Design of animal studies:

Increasing reproducibility and animal welfare

norecopa.no/ISAE2020

Adrian Smith
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https://norecopa.no

Norecopa

Norway's National Consensus Platform for the

Three Rs: Replacement, Reduction and Refinement

and a source of global 3R resources

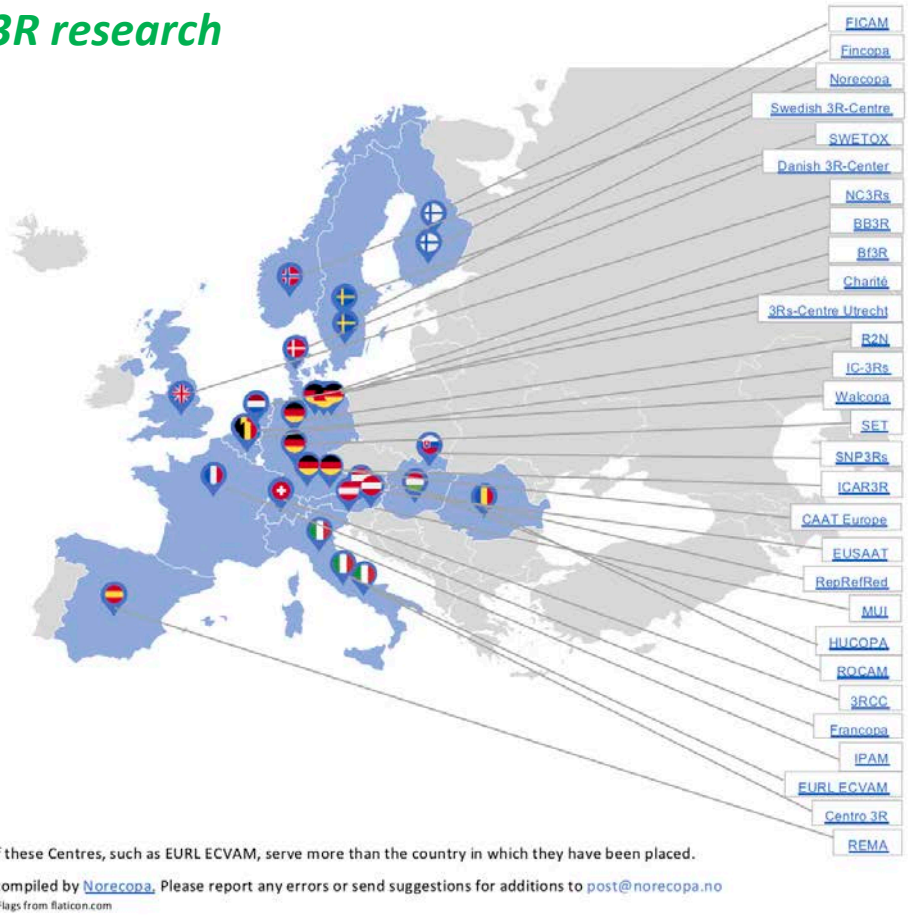
https://norecopa.no

Norecopa: PREPARE for better Science

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

Interactive map:
norecopa.no/3REuropeOverview

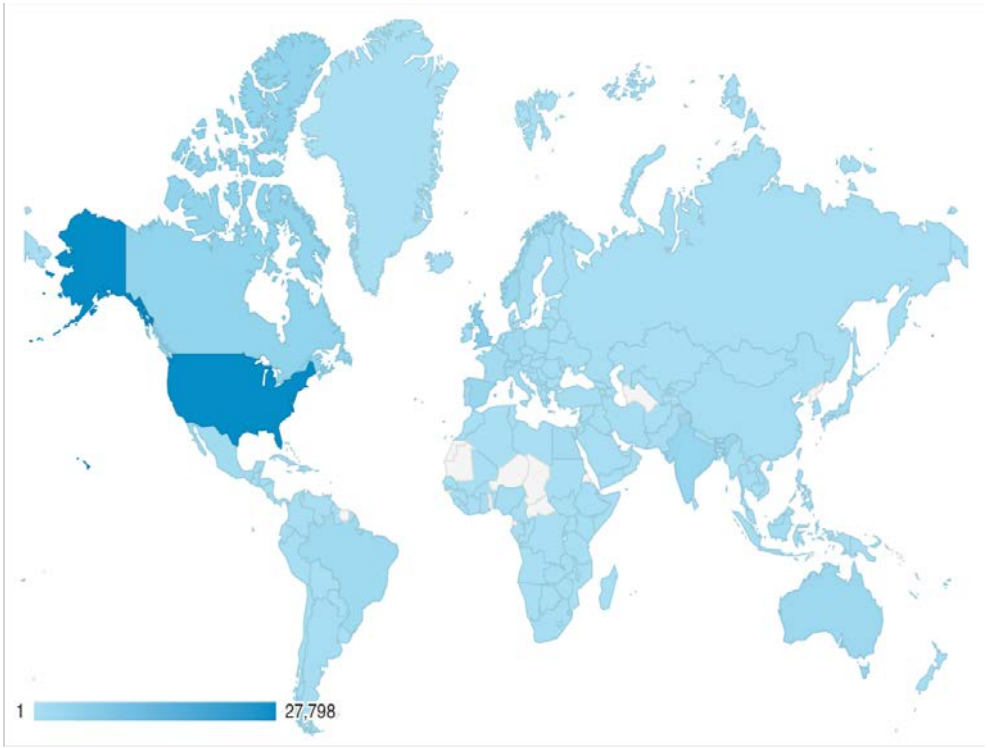
List of 3R centres:
norecopa.no/3REurope



norecopa.no : constructed for those involved in animal research and testing

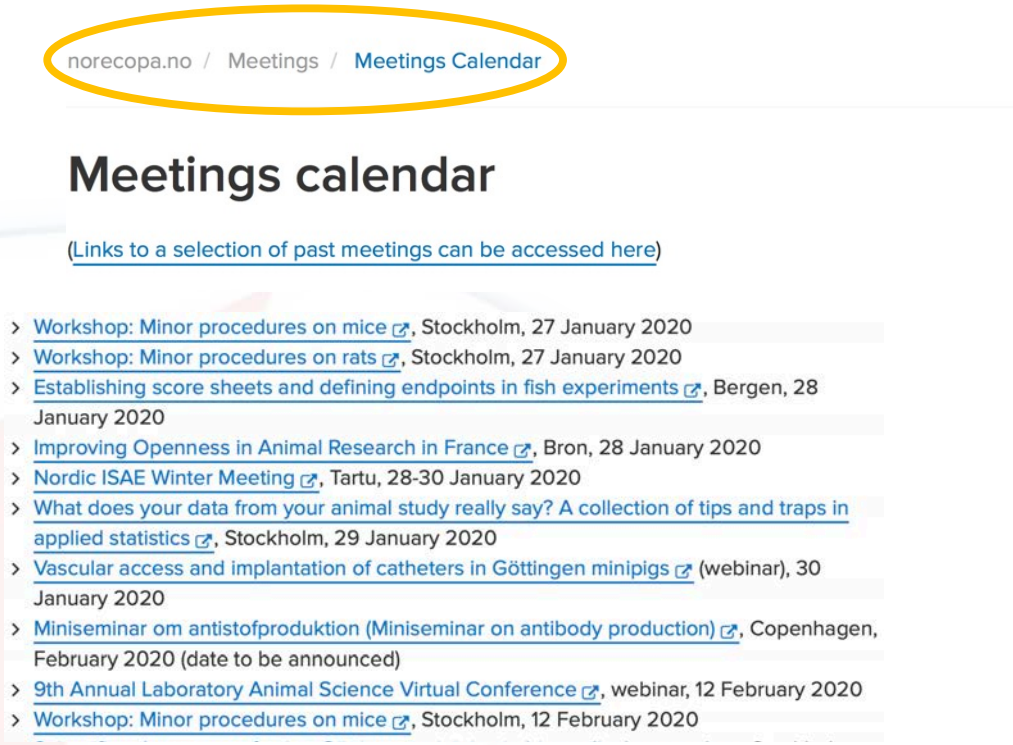
Video presentation of contents:
norecopa.no/info

9,000 webpages
approx. 80,000 links



1.		United States
2.		United Kingdom
3.		Canada
4.		India
5.		Spain
6.		Norway
7.		Australia
8.		Brazil
9.		Germany
10.		Mexico

Averaging about 250,000 page views a year



Norecopa: PREPARE for better Science

International consensus meetings

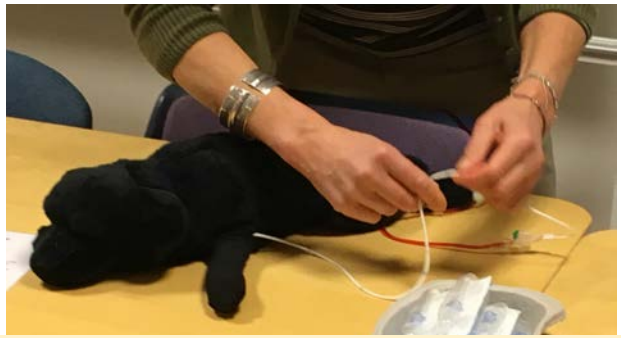
Harmonisation of the Care and Use of:

- *Fish (2005)*
- *Wildlife (2008)*
- *Fish (2009)*
- *Agricultural animals (2012)*
- *Wildlife (2017)*

https://norecopa.no/meetings

All presentations and consensus statements are on the internet: ***a lasting resource***

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



+ the NORINA database of 3,100 audiovisual aids for use in education and training
Established in 1991, updated weekly. norecopa.no/NORINA

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]

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Corresponding author: P Hawkins. Email: phawkins@rspca.org.uk

Abstract

The severity classification of procedures using animals is an important tool to help focus the implementation of refinement and to assist in reporting the application of the 3Rs (replacement, reduction and refinement). The recently revised Directive that regulates animal research and testing within the European Union requires Member States to ensure that all procedures are classified as 'non-recovery', 'mild', 'moderate' or 'severe', using assignment criteria set out by the European Commission (EC). However, these are focused upon terrestrial species, so are of limited relevance to fish users. A Working Group set up by the Norwegian Consensus-Platform for the 3Rs (Norecopa) has produced guidance on the classification of severity in scientific procedures involving fish, including examples of 'subthreshold', 'mild', 'moderate', 'severe' and 'upper threshold' procedures. The aims are to complement the EC guidelines and help to ensure that suffering in fish is effectively predicted and minimized. Norecopa has established a website (www.norecopa.no/categories) where more information on severity classification for procedures using fish, including field research, will be made available.

