How to plan studies well

norecopa.no/CBMR

Adrian Smith
adrian.smith@norecopa.no

Norecopa

Norway's National Consensus Platform for the

Three Rs: Replacement, Reduction and Refinement

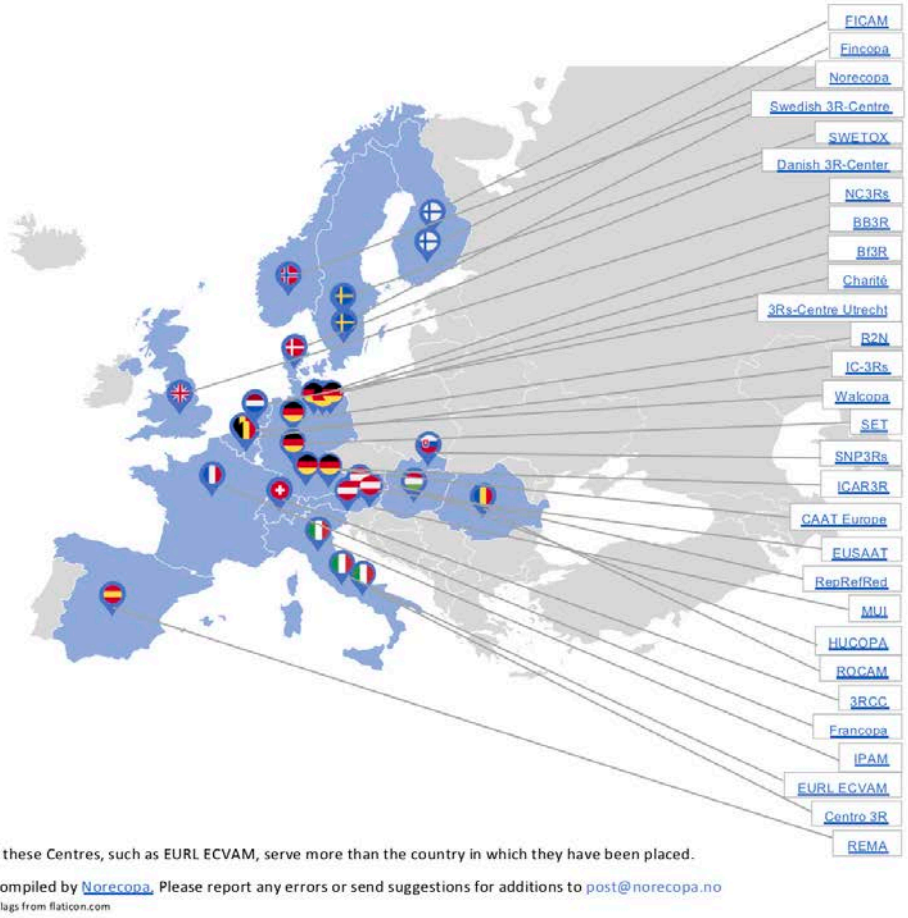
and a source of global 3R resources

https://norecopa.no

Norecopa: PREPARE for better Science

European network of 3R Centres established in 2018 - many with money for 3R research

Interactive map:
norecopa.no/3REuropeOverview



Content of this webinar (30 mins + Q&A)

- 'How to plan studies well': *am I suggesting that you are not?* An introduction to the topic and some personal views
- Where to go for more information
- *Disclosure*: I have written many of the webpages I will present, but most of them are links to other peoples' work, and were collected for teaching scientists and for managing lab animal facilities.

  Novo Nordisk are among the sponsors who financed the building of Norecopa's current website
- What's missing, and how you can help

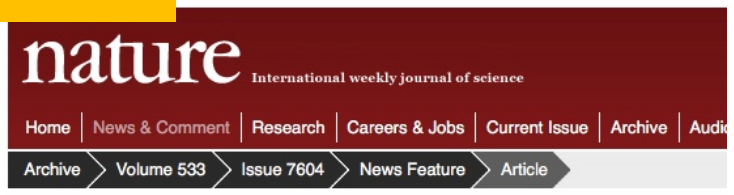
The views expressed in this webinar are my own and not necessarily those of Norecopa.

"The Reproducibility Crisis"

NATURE | NEWS

Swiss survey highlights potential flaws in animal studies

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



NATURE | NEWS FEATURE

1,500 scientists lift the lid on reproducibility

Survey sheds light on the 'crisis' rocking research.

Monya Baker

25 May 2016 | Corrected: 28 July 2016

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

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Frequently highlighted causes of the "reproducibility crisis"

1. Publication bias (reporting only positive results)
2. Low statistical power
3. P-value hacking (manipulating data to obtain significance)
4. HARKing (Hypothesizing after the results are known)
5. Lack of randomisation and blinding

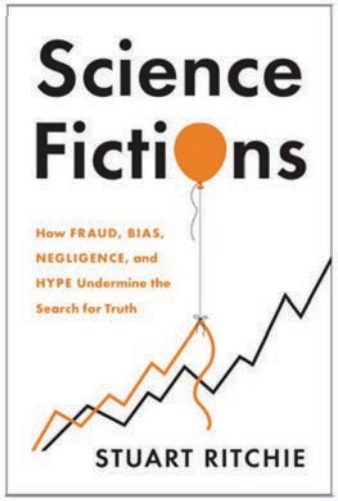
norecopa.no/concerns

Science Fictions

By Stuart Ritchie

Record number: 139853

An insider's view of science reveals why many scientific results cannot be relied upon – and how the system can be reformed.



Science is how we understand the world. Yet failures in peer review and mistakes in statistics have rendered a shocking number of scientific studies useless – or, worse, badly misleading. Such errors have distorted our knowledge in fields as wide-ranging as medicine, physics, nutrition, education, genetics, economics, and the search for extraterrestrial life. As *Science Fictions* makes clear, the current system of research funding and publication not only fails to safeguard us from blunders but actively encourages bad science – with sometimes deadly consequences.

Stuart Ritchie's own work challenging an infamous psychology experiment helped spark what is now widely known as the "replication crisis," the realization that supposed scientific truths are often just plain wrong. Now, he reveals the very human biases, misunderstandings, and

norecopa.no/textbase/science-fictions

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norecopa.no/meetings/meetings-calendar

Webinar and Meetings calendar

November 2020

- Aquatic Animal Welfare Conference 2020, 2-6 November 2020 (virtual event)
- Improving the reproducibility of cell line research, webinar, 3 November 2020
- FSVO/UFAW/HSA Online Symposium: Humanely Ending the Life of Animals, 3-4 November 2020
- Symposium and Workshop: Replacing Fetal Bovine Serum (FBS) in Research and Testing, Munich, 3-4 November 2020
- EARA Media Training Workshop (for Spain), online workshop, 4 November 2020
- ABSA 63rd Annual Biosafety and Biosecurity Conference, 4 - 6 November 2020 (virtual event)
- EARA Media Training Workshop (for Switzerland), online workshop, 5 November 2020
- Minipigs in translational immunosafety assessment, webinar, 5 November 2020
- Responsible Research 101 Course: 9-19 November 2020
- Anaesthesia, analgesia and surgery in mice and rats, online/Stockholm, 9-13 November 2020
- Do's and don'ts in rodent surgery aseptic technique, webinar, 10 November 2020
- EPAA Annual Conference, 10 November 2020 (virtual event)
- Fondamenti di Gestione di un Moderno Stabulario per Roditori, webinar in Italian, 10-11 November 2020

Two frustrations:

We can solve the reproducibility crisis by

1) courses in Experimental Design that focus primarily on elimination of bias and the more "mathematical" aspects (e.g. randomisation, experimental units, blinding, statistical methods)

nature human behaviour

Perspective | Open Access | Published: 10 January 2017

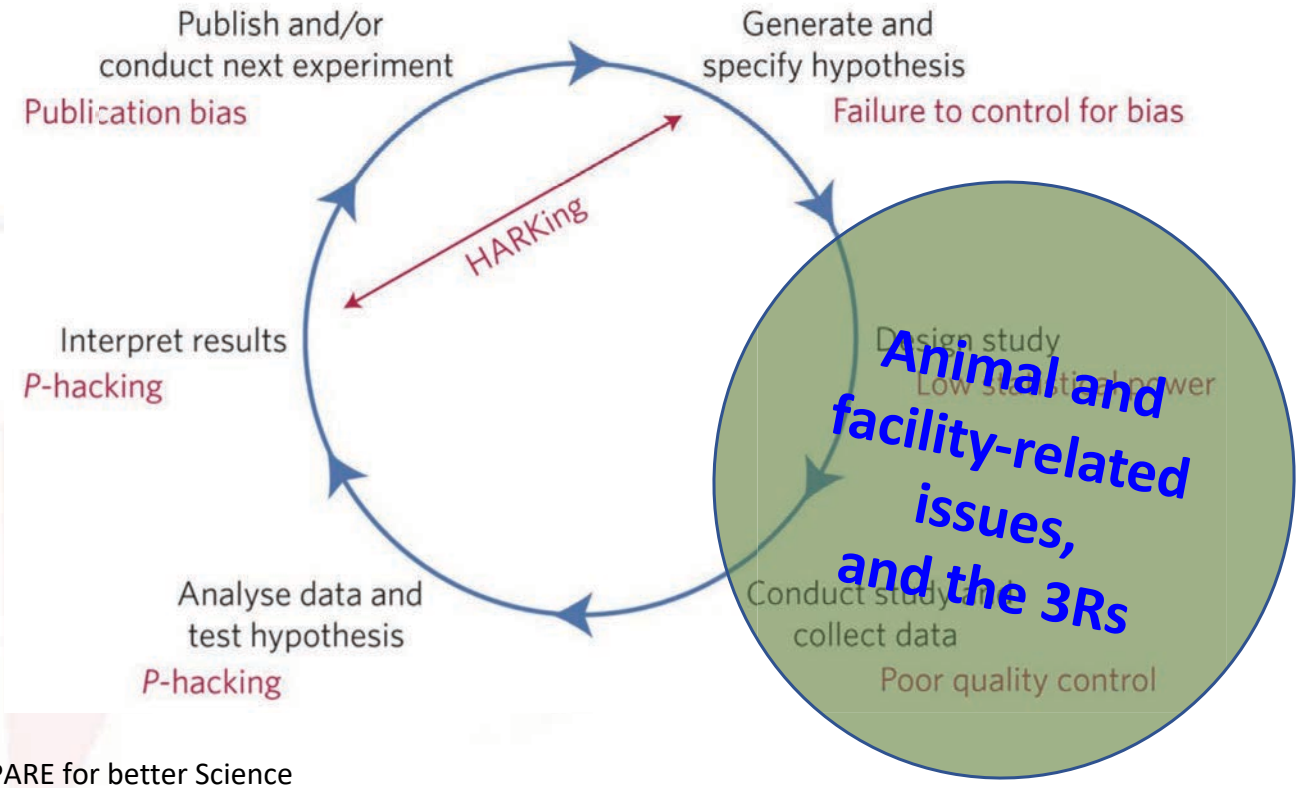
A manifesto for reproducible science

Marcus R. Munafò, Brian A. Nosek, Dorothy V. M. Bishop, Katherine S. Button, Christopher D. Chambers, Nathalie Percie du Sert, Uri Simonsohn, Eric-Jan Wagenmakers, Jennifer J. Ware & John P. A. Ioannidis

Nature Human Behaviour 1, Article number: 0021 (2017) |

Figure 1: Threats to reproducible science.

From: A *manifesto* for reproducible science



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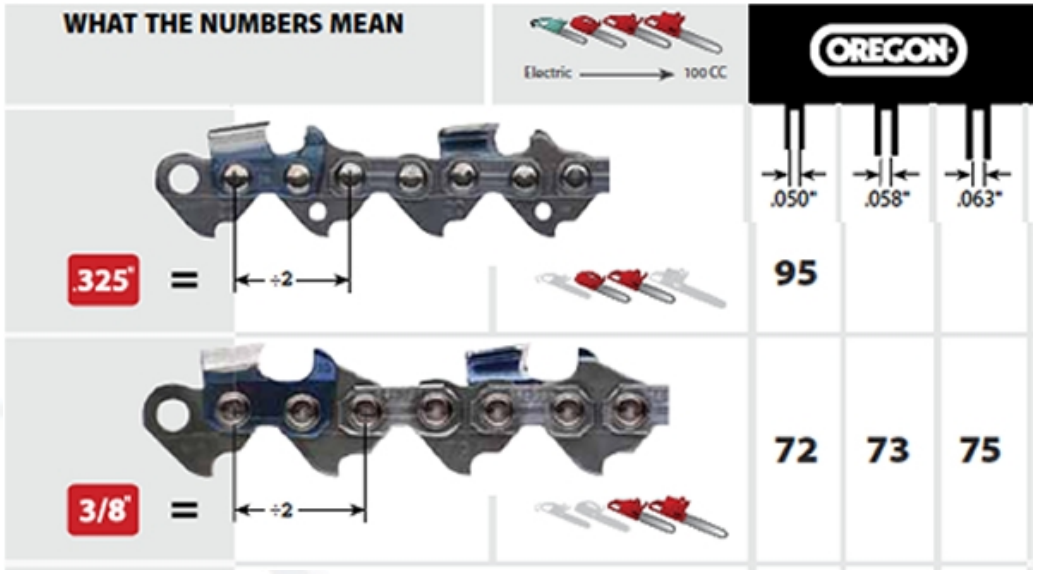
The easy parts of design and reporting:



arborist101.com

- Chainsaw
  - Blade characteristics
  - Sparkplug type
  - Petrol/oil mixture
  - Service history
- Angle of cut in tree
- Length of tree logs

Critical issues, behind the scenes:

- Experience of the workers
- Inspection for signs of rot and to decide felling direction
- Routines and equipment for maintenance of the chainsaw
- Additional equipment (winch, chains, straps, wedges)
- Clearing-up and transport of logs

- **Health and safety precautions – clothing, onlookers**
- **Division of labour and costs**

These issues may never get reported.

