
Assessment and justify any likely animal harm.	
3f	Discuss the learning objectives, if the animal use is for educational or training purposes.
3g	Allocate a severity classification to the project.
3h	Define objective, easily measurable and unequivocal humane endpoints.
3i	Discuss the justification, if any, for death as an end-point.
4-Experimental design and statistical analysis	

5. Have the experiments been carried out before, and is any repetition justifiable?
6. What [approaches to reduce distress](#) have been considered?

### 3a Construct a lay summary.

#### General principles

#### For fish researchers

1. Have national or local research ethics committees already produced statements relevant to the research being planned? Consideration should also be paid to the broader context of the research. For example, research directed at increasing the productivity of farming at the expense of (or without improving) individual animal welfare, or wildlife research whose primary aim is population management.

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

2. Will any advances in this research only index the title and abstract be rejected?
  3. Have the Three S's ([Good Science, Good Sense and Good Sensibilities](#)) been addressed? Sufficient time should be allocated to this point, since two of the three S's are highly subjective, but equally important. The use of commonsense and critical anthropomorphism are justifiably part of the work to assess the impact of research on animals, not least when a scientific evidence base does not exist.
  4. Does the proposed study have a clear rationale and scientific relevance, and what will be the next step if the hypothesis is supported or rejected?
  5. Have the experiments been carried out before and is any repetition justifiable?
  6. What [approaches to reduce distress](#) have been considered?
  7. Will the project undergo [pre-registration](#) and will negative results be published, to avoid publication bias?
- Many more [links to resources on ethics are available here](#).
- Details about pre-registration of animal studies and reporting of critical incidents are to be found in the section on [Experimental Design and Statistical Analysis](#).

#### Harm-Benefit Assessment



## PREPARE

PREPARE checklist

Comparison with ARRIVE

Endorsements

Film

1-Literature searches

2-Legal issues

3-Ethical issues, harm-benefit assessment and humane endpoints

## 3-Ethical issues, harm-benefit assessment and humane endpoints

Each research project has its own set of ethical challenges, but the following general questions should be raised for all projects:

1. Have national or local research ethics committees already produced statements relevant to the research being planned?
2. Have the Three Rs ([Replacement, Reduction, Refinement](#)) been addressed, and will any advances in this area be mentioned in publications of the study (remembering that many databases only index the title and abstract of papers)? Which [non-animal alternatives](#) have been considered but rejected?
3. Have the Three S's ([Good Science, Good Sense and Good Sensibilities](#)) been addressed?
4. Does the proposed study have a clear rationale and scientific relevance, and what will be the next step if the hypothesis is supported or rejected?
5. Have the experiments been carried out before, and is any repetition justifiable?
6. What [approaches to reduce distress](#) have been considered?

## Laboratory Animals

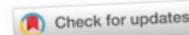


Affiliated Societies

### Clarification of early end-points for refinement of animal experiments, with specific reference to fish

Tim Ellis , Ioanna Katsiadaki

First Published December 1, 2020 | Review Article | [Find in PubMed](#)



<https://doi.org/10.1177/0023677220971002>

Sensibilities).

3d Consider pre-registration and the publication of negative results.

3e Perform a Harm-Benefit Assessment and justify any likely animal harm.

based on ecotoxicological data is not necessarily consistent with the real-life situation, and that [changes in body and fin shape](#) may good sub-lethal endpoints when performing *in vivo* studies.

The website <https://www.humane-endpoints.info/en> has sections dedicated to [zebrafish](#).

- > Heather Ikert *et al.* (2021) have investigated the possible [use of circulating plasma microRNAs as biomarkers for the identification of acute stress responses in fish](#).
- > [Video-based identification of surrogate endpoints in experimental bacterial infections of rainbow trout \(\*Oncorhynchus mykiss\*\)](#) (Keeling, 2018, Doctoral Thesis).
- > Ellis and Katsiadaki (2020) [Clarification of early end-points for refinement of animal experiments, with specific reference to fish](#). *Laboratory Animals*, 55(3): 244-253.

