The Path to Better Science: 3Rs and more

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norecopa.no/path

”...better science and more than 3Rs?"

- replacement if possible
- reduction and refinement if not possible to replace
- valid data (a true treatment effect)
- reproducible and translatable experiments
- best possible animal welfare
- health & safety (of animals and people)
- a culture of care at the animal facility
- communication of best practice to others

colourbox.com

animal welfarists

scientists

animal care staff

lab animal veterinarians

regulators

Harm-Benefit analyses

European Consensus-Platform for Alternatives

ecopa.eu

- Established in 2000
- Recognises National Consensus Platforms (NCPs) with 4 stakeholders equally represented:

Heard in 2023:

“Scientists don’t use alternatives because they are ignorant of them”

“The concept of improving animal research is obstructive. There is very little evidence that improving scientific rigor will improve translation.”

“The 3Rs are an impediment to progress...there is so little emphasis on replacement and reduction.”

“The perception that animal research is the gold standard....”

WC12Canada @WC12Canada · Jul 28

Did you know? The World Congress on Alternatives and Animal Use in the Life Sciences was established by the Alternatives Congress Trust in 1993 to bring together academia, industry, government, and non-profit sectors to advance the 3Rs.

#WC12niagarafalls #nonanimalmethods

We cannot improve our research by

better reporting alone...

reddit.com

reddit.com

about Science: ***'There is no point in even starting to do it, if you intend to do something which is sloppy, or deliberately misleading', and yet we know that that happens'.***

https://www.youtube.com/watch?v=KSOke8tImbc

Publish or perish

My project has been funded, and the method has been published before, so why should I change the protocol?


No thanks!

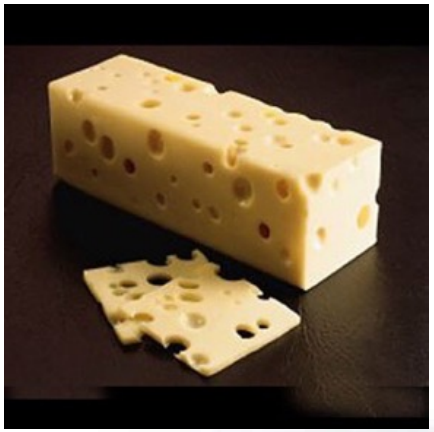

No thanks!
We are too busy

The pathway to better science

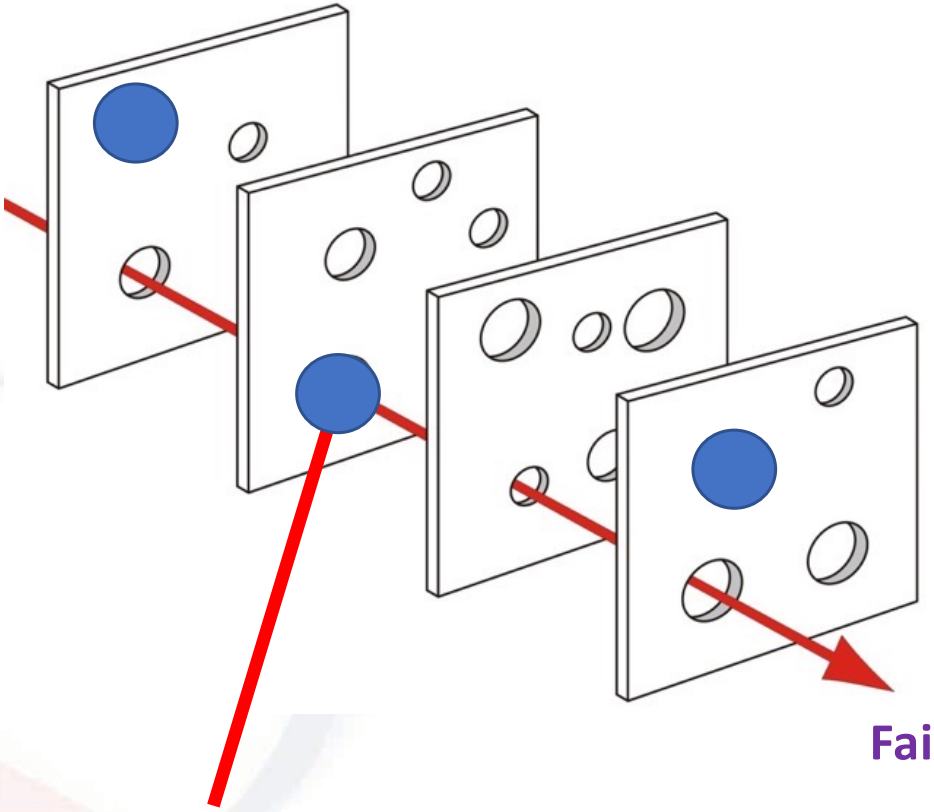
Norecopa: PREPARE for better Science

norecopa.no/PREPARE *and*
ivd-utrecht.nl/en/news/better-animal-research-through-open-science-1

Threat and Error Management



eaugallecheese.com/Swiss-Cheese



wikipedia.org/wiki/Swiss_cheese_model

Dagbladet.no Publisert fredag 23.04.2010 kl. 18:26

Solveig (38) forsket på kreft, ble selv uhelbredelig syk

Slår alarm om arbeidsforholdene på Radiumhospitalet. Sykehuset innrømmer rutinesvikt.


ASLE HANSEN
ash@dagbladet.no


DIANA BADI
dba@dagbladet.no

HELSEFARLIG ARBEIDSMILJØ: Solveig Garman-Vik (38) har fått diagnosen akutt myelogen leukemi (AML) etter å ha jobbet med kreftforskning på Radiumhospitalet i elleve år. Her får hun en klem av sykepleier Elisabeth A. Saghaug før hun går hjem for helgen. Få med hvor fantastiske alle her på Lovisenberg er mot meg, sier Solveig. Foto: LARS EIVIND BONES/DAGBLADET

Culture of Care facilitates honest discussion along the path

"because we've always done it that way"

"as often as necessary"

"there are no alternatives"

Closely related to a culture of care is

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

Look for the acceptable, rather than choosing the accepted.