Two frustrations:

We can solve the reproducibility crisis by

2) better reporting!!

reddit.com

Reporting guidelines are not new

- Guidelines for specification of animals and husbandry methods when reporting the results of animal experiments (GV-SOLAS, 1985)
- Reporting animal use in scientific papers (Jane Smith *et al.)*, 1997
- Öbrink & Rehbinder: Animal definition: a necessity for the validity of animal experiments? *Laboratory Animals,* 2000
- Guidelines for reporting the results of experiments on fish (2000)
- ARRIVE Guidelines, 2010 (Kilkenny *et al.*, NC3Rs) and ARRIVE v.2 2019
- Gold Standard Publication Checklist, 2010 (SYRCLE)
- Institute for Laboratory Animal Research, NRC, 2011
- Instructions to authors, in many journals

  e.g. Nature's Reporting Checklist

bmc.uu.se/digitalAssets/139/c_139643-l_3-k_kjo.jpg

Karl Johan Öbrink
1918-1998

norecopa.no/textbase/foersoeksdjurskunskap

1996

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Hurni 1969, quoted in Öbrink and Rehbinder

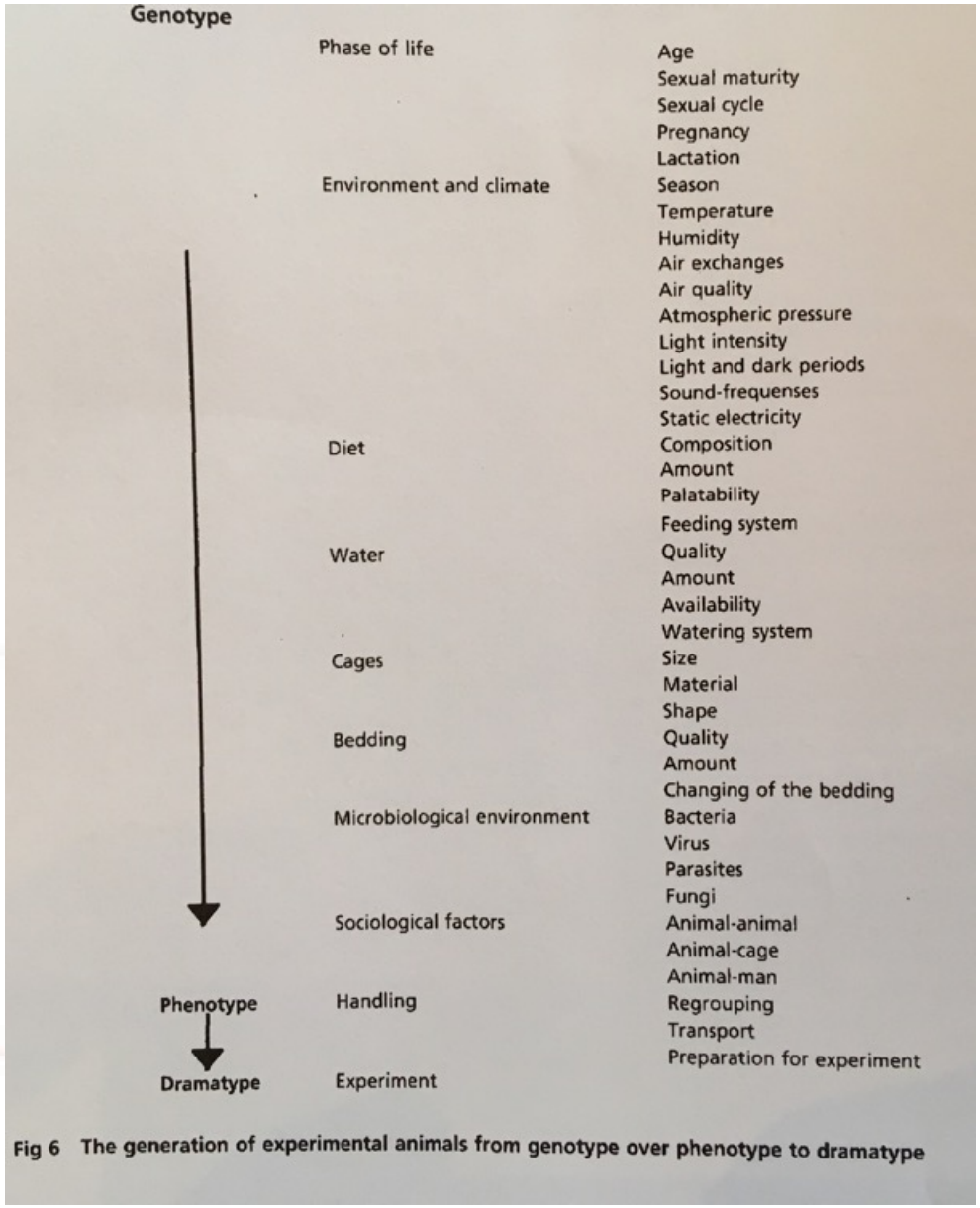

Fig 6 The generation of experimental animals from genotype over phenotype to dramatype

arriveguidelines.org

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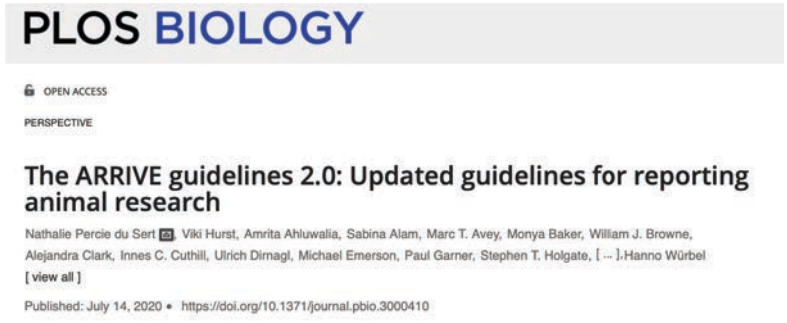


PLOS BIOLOGY

OPEN ACCESS

PERSPECTIVE

The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research

Nathalie Percie du Sert, Viki Hurst, Amrita Ahluwalia, Sabina Alam, Marc T. Avey, Monya Baker, William J. Browne, Alejandra Clark, Innes C. Cuthill, Ulrich Dirnagl, Michael Emerson, Paul Garner, Stephen T. Holgate, [...], Hanno Würbel

[view all]

Published: July 14, 2020 • https://doi.org/10.1371/journal.pbio.3000410

journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000410

ARRIVE (2010) 'endorsed by more than a thousand journals'
but
'only a small number of journals actively enforce compliance'

(Swiss study in 2016: 51% of researchers publishing in journals that had endorsed ARRIVE had never heard of them)

'Important information as set out in the ARRIVE guidelines is still missing from most publications sampled:

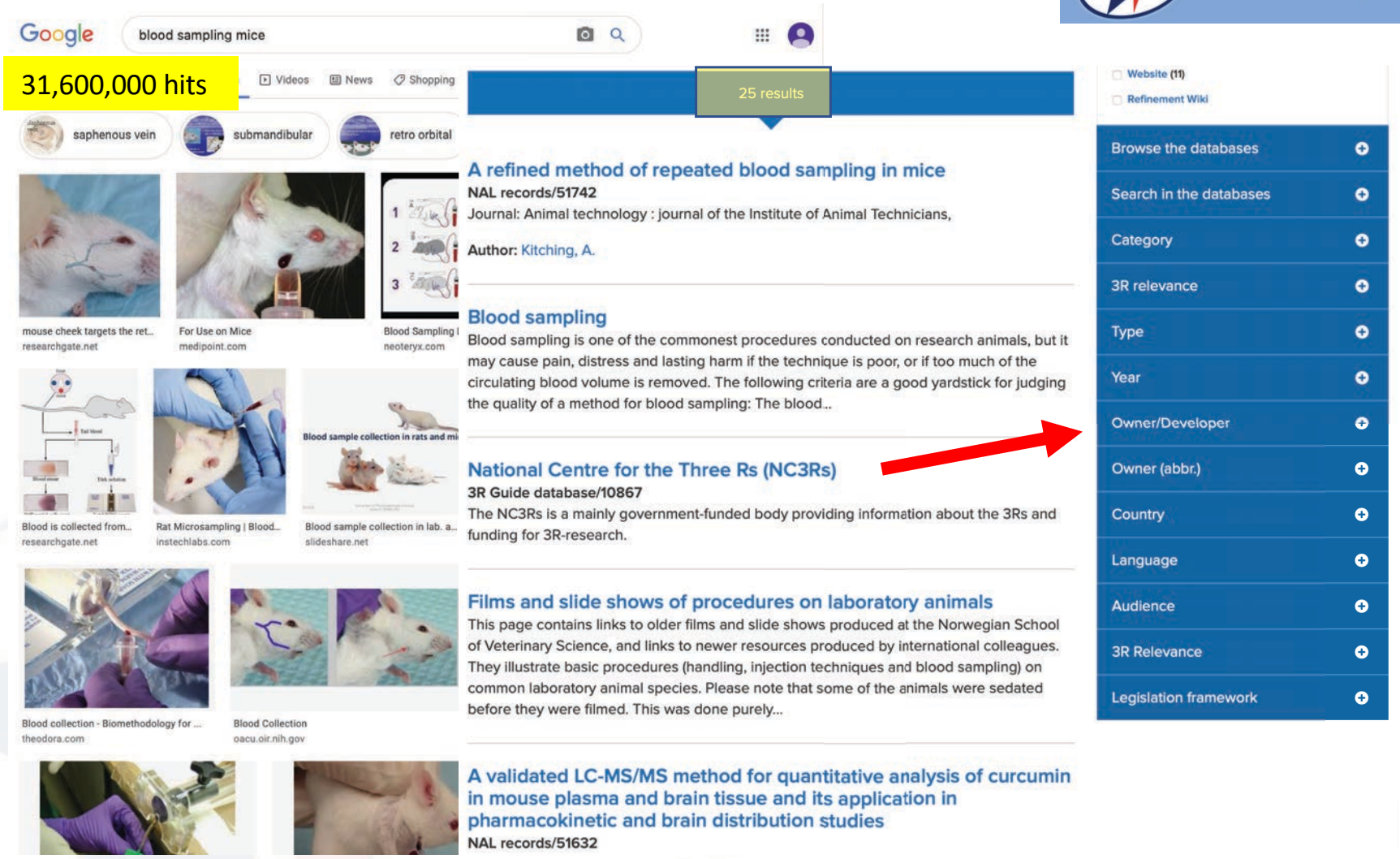
- randomisation 30-30%
- blinding 20%
- sample size justification <10%
- all basic animal characteristics <10%'

'It is likely that the level of resource required from journals and editors currently prohibits the implementation of all the items of the ARRIVE guidelines'

Where can we find guidelines for good planning?

- Bibliographic databases are often not used adequately (poor overlapping between the databases)
- Too few scientists are aware of the specialist 3R-databases
- Scientists rarely use ”3R” words when they write titles/abstracts/keywords for their papers
- Databases rarely flag papers with explicit 3R terms
- We have no single ”Journal of 3R-resources”

Search for ‘blood sampling mice’ on Google and Norecopa.



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"All I need is a blood sample…"

medipoint.com/html/for_use_on_mice.html

theodora.com/rodent_laboratory/blood_collection.html

Photo: NMBU

The best blood sampling techniques are those where you can:

- ✓ see the blood vessel
- ✓ regulate the amount of blood you remove
- ✓ stop the bleeding easily (including internal bleeding)
- ✓ avoid damage to the surrounding tissue
- ✓ collect samples rapidly, to avoid artefacts due to mechanical stress, temperature shock, metabolic changes due to differing storage times before centrifugation, etc.
- ✓ agree that they are feasible in the time available

norecopa.no/more-resources/blood-sampling

Artefacts and unnecessary suffering caused by poor technique

Photo: NMBU

- *Are you sure that your injection ends up in the same place each time?*
- *Are the injections painful?*
- *Are they realistic? (intramuscular injections in small animals)*

What if we can't find guidelines for best practice?