PREPARE (*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 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

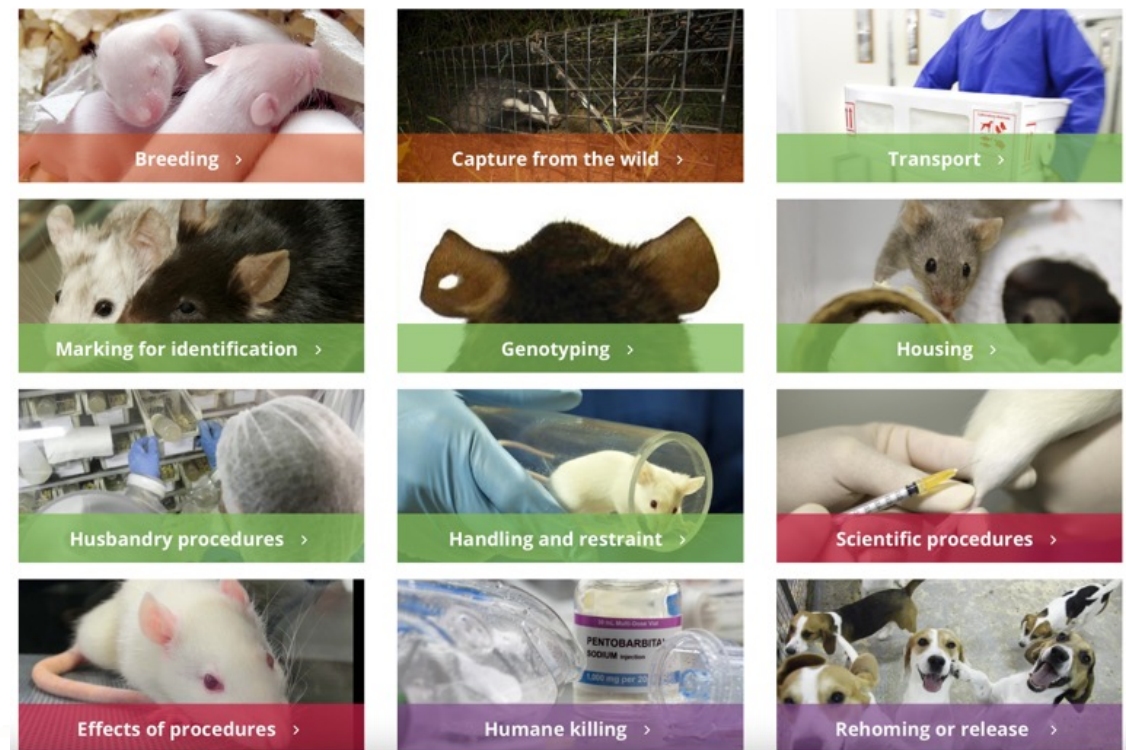
9. [Test substances and procedures](#)
10. [Experimental animals](#)
11. [Quarantine and health monitoring\\*](#)
12. [Housing and husbandry](#)
13. [Experimental procedures](#)
14. [Humane killing, release, reuse or rehoming\\*](#)
15. [Necropsy](#)

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[focusonseveresuffering.co.uk](http://focusonseveresuffering.co.uk)

### THINKING ABOUT THE LIFETIME EXPERIENCES OF EACH ANIMAL

There is huge potential for reducing suffering and improving welfare by thinking carefully about how every event might be experienced by the animal and how each one can be optimally refined.



***PREPARE encourages scientists to collaborate with animal carers and 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
- lack of involvement creates anxiety, depression and opposition to animal research, as well as limiting creativity which might improve the experiments



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. Advice on how to conduct a harm-benefit analysis [is available here](#).

[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. Extensive guidance on how to manage severe suffering [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.

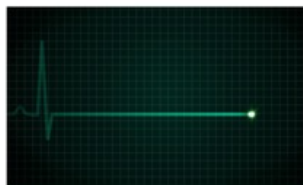




The [EU Directive 2010/63](#) on the protection of animals used for scientific purposes states that all procedures are to be classified into categories according to their severity (Article 15). This webpage lists resources designed to aid this process.