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


Map
Satellite
Greenland
Iceland
Finland
Sweden
Norway
Russia
United Kingdom
Poland
Germany
Ukraine
Kazakhstan
Mongolia
France
Italy
Spain
Turkey
China
Japan
South Korea
Canada
United States
North Atlantic Ocean
Iraq
Iran
Afghanistan
Pakistan
Algeria
Libya
Egypt
Saudi Arabia
India
Thailand
Mexico
Mali
Niger
Sudan
Chad
Nigeria
Ethiopia
Venezuela
Colombia
Kenya
DRC
Tanzania
Indonesia
Papua New Guinea
Brazil
Peru
Angola
Bolivia
Namibia
Botswana
Madagascar
Indian Ocean
South Pacific Ocean
South Atlantic Ocean
Chile
Australia
South Africa
Argentina
New Zealand
norecopa.no/coc
Culture of Care Network
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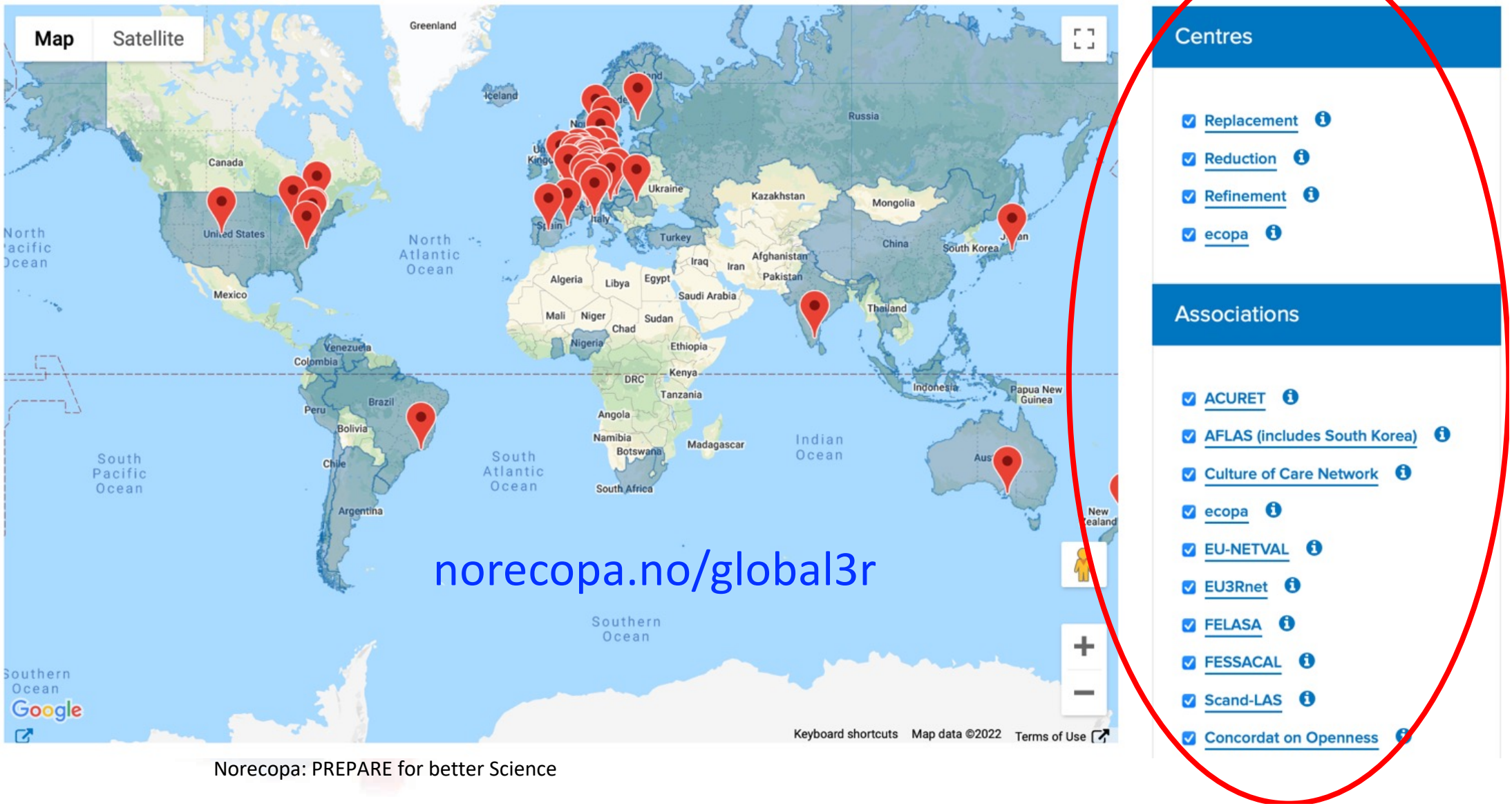


Centres

- Replacement
- Reduction
- Refinement
- ecopa

Associations

- ACURET
- AFLAS (includes South Korea)
- Culture of Care Network
- ecopa
- EU-NETVAL
- EU3Rnet
- FELASA
- FESSACAL
- Scand-LAS
- Concordat on Openness

