

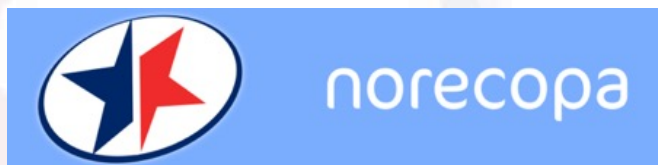
# An overview of guidelines for better preclinical research

**Adrian Smith**

*adrian.smith@norecopa.no*

*@adrian\_3r*

***[norecopa.no/EUSAAT2022](https://norecopa.no/EUSAAT2022)***



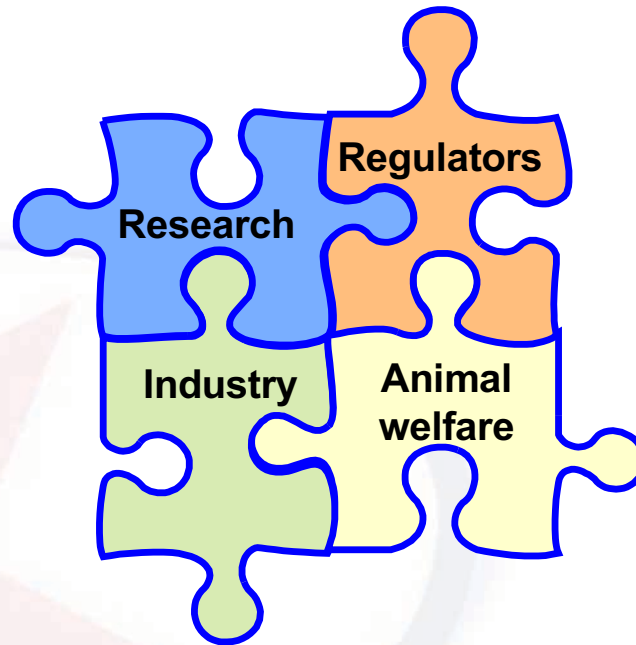
**<https://norecopa.no>**

Norecopa is Norway's National **Consensus**-platform,

working to advance ***all the three R's***:

Replacement, Reduction and Refinement

Its Board represents:



*Established in 2007*

## ***Disclosures***

- ***Webmaster for the Norecopa site whose purpose is to spread information about global guidelines***
- ***Lead author of the 3R Guide database of guidelines***
- ***Lead author of the PREPARE guidelines for better preclinical research***
- ***Manager of the Refinement Wiki***



norecopa



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Norecopa: PREPARE for better Science



## **PREPARE:**

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

PREPARE covers 15 topics:

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

### **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

Systematic Reviews

Synthesis of Evidence from published papers

In vitro / in silico research

# The pathway to better science



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)

***“...better preclinical research”***

***If it involves animals:***

- valid data (a true treatment effect)
- reproducible and translatable experiments
- best possible animal welfare
- health & safety (of animals and people)
- a culture of care in the research group
- communication of best practice to others



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[norecopa.no/PREPARE/film](http://norecopa.no/PREPARE/film)  
3-minute whiteboard film



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## *How do others achieve reproducibility?*



<https://www.meonuk.com/runway-markings-explained>





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*...and precision in a variable environment?*



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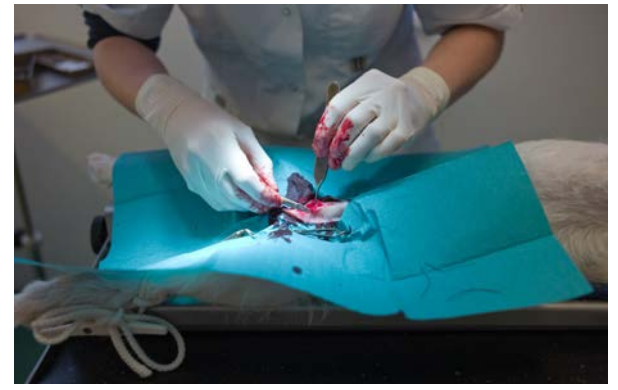
**EU / National**