- › In July 2009 an expert group commissioned by the EU met to produce guidelines for the severity classification of procedures used on animals in research. [Their report is available here](#). It focuses on procedures that are used on the traditional laboratory animals (see the separate section below for complementary guidelines for fish).
- › The [FELASA/ECLAM/ESLAV Report on the classification and reporting of severity experienced by animals used in scientific procedures \(Smith et al., 2018\)](#) aims to deliver guidance on the assignment of severity, both prospectively and at the end of a procedure. The rationale behind this report is based upon the fact that the examples of severity classification given in [Annex VIII of the EU Directive](#) are limited in number and often relate to the procedure rather than assessing its outcome.
- › A Working Group convened by AALAS and FELASA has published two papers on a related topic: Harm-Benefit Assessment (HBA). These and other resources on HBA [can be accessed here](#).
- › Zintzsch et al. (2017): [Guidelines on severity assessment and classification of genetically altered mice and rat lines](#)
- › [UK Home Office advisory notes on recording and reporting the actual severity reporting of regulated procedures](#)
- › [UK Home Office advice on severity assessment of genetically altered animals](#)
- › Tappe-Theodor et al. (2022): [The “WWHow” Concept for Prospective Categorization of Post-operative Severity Assessment in Mice and Rats](#)
- › The RSPCA has constructed a comprehensive website entitled [Focus on Severe Suffering](#), in collaboration with LASA, LAVA and IAT, with ideas on how to reduce or avoid severe suffering. The website is designed to help all those involved with research animal use, care or regulation, but on some topics information is tailored for researchers, animal technologists or veterinarians, and members of ethics or animal care and use committees.

## Links to these reports within PREPARE



### Avoiding mortality

Hawkins et al. (2019)

Avoiding mortality in animal research and testing.  
ISBN: 978-0-901098-17-7A



### Experimental Autoimmune Encephalomyelitis (EAE)

Wolfensohn et al. (2013)

Reducing suffering in experimental autoimmune encephalomyelitis (EAE).  
Journal of Pharmacological & Toxicological Methods 67, 169-176



### Rheumatoid arthritis

Hawkins et al. (2015)

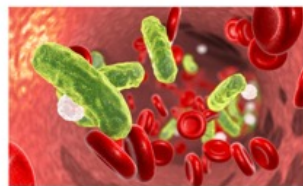
Applying refinement to the use of mice and rats in rheumatoid arthritis research.  
Inflammopharmacology 23, 131-150



### Seizures, convulsions and epilepsy

Wolfensohn et al. (2013)

Reducing suffering in animal models and procedures involving seizures, convulsions and epilepsy.  
Journal of Pharmacological & Toxicological Methods 67, 9-15



### Sepsis

Lilley et al. (2015)

Refinement of animal models of sepsis and septic shock.  
Shock 43, 304-316



### Spinal cord injury

Lilley et al. (2020)

Refining rodent models of spinal cord injury.  
Experimental Neurology 328, 113273

## OTHER RELEVANT REPORTS

### Cancer research

Workman P et al. (2010)

Guidelines for the welfare and use of animals in cancer research.  
British Journal of Cancer 102, 1555-1577  
<https://doi.org/10.1038/sj.bjc.6605642>

### Cardiovascular research

Emerson (2010)

Refinement, reduction and replacement approaches to in vivo cardiovascular research.  
British Journal of Pharmacology 161, 749-754

### Epilepsy

Modebadze et al. (2016)

A low mortality, high morbidity Reduced Intensity Status Epilepticus (RISE) model of epilepsy and epileptogenesis in the rat.  
PLoS ONE 11(2), e0147265  
[doi:10.1371/journal.pone.0147265](https://doi.org/10.1371/journal.pone.0147265)

### Epilepsy and seizures

Lidster et al. (2016)

Opportunities for improving animal welfare in rodent models of epilepsy and seizures.  
Journal of Neuroscience Methods 260, 2-25

### Fish acute toxicity test

Katsiadaki et al. (2021)

Dying for change: A roadmap to refine the fish acute toxicity test after 40 years of applying a lethal endpoint.  
Ecotoxicology and Environmental Safety 223, 112585

### Ischaemic stroke

Percie du Sert N et al. (2017)

The IMPROVE Guidelines (Ischaemia Models: Procedural Refinements Of in Vivo Experiments).  
Journal of Cerebral Blood Flow and Metabolism 37(11), 3488-3517  
[doi:10.1177/0271678X17709185](https://doi.org/10.1177/0271678X17709185)

### Liver fibrosis models

Probert PME et al. (2014)

A reversible model for periportal fibrosis and a refined alternative to bile duct ligation.  
Toxicology Research 3, 98-109  
[doi: 10.1039/C3TX50069A](https://doi.org/10.1039/C3TX50069A)

### Neuropathic pain

Percie du Sert & Rice (2014)

Improving the translation of analgesic drugs to the clinic: animal models of neuropathic pain.  
British Journal of Pharmacology 161, 749-754

### Vaccine potency testing

Hendriksen (2009)

Replacement, reduction and refinement alternatives to animal use in vaccine potency measurement.  
Expert Review of Vaccines 8, 313-322

### Vaccine studies using NHPS

Prescott et al. (2021)

Opportunities for refinement of non-human primate vaccine studies.  
Vaccines 9, 284

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## The Refinement Wiki

Born from the knowledge that a lot of good ideas on refinement circulate on discussion forums, but never get published.