Norecopa: PREPARE for better Science

Program Description

A. **Animal Care and Use Program**

B. **Animal environment, Housing and Management**

C. **Veterinary Care**

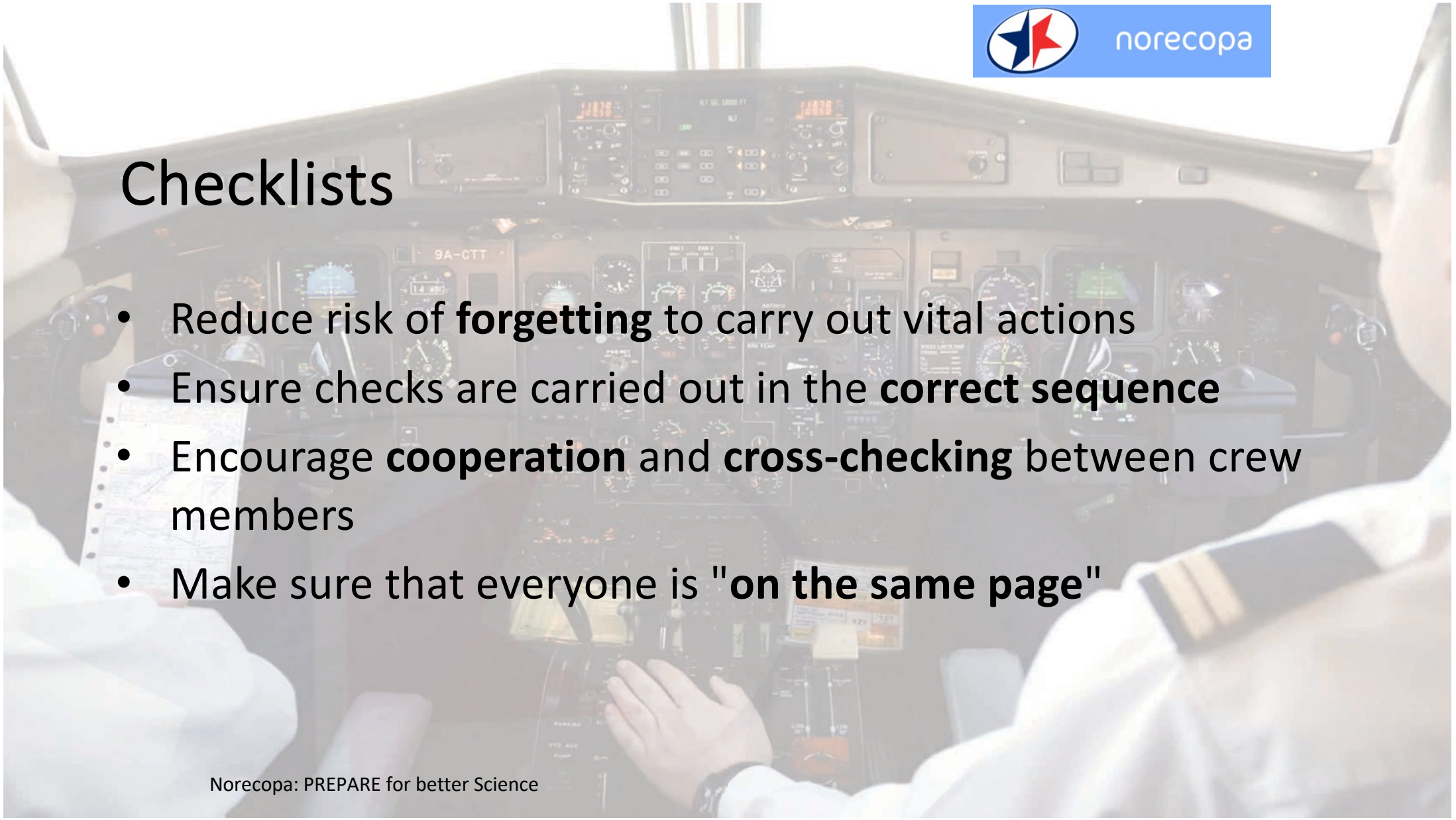
D. **Physical plant**

63 pages

norecopa.no/prepare/6-facility-evaluation/6a/general-principles

Precision and replicability in the aviation industry:

10-15 checklists even on short routine flights



Checklists

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

norecopa

Surgical Safety Checklist

World Health Organization | Patient Safety
A World Alliance for Safer Health Care

Before induction of anaesthesia
(with at least nurse and anaesthetist)

Has the patient confirmed his/her identity, site, procedure, and consent?
- ☐ Yes

Is the site marked?
- ☐ Yes
- ☐ Not applicable

Is the anaesthesia machine and medication check complete?
- ☐ Yes

Is the pulse oximeter on the patient and functioning?
- ☐ Yes

Does the patient have a:

Known allergy?
- ☐ No
- ☐ Yes

Difficult airway or aspiration risk?
- ☐ No
- ☐ Yes, and equipment/assistance available

Risk of >500ml blood loss (7ml/kg in children)?
- ☐ No
- ☐ Yes, and two IVs/central access and fluids planned

Before skin incision
(with nurse, anaesthetist and surgeon)

- ☐ **Confirm all team members have introduced themselves by name and role.**
- ☐ **Confirm the patient's name, procedure, and where the incision will be made.**

Has antibiotic prophylaxis been given within the last 60 minutes?
- ☐ Yes
- ☐ Not applicable

Anticipated Critical Events

To Surgeon:
- ☐ What are the critical or non-routine steps?
- ☐ How long will the case take?
- ☐ What is the anticipated blood loss?

To Anaesthetist:
- ☐ Are there any patient-specific concerns?

To Nursing Team:
- ☐ Has sterility (including indicator results) been confirmed?
- ☐ Are there equipment issues or any concerns?

Is essential imaging displayed?
- ☐ Yes
- ☐ Not applicable

Before patient leaves operating room
(with nurse, anaesthetist and surgeon)

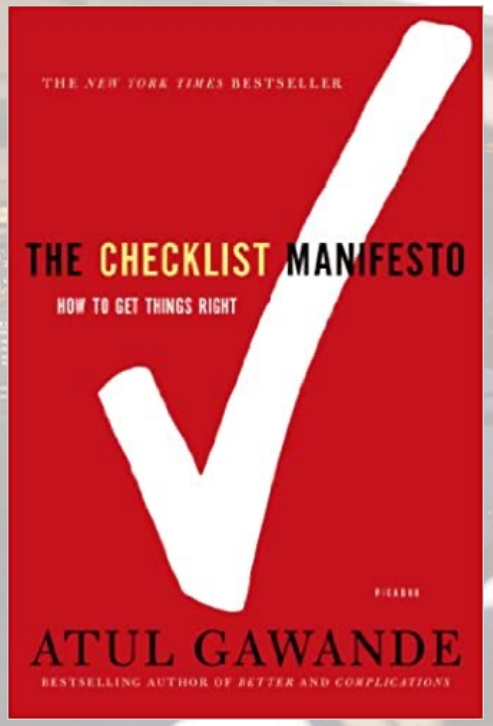
Nurse Verbally Confirms:
- ☐ The name of the procedure
- ☐ Completion of instrument, sponge and needle counts
- ☐ Specimen labelling (read specimen labels aloud, including patient name)
- ☐ Whether there are any equipment problems to be addressed

To Surgeon, Anaesthetist and Nurse:
- ☐ What are the key concerns for recovery and management of this patient?