Apply critical anthropomorphism (empathy + an objective, knowledge-based consideration of what is likely to be significant to the animal)

Carol M. Newton (1925-2014)

National Library of Medicine

The three S's

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

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over 9,000 webpages
over 300,000 hits / year
1. United States
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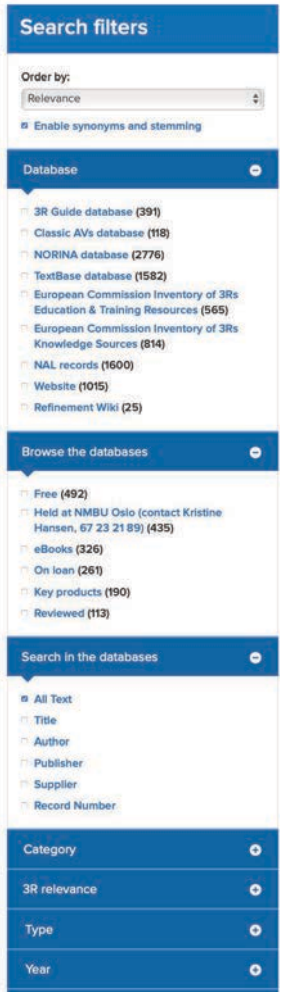
3. Canada
4. India
5. Norway
6. Spain
7. Australia
8. Germany
9. Brazil
10. France

Some of the content of norecopa.no

- Embedded databases, among others:
  - NORINA (2,700 animal alternatives in teaching)
  - TextBase (1,500 books on Laboratory Animal Science)
  - 3R Guide (390 guidelines for animal research)
  - EU Commission datasets of 3R resources and training courses
- A Refinement Wiki
- Webpages for two European networks (3R Centres & Culture of Care)
- The PREPARE guidelines for planning animal studies
- A Webinars and Meetings Calendar
- Overview of courses in Laboratory Animal Science
- Newsletters (7-8 per year in English)

All searchable by one search engine and its filters



norecopa.no/NORINA

NEW:
overview of resources suitable for home learning
during the Covid-19 pandemic:

norecopa.no/norina-database/resources-for-home-learning

rescuecritters.com

limbsandthings.com

norecopa.no/education-training/homemade-educational-materials

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

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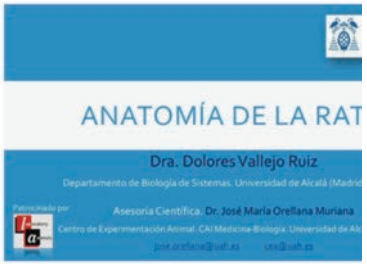
Rat s.c. injection
Norecopa | 1,380 views

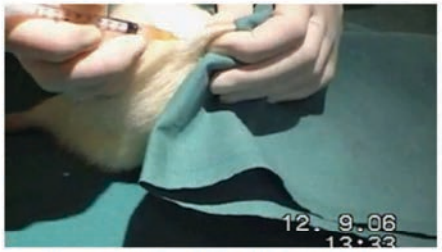
Rat i.p. injection (method 2)
Norecopa | 1,280 views



Anatomia de la rata
Norecopa | 977 views

Testing anaesthetic depth in the chicken
Norecopa | 598 views

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



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

Blood san
Norecop

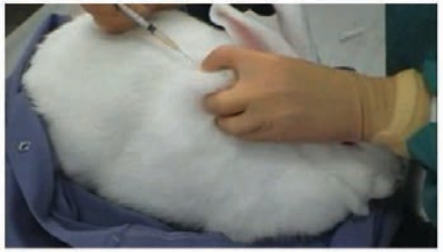
Blood san
Norecop

Blood san
Norecop

Blood sampling from the pig
Norecopa | 3,914 views

Intravenous injection in a rabbit
Norecopa | 2,025 views

Lifting a rabbit
Norecopa | 2,420 views



Subcutaneous injection in the rabbit
Norecopa | 1,479 views

Subcutaneous injection in the chicken
Norecopa | 1,806 views

Immobilisation of the rabbit
Norecopa | 2,072 views

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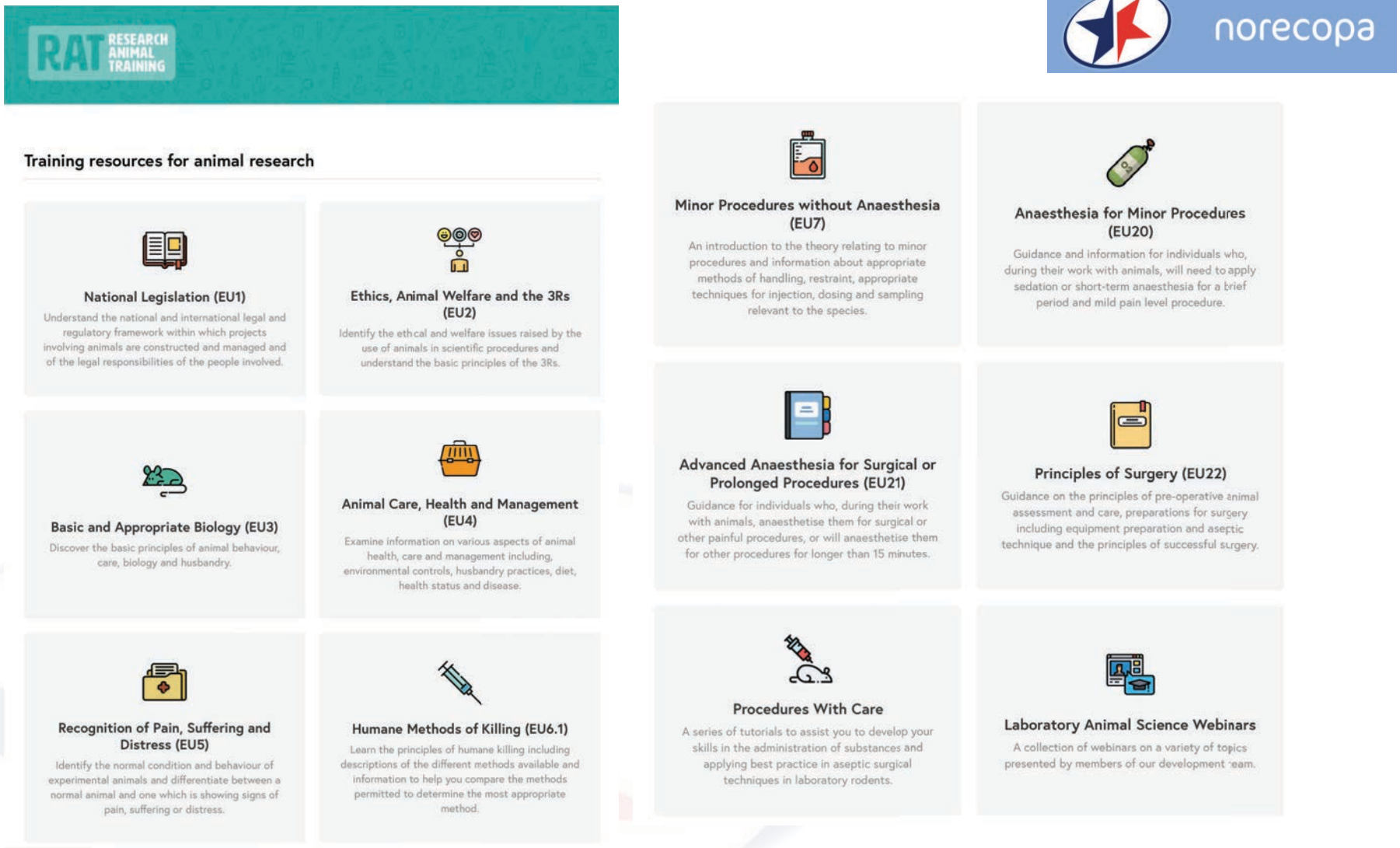

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researchanimaltraining.com

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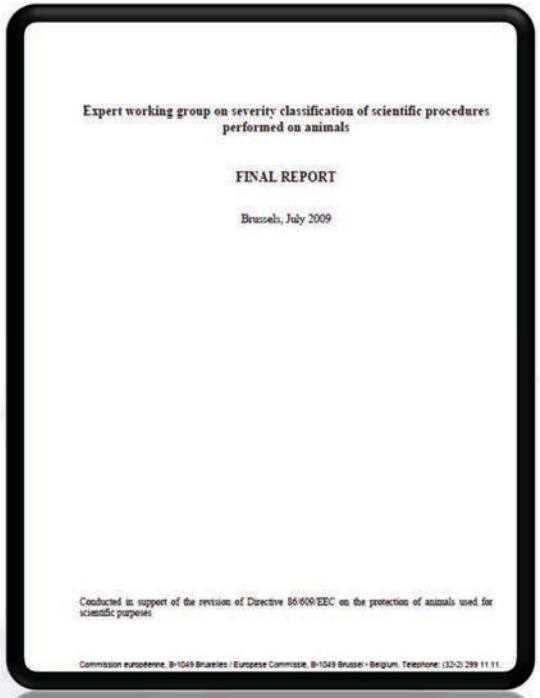
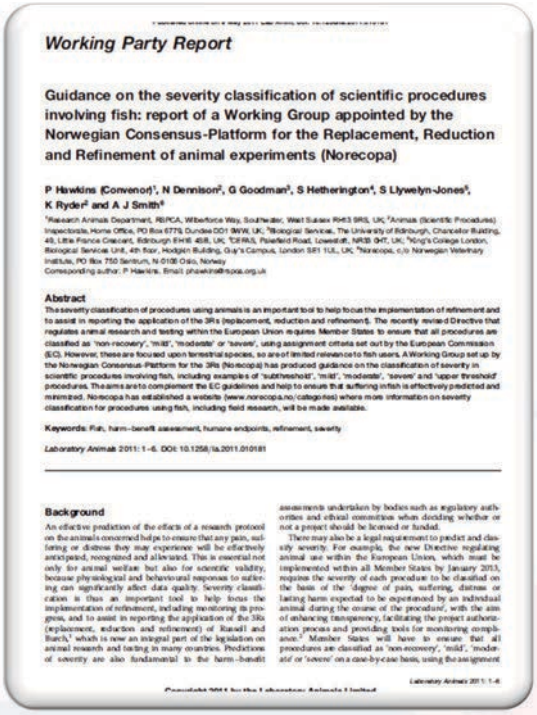
ec.europa.eu/environment/chemicals/lab_animals



Expert Working Group report on severity classification

http://ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf

From **3R-Guide** (390 guidelines for animal research and testing): norecopa.no/3r-guide



Working Party Report

Guidance on the severity classification of scientific procedures involving fish: report of a Working Group appointed by the Norwegian Consensus-Platform for the Replacement, Reduction and Refinement of animal experiments (Norecopa)

P Hawkins (Convenor)[1], N Dennison[2], G Goodman[3], S Hetherington[4], S Llywelyn-Jones[5], K Ryder[2] and A J Smith[6]

Abstract

Keywords: Fish, harm–benefit assessment, humane endpoints, refinement, severity

Laboratory Animals 2011; 1–6. DOI: 10.1258/la.2011.010181

Background

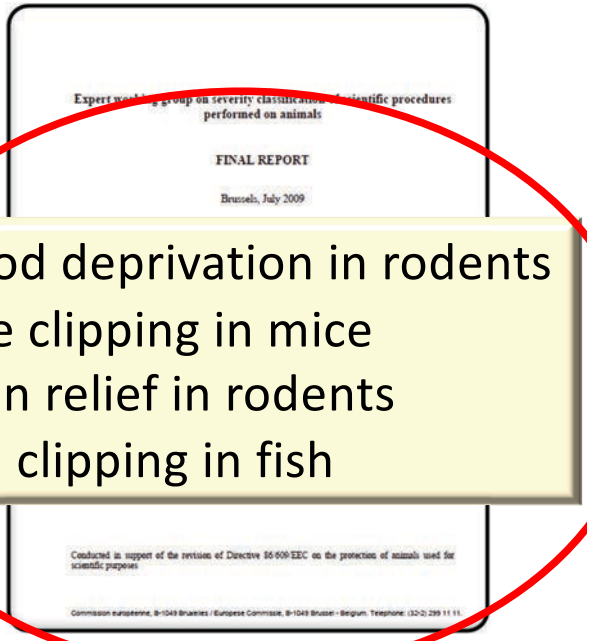
Guidance on the severity classification of procedures involving fish

Report from a Working Group convened by Norecopa

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

Laboratory Animals, 45: 219-224, 2011

norecopa.no/categories



Expert working group on severity classification of scientific procedures performed on animals

FINAL REPORT

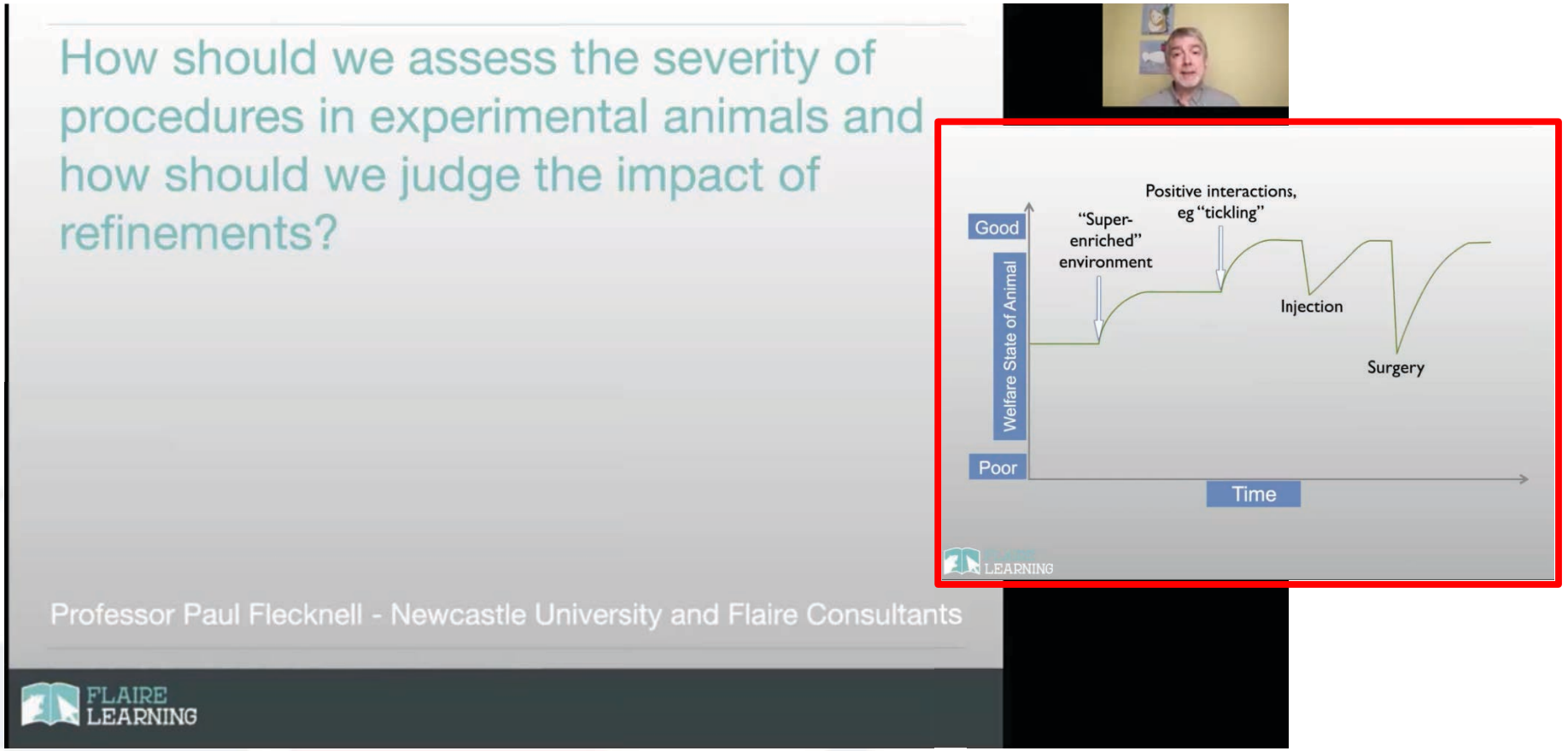
Brussels, July 2009

Conducted in support of the revision of Directive 86/609/EEC on the protection of animals used for scientific purposes

