Resources for AWERBs on assessing replacement available on the Norecopa website

Adrian Smith

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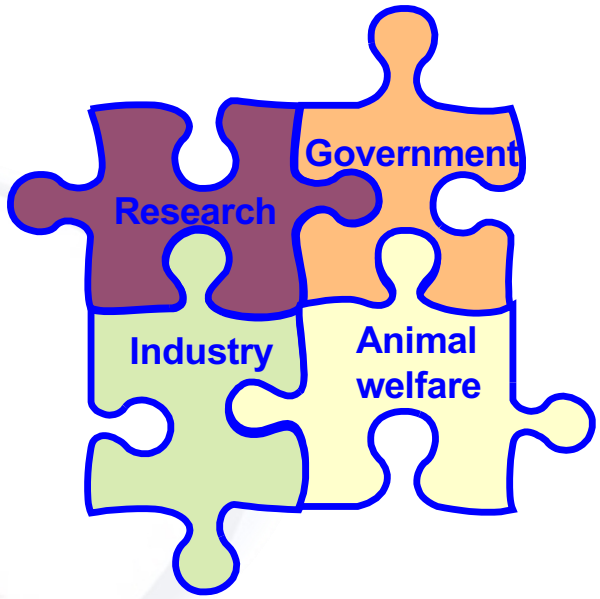
@adrian_3r

norecopa.no/ASC

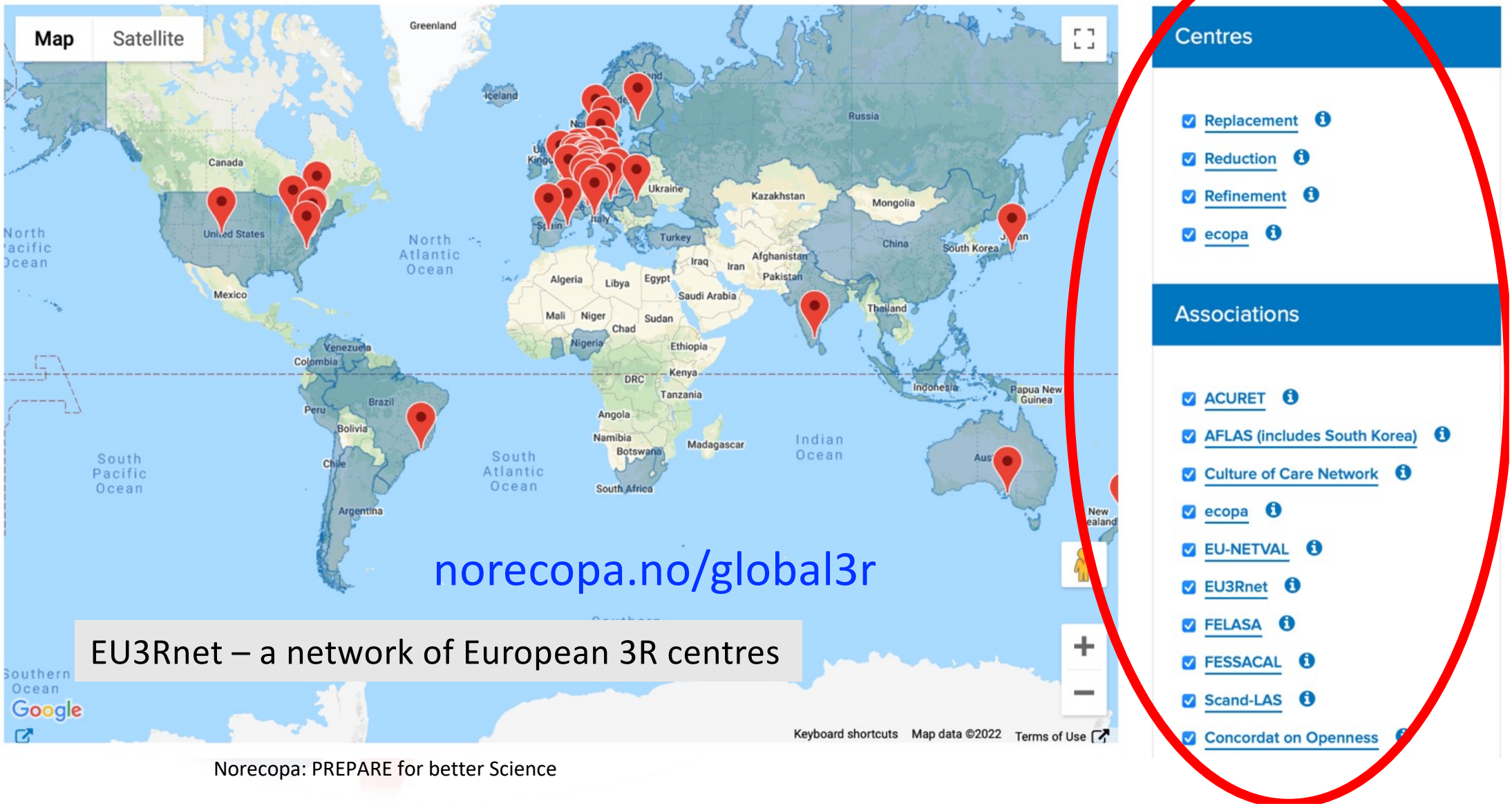
Norecopa is a National Consensus Platform for the 3Rs:
Replacement*, *Reduction *and* ***Refinement*** *of animal experiments*