This checklist is not intended to be comprehensive. Additions and modifications to fit local practice are encouraged.

Revised 1 / 2009

who.int/patientsafety/topics/safe-surgery/checklist/en



amazon.com/gp/product/0312430000

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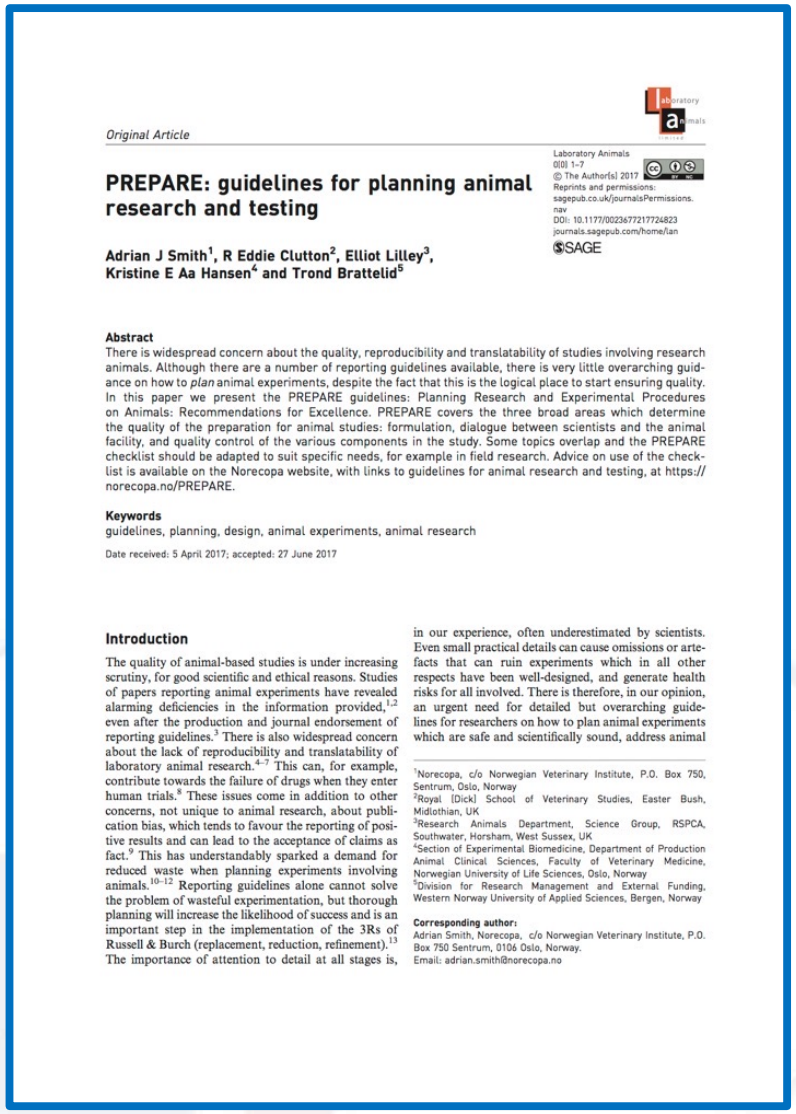

Original Article

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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Norecopa: PREPARE for better Science

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https://doi.org/10.1177/0023677217724823

Over 31,000 downloads from the journal website so far

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 typically highlighted in reporting guidelines

norecopa.no/PREPARE/prepare-checklist

PREPARE

norecopa

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]

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PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines [2].
PREPARE covers the three broad areas which determine the quality of [illegible]:

1. Formulation of the study
2. Dialogue between [illegible]

[illegible] will evolve as more species- and situation-specific guidelines are produced, [illegible] guidelines is available on the Norecopa [illegible] 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.

Animal welfare and all 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.
	[illegible]ection of the facilities, to evaluate building and equipment standards and needs. [illegible]t times of extra risk.
	[illegible]etence of staff members and the need for further education or training prior
8. Health 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