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

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

Background

An effective prediction of the effects of a research protocol on the animals concerned helps to ensure that any pain, suffering or distress they may experience will be effectively anticipated, recognized and alleviated. This is essential not only for animal welfare but also for scientific validity, because physiological and behavioural responses to suffering can significantly affect data quality. Severity classification is thus an important tool to help focus the implementation of refinement, including monitoring its progress, and to assist in reporting the application of the 3Rs (replacement, reduction and refinement) of Russell and Burch,[1] which is now an integral part of the legislation on animal research and testing in many countries. Predictions of severity are also fundamental to the harm–benefit assessments undertaken by bodies such as regulatory authorities and ethical committees when deciding whether or not a project should be licensed or funded.

There may also be a legal requirement to predict and classify severity. For example, the new Directive regulating animal use within the European Union, which must be implemented within all Member States by January 2013, requires the severity of each procedure to be classified on the basis of the 'degree of pain, suffering, distress or lasting harm expected to be experienced by an individual animal during the course of the procedure', with the aim of enhancing transparency, facilitating the project authorization process and providing tools for monitoring compliance.[2] Member States will have to ensure that all procedures are classified as 'non-recovery', 'mild', 'moderate' or 'severe' on a case-by-case basis, using the assignment

Laboratory Animals 2011; 1–6

Guidance on the severity classification of procedures involving fish

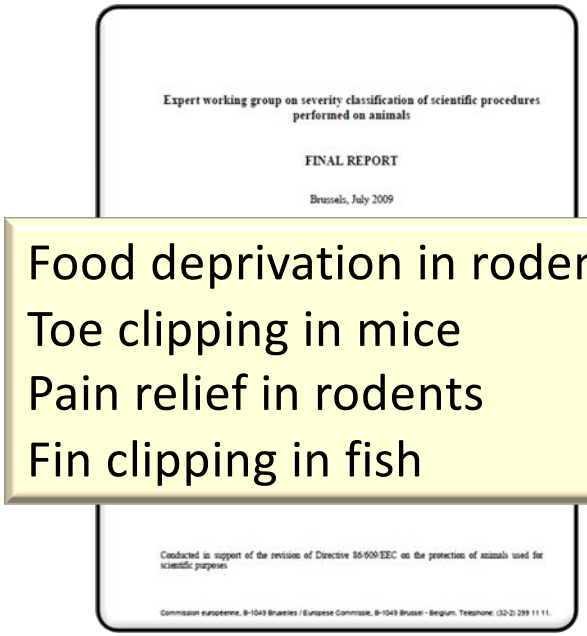
Report from a Working Group
convened by Norecopa

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

Norecopa: PREPARE for better Science



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

FINAL REPORT

Brussels, July 2009

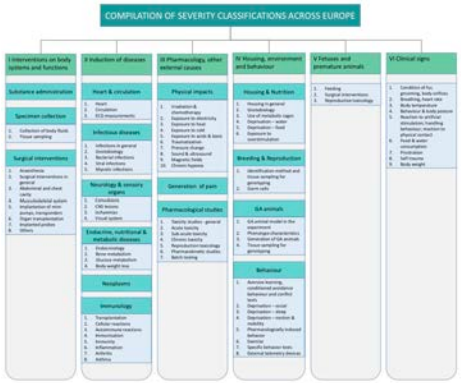
Conducted in support of the revision of Directive 86/609/EEC on the protection of animals used for scientific purposes

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

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

Mild, Moderate or Severe? A compilation of severity classification



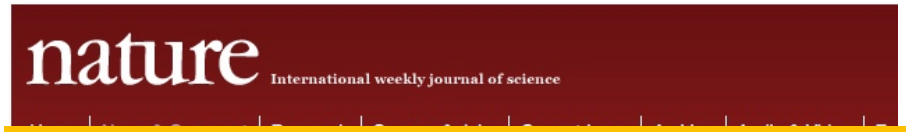
norecopa.no/severity

The compilation covers

- Interventions on body systems and functions
  - Substance administration
  - Specimen collection
  - Surgical interventions
- Induction of diseases
  - Heart and circulation
  - Infectious diseases
  - Neurology and sensory organs
  - Endocrine, nutritional and metabolic diseases
  - Neoplasms
  - Immunology
- Pharmacology and other external causes
  - Physical impacts
  - Generation of pain
  - Pharmacological studies
- Housing, environment and behaviour
  - Housing and nutrition
  - Breeding and Reproduction
  - GA animals
  - Behaviour
- Foetuses and premature animals
- Clinical signs

Source	Non-harmful / below threshold / severity degree 0	Mild / severity degree 1	Moderate / severity degree 2	Severe / severity degree 3
Directive 2010/63/EU, Annex VIII		Administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal.	Frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (> 10 % of circulating volume) in a conscious animal within a few days without volume replacement.	
Home Office (2014 a)		Injection by conventional routes, i.e. subcutaneous, intravenous, intraperitoneal or intramuscular (assuming competence of the person performing the procedure and that best practice guidelines for volume, pH, needle size, etc. are followed). Multiple injections by these routes may remain in the mild category if there are no cumulative effects.		
Federal Food Safety and Veterinary Office FSVO (2018)	Single injection of small volumes s.c. and i.v. (species-specific), including repeated injections at long intervals (at least 24 hours).	I.v. or i.p. injections in sedated animals by catheter or tube and substances introduced into the body such as enemas. Implants and permanent accesses that can be created and used by means of a minimally invasive (superficial) procedure. Examples: Repeated iv or sc injection of small volumes (species-specific). Insertion of cannulae into peripheral blood vessels. Subcutaneous injection of tumour tissue. Single subcutaneous implantations of osmotic minipumps and transponders. Subcutaneously channelled venous catheters.	Repeated injections at short intervals (several times within 24 hours). Implants and permanent accesses that have to be created by means of a deep surgical procedure or causing mild long-term constraint on an animal. Examples: Chronic iv catheters. Duodenal infusion cannula. Hepatic portal vein catheter. Gastric tube or chronic intragastric infusion cannula. Intraperitoneal or intravenous osmotic minipumps. Gavage. Telemetry transmitters. Implanted iv catheters with pumps in a jacket worn by dogs. Implantation of indwelling catheters in	Implants and permanent accesses that have to be created by means of a deep surgical procedure and causing severe long-term strain on an animal. Examples: Attachment of implants on the locomotor apparatus or other large implants that restrict movement (e.g. dorsal skinfold chamber in mice). Implantation of catheters in the abdominal aorta or bile duct. Implantation of an arterial blood-pressure catheter in the aortic arch via the left carotid artery or in the abdominal aorta via the femoral artery. Implantation of a combination of a venous and arterial catheter.

Scientists are becoming increasingly concerned about the validity of animal experiments



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.

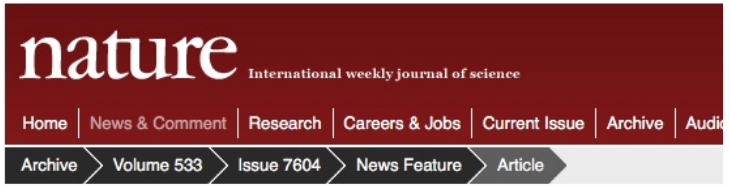
Pain management in pigs undergoing experimental surgery; a literature review (2012–4)

FREE

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

Br J Anaesth (2016) 116 (1): 37-45. **DOI:** https://doi.org/10.1093/bja/aev301
Published: 03 October 2015

selection criteria. Most articles (193/233, 83%) described use of drugs with analgesic properties, but only 87/233 (37%) described postoperative analgesia. No article provided justification for the analgesic chosen, despite the lack of guidelines for analgesia in porcine surgical models and the lack of formal studies on this subject. Postoperative pain assessment was reported in only 23/233 (10%) articles. It was found that the reporting of postoperative pain management in the studies was remarkably low, reflecting either under-reporting or under-use. Analgesic description, when given, was frequently too limited to enable reproducibility. Development of a