A member of ***ecopa****:*
European Consensus-Platform for Alternatives
which recognises National Consensus Platforms with
4 stakeholders equally represented:

ecopa.eu



Norecopa: PREPARE for better Science



norecopa.no/global3r

EU3Rnet – a network of European 3R centres

"the most comprehensive, up-to-date, website for global 3R resources"

over 10,000 webpages
approx. 1,000 hits per day

7-8 detailed newsletters per year

norecopa.no

April 2025

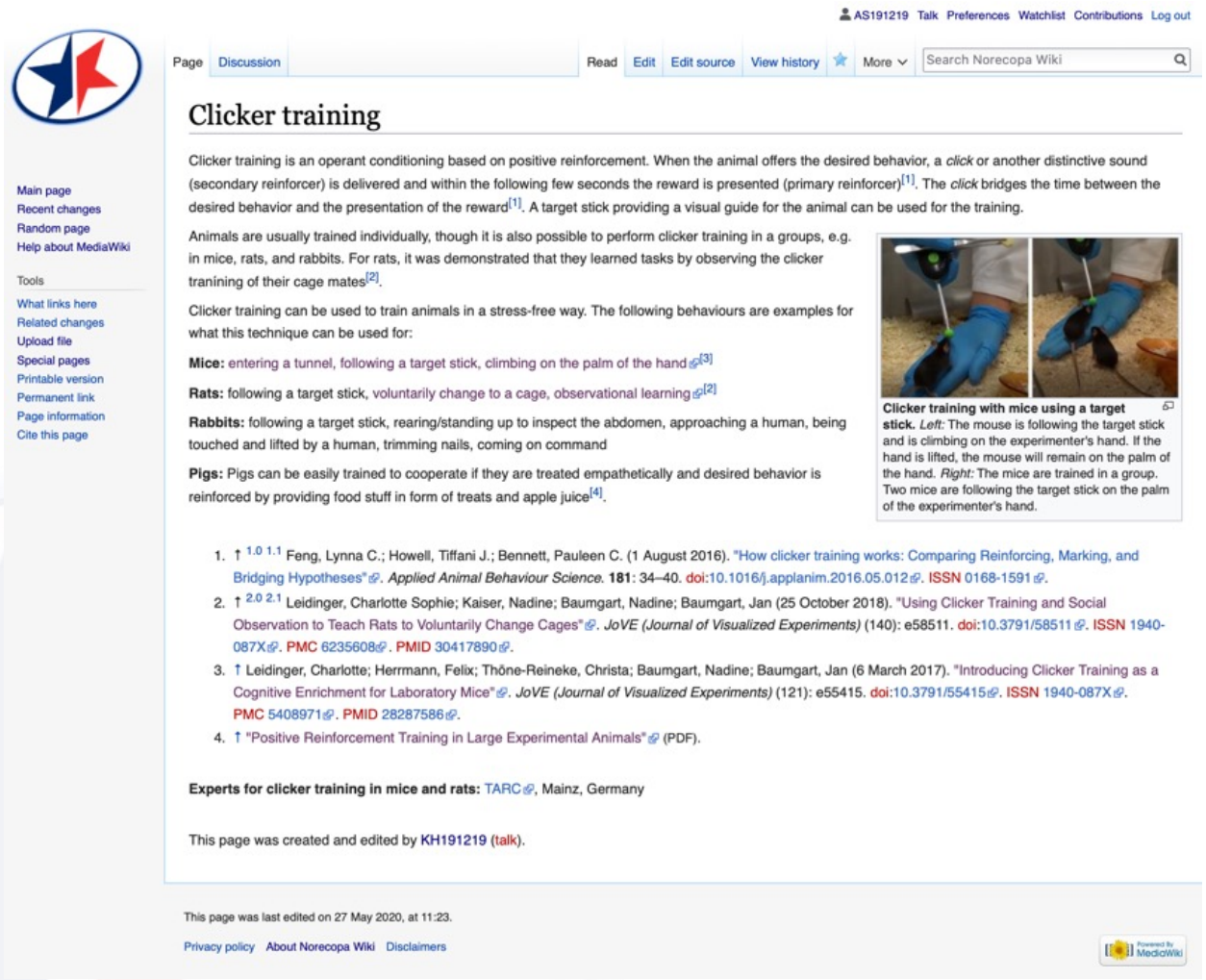
- *Ex vivo, de novo & in silico* models in biomedical research , Stuttgart, 1-2 April 2025
- EBVS Congress, Belgrade, 2-4 April 2025
- The Transparent Transition - The future of animal and animal-free research, Amsterdam, 3 April 2025
- From crisis to opportunity - systematic heterogenization as a tool to improve reproducibility and reduce animal use, webinar (Helene Richter), 8 April 2025
- 1st Finnish Culture of Care Symposium, H[illegible]
- Antibodies and Beyond: The Power of Anim
- Wenzel), 9 April 2025
- Meeting the Requirements of the US Animal Welfare Act, workshop, 9-10 April 2025
- 17th Minipig Research Forum, Amsterdam, 9-11 April 2025
- Care-Full Stories workshop, Helsinki, 10 April 2025
- Lussier), 10 April 2025
- Stress-reduced handling of rats and mice, webinar (Therése Ahlström), 11 April 2025
- 41st LAMA/ATA Annual Conference, Fort Walton Beach, 14-17 April 2025
- Environmental Monitoring & Database Management, webinar (Zoltan Varga & TBC), 18 April 2025
- Course in Fish Diseases part 1, Copenhagen, 21-25 April 2025
- 3Rs Sharing Conference, Seattle, 23 April 2025
- 46th Annual BCLAS Symposium: Stress and emotions in animals, Namur, 23-24 April 2025
- Replication of null results: Absence of evidence or evidence of absence?, webinar (Samuel Pawel), 29 April 2025
- All you ever wanted to know about registered reports, webinar (Nonia Pariente), 30 April 2025