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

norecopa.no/PREPARE

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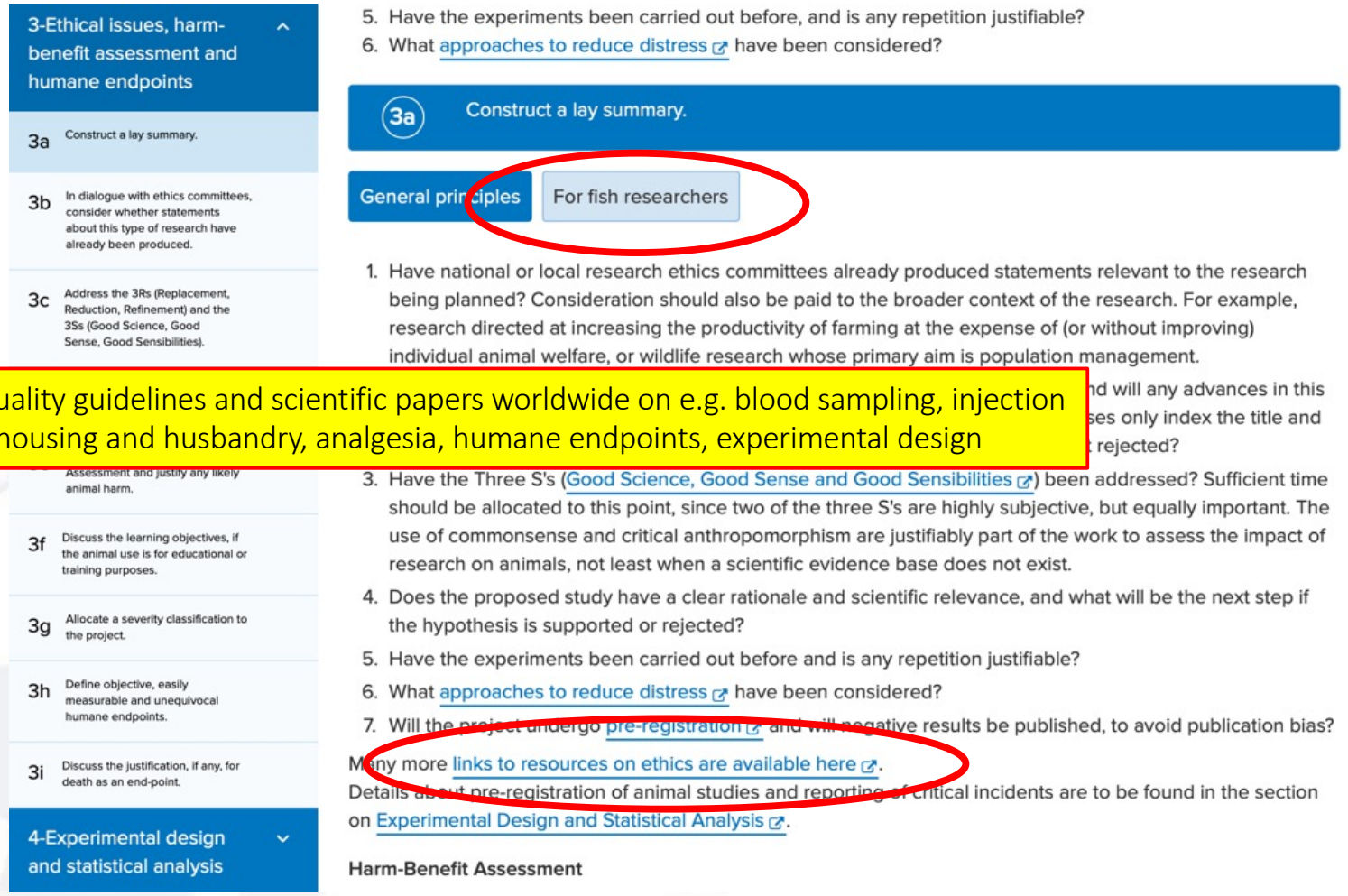

Links to quality guidelines and scientific papers worldwide on e.g. blood sampling, injection volumes, housing and husbandry, analgesia, humane endpoints, experimental design

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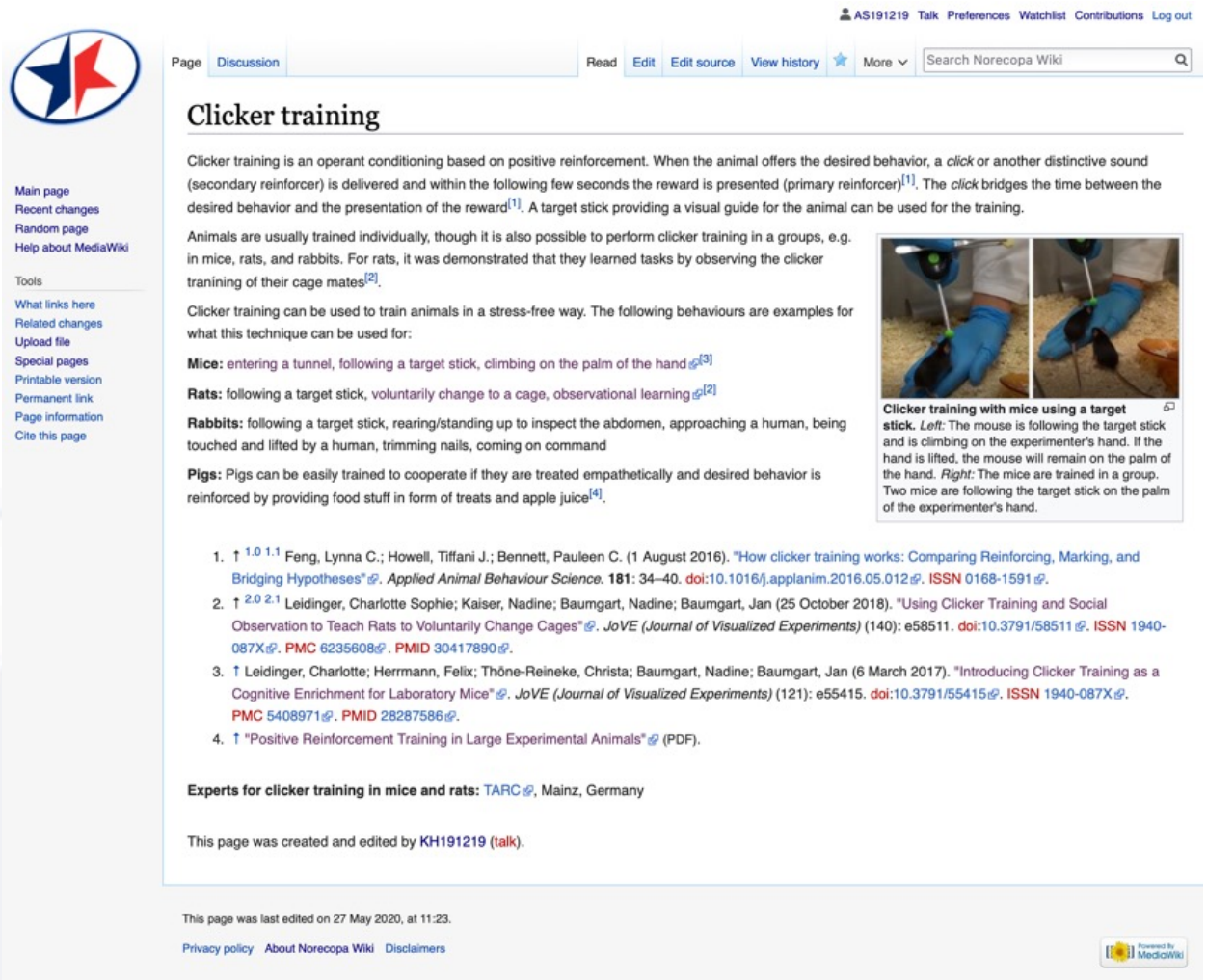
Refinement Wiki: wiki.norecopa.no



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Clicker training

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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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This page was last edited on 27 May 2020, at 11:23.