**Facility**

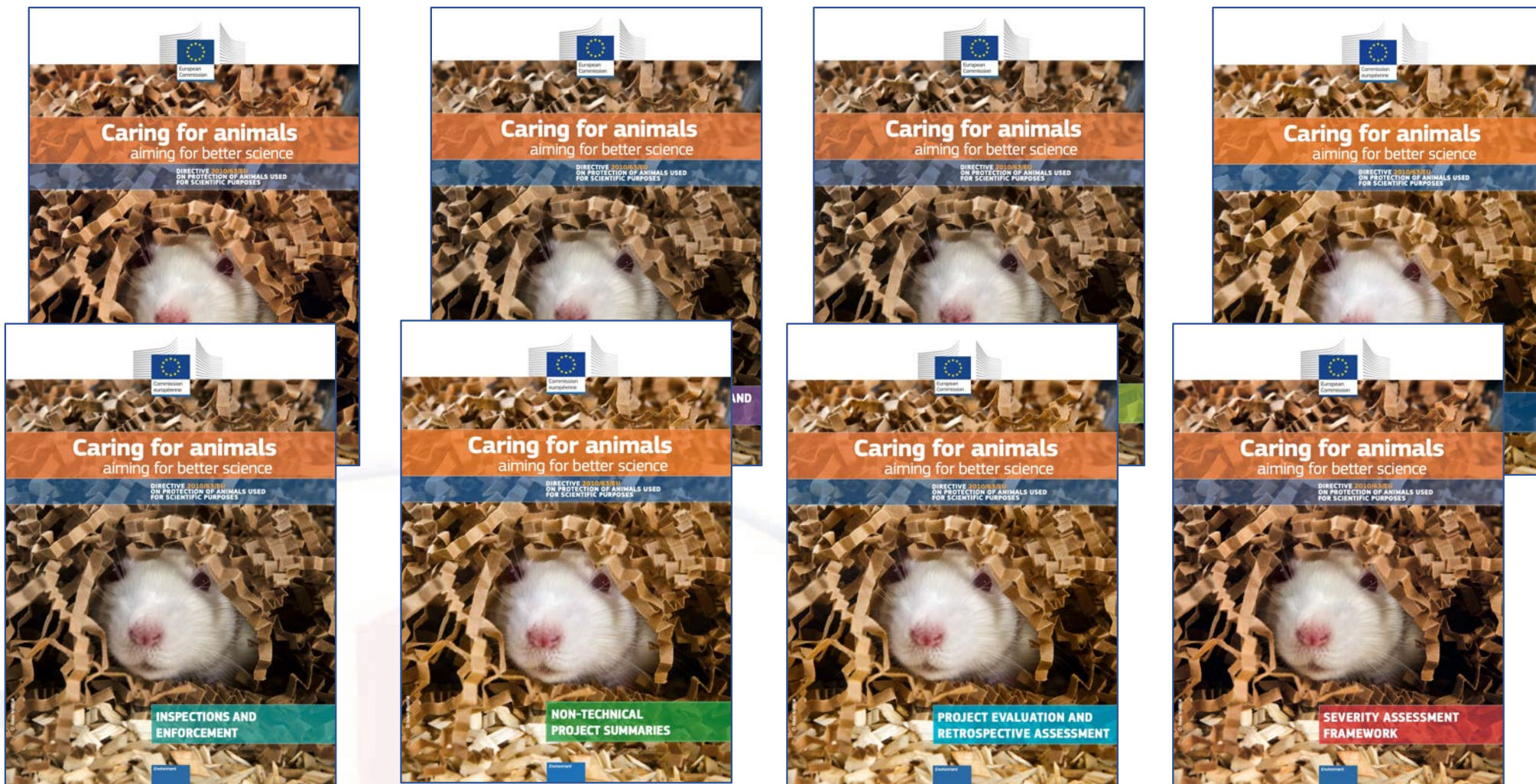


**Project**



**Procedure**





[ec.europa.eu/environment/chemicals/lab\\_animals/pubs\\_guidance\\_en.htm](https://ec.europa.eu/environment/chemicals/lab_animals/pubs_guidance_en.htm)

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ec.europa.eu/animals-in-science

**ENVIRONMENT**

European Commission

European Commission > Environment > Chemicals > Animals used for scientific purposes

Home About us Policies Funding Legal compliance News & outreach

**Animals used for scientific purposes**

**Retrieval and provision of information on the "Three Rs" and alternatives**

Accessing accurate, relevant and up-to-date information on the Three Rs is a challenge for all those use of animals.

**Legislation and implementation**

- EU legislative framework
- Implementation of Directive 2010/63/EU
- Q&A and guidance documents**

**The "Three Rs" and alternative approaches**

- Replacement, Reduction and Refinement – the "Three Rs"
- Validation, acceptance and use
- EU activities to advance alternatives
- Member State activities to advance alternatives
- Finding and distributing information on alternatives
- Key resources**

  - Search Tools
  - Databases
  - Portals and web-sites
  - Journals
  - Other resources and organisations

**Animals used for scientific purposes**

**Opinions of European Commission Expert Committees related to the use of animals in experiments**

f t





### ***Coming soon:***

Updated general guidance from the EU on animal research in, for example, Horizon Europe programmes

#### ***Examples:***

*'Higher' animals can be replaced by 'lower' animals: microorganisms, plants, eggs, reptiles, amphibians, and invertebrates may be used in some studies to replace warm-blooded animals.*

[ec.europa.eu/info/funding-tenders/opportunities/docs/2021-2027/common/guidance/how-to-complete-your-ethics-self-assessment\\_en.pdf](https://ec.europa.eu/info/funding-tenders/opportunities/docs/2021-2027/common/guidance/how-to-complete-your-ethics-self-assessment_en.pdf)



## Facility



- A. Animal Care and Use Program**
- B. Animal environment, Housing and Management**
- C. Veterinary Care**
- D. Physical plant**

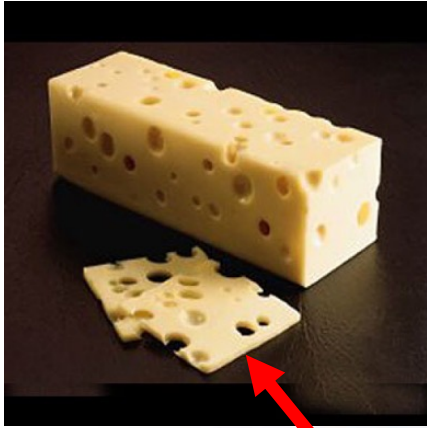
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III. Veterinary Care.....	29
A. Animal Procurement and Transportation .....	29
1. Animal Procurement.....	29
2. Transportation of Animals .....	29
B. Preventive Medicine.....	29
1. Animal Biosecurity.....	29
2. Quarantine and Stabilization .....	30
3. Separation by Health Status and Species.....	30
C. Clinical Care and Management.....	30
1. Surveillance, Diagnosis, Treatment and Control of Disease .....	30
2. Emergency Care .....	31
3. Clinical Record Keeping.....	31
4. Diagnostic Resources .....	32
5. Drug Storage and Control .....	32
D. Surgery.....	32
1. Pre-Surgical Planning.....	32
2. Surgical Facilities .....	33
3. Surgical Procedures .....	33
4. Aseptic Technique.....	33
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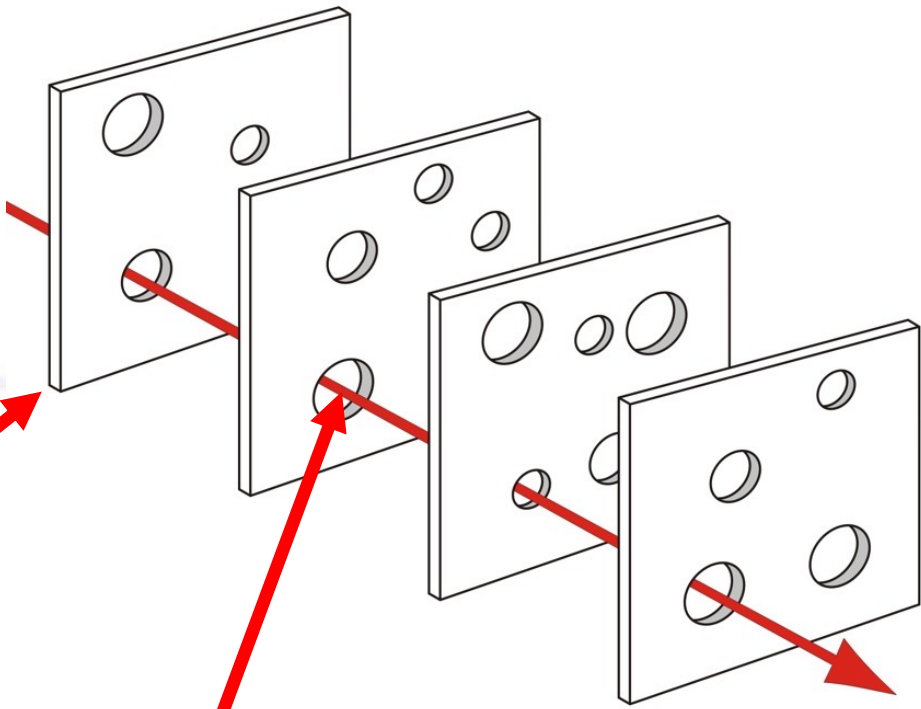
63 pages