NATURE | NEWS FEATURE

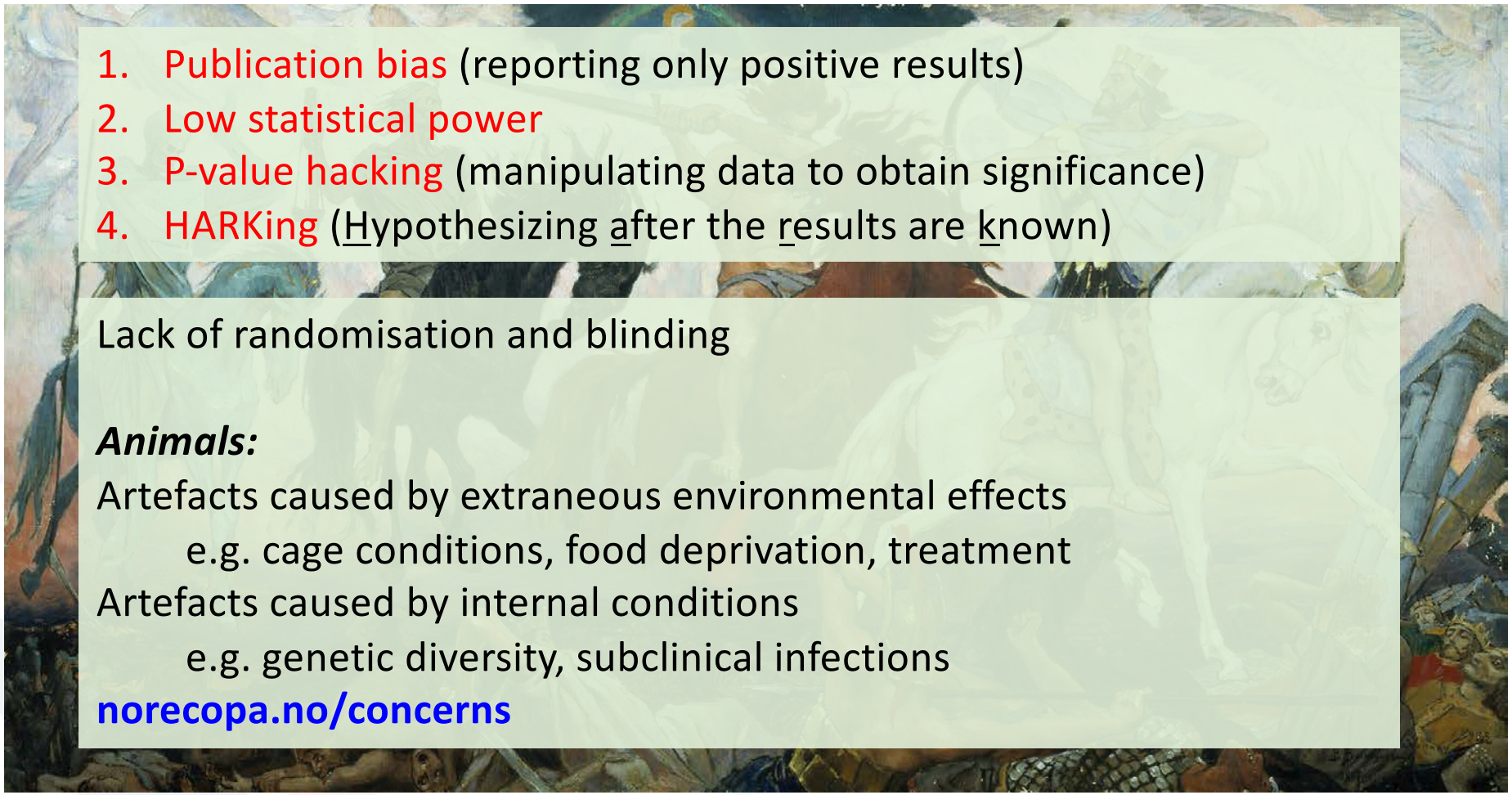
1,500 scientists lift the lid on reproducibility

Survey sheds light on the 'crisis' rocking research.

Monya Baker

25 May 2016 | Corrected: 28 July 2016

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



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)

Lack of randomisation and blinding

Animals:

Artefacts caused by extraneous environmental effects

e.g. cage conditions, food deprivation, treatment

Artefacts caused by internal conditions

e.g. genetic diversity, subclinical infections

norecopa.no/concerns

Two frustrations:

"We can solve the reproducibility crisis by"

- better reporting
- courses in Experimental Design that focus on the "mathematical" elements (e.g. group size, randomisation, blinding, bias, statistical analysis) and ignore the animal/human-related issues

nature human behaviour

Perspective | Open Access | Published: 10 January 2017

A manifesto for reproducible science

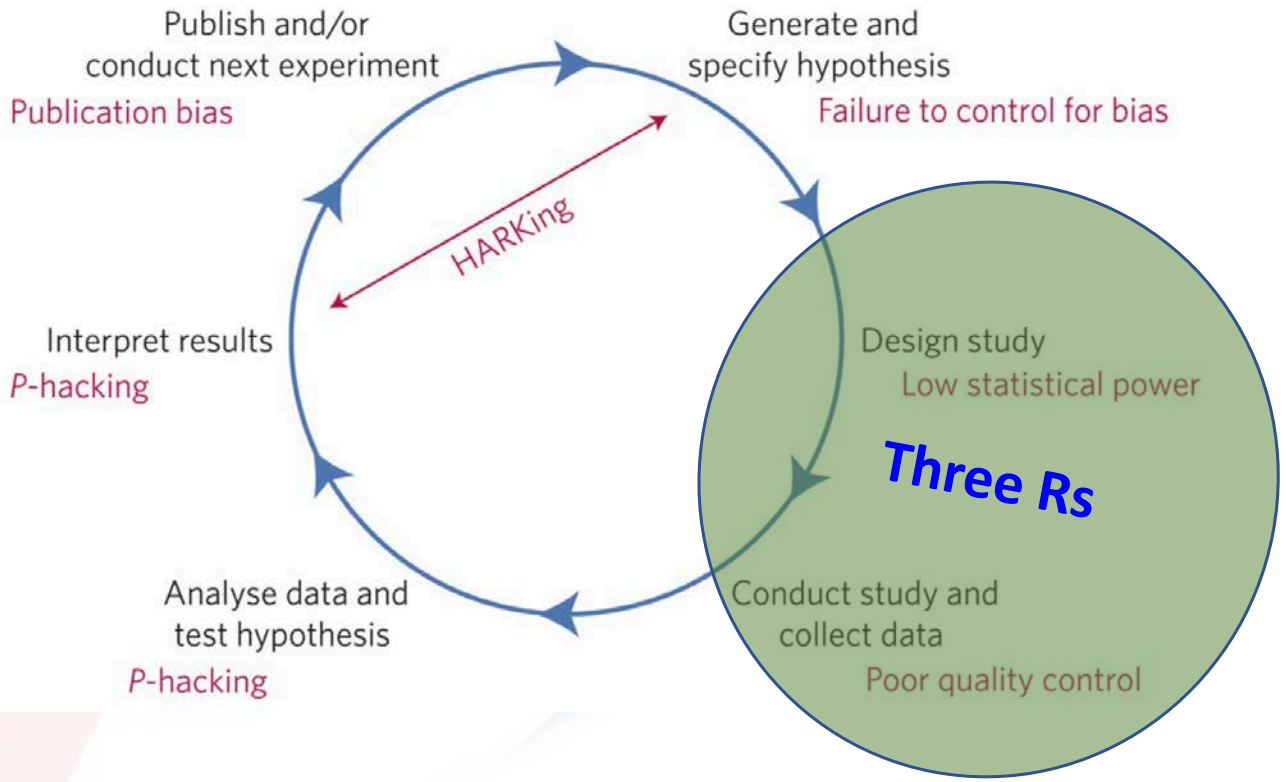
Marcus R. Munafò, Brian A. No
Button, Christopher D. Chambers,
Jan Wagenmakers, Jennifer J. Wa

Nature Human Behaviour 1, Artic

33k Accesses | **518** Citations |

Figure 1: Threats to reproducible science.

From: A manifesto for reproducible science



We need guidelines for *reporting* animal studies, and we have been trying to solve the reproducibility problem for a long time!