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Pages created (August 2023)

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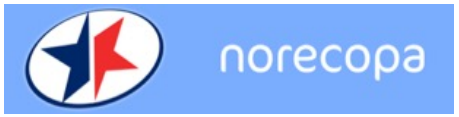

- Acclimatisation
- Adrian Smith
- Alphaxalone
- Anaesthesia in neonates
- Analgesia
- Asepsis
- Blood sampling of hamsters
- Blood sampling of pigs
- Blood sampling of rainbow trout
- Breeding strategies for mice
- Clicker training
- Contingency plans
- Decapitation
- Detecting early onset of clinical signs in the mouse model of Covid-19
- Detection of pain and distress in mice
- EMLA cream
- Embryo transfer
- Experimental Autoimmune Encephalomyeltis (EAE)
- Facial expression analysis
- Food crunchers
- General discusson on use of analgesics
- Genotyping mice
- Habituation training
- High-fat diets
- Hot Bead Sterilisers
- Housing nude mice
- Housing research fish
- Humane endpoints
- Hydrodynamic gene delivery
- Intra-ocular injections
- Intranasal administration
- Intraperitoneal injection
- Intraperitoneal pentobarbitone
- Ketamine and alpha-2 agonist combinations
- Long-term anaesthesia in rodents
- Lumpfish
- Main Page
- Marble Burying Test
- Metabolic cages
- Minipumps
- Montanide adjuvant
- Mouse Grimace Scale
- Mouse handling
- Nest building material
- Oestrus suppression in ferrets
- Pneumocystis murina
- Recapping needles
- Rotarod Test
- Screening cell lines
- Sedation of cattle
- Splenectomy
- Sterilisation of instruments
- TTEAM and TTouch
- Tail vein injection
- Tramadol
- Transport stress
- Tumour cell implant into mammary fat pad
- Ulcerative Dermatitis in Mice
- Water quality
- Xenopus laevis
- Zebrafish swabbing

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norecopa.no/PREPARE *and*
ivd-utrecht.nl/en/news/better-animal-research-through-open-science-1

arriveguidelines.org

The ARRIVE guidelines 2.0

This section of the website provides detailed explanations about each item of the guidelines. Use the left-hand side menu to navigate to each item.

To facilitate a step-wise approach to improving reporting, the guidelines are organised into two prioritised sets:

ARRIVE Essential 10

These ten items are the basic minimum that must be included in any manuscript describing animal research. Without this information readers and reviewers cannot assess the reliability of the findings.

Recommended Set

These items complement the Essential 10 set and add important context to the study described. Reporting the items in both sets represents best practice.

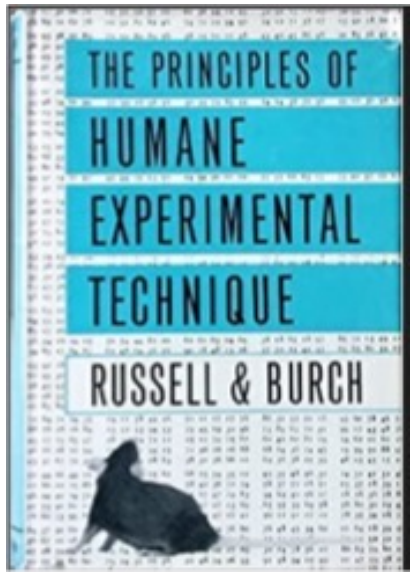
Norecopa: PREPARE for better Science

Poor reporting

Russell & Burch quote Visscher (1951):

"In general, methodology is usually relegated to a place of smaller type and sharply abbreviated importance in journal publication of research.

Numerous essential details are customarily omitted, either because they are considered to be common knowledge, or simply for lack of space."



Russell WMS & Burch RL (1959)

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"We ARRIVED, because we were PREPARED"

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

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More than 3Rs

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

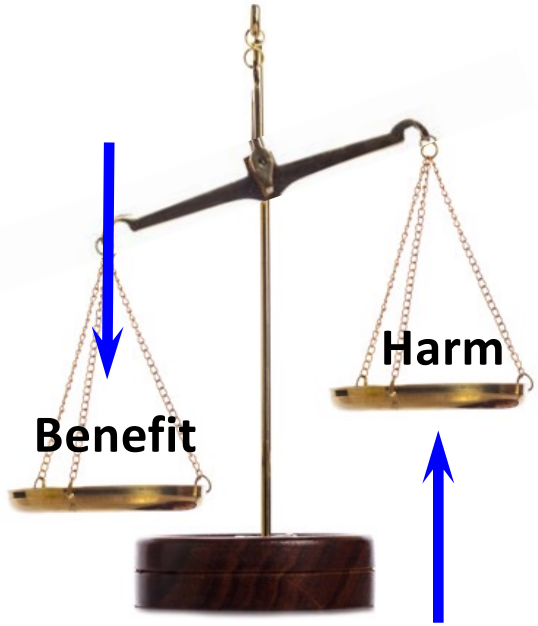
- *Replace the unnecessary experiments*
- *Reduce the number of animals used*
- *Refine the conditions for the animals*

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

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

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

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



norecopa.no/3R
norecopa.no/3S
norecopa.no/3V

Additional Rs have been proposed...