# Threat and Error Management



[eaugallecheese.com/Swiss-Cheese](http://eaugallecheese.com/Swiss-Cheese)



"Layer of defence"  
or redundancy

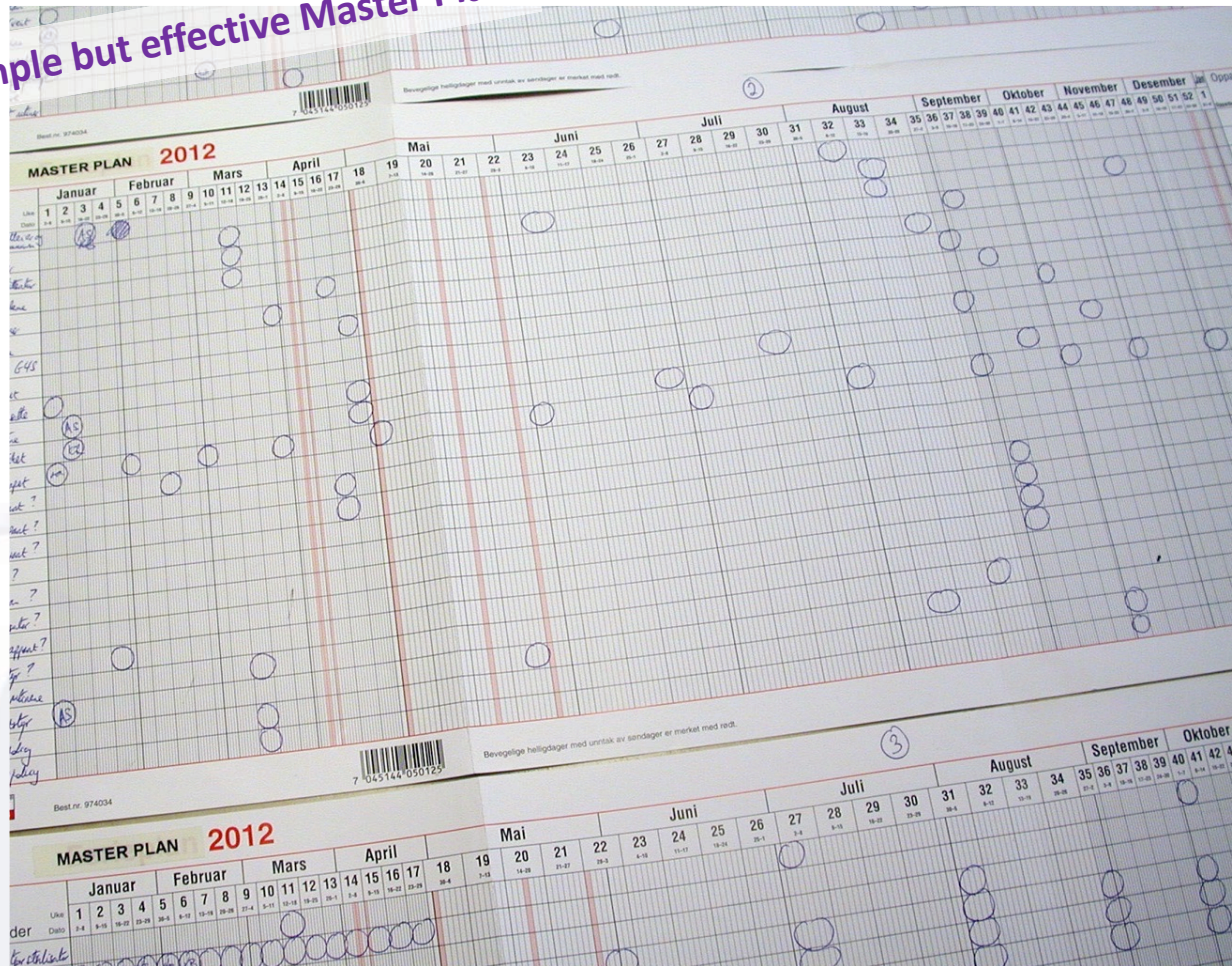
Weakness / hazard

**Loss**

[wikipedia.org/wiki/Swiss\\_cheese\\_model](http://wikipedia.org/wiki/Swiss_cheese_model)



A simple but effective Master Plan



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## Contingency and redundancy

***Anything that can go wrong, will go wrong*** (Murphy's Law)  
***when it's least convenient*** (Sod's Law)

Work in the spirit of AAALAC,  
even if not accredited!