Commission européenne, B-1049 Bruxelles / Europese Commissie, B-1049 Brussel - Belgium. Telephone: (32-2) 299 11 11

Food deprivation in rodents
Toe clipping in mice
Pain relief in rodents
Fin clipping in fish

ec.europa.eu/environment/chemicals/lab_animals/pdf/report_ewg.pdf



researchanimaltraining.com/articles/webinar-how-should-we-assess-the-severity-of-procedures-in-experimental-animals-and-the-impact-of-refinements

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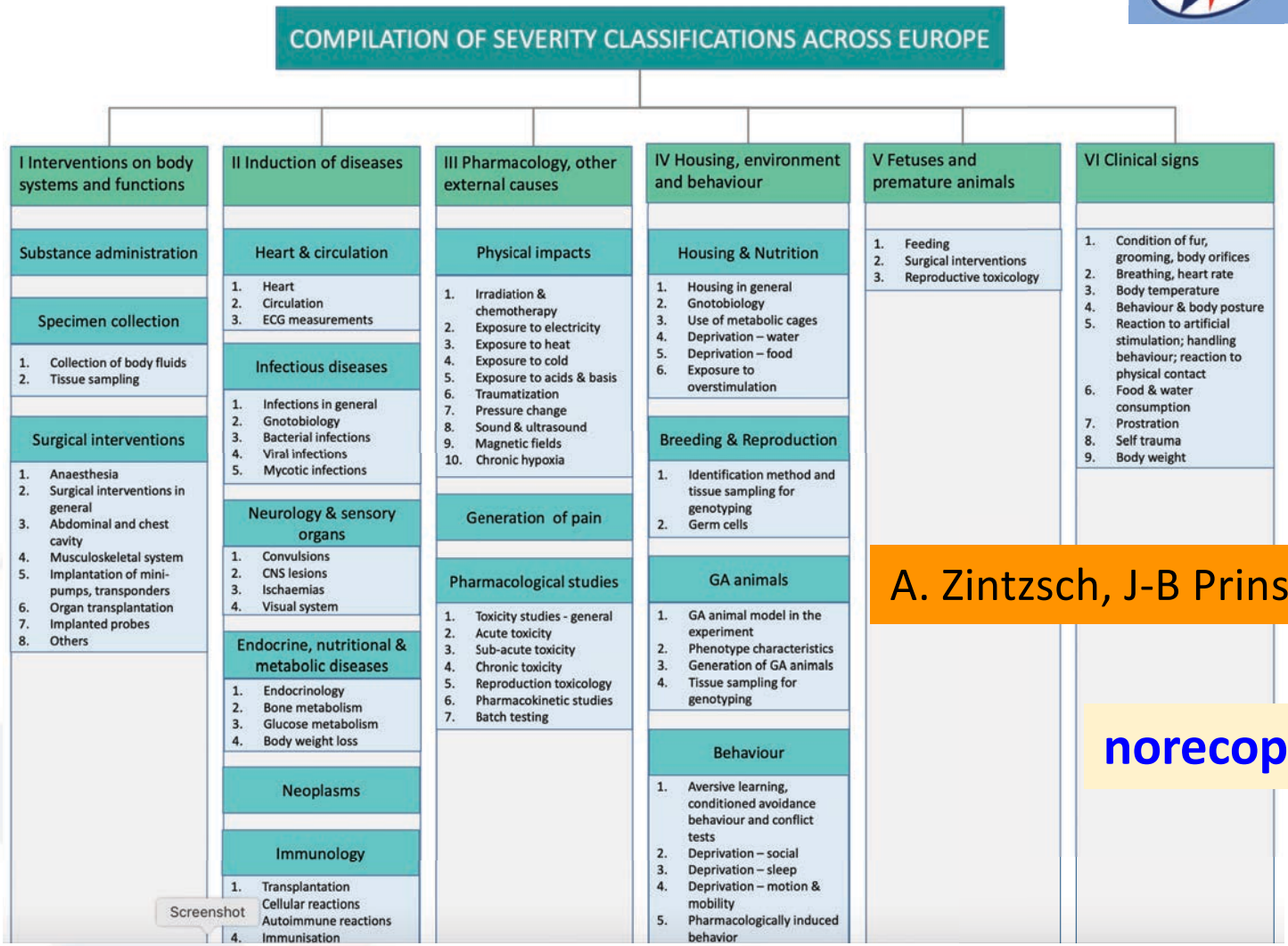

A. Zintzsch, J-B Prins & N Kostomitsopolous

norecopa.no/severity

Neglected Factors in Pharmacology and Neuroscience Research: Biopharmaceutics, Animal Characteristics, Maintenance, Testing Conditions

By Claassen, Volkert

Record number: 13335 (legacy id: 6153)

The objective of this book is to indicate those variables which in general may need a better control. Examples, gathered from the literature, are presented to illustrate the impact that those neglected variables may have on various characteristics. The book presents a series of representatives studies from a broad field of interest so that insight can be obtained about the potential effects of these parameters in experimental outcomes. In this way, an impetus should be given to the critical consideration of test design and limitations of conclusions from experimental results. In part, the book is written as a reaction to frustrations endured during pharmacological research of many years' standing, and therefore the choice of examples from the literature is largely related to this discipline. As pharmacological research is to a large extent based on the other life sciences, this volume may be of interest to a much broader audience. This may certainly be the case for pharmacokineticists and toxicologists for whom drugs are the main object of study. This book may also help to improve test designs for biochemists and physiologists, not only when using drugs as tools in their experiments, but also to improve generally the control of animal characteristics and test conditions. This book is Volume 12 in a series entitled *Techniques in the Behavioral and Neural Sciences*.

Comments & References: First Edition. 496 pages. Paperback. A review is available in Laboratory Animals, April 1996, Volume 30 (2).

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Review:

'This book is essential reading for anybody that wishes to take the problem of experimental variability seriously. There are no magic cures offered for experimental problems, but there are many explanations offered within this book. A worthwhile addition to any library.'

norecopa.no/textbase/neglected-factors-in-pharmacology-and-neuroscience-research-biopharmaceutics-animal-characteristics-maintenance-testing-conditions

DEPART guidelines for osteoarthritis research

3R Guide database/74528

DEPART is a set of planning guidelines which emphasise specific issues that may be particularly relevant in pre-clinical studies of osteoarthritis.

CCAC Guidelines on transgenic animals

3R Guide database/10676

Topics covered include investigator and animal care committee responsibilities, proposals to create new or use existing strains, accounting, containment, reporting, responsibility of the CCAC and literature references.

Supplier: Canadian Council on Animal Care (CCAC)

Guidelines for biosafety laboratory competency

3R Guide database/10778

AAALAC International cites these guidelines as one of its reference resources.

Guidelines on Adequate Veterinary Care

3R Guide database/10688

This paper, written by ACLAM, an organisation consisting of veterinarians specialising in laboratory animal medicine, is a detailed description of adequate veterinary care and is intended to be applied to animals used, or intended to be used, in research, teaching or testing.

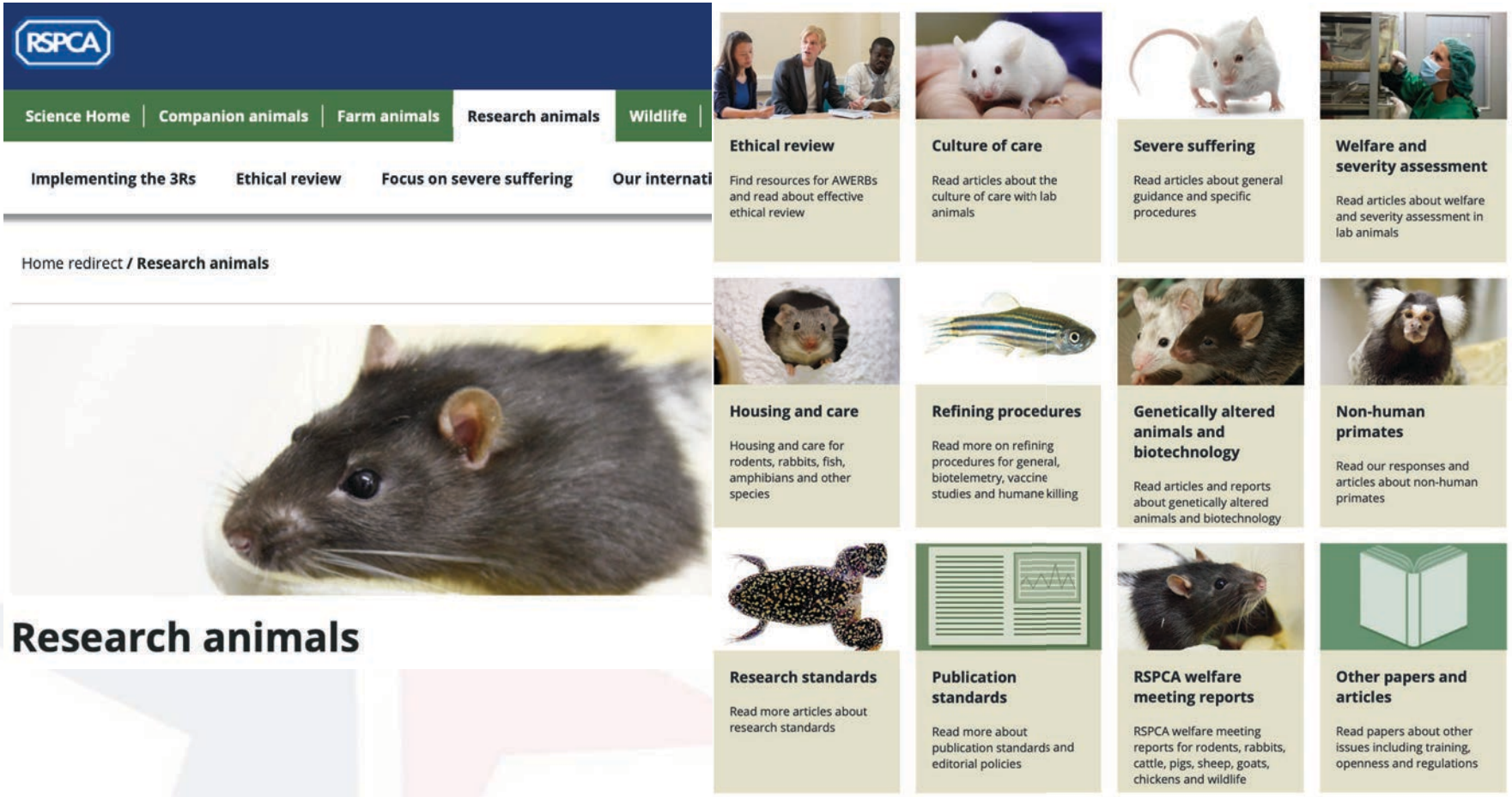
CCAC Guidelines on antibody production

3R Guide database/10638

The Canadian Council on Animal Care (CCAC) produced guidelines on antibody production in 2002.

From the 3R Guide database

Guideline collections on other 3R Centre websites



science.rspca.org.uk/sciencegroup/researchanimals

nc3rs.org.uk/3rs-resources

Pdf files of 80+ presentations held at Norecopa's meetings

norecopa.no/meetings/presentations

Most of the presentations on this page are from events arranged by Norecopa. A few of them are from external events where Norecopa's staff have lectured.
They are grouped into

Koenig 101017.pdf

- General presentations
- Care and use of animals in field research
- Care and use of farm animals in research
- Care and use of fish in research

Title	Speaker	Affiliation	Year
General presentations			
Design of animal studies: Increasing reproducibility and animal welfare	Adrian Smith	Norecopa	2020
PREPARE before you ARRIVE: Good reporting relies on good planning	Adrian Smith	Norecopa	2019
Animal-free testing and humans-on-a-chip: How far have we come?	Leopold Koenig	TissUse GMBH, Berlin, Germany	2017
Nordic 3R-Centres: What can we offer?	Tom Bengtsen	Denmark's 3R-Center	2017
Prize-winning 3R activity in Norway	Gøril Eide	University of Tromsø, Norway	2017
Have the 3Rs made any difference?	Elliot Lilley	RSPCA, UK	2017

Guidelines for research using fish

Please also see the PREPARE guidelines for planning and conducting animal research and testing.