- 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)
- 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

The ARRIVE Guidelines, 2010

	ITEM	RECOMMENDATION
Title	1	Provide as accurate and concise a description of the content of the article as possible.
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.
INTRODUCTION		
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
METHODS		
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free (SPF); type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.
RESULTS		
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often be tabulated).
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.
DISCUSSION		
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the [illegible]n (the 3Rs) of the use of animals in research.
		findings of this study are likely to translate to any relevance to human biology.
		rant number) and the role of the funder(s)

ARRIVE The ARRIVE Gu… Experiments. Or…

https://www.nc3rs.org.uk/arrive-guidelines

Norecopa: PREPARE for better Science

The ARRIVE guidelines

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

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

These items **improve**

- **study quality**
- **animal welfare**
- and therefore **reproducibility**

- and also the **safety of humans and animals** affected directly or indirectly by the work

The ARRIVE guidelines 2019: updated guidelines for reporting animal research

Nathalie Percie du Sert[1], Viki Hurst[1], Amrita Ahluwalia[2], Sabina Alam[3], Marc T. Avey[4], Monya Baker[5], William J. Browne[6], Alejandra Clark[7], Innes C. Cuthill[6], Ulrich Dirnagl[8], Michael Emerson[9], Paul Garner[10], Stephen T. Holgate[11], David W. Howells[12], Natasha A. Karp[13], Katie Lidster[1], Catriona J. MacCallum[14], Malcolm Macleod[15], Ole Petersen[16], Frances Rawle[17], Penny Reynolds[18], Kieron Rooney[19], Emily S. Sena[15], Shai D. Silberberg[20], Thomas Steckler[21], Hanno Würbel[22]

biorxiv.org/content/10.1101/703181v1

'Endorsed by more than a thousand journals'
but:
'only a small number of journals actively enforce compliance'

(Swiss study in 2016: 51% of researchers using 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%'

'Providing the level of journal or editorial input to ensure compliance with all the items of the ARRIVE guidelines is unlikely to be sustainable for most journals because of the resources needed'

The ARRIVE guidelines 2019: updated guidelines for reporting animal research

ARRIVE Essential 10		
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.
Inclusion and exclusion criteria	3	a. Describe any criteria established *a priori* for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. c. For each analysis, report the exact value of N in each experimental group.
Randomisation	4	Describe the methods used: a. To allocate experimental units to control and treatment groups. If randomisation was used, provide the method of randomisation. b. To minimise potential confounding factors such as the order of treatments and measurements, or animal/cage location.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis. b. Specify the experimental unit that was used for each statistical test. c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.
Experimental animals	8	a. Provide details of the animals used, including species, strain and substrain, sex, age or developmental stage, and weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimation periods). d. Why (provide rationale for procedures).
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable. b. If applicable, the effect size with a confidence interval.

biorxiv.org/content/10.1101/703181v1

Norecopa: PREPARE for better Science

The ARRIVE guidelines 2019: updated guidelines for reporting animal research

Recommended Set		
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study and the frequency of monitoring.
Interpretation /scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.
Generalisability /translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.
Data access	20	Provide a statement describing if and where study data are available.
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.

biorxiv.org/content/10.1101/703181v1

Norecopa: PREPARE for better Science

How do they do it?

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


norecopa
Norecopa: PREPARE for better Science

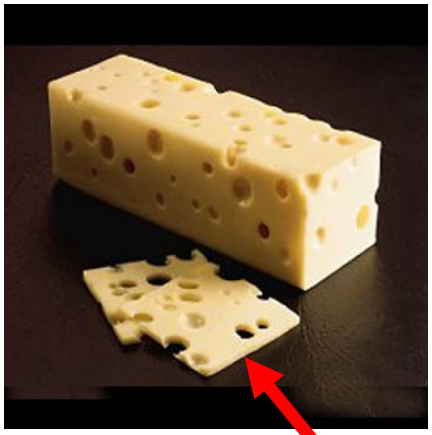
Aviation and Animal Research: Human Factors

A Pilot's Perspective

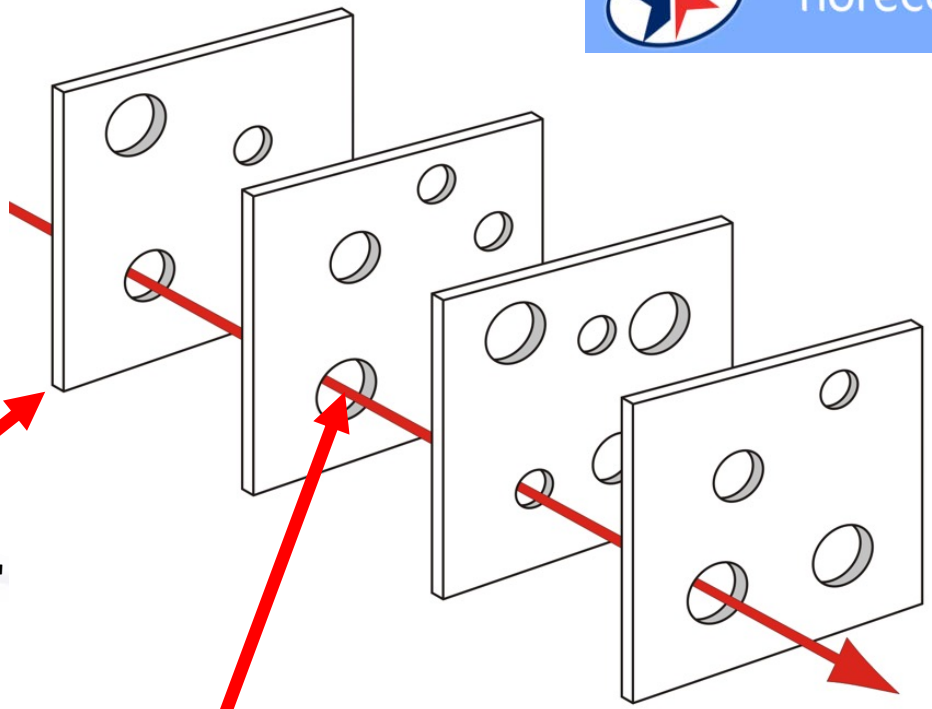
By Jake Hannabuss

Accident Rate for commercial flights is one fatal accident per 16 million flights

10-15 checklists on short European flights



eaugallecheese.com/Swiss-Cheese



"Layer of defence"
or redundancy

Weakness / hazard

Loss

wikipedia.org/wiki/Swiss_cheese_model

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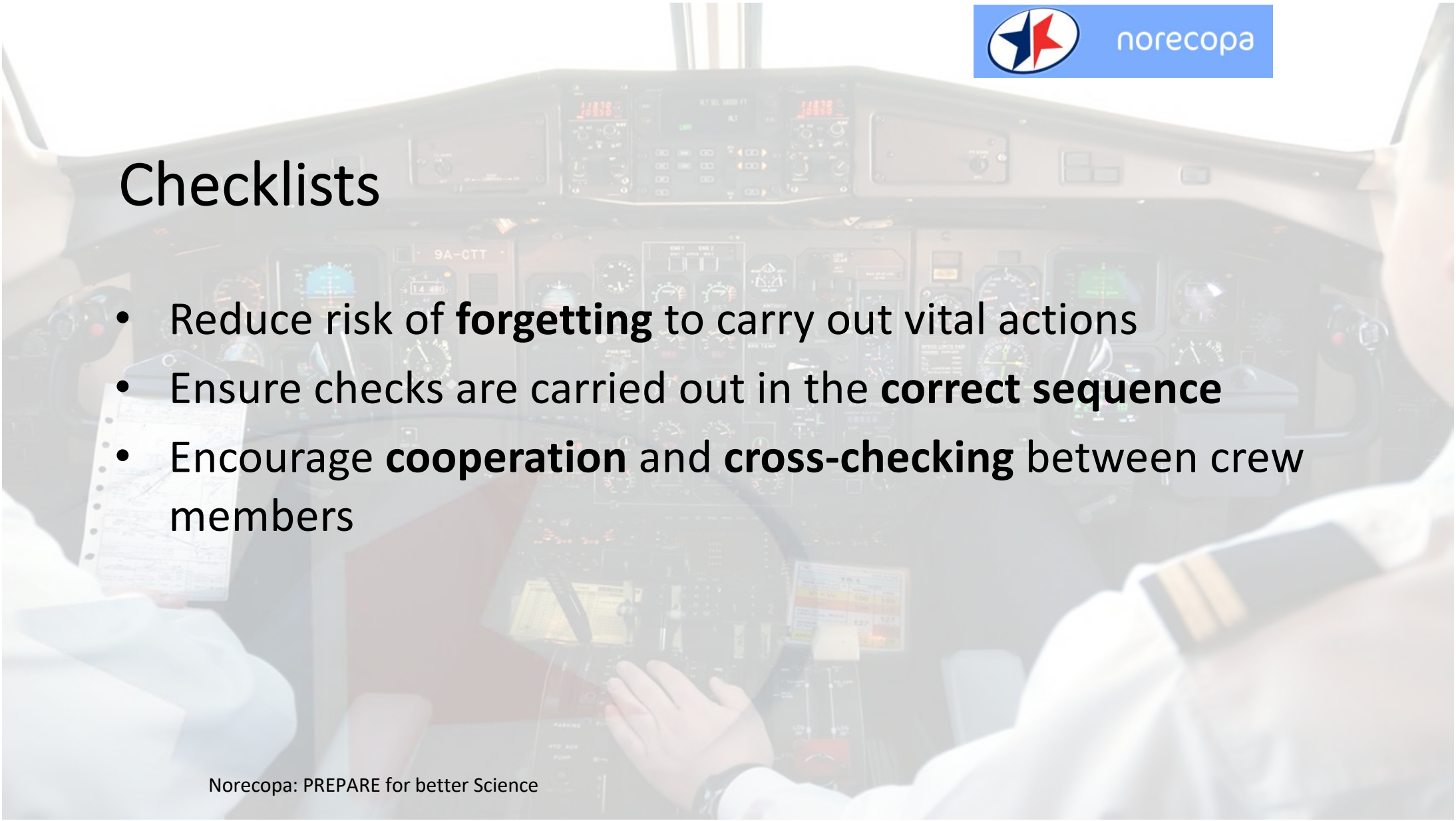
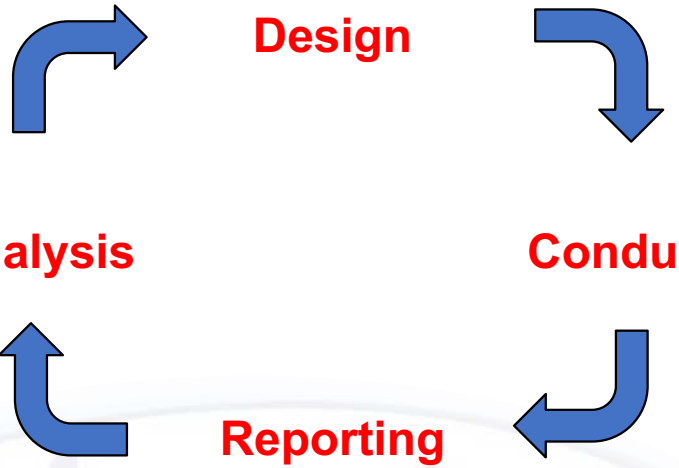


Checklists

- 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

Too late to read the checklists when you have arrived!

colourbox.com



Space Shuttle, NASA

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

1) Columbia

Photo: gettyimages.no

White insulating tiles were glued to the shuttle to prevent it from burning up on re-entry (the black areas on this photo are areas where tiles have not yet been installed).