Photo: NMBU





[wikipedia](#)

Norecopia: PREPARE for better Science



# CIRS-LAS Portal

Critical incident reporting system in laboratory animal science

**Refine - Reduce - Replace**

Homepage

Project

Team

FAQ



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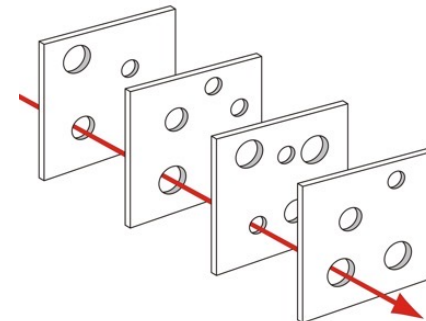
## A Contingency Plan, based upon risk assessment

- Access to emergency services (police, fire, medical and veterinary help, security guards, personnel transport in cases of acute illness)
- Means of communication with staff members at all levels
- SOPs for acute illness, including
  - serious haemorrhages
  - fainting
  - allergic reactions

Many of these needed revision in the light of Covid-19  
[norecopa.no/be-prepared](https://norecopa.no/be-prepared)

- corrosive injuries
- and forms for reporting such injuries
- Firefighting, evacuation of personnel and animals
- Access to specialist services (e.g. ventilation system, plumbing, electrical installations, suppliers of equipment)
- Routines in cases of power failure, water leaks and (if applicable) natural disasters such as flooding
- Routines for emergency killing of animals
- Routines in cases of threats to the facility or personnel

<https://norecopa.no/prepare/6-facility-evaluation/master-plan-and-sops/contingency-plan>



Temporary staff at weekends and holidays





## Project

A contract between the animal facility and the research group

Division of labour, responsibilities and cost

Clarifying all stages of the experiment

Ensuring that all necessary data are recorded



	Animal facility	Researcher	Not applicable
<b>Animal:</b>			
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)			
<b>Environment:</b>			
Type of housing: barrier/conventional			
Temperature (mean ± variation)			
Light schedule			
Relative humidity (mean ± variation)			
Number of air changes in the animal room/cabinet per hour			
Environmental enrichment			
<b>Housing:</b>			
Free-range, shelf, cabinet, isolator			
Cage type and size			
Number and method of distribution of animals per cage			





**Procedure**

## ***Brain and chest surgery with minimal analgesia***

Experiments were performed on spontaneously breathing adult male Wistar rats (anesthetized with sodium thiopentone 100 mg/kg i.p.) Two trephinations were made over the left parieto-occipital cortex, the dura mater was opened, and the exposed brain areas were superfused with regular artificial cerebrospinal fluid (ACSF, warmed to

experimental protocol, thoracotomy was performed under 50 mg/kg sodium thiopental anesthesia.

[norecopa.no/3RGuide](http://norecopa.no/3RGuide)

Links to 415 guidelines

**A good practice guide to the administration of substances and removal of blood, including routes and volumes**

3R Guide database/c6721 (legacy id: 15079)

This paper provides the researcher in the safety evaluation laboratory with an up-to-date, easy-to-use set of data sheets to aid in the study design process whilst at the same time affording maximum welfare considerations to the experimental animals.

**A guide to defining and implementing protocols for the welfare assessment of laboratory animals**

3R Guide database/68ba4 (legacy id: 15065)

Eleventh report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement

**A guide to the care and use of native Australian mammals in research and teaching**

3R Guide database/502ff (legacy id: 15377)

The Guide supports implementation of the Australian Code for the care and use of animals for scientific purposes (8th edition, 2013) and ensures that the specific and unique needs of Australian native mammals are met when these animals are used for scientific purposes.

**AAALAC Position Statements**

3R Guide database/ef566 (legacy id: 15155)

In connection with its work of accreditation of animal care and use programmes, AAALAC International has issued position statements on a number of key elements in such a programme.



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## *10-15 checklists even on short routine flights*



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# Checklists

- Reduce risk of **forgetting** to carry out vital actions
- Ensure checks are carried out in the **correct sequence**
- Encourage **cooperation** and **cross-checking** between crew members
- Make sure that everyone is "**on the same page**"





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***Hudson River, 2009***

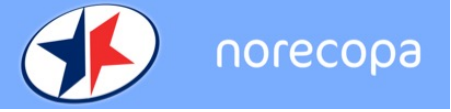
[en.wikipedia.org](https://en.wikipedia.org)

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[travelandleisure.com/airlines-airports/what-happens-when-planes-hit-birds](https://travelandleisure.com/airlines-airports/what-happens-when-planes-hit-birds)

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15.25.33	-01.38	Kaptein	Cockpit	V one, rotate
15.25.38	-01.33	Kaptein	Cockpit	positive rate
15.25.39	-01.32	Styrmann	Cockpit	Gear up please
15.25.39	-01.32	Kaptein	Cockpit	Gear up
15.26.37	-00.34	Kaptein	Cockpit	Uh what a view of the Hudson today
15.26.42	-00.29	Styrmann	Cockpit	Yeah
15.27.07	-00.04	Kaptein	Cockpit	After takeoff checklist complete
15.27.10	-00.01	Kaptein	Cockpit	Birds
15.27.11	-00.00	Styrmann	Cockpit	Whoa
15.27.11	00.00			
15.27.12	+00.01	Kaptein	Cockpit	Oh ---
15.27.13	+00.02	Styrmann	Cockpit	Oh yeah
15.27.14	+00.03	Styrmann	Cockpit	Uh oh
15.27.15	+00.04	Kaptein	Cockpit	We got one rol... both of 'em rolling back
15.27.18	+00.07	Kaptein	Cockpit	Ignition, start
15.27.21	+00.10	Kaptein	Cockpit	I'm starting the APU
15.27.23	+00.12	Kaptein	Cockpit	My aircraft
15.27.24	+00.13	Styrmann	Cockpit	Your aircraft
15.27.28	+00.17	Kaptein	Cockpit	Get the QRH... loss of thrust on both engines
15.27.32	+00.21	Kaptein	Radio	Mayday mayday mayday. Uh this is Cactus fifteen thirty [sic] nine, hit birds. We've lost thrust on both engines. We're turning back towards LaGuardia.