- Revised version of Appendix A of the "European Convention for the protection of animals used for scientific purposes". The revision came into force on 15 June 2007 and contains species-specific guidelines for a number of fish species.
- Guidelines for the severity classification of procedures on fish
- Wootten R (2005) Species-specific provisions for fish in the European Convention for the protection of animals used for experimental and scientific purposes (presentation at a meeting in Oslo in May 2005)
- Griffin G (2005) Canadian Guidelines for the care & use of fish in research, teaching and testing (presentation at a meeting in Oslo in May 2005)
- Guidelines on: the care and use of fish in research, teaching and testing (2005) Canadian Council on Animal Care (CCAC)
- Johansen R., Needham JR, Colquhoun D., Poppe TT & Smith AJ. (2006): Guidelines for health and welfare monitoring of fish used in research. Laboratory Animals 40(4): 323-340
- Considerations for a European animal welfare standard to evaluate adverse phenotypes in teleost fish
- Guidelines for the Use of Fishes in Research (2004) American Fisheries Society (AFS)
- Ostrander GK (2000) The Laboratory Fish. Academic Press, Baltimore, USA.
- Poole T (1999) UFAW Handbook on the Care and Management of Laboratory Animals. Volume 2: Amphibious & Aquatic Vertebrates & Advanced Invertebrates. Blackwell Science.
- Casebolt DB, Speare DJ, Horney BS (1998) Care and use of fish as laboratory animals: Current state of knowlege. Laboratory Animal Science 48, 124-136
- DeTolla LJ et al. (1995) Guidelines for the Care and Use of Fish in Research ILAR Journal 37 (4)
- Borski RG, Hodson RJ (2003) Fish Research and the Institutional Animal Care and Use Committee ILAR journal 44 (4)
- Brattelid T, Smith AJ (2000) Guidelines for reporting the results of experiments using fish Laboratory Animals 34 131-135
- The welfare of farmed fish at slaughter (concerns about carbon dioxide use) (HSUS report)
- Schaeffer et al. (1992) The Care and Use of Amphibians, Reptiles and Fish in Research Scientists for Animal Welfare (SCAW)
- Documents for laypeople on ethics committees, Royal Society for the Protection of Animals (RSPCA), UK

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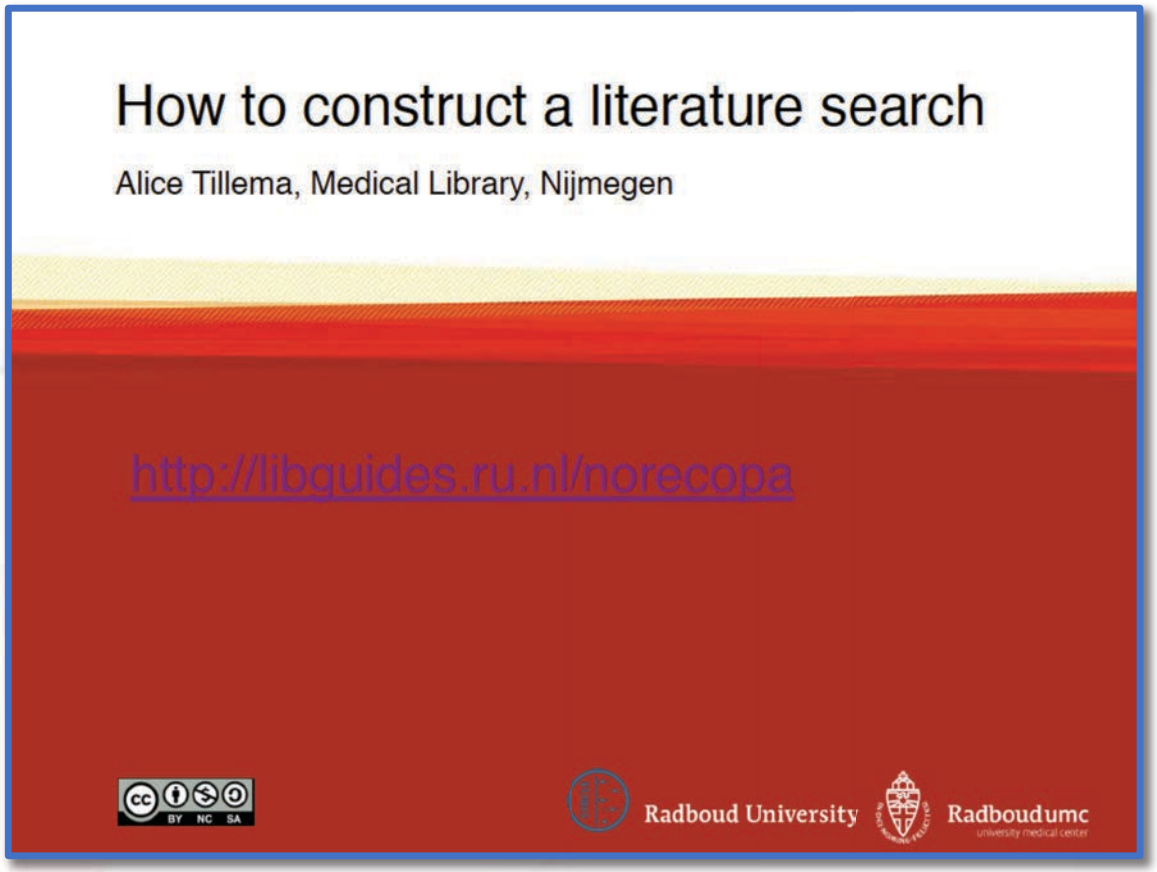
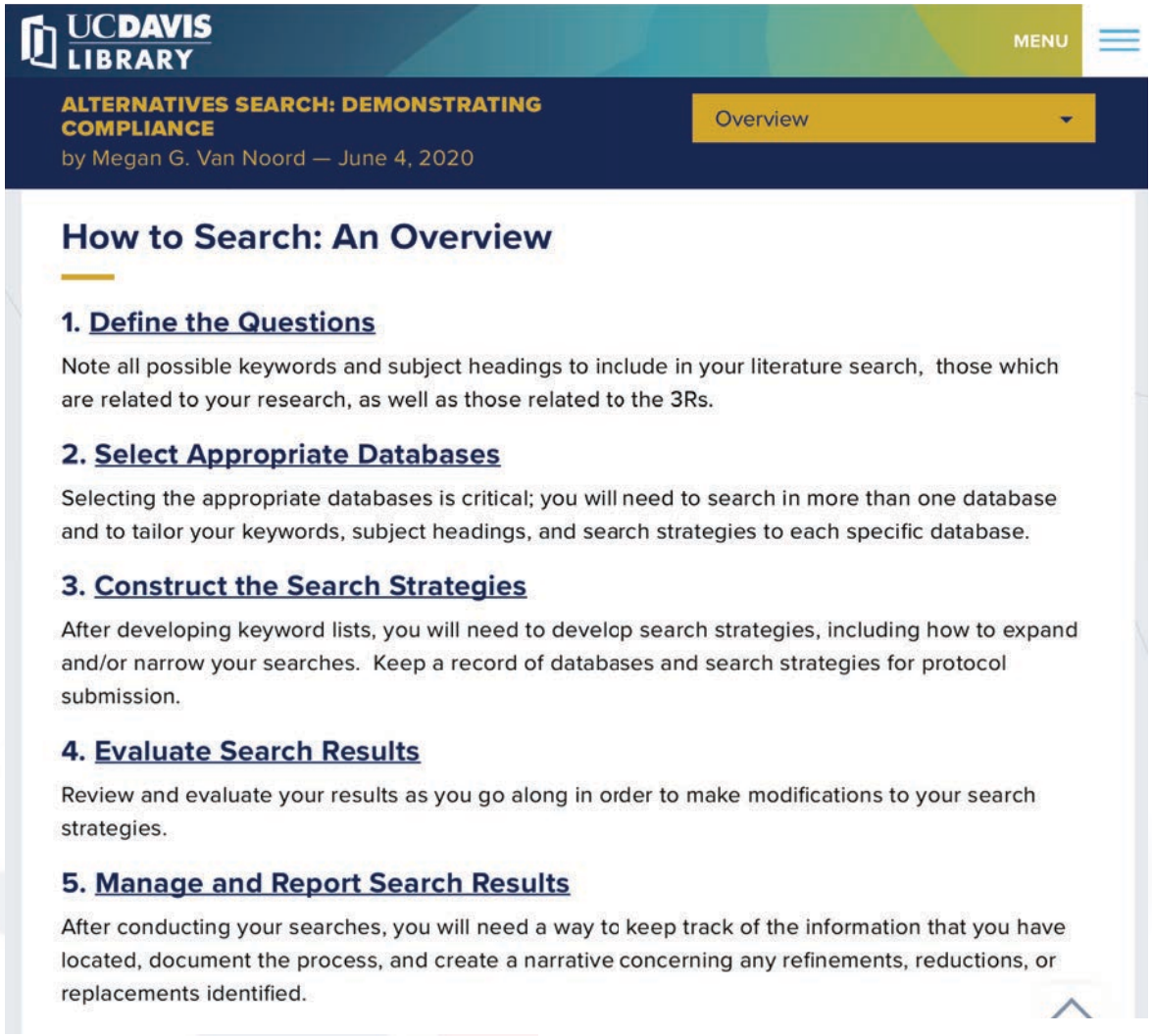
norecopa.no/fish/guidelines

norecopa.no/species

Alice Tillema, Radboud University:
How to construct a literature search

http://norecopa.no/how-to-construct-a-literature-search.pdf



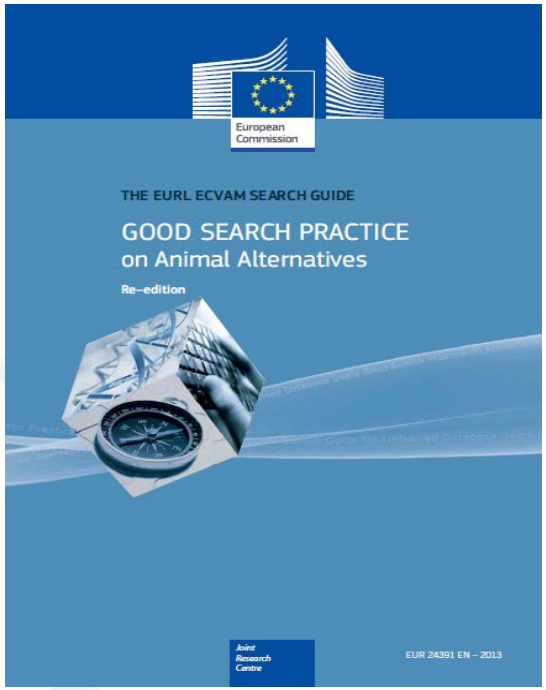


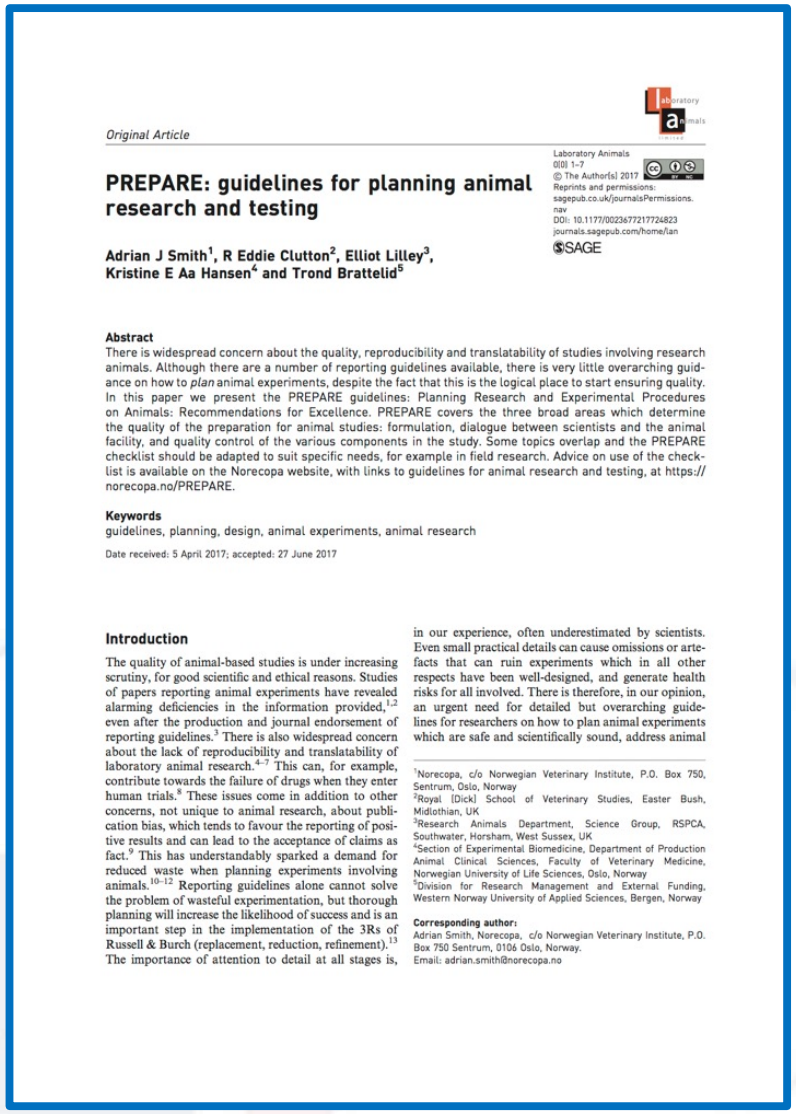
library.ucdavis.edu/guide/animal-alternatives-searching