... but many of these concepts are actually explicitly or implicitly discussed by Russell & Burch:

- **Reproducibility** and **Replicability** of animal experiments
- **Responsibility** when planning and conducting procedures on sentient animals
  - toward the animals
  - towards our colleagues (Culture of Care)

- A 6R concept: 3Rs + **Robustness**, **Registration** & **Reporting** (Strech & Dirnagl, 2019)

Responsible animal research: a riff of Rs. Rowan A & Goldberg A (1995), *Altern Lab Anim*. 23(3):306-11. doi.org/10.1177/026119299502300307

Aryelle Canedo Pereira *et al*. (2022)

Zebrafish (*Danio rerio*) meets bioethics: the 10Rs ethical principles in research

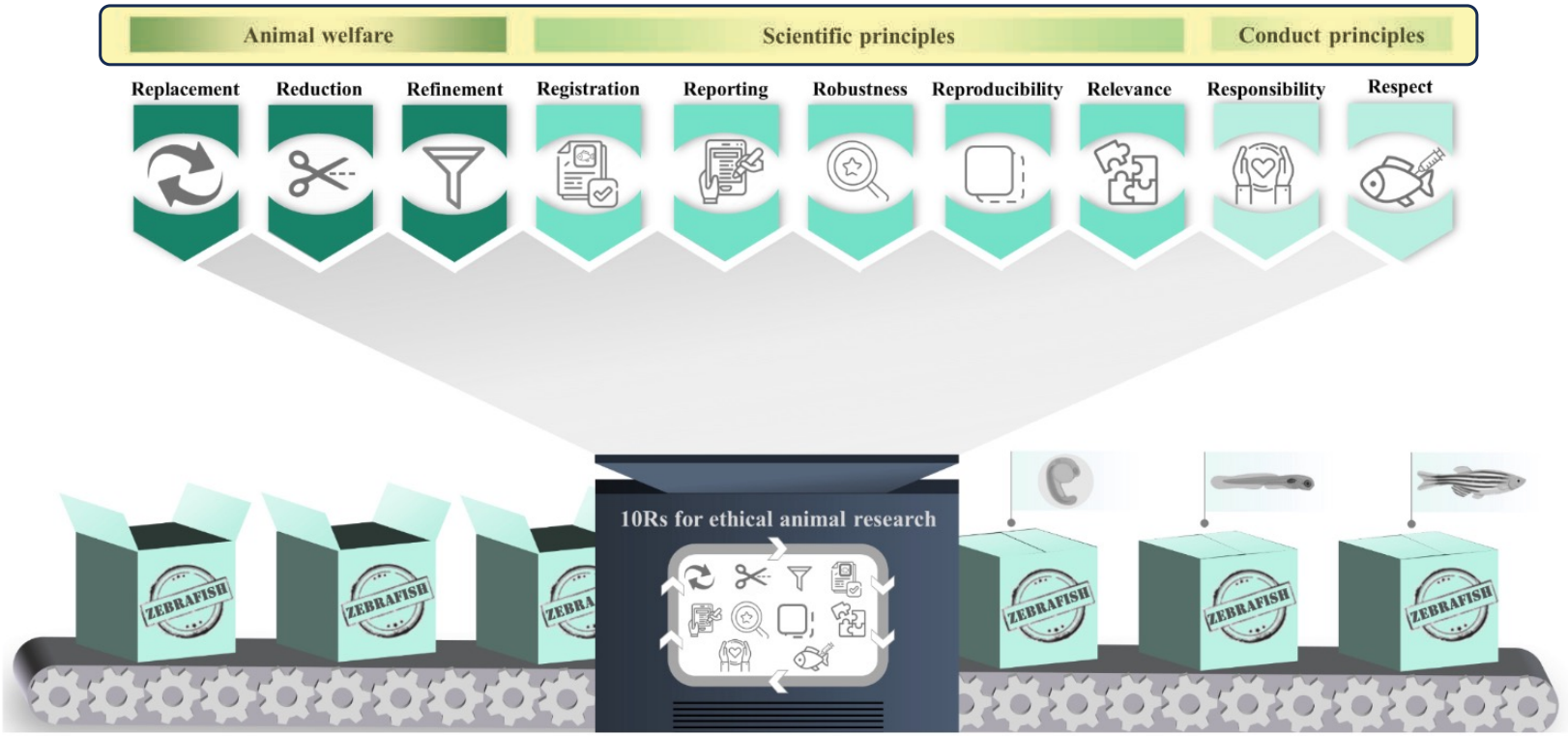
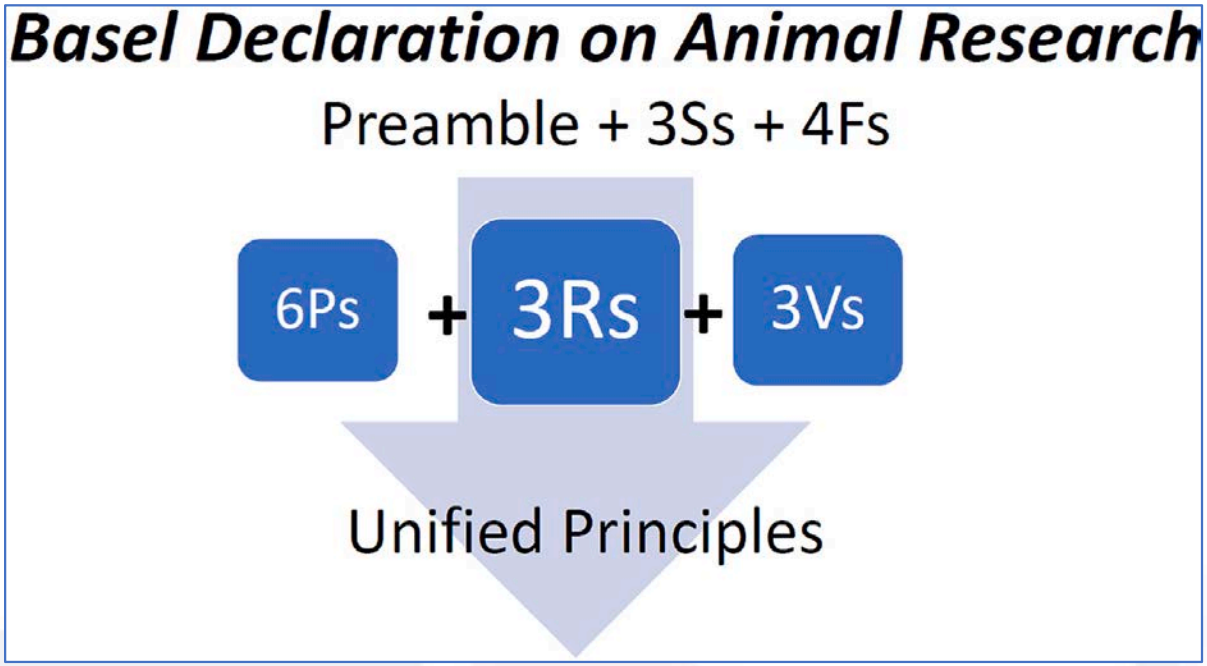