**All 155 passengers and crew saved**



**Rapid evacuation by trained cabin crew saved many lives**

***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





*Culture of Care*

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!*

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## 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

## *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.**

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

The image shows a screenshot of the norecopa.no website. The header features the norecopa logo and navigation links for 'NORSK' and 'ENGLISH'. A search bar is located in the top right, with a yellow arrow pointing to it. Below the header is a navigation menu with categories like 'About Norecopa', 'Alternatives', 'Databases & Guidelines', 'Education & training', 'Legislation', 'Meetings', 'More resources', 'News', 'PREPARE', 'Species', and 'Wiki'. A secondary menu lists various topics such as '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 callout box with the text 'we welcome more from you!' is overlaid on the page. 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, a search filter sidebar is visible, containing sections for 'Search filters', 'Database', 'Browse the databases', and 'Search in the databases'. The 'Database' section lists various resources with their respective counts, such as '3R Guide database (403)', 'Classic AVs database (118)', and 'European Commission Inventory of 3Rs Education & Training Resources (567)'. The 'Browse the databases' section lists categories like 'eBooks (286)', 'Free (199)', and 'Reviewed (85)'. The 'Search in the databases' section lists search criteria like 'All Text', 'Title', 'Author', 'Publisher', 'Supplier', and 'Record Number'. A 'Privacy' icon is visible at the bottom right of the sidebar.

**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).

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**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)
- 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)
- Website (761)

**Browse the databases**

- eBooks (286)
- Free (199)
- Held at NMBU Oslo (contact Kristine Hansen, 67 23 21 89) (431)
- Key products (68)
- On loan (6)
- Reviewed (85)

**Search in the databases**

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



## 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

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## Databases & Guidelines

Published lists of resources are difficult to search and quickly become outdated. Lists on a website are easier to search, but do not enable the use of filters or intelligent search engines.

***Norecopa has therefore constructed four databases, which together with all the text on this website can be searched simultaneously using the search field at the top of every page.***

- > [3R Guide](#): a global overview of **databases, guidelines, information centres, journals, email lists, regulations and policies** which may be of use when planning experiments which might include animals. [A quick overview of all the guidelines can be accessed here.](#) Norecopa has written several of these, including [the PREPARE guidelines for planning animal research and testing.](#)
- > [NORINA](#): a global overview of audiovisual aids and other items which may be used as **alternatives or supplements to animals in education and training** at all levels from junior school to University, including [dissection alternatives](#) and surgical simulators.
- > [TextBase](#): a global overview of **textbooks and other literature within laboratory animal science** and related topics.
- > [Classic AVs](#): a subset of NORINA covering **audiovisual aids that are based on older technology.**

These databases are updated regularly. [Please give us feedback](#) if you discover errors or omissions.

The Norecopa website also includes four other collections:

- > [NAL](#): a collection of literature references relating to [the 3Rs](#) from the US National Agricultural Library
- > European Commission datasets:
  - ▶ [3Rs Knowledge Sources](#): over 800 resources collected by the Commission in 2016
  - ▶ [3Rs Education and Training Resources](#), over 560 items collected in 2018
  - ▶ [Non-animal models for respiratory tract diseases](#), over 280 models identified in a literature review of over 21,000 publications

Here is [an alphabetical global list of all the databases](#) cited on the Norecopa website.

[norecopa.no/databases-guidelines](https://norecopa.no/databases-guidelines)

[links to over 70 other databases](#)

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# 3rswildlife.info

**3Rs** PRINCIPLES IN WILDLIFE RESEARCH

BACKGROUND ▾ EXAMPLES OF 3RS IMPLEMENTATION ▾ FAQ LINKS AUTHOR CONTACT

## 3Rs PRINCIPLES IN WILDLIFE RESEARCH

This site has been created to provide information about the 3Rs principles of animal use and guide their application in wildlife research. It contains examples of peer-reviewed studies that implemented non-lethal or non-invasive methods and that could be used as a guidance. It is the first online resource of its kind developed specifically for wildlife biologists, ecologists, and conservation managers.

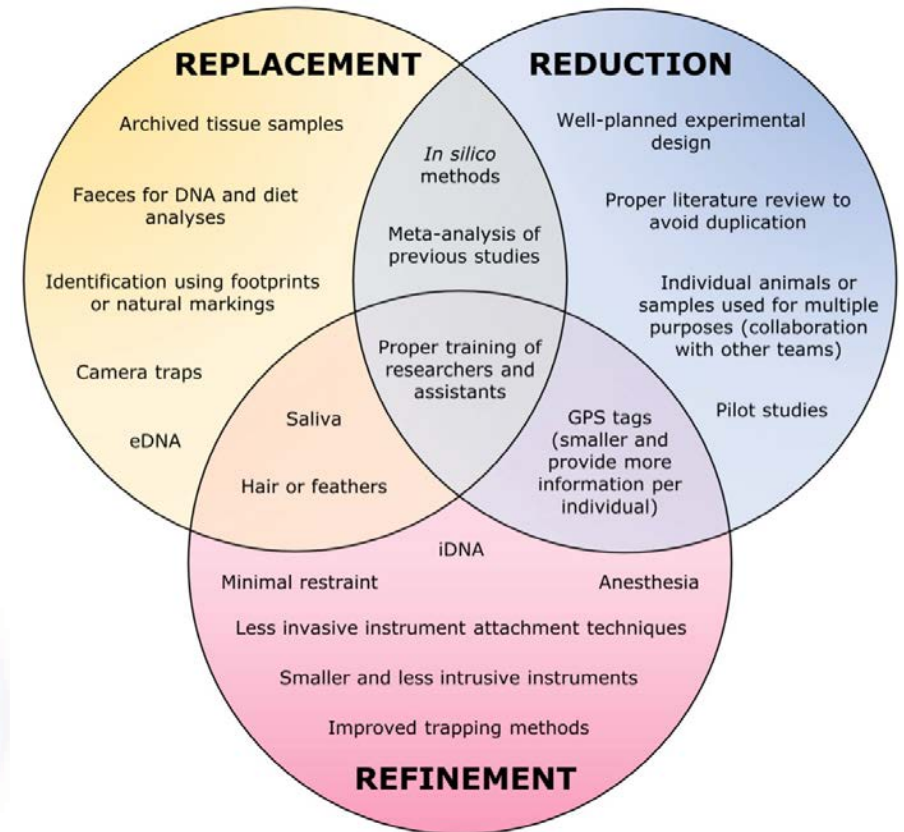


**Featured so far:**

<b>71</b>	<b>937</b>	<b>603</b>
NON-INVASIVE METHODS	SPECIES	PEER-REVIEWED STUDIES

THIS WORK HAS BEEN KINDLY SUPPORTED BY:

Animalfree Research  
Eva Husi-Stiftung für Tierschutz



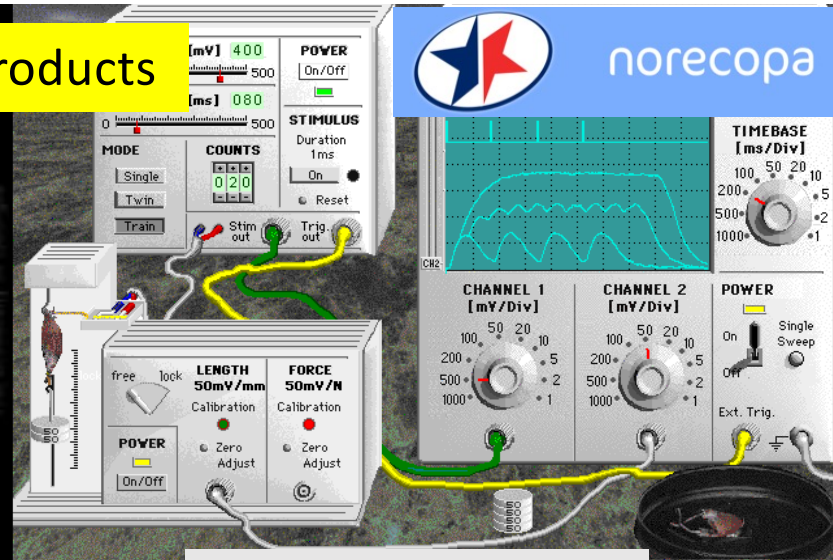
Source: Zemanova 2020

# Miriam Zemanova

NORINA database: approx. 3,000 products



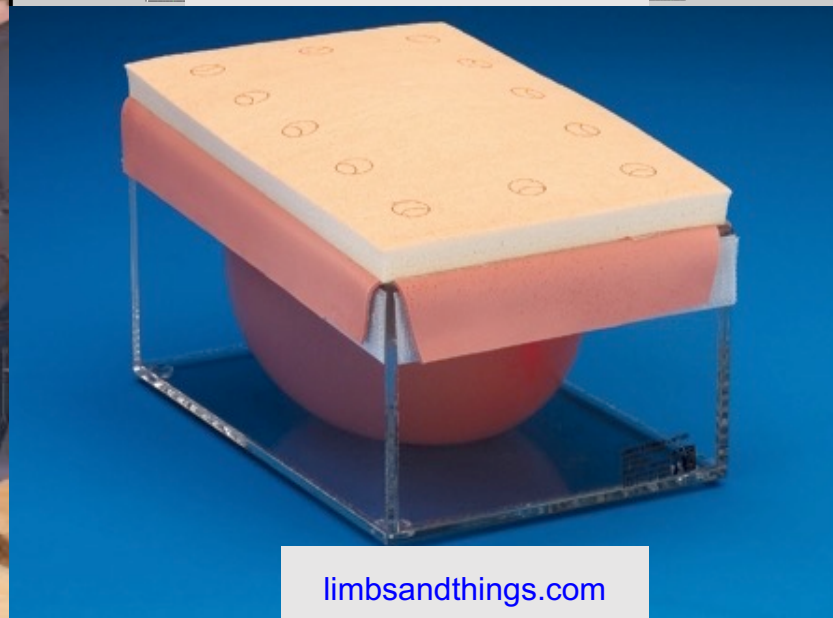
[3dglasshorse.com](http://3dglasshorse.com)



[virtualphysiology.com](http://virtualphysiology.com)



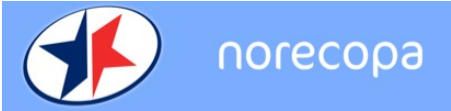
[rescuecritters.com](http://rescuecritters.com)



[limbsandthings.com](http://limbsandthings.com)



norecopa.no/education-training/films-and-slide-shows



Rat s.c. injection  
Norecopa | 1,380 views



Testing anaesthetic depth in the chicken  
Norecopa | 598 views



Blood sampling from the pig  
Norecopa | 3,914 views



Subcutaneous injection in the rabbit  
Norecopa | 1,479 views



Rat i.p. injection (method 2)  
Norecopa | 1,280 views



Blood collection from the saphenous vein in the mouse  
Norecopa | 6,777 views



Intravenous injection in a rabbit  
Norecopa | 2,025 views



Subcutaneous injection in the chicken  
Norecopa | 1,806 views

**ANATOMÍA DE LA RATA**

Dra. Dolores Vallejo Ruiz  
Departamento de Biología de Sistemas, Universidad de Alcalá (Madrid)

Patrocinado por:  
Asesoría Científica: Dr. José María Orellana Moriana  
Centro de Experimentación Animal, CAI Medicina-Biología, Universidad de Alcalá

Anatomía de la rata  
Norecopa | 977 views



Subcutaneous injection in the rat - Technique 1  
Norecopa | 2,249 views



Lifting a rabbit  
Norecopa | 2,420 views











Immobilisation of the rabbit  
Norecopa | 2,072 views














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Training resources for animal research