The EURL ECVAM **Search** Guide

Can be ordered free of charge from

bookshop.europa.eu





Original Article

PREPARE: guidelines for planning animal research and testing

Adrian J Smith[1], R Eddie Clutton[2], Elliot Lilley[3], Kristine E Aa Hansen[4] and Trond Brattelid[5]

Laboratory Animals
0(0) 1–7

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journals.sagepub.com/home/lan
SAGE

Abstract

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

Keywords

guidelines, planning, design, animal experiments, animal research

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,[1,2] even after the production and journal endorsement of reporting guidelines.[3] There is also widespread concern about the lack of reproducibility and translatability of laboratory animal research.[4–7] This can, for example, contribute towards the failure of drugs when they enter human trials.[8] These issues come in addition to other concerns, not unique to animal research, about publication bias, which tends to favour the reporting of positive results and can lead to the acceptance of claims as fact.[9] This has understandably sparked a demand for reduced waste when planning experiments involving animals.[10–12] Reporting guidelines alone cannot solve the problem of wasteful experimentation, but thorough planning will increase the likelihood of success and is an important step in the implementation of the 3Rs of Russell & Burch (replacement, reduction, refinement).[13] The importance of attention to detail at all stages is, in our experience, often underestimated by scientists. Even small practical details can cause omissions or artefacts that can ruin experiments which in all other respects have been well-designed, and generate health risks for all involved. There is therefore, in our opinion, an urgent need for detailed but overarching guidelines for researchers on how to plan animal experiments which are safe and scientifically sound, address animal

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Norecopa: PREPARE for better Science

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Smith, AJ, Clutton, RE, Lilley, E, Hansen KEAa, Brattelid, T. (2018): PREPARE: Guidelines for planning animal research and testing. Laboratory Animals, 52(2): 135-141.
DOI: 10.1177/0023677217724823

norecopa.no/PREPARE/prepare-checklist

PREPARE

The PREPARE Guidelines Checklist

Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith[a], R. Eddie Clutton[b], Elliot Lilley[c], Kristine E. Aa. Hansen[d] & Trond Bratteli[d]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; [d]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; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE[2].
PREPARE covers the three broad areas which determine the quality of the preparation for animal st

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

The topics will not always be addressed in the order in which they are presented here, and some topi
checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidance on
facilities, since in-house experiments are dependent upon their quality. The full version of the guideline
website, with links to global resources, at **https://norecopa.no/PREPARE**.
The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ 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. ☐ Assess the reproducibility and translatability of the project.
2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, harm-benefit assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (replacement, reduction, refinement) and the 3Ss (good science, good sense, good sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a harm-benefit assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.
4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.

Three Rs!

Topic	Recommendation
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. ☐ Discuss and disclose all expected and potential costs. ☐ Construct a detailed plan for division of labour and expenses at all stages of the study.
6. Facility [illegible]tion	☐ Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. ☐ Discuss staffing levels at times of extra risk.
[illegible]ation and	☐ Assess the current competence of staff members and the need for further education or training prior to the study.
[illegible] risks, waste disposal and decontamination	☐ Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study. ☐ Assess, and if necessary produce, specific guidance for all stages of the project. ☐ Discuss means for containment, decontamination, and disposal of all items in the study.
(C) Quality control of the components in the study	
9. Test substances and procedures	☐ Provide as much information as possible about test substances. ☐ Consider the feasibility and validity of test procedures and the skills needed to perform them.
10. Experimental animals	☐ Decide upon the characteristics of the animals that are essential for the study and for reporting. ☐ Avoid generation of surplus animals.
11. Quarantine and health monitoring	☐ Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.
12. Housing and husbandry	☐ Attend to the animals' specific instincts and needs, in collaboration with expert staff. ☐ Discuss acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing).
13. Experimental procedures	☐ Develop refined procedures for capture, immobilisation, marking, and release or rehoming. ☐ Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, reuse or rehoming	☐ Consult relevant legislation and guidelines well in advance of the study. ☐ Define primary and emergency methods for humane killing. ☐ Assess the competence of those who may have to perform these tasks.
15. Necropsy	☐ 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 & Brattelid 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* / @norecopa

Norecopa: PREPARE for better Science

PREPARE:

Planning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence

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

Maybe the study shouldn't go ahead

Systematic review of published research?

PREPARE:

Planning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence

PREPARE covers 15 topics:

Formulation of the study

1. Literature searches
2. Legal issues
3. Ethical issues, harm-benefit assessment and humane endpoints
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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

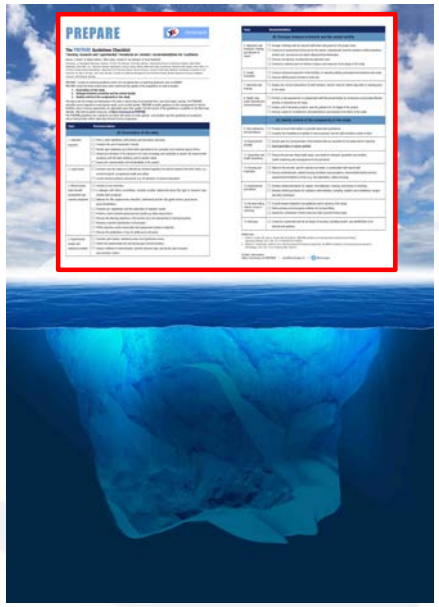
Methods

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

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

norecopa.no/PREPARE



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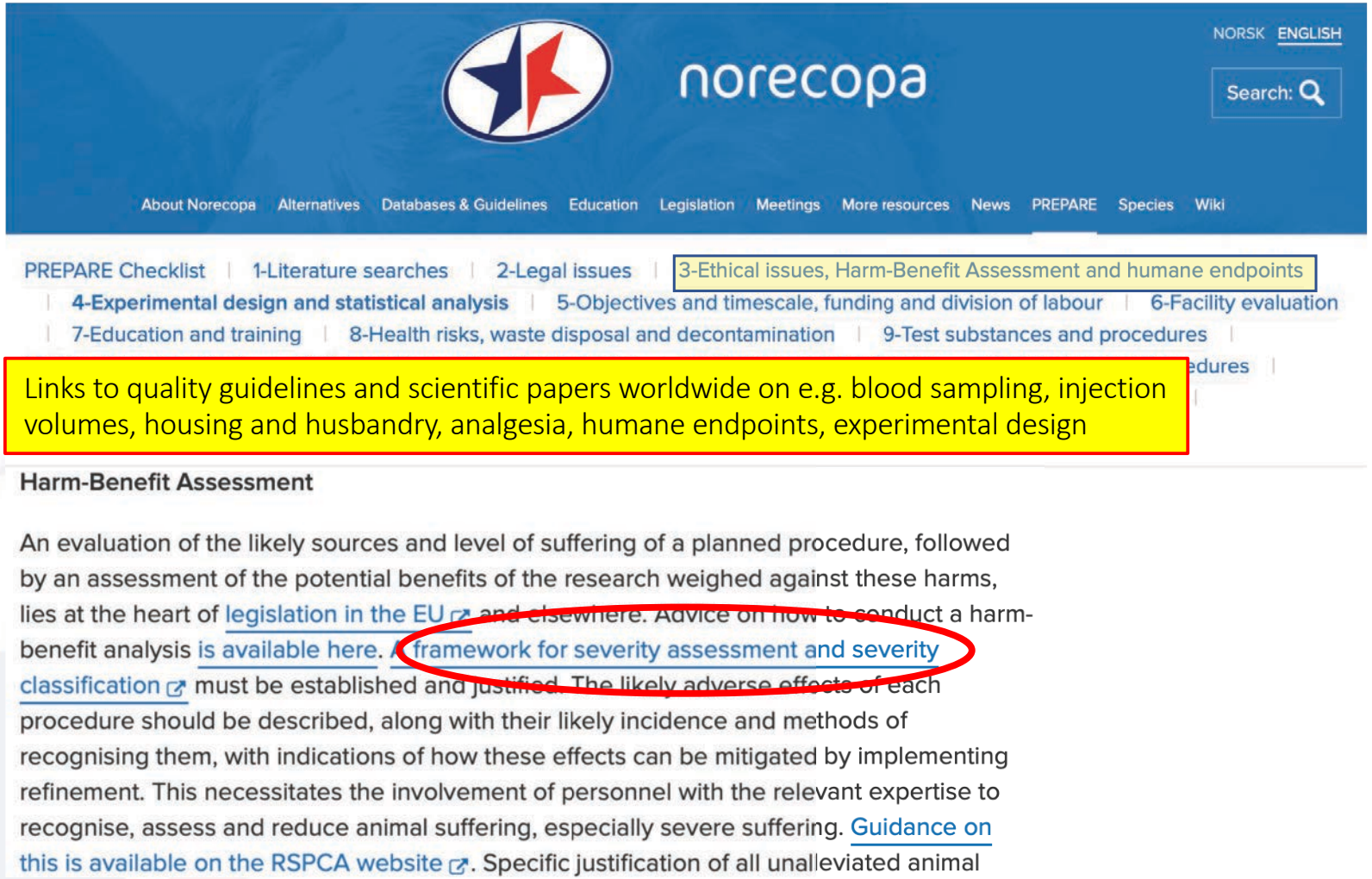


Pre-registration of a study

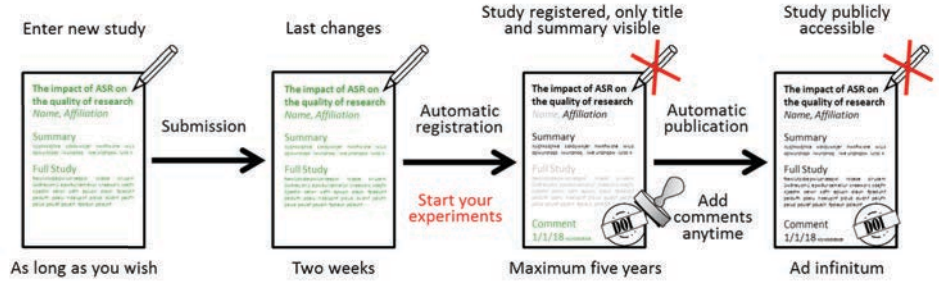
Prevents p-hacking and HARKING

Encourages the publication of negative results

Ensures a detailed description of the study

PRECLINICALTRIALS.EU

International register of preclinical trial protocols



Pre-registration of animal research

The pre-registration of protocols for animal research is gaining momentum, enabling peer review and as part of the work to reduce bias:

- Preclinicaltrials.eu
- The Animal Study Registry (animalstudyregistry.org), Germany (see also Bert *et al.*, 2019)
- PROSPERO: An international prospective register of systematic reviews, established by the National Institute for Health Research (NIHR) in the UK
- Should preclinical studies be registered? (Anderson & Kimmelman, 2015)
- Further advice on protocol registration

Depositories for online protocols

- Protocol Exchange from Nature.com
- protocols.io
- protocol-online.org
- Open Wetware

https://norecopa.no/prepare/4-experimental-design-and-statistical-analysis

A simple but effective Master Plan

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A contract between the animal facility and the research group

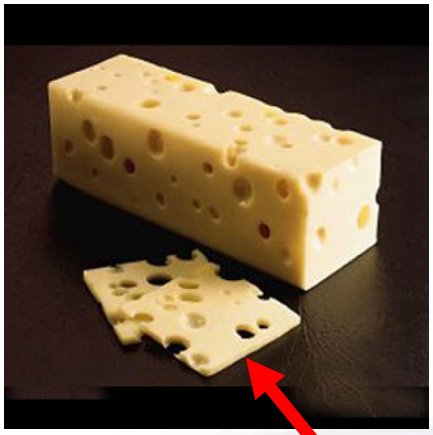
The division of labour and responsibilities

Clarifying all stages of the experiment

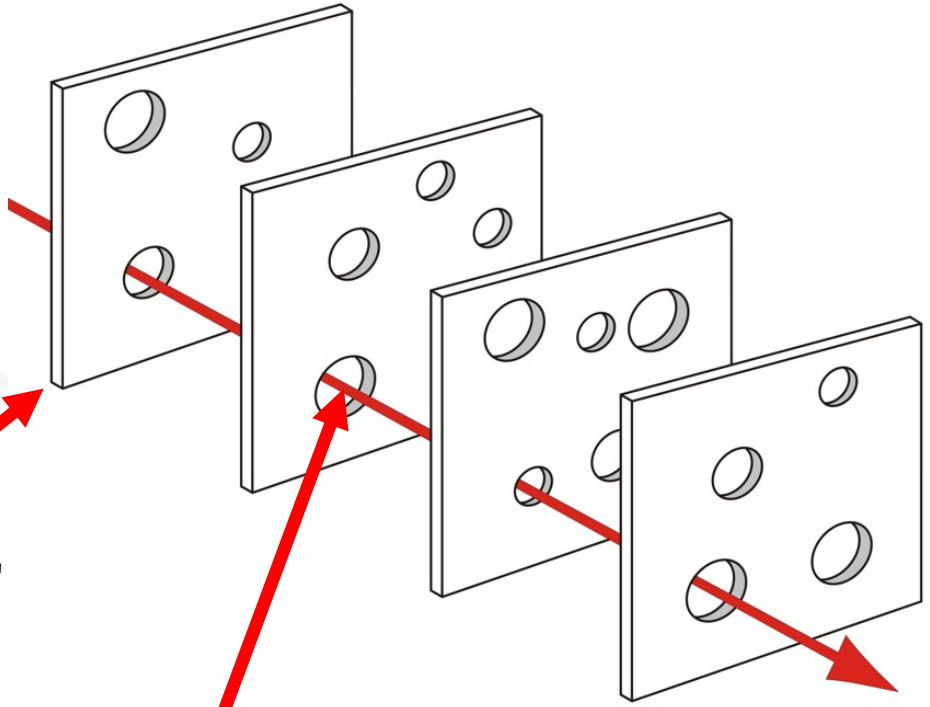
Ensuring that all necessary data are recorded