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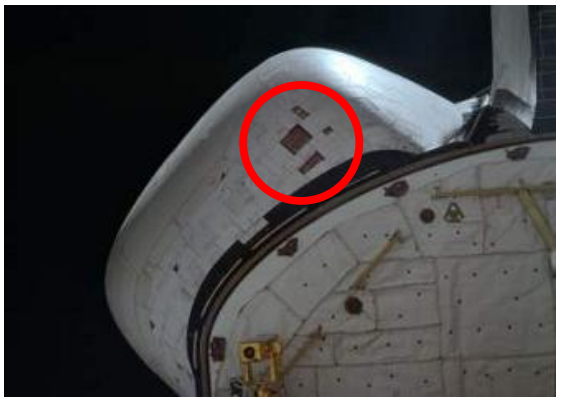


First shuttle flight, Columbia, in April 1981.
Some tiles fell off at take-off, but these were not on a critical part of the vehicle.
Photo: nasaspaceflight.com

Columbia burnt up in 2003, killing all 7 crew members, because tiles on a critical area (the leading edge of the wing) fell off.
Photo: cbsnews.com

2) Challenger

www.mahal.org

Flexible rubber O-rings (seen in cross-section as black dots) prevent hot gases from escaping between the joints of the solid rocket boosters. These rings lose some of their flexibility at low temperatures.

Challenger was launched in cold weather in January 1986. The O-rings on one booster rocket malfunctioned, allowing hot gases to ignite the contents of the liquid fuel tank. The vehicle subsequently disintegrated, killing all 7 crew members.
Photo: no.wikipedia.org

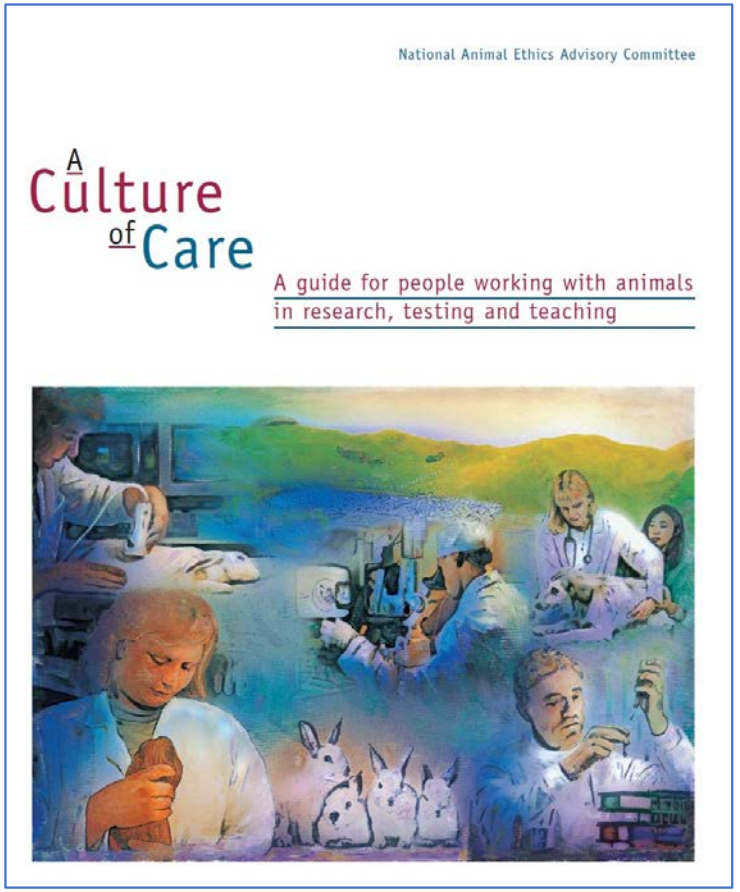
Details are important!!

An International Culture of Care Network

norecopa.no/CoC

- Pro-active approach to improving standfards
- Effective communication on animal welfare
- Roles of animal care and technical staff respected and listened to
- A no-blame culture
- Mentioned in the EU Directive recitals
- The National Committee and the local Animal Welfare Bodies should encourage this culture

35 members from user establishments, competent authorities and stakeholder organisations, in 16 countries – sharing experiences
A 3R Prize is a good incentive

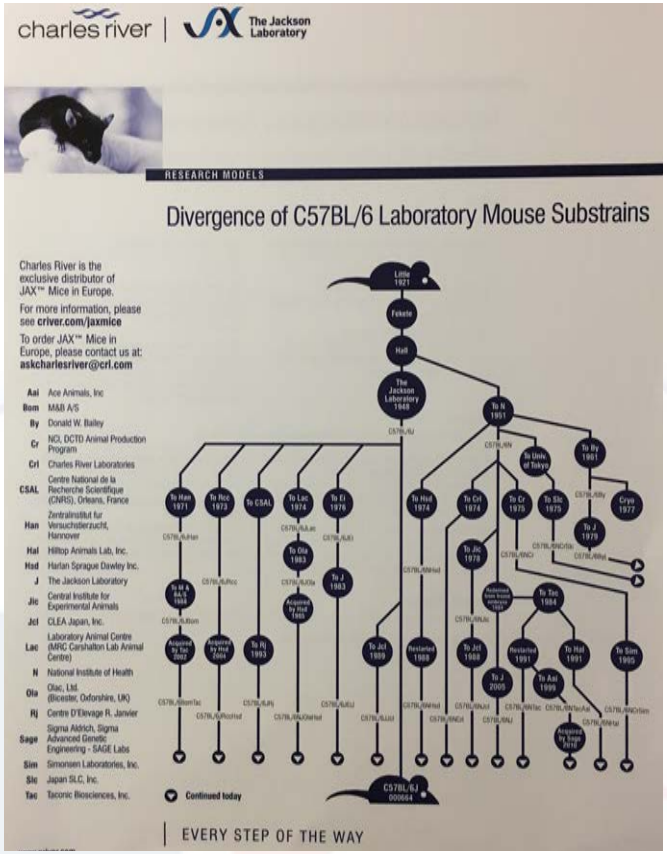


mpi.govt.nz/dmsdocument/1473

How do we perform?

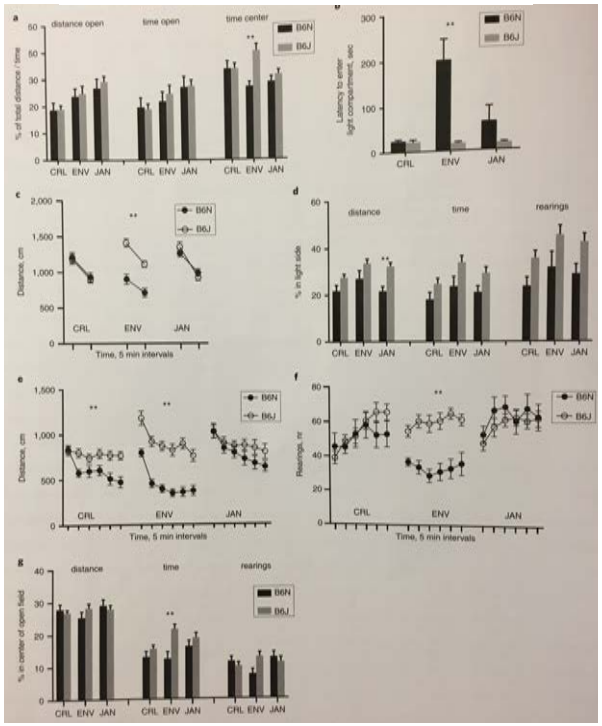
https://www.meonuk.com/runway-markings-explained

The C57BL/6 mouse



Åhlgren & Voikar (2019): Behavioural differences between /6J and /6N mice

nature.com/articles/s41684-019-0288-8



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Contingent suffering

animalcaresystems.com

(not just the direct suffering caused by the procedure)

Fear, boredom and discomfort

Caused by, for example:

Transport, or changes in housing, husbandry and social groups

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

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

photo: colourbox.com

Stress caused by capture and handling

INDEPENDENT

News › Science

Scores of scientific studies based on mice thrown into doubt because they wer

Mice pic
naturall

't act

Ian Johnstor

https://www.nc3rs.org.uk/how-to-pick-up-a-mouse

Stress caused by capture and handling

http://bitly.com/scruff-technique

Artefacts caused by poor administration techniques

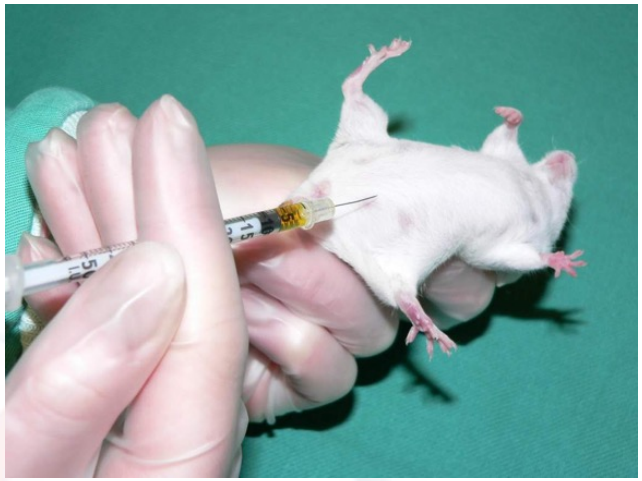


Photo: NMBU

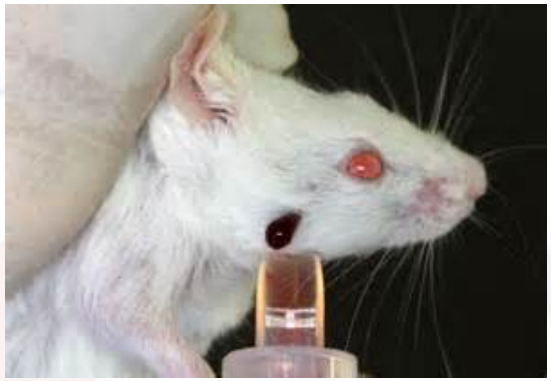
- *Are you sure that your injection ends up in the same place each time?*
- *Are the injections painful?*
- *Are they realistic? (intramuscular injections in small animals)*

'Simple' blood sampling techniques?

At the doctor:
I think I'll take a blood sample from you tomorrow.

I take my blood samples by sticking a knife into your neck, without anaesthesia.

But don't worry, I'll inject 2 litres of liquid into your abdomen first so you don't die from fluid loss.



?

medipoint.com/html/for_use_on_mice.html

'Simple' blood sampling techniques?

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 and
- ✓ not damage the surrounding tissue
- ✓ collect samples rapidly to avoid artefacts due to mechanical stress, temperature changes, length of sampling

Carol M. Newton (1925-2014)

National Library of Medicine

The three S's

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

norecopa.no/3S

Carol M Newton, quoted in Rowsell HC (1977): The Ethics of Biomedical Experimentation in The Future of Animals, Cells, Models, and Systems in Research, Development, Education, and Testing pp. 267-281, National Academy of Sciences, Washington, D.C., ISBN 0-309-02603-2.

3R methods are often not highlighted in the scientific literature

http://www.theodora.com/rodent_laboratory/blood_collection.html

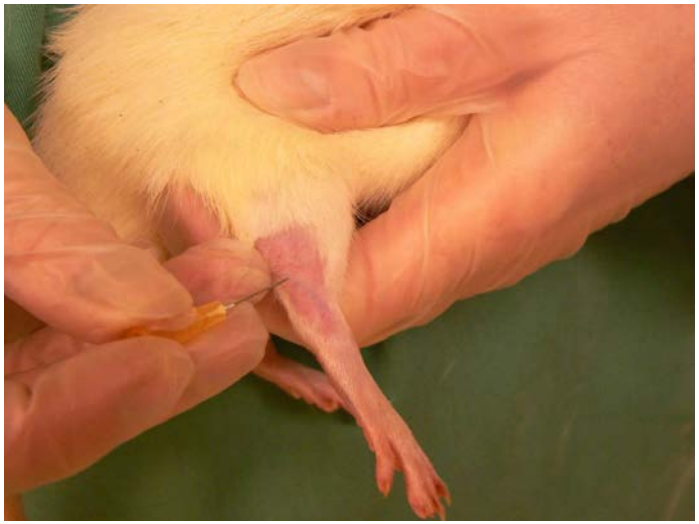


photo:NMBU

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

Saphenous vein puncture for

blood sampling of the mouse, rat, hamster,

gerbil, guinea-pig,

ferret and mink

Visibility! Not necessarily in a high-impact journal.

The title and abstract are critical, because they are often the only parts that are indexed. They must be informative and contain 3R-terms!

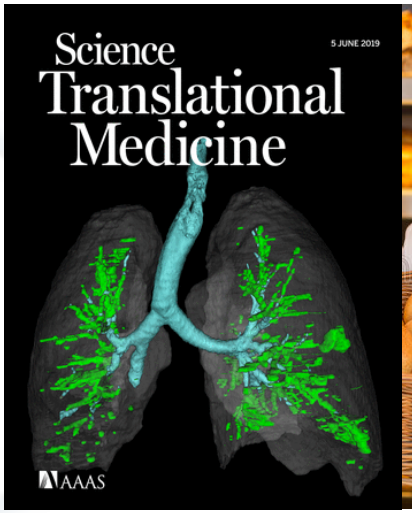
The development of Response Surface Pathway Design in toxicity studies

The development of Response Surface Pathway Design to reduce animal numbers in toxicity studies

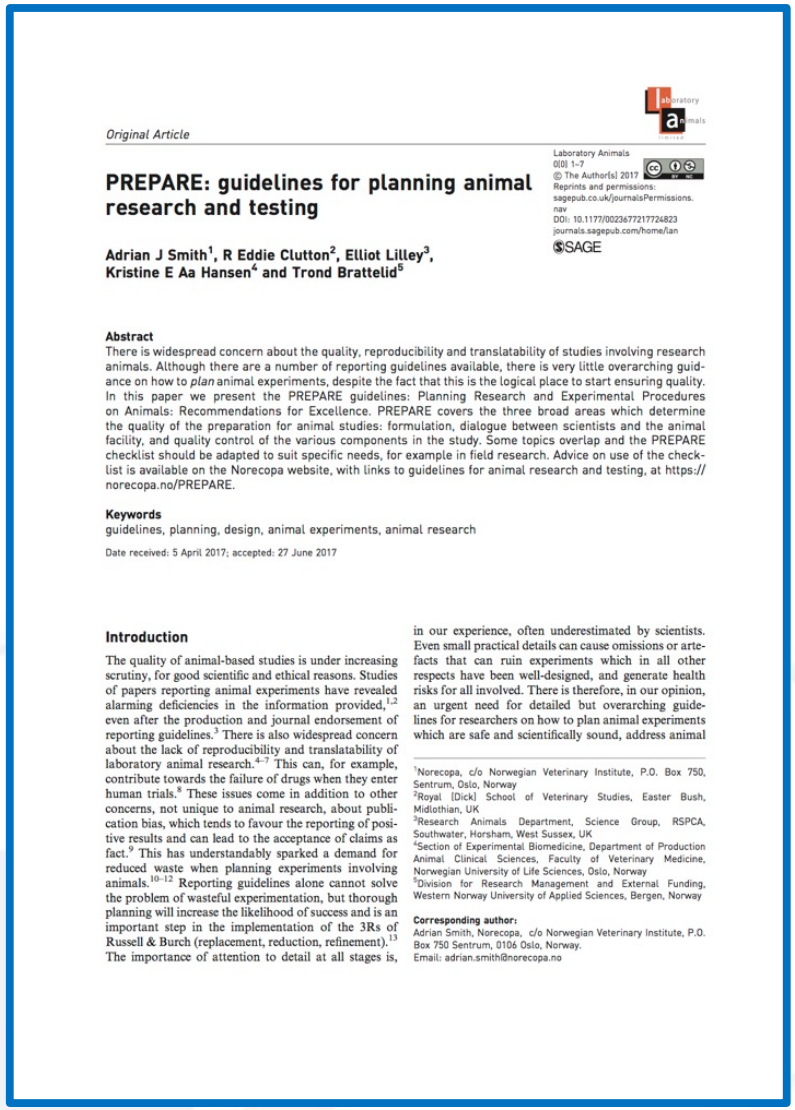
https://www.bls.gov/ooh/images/3077.jpg

PREPARE *from day 1*

ARRIVE



https://www.dreamstime.com



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

DOI: 10.1177/0023677217724823
journals.sagepub.com/home/lan
SAGE

Abstract

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

Keywords

guidelines, planning, design, animal experiments, animal research

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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https://doi.org/10.1177/0023677217724823