 <p><b>National Legislation (EU1)</b> Understand the national and international legal and regulatory framework within which projects involving animals are constructed and managed and of the legal responsibilities of the people involved.</p>	 <p><b>Ethics, Animal Welfare and the 3Rs (EU2)</b> Identify the ethical and welfare issues raised by the use of animals in scientific procedures and understand the basic principles of the 3Rs.</p>
 <p><b>Basic and Appropriate Biology (EU3)</b> Discover the basic principles of animal behaviour, care, biology and husbandry.</p>	 <p><b>Animal Care, Health and Management (EU4)</b> Examine information on various aspects of animal health, care and management including: environmental controls, husbandry practices, diet, health status and disease.</p>
 <p><b>Recognition of Pain, Suffering and Distress (EU5)</b> Identify the normal condition and behaviour of experimental animals and differentiate between a normal animal and one which is showing signs of pain, suffering or distress.</p>	 <p><b>Humane Methods of Killing (EU6.1)</b> Learn the principles of humane killing including descriptions of the different methods available and information to help you compare the methods permitted to determine the most appropriate method.</p>
 <p><b>Minor Procedures without Anaesthesia (EU7)</b> An introduction to the theory relating to minor procedures and information about appropriate methods of handling, restraint, appropriate techniques for injection, dosing and sampling relevant to the species.</p>	 <p><b>Anaesthesia for Minor Procedures (EU20)</b> Guidance and information for individuals who, during their work with animals, will need to apply sedation or short-term anaesthesia for a brief period and mild pain level procedure.</p>

eModules

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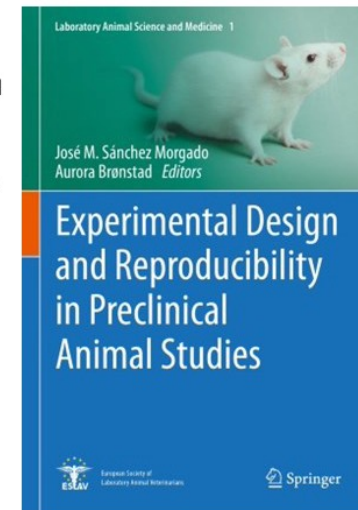
## Experimental Design and Reproducibility in Preclinical Animal Studies

By José M. Sánchez Morgado & Aurora Brønstad (Eds.)

Record number: 8619d

This book provides grounds on how to plan and conduct animal experiments that can be reproduced by others. It touches on factors that may impact the reproducibility of animal studies including: the animal genetic background, the animal microbial flora, environmental and physiological variables affecting the animal, animal welfare, statistics and experimental design, systematic reviews of animal studies, and the publishing process.

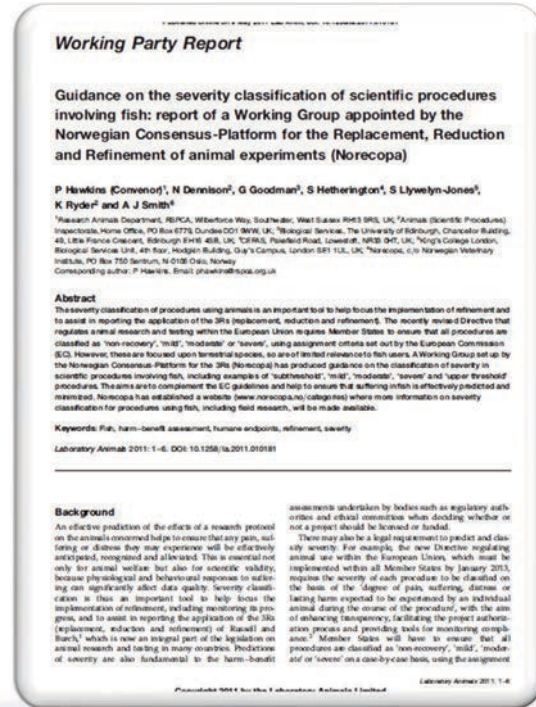
The book addresses advanced undergraduates, graduate students and all scientists working with animals.



[norecopa.no/textbase/experimental-design-and-reproducibility-in-preclinical-animal-studies](http://norecopa.no/textbase/experimental-design-and-reproducibility-in-preclinical-animal-studies)

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

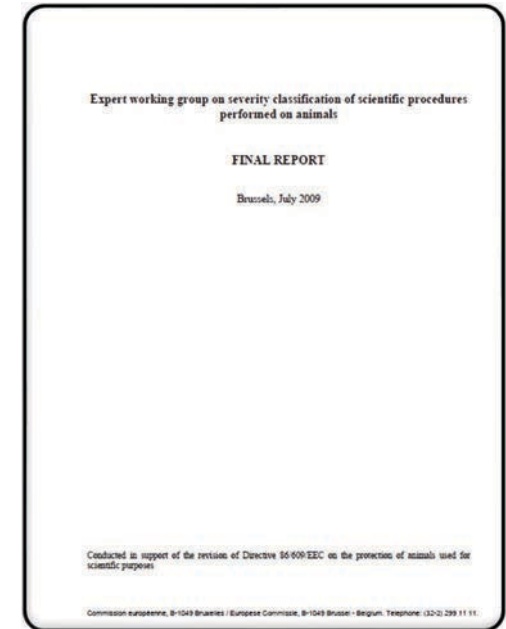
[norecopa.no/3r-guide](http://norecopa.no/3r-guide)



## Guidance on the severity classification of procedures involving fish

Report from a Working Group convened by Norecopa

Laboratory Animals, 2011  
[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)

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# Harm-Benefit Analysis

- *The harm* is experienced **NOW**, and is certain
- The benefit is *in the future*, for *other animals or humans*, and *is uncertain*
- HBA analysis is meaningless if the data from the experiment are unreliable
- So we need *guidelines* both *for HBA* and for *experimental design & statistical analysis*



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



## **We can work to tip the balance**

### **The 3 Rs to minimise the harm:**

- *Replace the unnecessary experiments*
- *Reduce the number of animals used*
- *Refine the conditions for the animals*