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

Threat and Error Management



eaugallecheese.com/Swiss-cheese

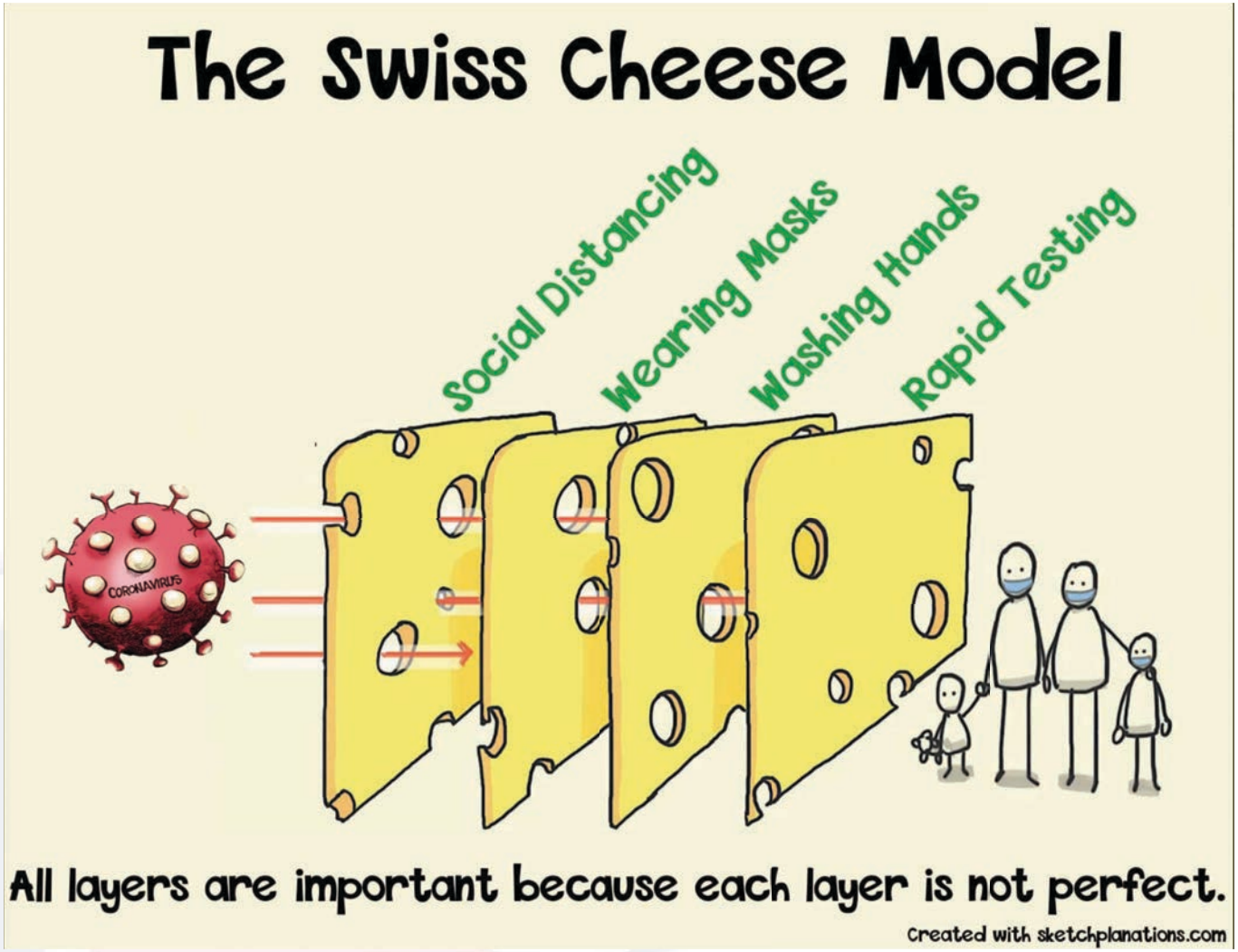


"Layer of defence"
or redundancy

Weakness / hazard

Loss

wikipedia.org/wiki/Swiss_cheese_model



twitter.com/jkwan_md/status/1313328317718843394/photo/1

Contingency and redundancy

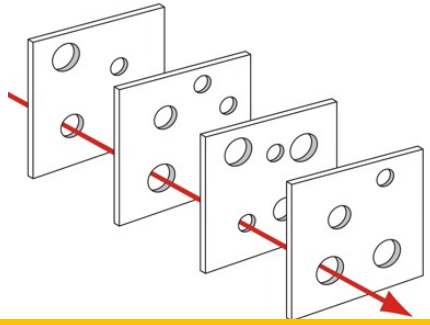
Anything that can go wrong, will go wrong (Murphy's Law)
when it's least convenient *(Sod's Law)*

Photo: NMBU

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 and anaph
  - bites
  - 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

These need to be revised or supplemented in the light of Covid-19



Temporary staff at weekends and holidays

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

Those who plan animal studies should ask relevant questions about quality assurance of the animal facility itself

The AAALAC Program Description Template is a good checklist for the facility as a whole

https://www.aaalac.org/program-description

Disclosure:

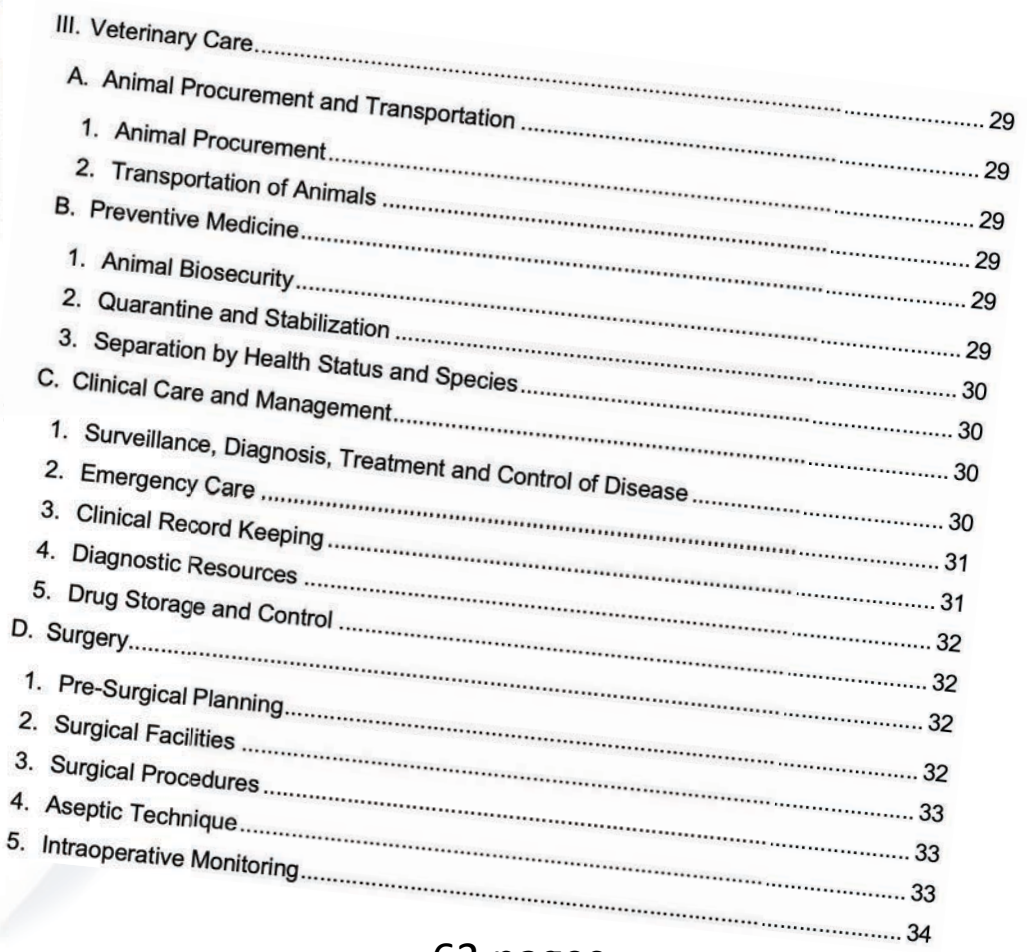
I have prepared for and managed an animal facility with AAALAC accreditation for 10 years

No other connection to AAALAC International

Program Description

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

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



63 pages

"because we've always done it that way"

"as often as necessary"

"there are no alternatives"

A Culture of Care

The International Culture of Care Network

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

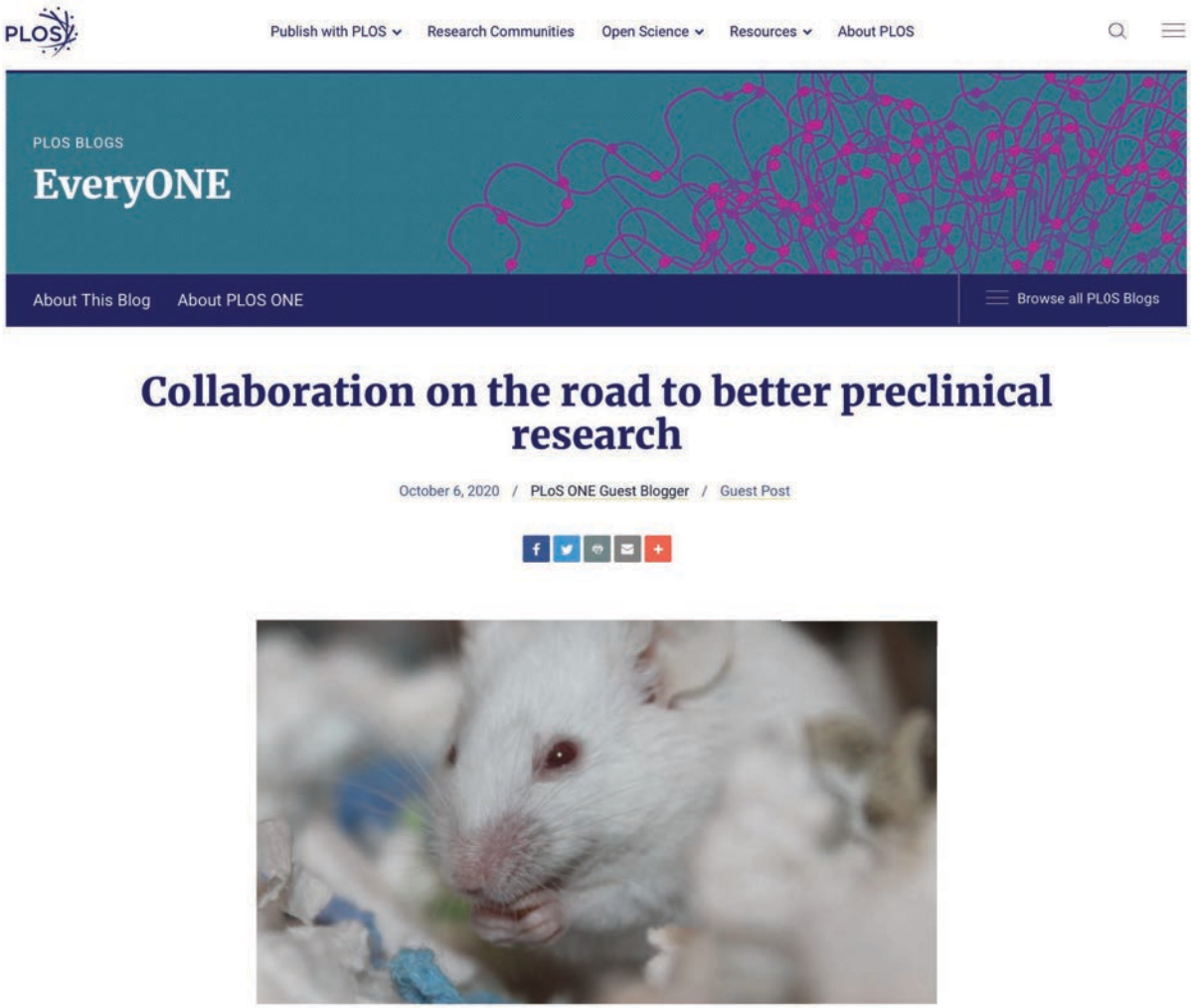
It goes beyond simply complying with the law!

Closely related to a culture of care is the concept of a **Culture of Challenge** (Louhimies, 2015)**.**

Look for the acceptable, rather than choosing the accepted.