Designed to be

- a portal for rapid publication and dissemination of these ideas
- a place to identify experts on specific refinement techniques
- an aid to finding collaborators for multi-lab studies on refinement

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## Clicker training

Clicker training is an operant conditioning based on positive reinforcement. When the animal offers the desired behavior, a *click* or another distinctive sound (secondary reinforcer) is delivered and within the following few seconds the reward is presented (primary reinforcer)<sup>[1]</sup>. The *click* bridges the time between the desired behavior and the presentation of the reward<sup>[1]</sup>. A target stick providing a visual guide for the animal can be used for the training.

Animals are usually trained individually, though it is also possible to perform clicker training in a groups, e.g. in mice, rats, and rabbits. For rats, it was demonstrated that they learned tasks by observing the clicker training of their cage mates<sup>[2]</sup>.

Clicker training can be used to train animals in a stress-free way. The following behaviours are examples for what this technique can be used for:

**Mice:** entering a tunnel, following a target stick, climbing on the palm of the hand<sup>[3]</sup>

**Rats:** following a target stick, voluntarily change to a cage, observational learning<sup>[2]</sup>

**Rabbits:** following a target stick, rearing/standing up to inspect the abdomen, approaching a human, being touched and lifted by a human, trimming nails, coming on command

**Pigs:** Pigs can be easily trained to cooperate if they are treated empathetically and desired behavior is reinforced by providing food stuff in form of treats and apple juice<sup>[4]</sup>.



**Clicker training with mice using a target stick.** *Left:* The mouse is following the target stick and is climbing on the experimenter's hand. If the hand is lifted, the mouse will remain on the palm of the hand. *Right:* The mice are trained in a group. Two mice are following the target stick on the palm of the experimenter's hand.

- <sup>1</sup> <sup>1.0</sup> <sup>1.1</sup> Feng, Lynna C.; Howell, Tiffani J.; Bennett, Pauleen C. (1 August 2016). "How clicker training works: Comparing Reinforcing, Marking, and Bridging Hypotheses". *Applied Animal Behaviour Science*. **181**: 34–40. doi:10.1016/j.applanim.2016.05.012. ISSN 0168-1591.
- <sup>2</sup> <sup>2.0</sup> <sup>2.1</sup> Leidinger, Charlotte Sophie; Kaiser, Nadine; Baumgart, Nadine; Baumgart, Jan (25 October 2018). "Using Clicker Training and Social Observation to Teach Rats to Voluntarily Change Cages". *JoVE (Journal of Visualized Experiments)* (140): e58511. doi:10.3791/58511. ISSN 1940-087X. PMC 6235608. PMID 30417890.
- <sup>3</sup> Leidinger, Charlotte; Herrmann, Felix; Thöne-Reineke, Christa; Baumgart, Nadine; Baumgart, Jan (6 March 2017). "Introducing Clicker Training as a Cognitive Enrichment for Laboratory Mice". *JoVE (Journal of Visualized Experiments)* (121): e55415. doi:10.3791/55415. ISSN 1940-087X. PMC 5408971. PMID 28287586.
- <sup>4</sup> "Positive Reinforcement Training in Large Experimental Animals" (PDF).

**Experts for clicker training in mice and rats:** TARC, Mainz, Germany

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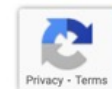
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A simple instruction manual to keep the threshold for adding new content as low as possible

This page supplements advice given in [Section 4 of the PREPARE guidelines](#). PREPARE covers all aspects of design (including animal and facility related issues).