### **The 3 Ss - your commonsense and your heart**

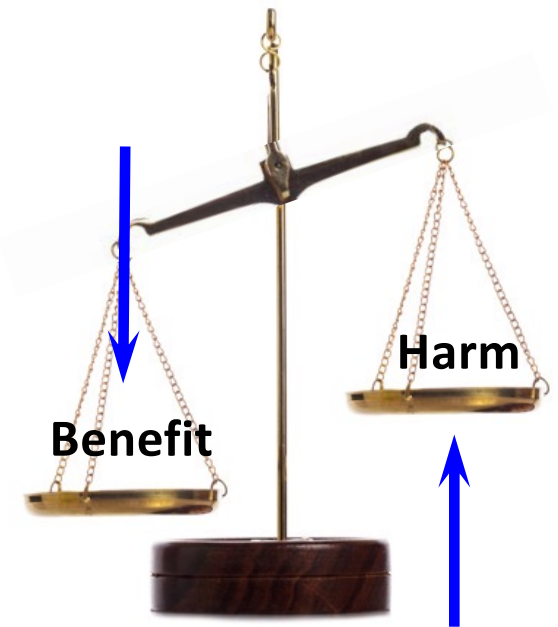
- *Good Science*
- *Good Sense*
- *Good Sensibilities*



### **The 3 Vs to increase the validity of the experiment:**

- *Construct Validity (can the model answer the question?)*
- *Internal Validity (has the experiment been correctly designed?)*
- *External Validity (are the results translatable to the target group?)*

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[norecopa.no/3R](https://norecopa.no/3R)

[norecopa.no/3S](https://norecopa.no/3S)

[norecopa.no/3V](https://norecopa.no/3V)



Original Article

Laboratory Animals  
0011-77  
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DOI: 10.1177/0023677217724823  
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**PREPARE: guidelines for planning animal research and testing**

Adrian J Smith<sup>1</sup>, R Eddie Clutton<sup>2</sup>, Elliot Litley<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 Norecoba website, with links to guidelines for animal research and testing, at <https://norecoba.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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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>



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## **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

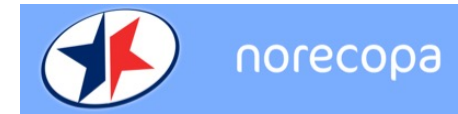
### **Dialogue between scientists and the animal facility**

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 typically  
highlighted in  
reporting guidelines



# 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>  
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PREPARE<sup>®</sup> consists of planning guidelines which are complementary to reporting guidelines. PREPARE covers the three broad areas which determine the quality of animal research: the 3Rs (Replacement, Reduction, Refinement) and the 3Ss (Good Science, Good Sense, Good Sensibilities).

1. Formulation of the study  
 2. Diagnostics  
 3. Health risks, waste disposal and decontamination  
 4. Experimental design and statistical analysis  
 5. Objectives and timescale, funding and division of labour

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 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.

Animal welfare and Three Rs!

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. 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 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 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)



# norecopa.no/PREPARE

- 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).
  - 3d 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 be published, to avoid publication bias? Will the project undergo pre-registration and will negative results be published, to avoid publication bias?
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

## The Refinement Wiki



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

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



Susanna Louihimies

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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.

1. <sup>↑</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 [↗](#).
2. <sup>↑</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 [↗](#).
3. <sup>↑</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 [↗](#).
4. <sup>↑</sup> "Positive Reinforcement Training in Large Experimental Animals" [↗](#) (PDF).

**Experts for clicker training in mice and rats:** [TARC](#) [↗](#), Mainz, Germany

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- Contingency plans
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- Detecting early onset of clinical signs in the mouse model of Covid-19
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- Oestrus suppression in ferrets
- Pneumocystis murina
- Recapping needles
- Rotarod Test
- Screening cell lines
- Sedation of cattle
- Splenectomy
- Sterilisation of instruments
- TTEAM and TTouch
- Tail vein injection
- Tramadol
- Transport stress
- Tumour cell implant into mammary fat pad
- Ulcerative Dermatitis in Mice
- Water quality
- Xenopus laevis
- Zebrafish swabbing





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*"We ARRIVED, because we were PREPARED"*

- ✓ *Better Science*
- ✓ *Improved animal welfare*
- ✓ *Advancement of the 3Rs*
- ✓ *Safer working environment*

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## Let's PREPARE together to ARRIVE in better shape: how to plan animal experiments

Adrian Smith, Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 64, 1431 Ås, Norway  
[adrian.smith@norecopa.no](mailto:adrian.smith@norecopa.no)

### What's the problem?

Preclinical *in vivo* research needs to be reproducible and translatable, while maximising the animals' welfare and replacing them with alternatives wherever possible. This can be summed up in the **3Rs** of Russell & Burch: **Replace, Reduce & Refine**.

Scientists are usually well aware of **reporting** guidelines when publishing research. These are important, but a sub-standard study, like a burnt cake, cannot be improved by a better description. Guidelines for **planning**, although not mandatory, are of great help in designing better experiments.



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

### What can Norecopa offer?

Norecopa maintains a comprehensive database of resources for scientists, which include:

- 8,900 searchable webpages of quality 3R resources, with filters to facilitate searching
- the PREPARE guidelines for planning animal experiments, with a checklist in over 30 languages
- links to recordings of webinars covering all aspects of animal research
- an International Webinars & Meetings Calendar
- a collection of 400 guidelines for planning and conducting animal research
- an English-language newsletter with the latest developments within experimental design
- the NORINA database of alternatives to animal use in education and training
- a Refinement Wiki



### Examples of Norecopa's resources:

**PREPARE covers:**

- ✓ Formulation of a study
- ✓ Dialogue between scientists and the animal facility
- ✓ Quality control of the components in the study



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

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Norecopa gratefully acknowledges financial support from:  
The Norwegian Parliament, the Ministry of Agriculture & Food and the Ministry of Trade, Industry & Fisheries; the Nordic Society against Painful Experiments (NSMSO), Novo Nordisk, the Norwegian Animal Protection Alliance (Dyrevernalliansen), the Norwegian Society for Protection of Animals (Dyreskytelseen Norge), the Research Council of Norway, Laboratory Animals Ltd., the Royal Society for the Prevention of Cruelty to Animals (RSPCA), Sanofi, the Scottish Accreditation Board, the Stånsen Foundation, the Universities Federation of Animal Welfare (UFAW) and the US Department of Agriculture (USDA).

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**Thank you for listening!**