Encourage collaboration 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



https://everyone.plos.org/2020/10/06/prepare

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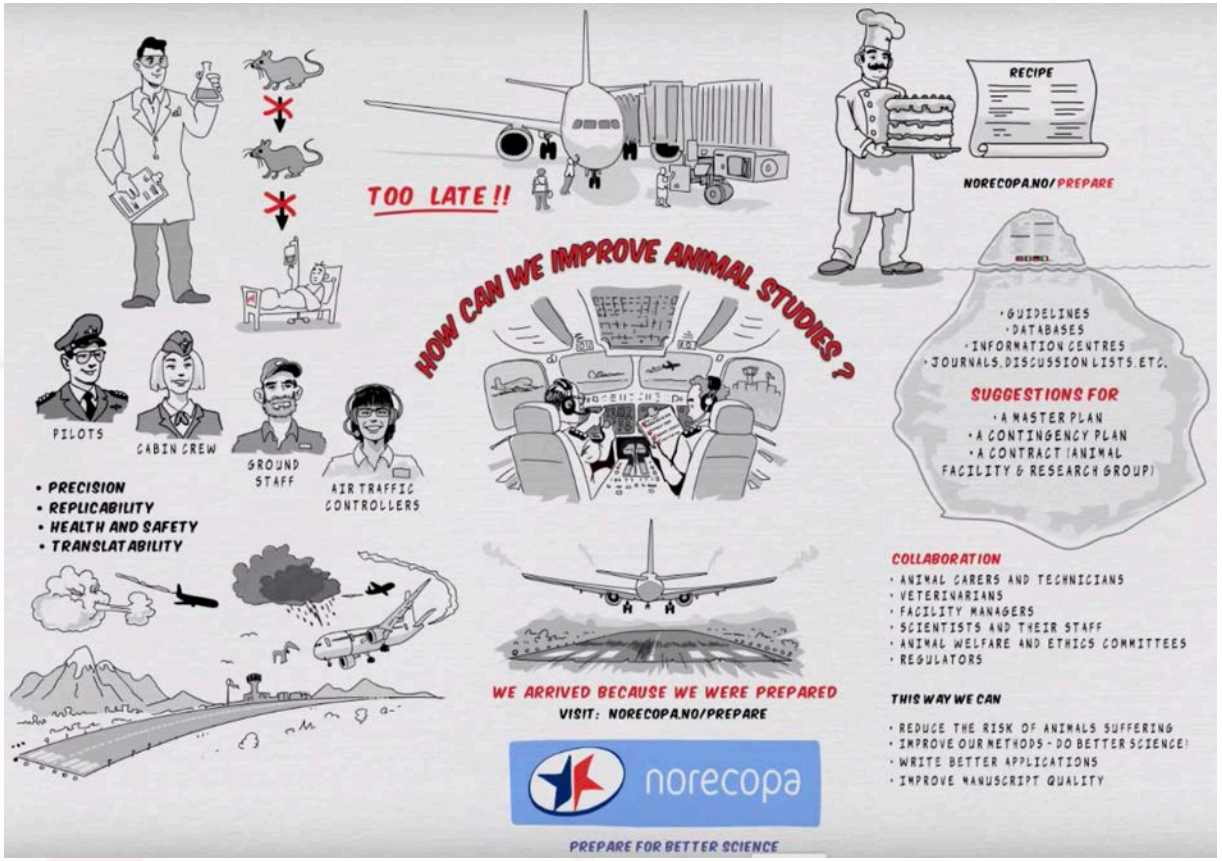
norecopa.no/PREPARE/film

a 3-minute cartoon film



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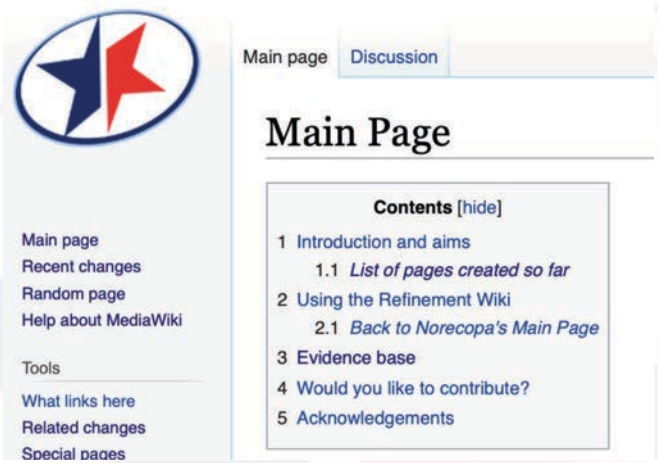
Another option to spread good practice:

norecopa.no/wiki

The Refinement Wiki



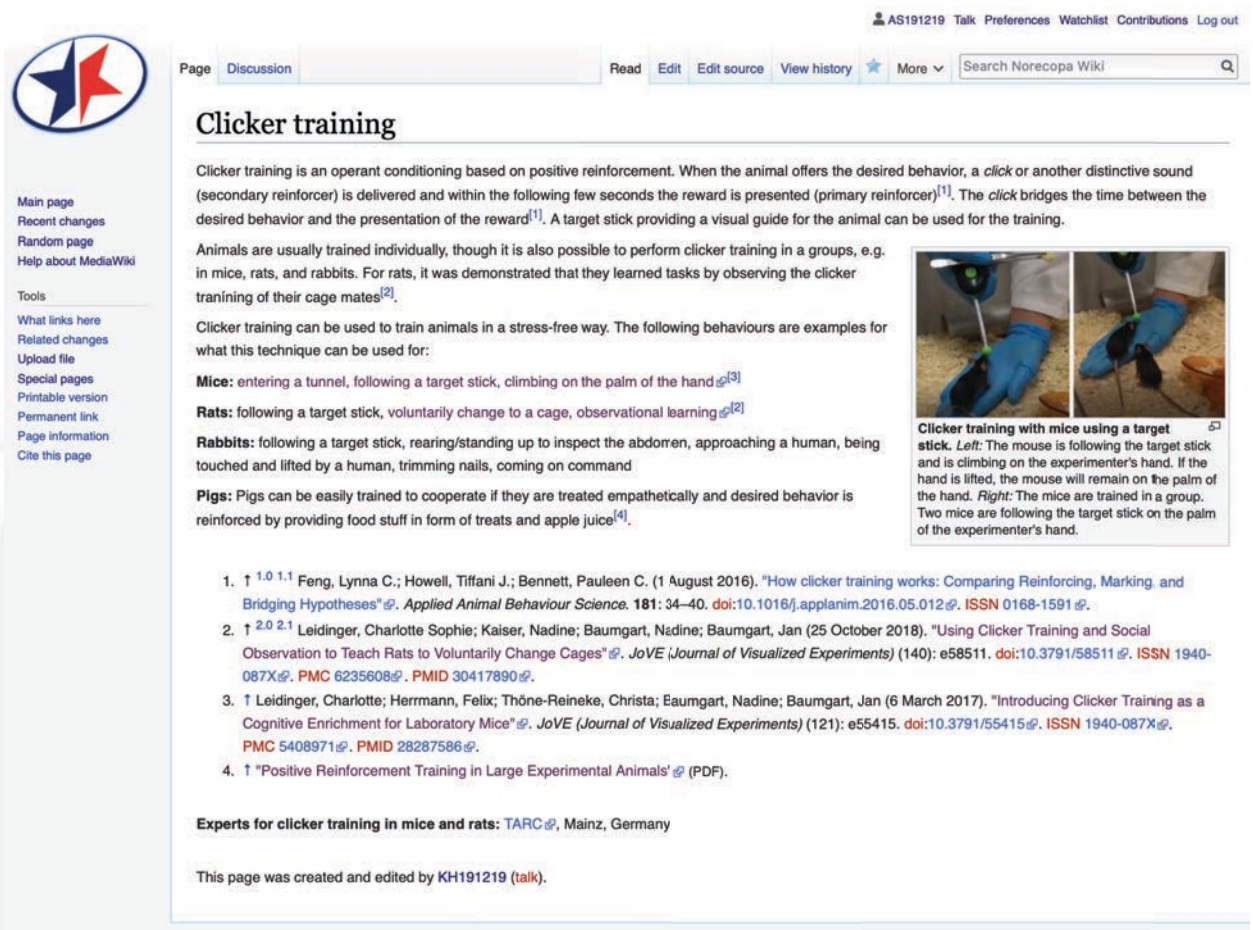
Many good ideas on refinement are posted on discussion forums, but never get published.

Designed to be

- a portal for rapid publication and dissemination of these ideas
- a place to identify experts or potential collaborators

norecopa

wiki.norecopa.no



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Clicker training

Main page
Recent changes
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Related changes
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Cite this page

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)[1]. The *click* bridges the time between the desired behavior and the presentation of the reward[1]. 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 tranining of their cage mates[2].

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[3]

Rats: following a target stick, voluntarily change to a cage, observational learning[2]

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[4].

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. ↑ [1.0] [1.1] 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. ↑ [2.0] [2.1] 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. ↑ 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. ↑ "Positive Reinforcement Training in Large Experimental Animals' (PDF).

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

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CIRS-LAS Portal

Critical incident reporting system in laboratory animal science

Refine
Reduce
Replace

Operating principles

Recent incidents

Injury of the mesentery by vertebral kyphoplasty

Mouse neonates exposed to CO2

Animal escapes during transportation

Kidney damage in mouse after surgery on heating mat

Soft tissue implant in rabbit

Summary

My **3-step recipe for better science** is therefore:

1. ***PLAN***, in collaboration with animal care staff from day one and consult the guidelines: *be PREPARED*

2. ***WRITE*** a good manuscript, showing that you have been aware of the potential causes of irreproducibility, and with enough detail that scientists can evaluate the model

3. ***FLAG*** any advances you have made within the 3Rs, preferably in the title or abstract (or write a separate method paper)

The title and abstract are critical, because they are often the only parts that are indexed by databases

"SCID-Hu mice immunized with a pneumococcal vaccine produce specific human antibodies and show increased resistance to infection"

Help others plan better!

Photo: NMBU

Published later in:

"Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea-pig, ferret and mink"

Not necessarily a high-impact factor journal.

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English-language newsletters

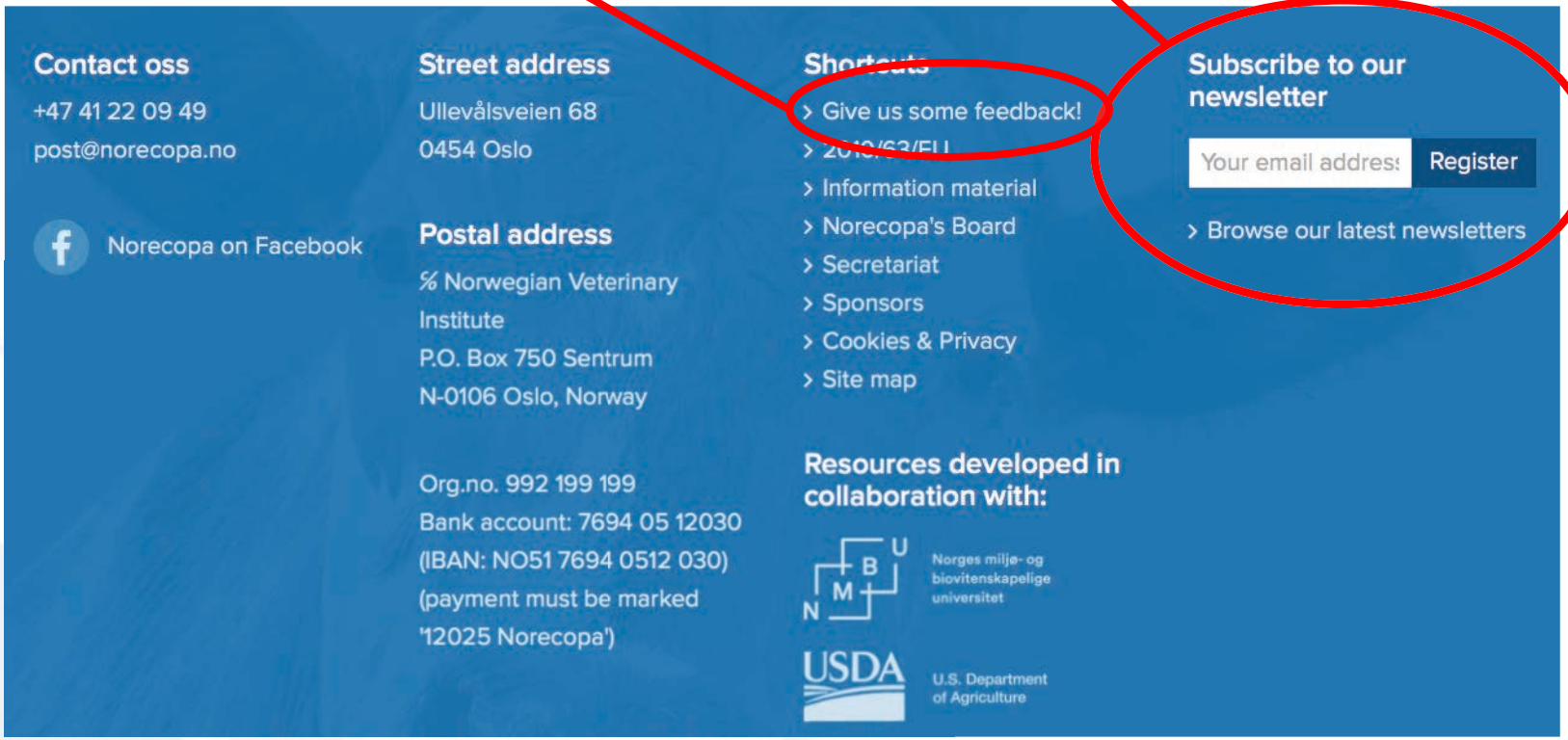
norecopa.no/news/newsletters

7-8 times a year

800 international subscribers

Feedback

English-language newsletters



Thanks to Norecopa's main sponsors:

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- Norwegian Soci
- Norwegian
- Novo No
- Sanofi
- Scottish Accre
- Stiansen Foundation
- Universities Federation for Animal Welfare (UFAW)
- US Department of Agriculture (USDA)

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