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# CIRS-LAS Portal

Critical incident reporting system in laboratory animal science

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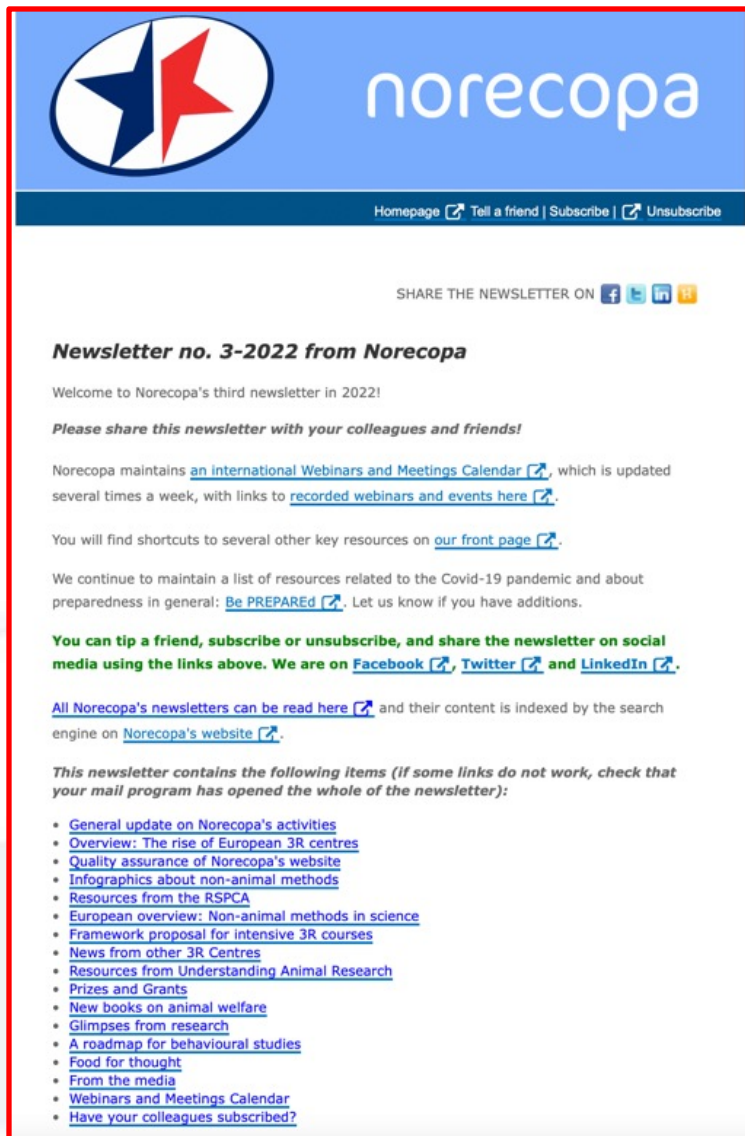
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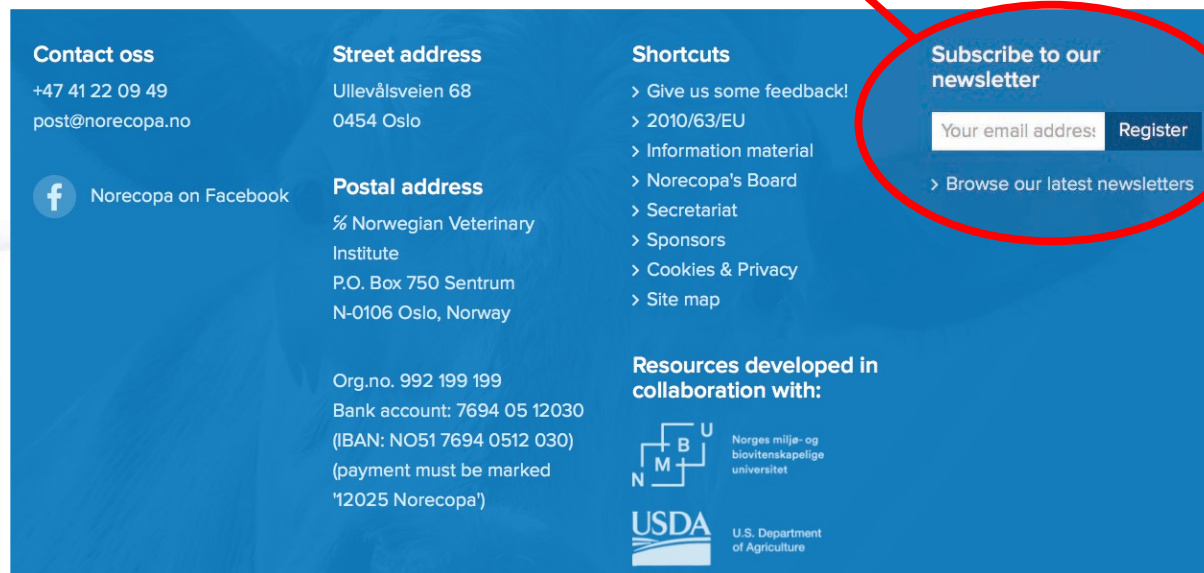
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