Over 12,000 downloads from the journal website so far

Also downloadable from norecopa.no/PREPARE

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

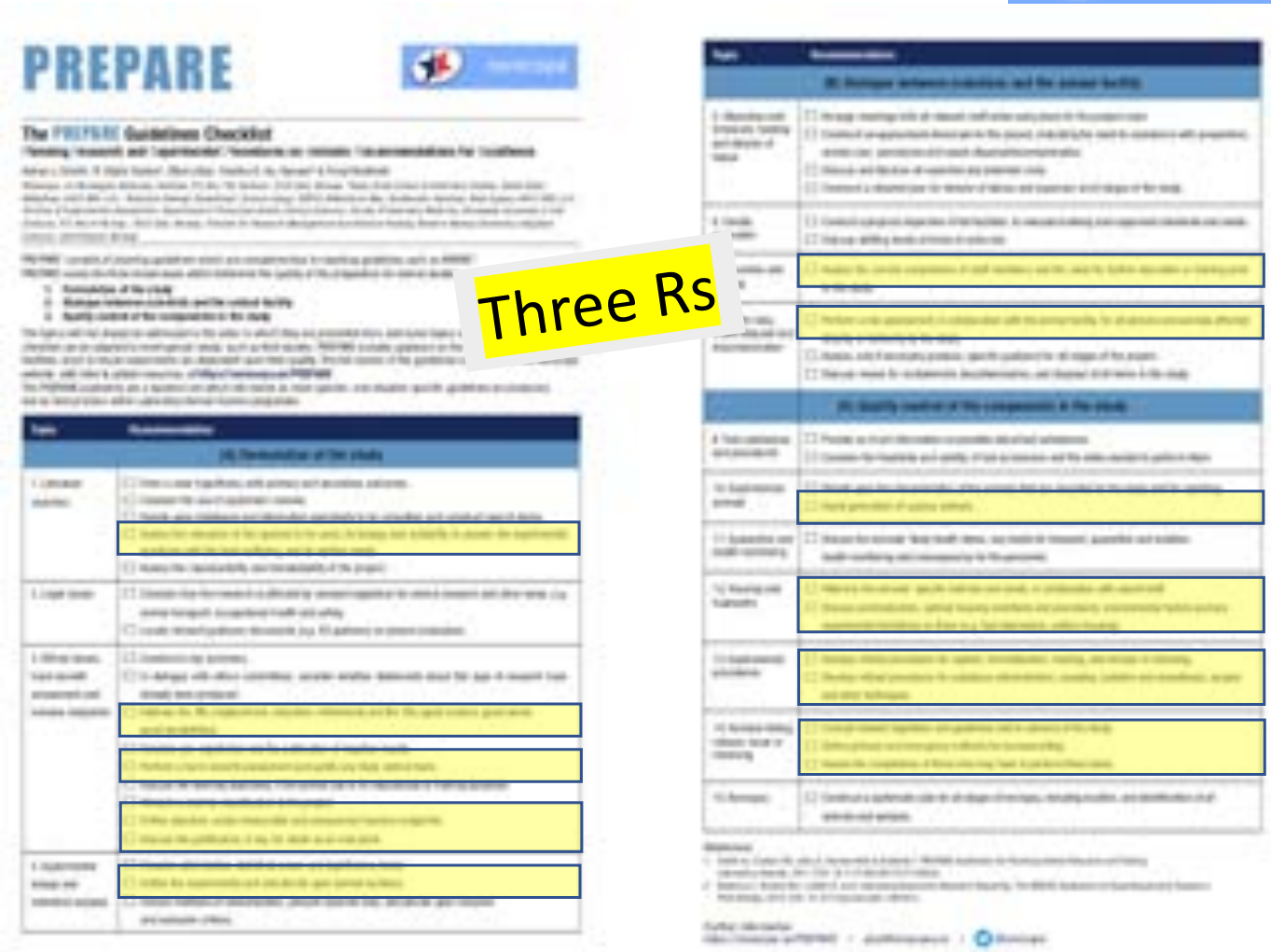
5. Objectives and timescale, funding and division of labour
6. Facility evaluation
7. Education and training
8. Health risks, waste disposal and decontamination

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

Items in pink are not highlighted in ARRIVE

A downloadable checklist



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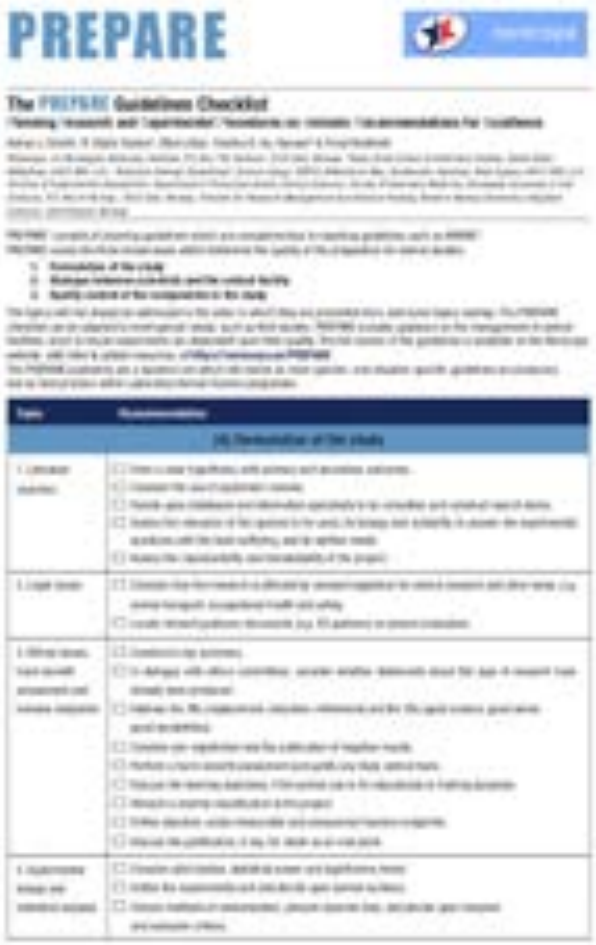
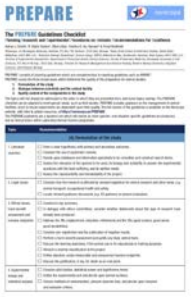
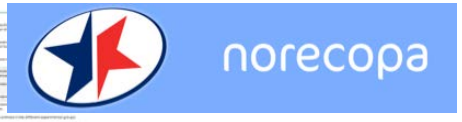
norecopa.no/PREPARE/prepare-checklist


PREPARE

The PREPARE Guidelines Checklist

Norecopa: PREPARE for better Science





Day 1 of planning

Experiment and data analysis

Manuscript

Submission

PREPARE

ARRIVE

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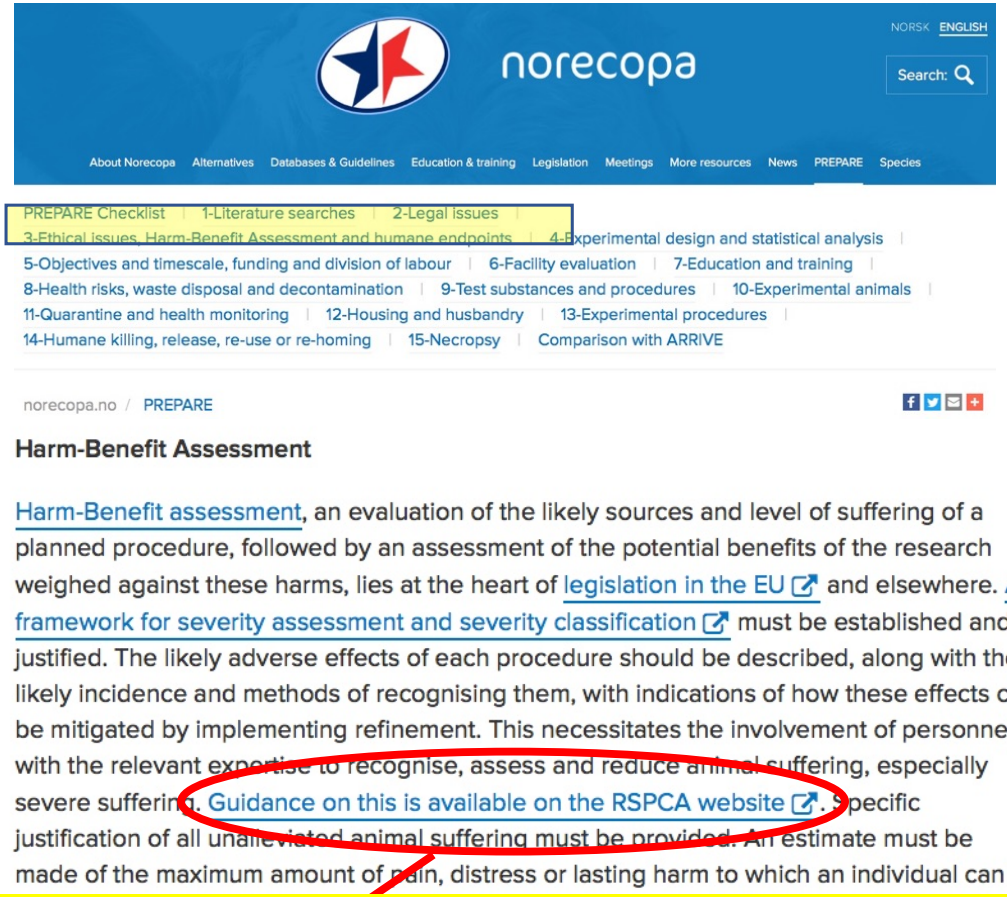
In addition to the checklist, much more information is available on:

norecopa.no/PREPARE

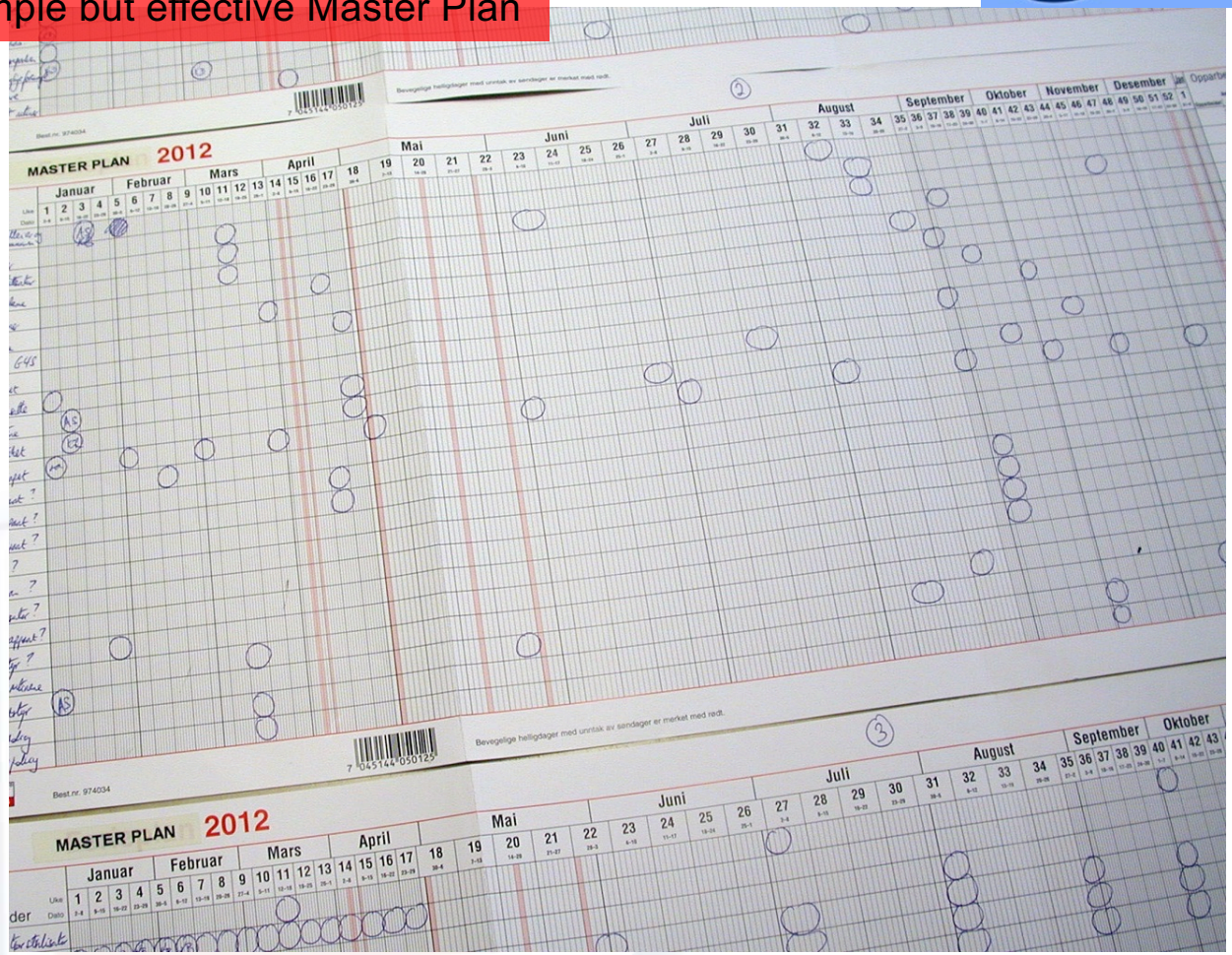
Norecopa: PREPARE for better Science

norecopa.no/PREPARE



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

A simple but effective Master Plan



Contract between the animal facility and the research group

The division of labour and responsibilities

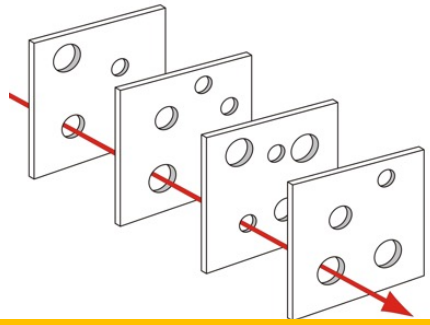
Clarifying all stages of the experiment

Ensuring that all necessary parameters are recorded

	Animal facility	Researcher	Not applicable
Animal:			
Arrival date			
Species			
Strain/stock and substrain			
Supplier (full name and address) or bred on the premises			
Number and sex			
Age, weight, stage of life cycle on arrival			
Pre-treatment (surgical or medical) from supplier			
Quality (e.g. SPF, germ-free, gnotobiotic, conventional)			
Acclimation time before the start of the experiment			
Time and duration of fasting (with/without water and bedding)			
Environment:			
Type of housing: barrier/conventional			
Temperature (mean ± 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			

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 anaphylactic reactions
  - burns
  - head injuries
  - 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



Temporary staff at weekends and holidays

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

Contingency and redundancy

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

Photo: NMBU

Consult the 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

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.

"as often as necessary"

"because we've always done it that way"

(a strange comment from scientists looking for novel events!)

An example: i.v. injection of a radioactive isotope:

proceduresWithCare.org.uk/intravenous-injection-in-the-mouse

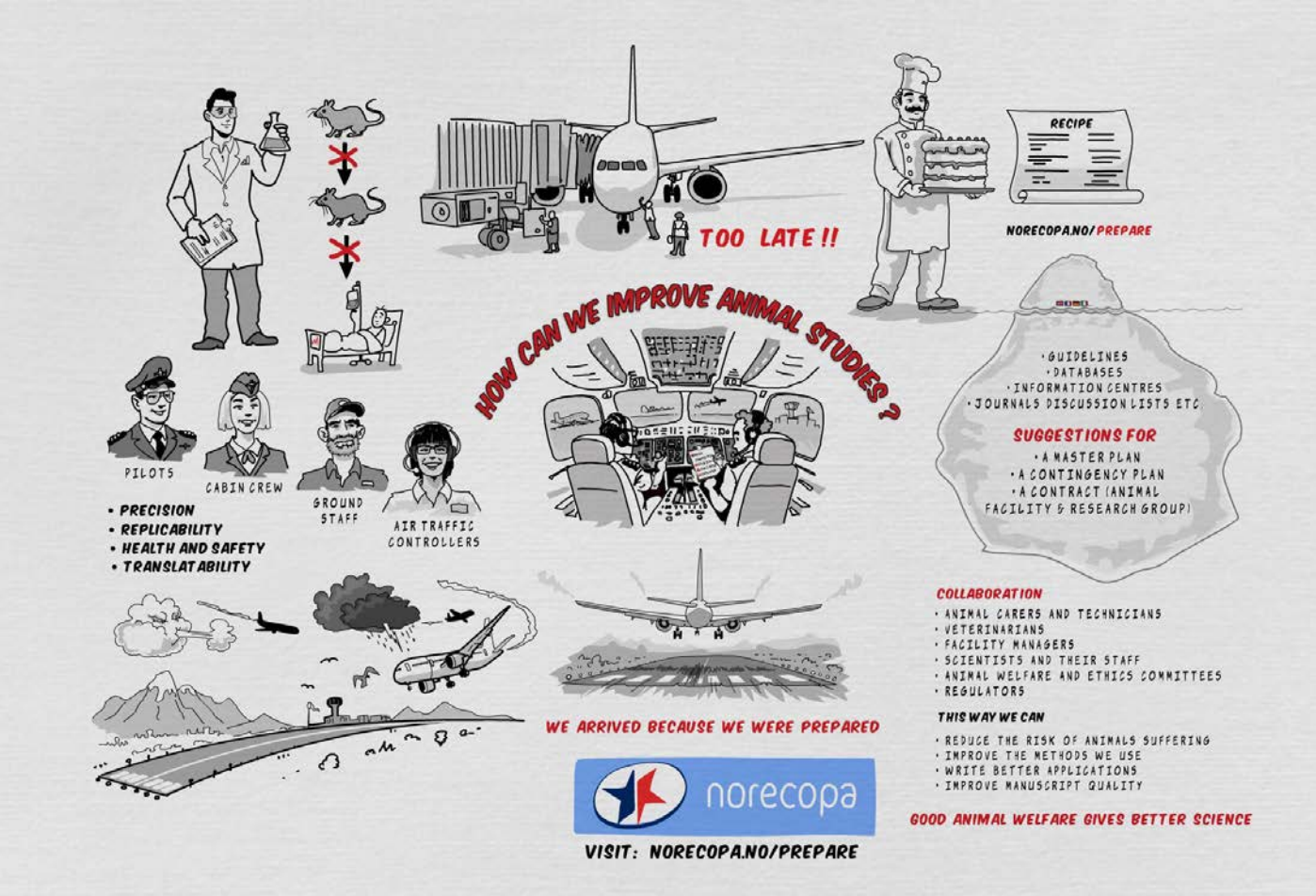
norecopa.no/PREPARE

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


norecopa
"We ARRIVED, because we were PREPARED"
Better Science
Improved animal welfare
Advancement of the 3Rs
Safer working environment
Norecopa: PREPARE for better Science

vimeo.com/358069203 or norecopa.no/PREPARE

3-minute cartoon film



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- Standing Committee on Business Affairs, Norwegian Parliament
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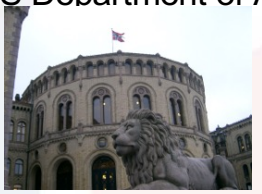
- Research Council of Norway
- Laboratory Animals Ltd.
- Architect Finn Rahns Legacy
- Nordic Society Against Painful Experiments (NSMSD)
- Norwegian Society for Animal Protection
- Animal Welfare Fund (Dyrevernfondet)
- Novo Nordisk
- Scottish Accreditation Board
- Stiansen Foundation
- Universities Federation for Animal Welfare (UFAW)
- US Department of Agriculture



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