Figure 2. The 10 Rs ethical principles for zebrafish using as model system in research. The principles were classified into three categories, namely: welfare (replacement, reduction and refinement), scientific (registration, reporting, robustness, reproducibility and relevance) and conduct principles (responsibility and respect).

www.scielo.br/j/cab/a/HsNhVXVRCSHTdYmLtqXfv5J/?lang=en

Christopher Petkov *et al*. (2022) propose an animal research 'Helsinki Declaration'



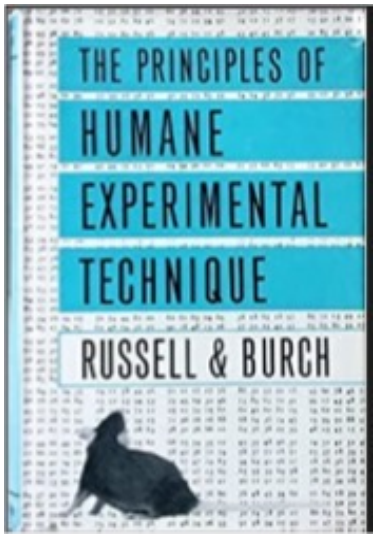
4Fs: Fundamental principles (Tannenbaum, 2017)

6Ps: Principles of research (Beauchamp & DeGrazia, 2019)

3Vs: Würbel

sciencedirect.com/science/article/pii/S2665945X2200033X

Russell & Burch's book is much more than the 3Rs...



Russell WMS & Burch RL (1959)

- The status, training and importance of animal care staff (even when they do not carry out procedures)
- Humanity is linked to experimental success ("efficiency")
- The "triumph of husbandry": more uniform and healthier animals. Emphasis first on good husbandry and then on good experimental technique. Crowding leads to disease outbreaks and poor reproduction.
- Well-being, not just an absence of distress: *Mens sana in corpore sano*
- The high fidelity fallacy – more important to have good correlation (even if the mechanism is not understood)
- The correct number of control animals
- Use a greater number of species – ones that are better suited for the model. Recruit species that have been better studied, or increase studies on the species being used
- 'Violence is the last refuge of the incompetent' (Asimov, 1953): use punishment-free models, even in fear research.

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- Norwegian Animal Protection Alliance (Dyrevernalliansen)
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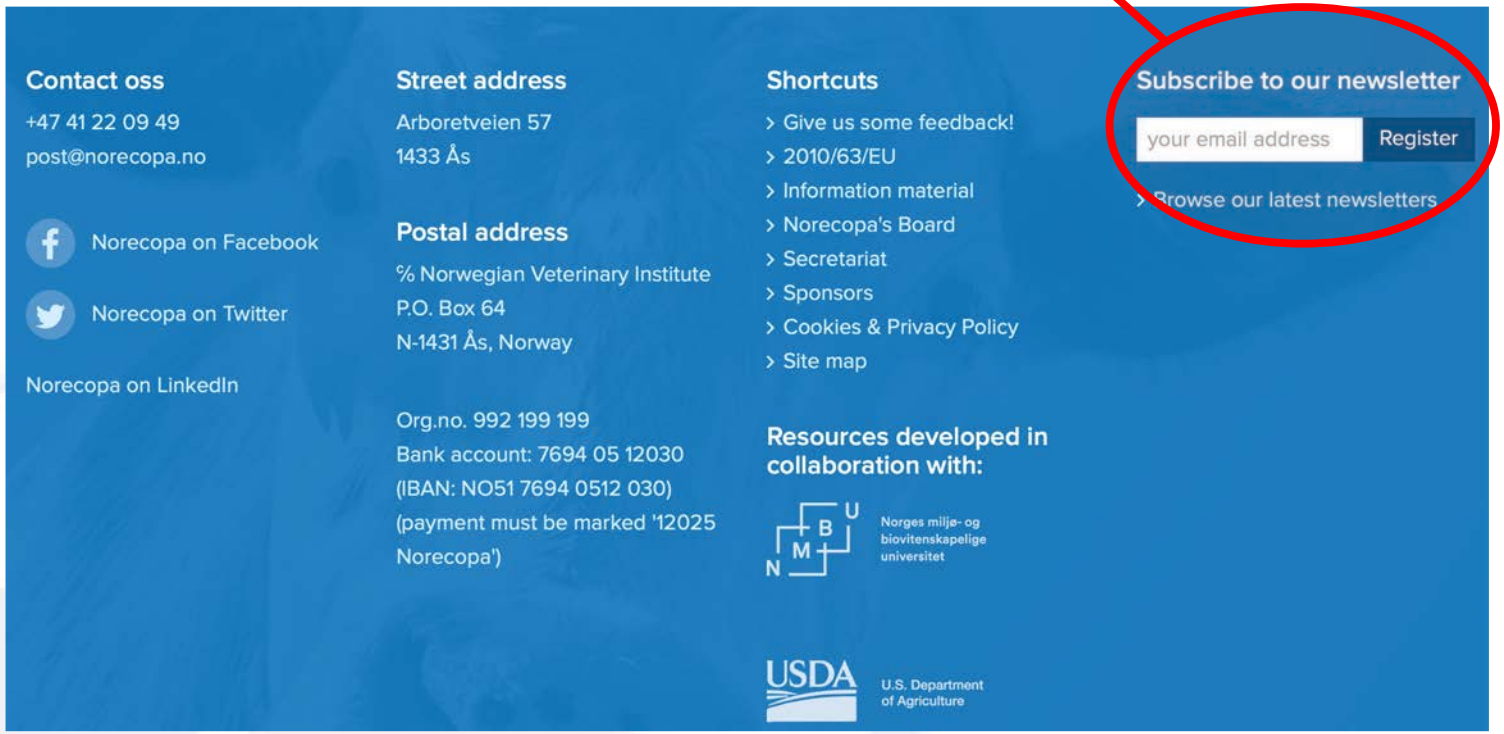

Thank you for listening!

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