norecopa.no/meetings/meetings-calendar

+ webpages for recorded meetings, including a page sorted by the PREPARE topics

The Refinement Wiki

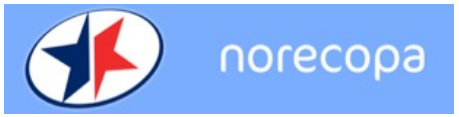
wiki.norecopa.no



Norecopa: PREPARE for better Science

Pages created (March 2025)

wiki.norecopa.no



- 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
- Dehydration
- Detecting early onset of clini… 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
- Forced swim test
- General discusson on use of analgesics
- Genotyping mice
- Habituation training
- Health monitoring
- High-fat diets
- Hot Bead Sterilisers
- Housing nude mice
- Housing research fish
- Humane endpoints
- Hydrodynamic gene delivery
- Intra-ocular injections
- Intranas…
- …arbitone
- …ilation for haematology studies
- Ketamine and alpha-2 agonist combinations
- Long-term anaesthesia in rodents
- Lumpfish
- MDA (micropipette-guided drug administration) Method
- Main Page
- Marble Burying Test
- Metabolic cages
- Microchipping rats and mice
- Minipumps
- Montanide adjuvant
- Mouse Grimace Scale
- Mouse handling
- Nest building material
- Non-invasive genetic sampling in wildlife research
- Oestrus suppression in ferrets
- Pneumocystis murina
- Recapping needles
- …of oral gavage
- …t
- …g cell lines
- Sedation of cattle
- Splenectomy
- Sterilisation of instruments
- TTEAM and TTouch
- Tail vein injection
- Tamoxifen
- Tamoxifen information sheet V4.pdf
- The use of DMSO
- Tramadol
- Transport stress
- Tumour cell implant into mammary fat pad
- Ulcerative Dermatitis in Mice
- Water quality
- Xenopus laevis
- Zebrafish swabbing

Do we also need a Replacement Wiki?

Norecopa: PREPARE for better Science

Guidelines for planning studies that look as if they may involve animal use

norecopa

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: PREPARE for better Science

PREPARE:

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6. Facility evaluation
7. Education and training
8. Health risks, waste disposal and decontamination

Replacement should be considered here

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

ARRIVE study plan

- Study details
- Experimental animals
- Experimental procedures
  What is done and how is it done, when and how often.
- Animal care and monitoring
- Risks
- Personnel involved in the experiment
- Study design and sample size
- Inclusion and exclusion criteria
- Randomisation and blinding/masking
- Outcome measures and statistical methods

https://arriveguidelines.org/news/arrive-study-plan

Before embarking on research involving the use of animals it is also critical to form a clear hypothesis, identify possible non-animal alternatives to all or part of the proposed study and assess the relevance of the chosen model to answer the experimental question. We therefore encourage researchers to consult the **PREPARE guidelines** before considering the use of animals in research. PREPARE provides researchers with an extensive overview to formulating an experiment and the requirements of using animals before carrying out the research.

https://arriveguidelines.org/news/arrive-study-plan

Norecopa: PREPARE for better Science

Stating the obvious: Replacement must be considered from day 1 of planning

Norecopa: PREPARE for better Science

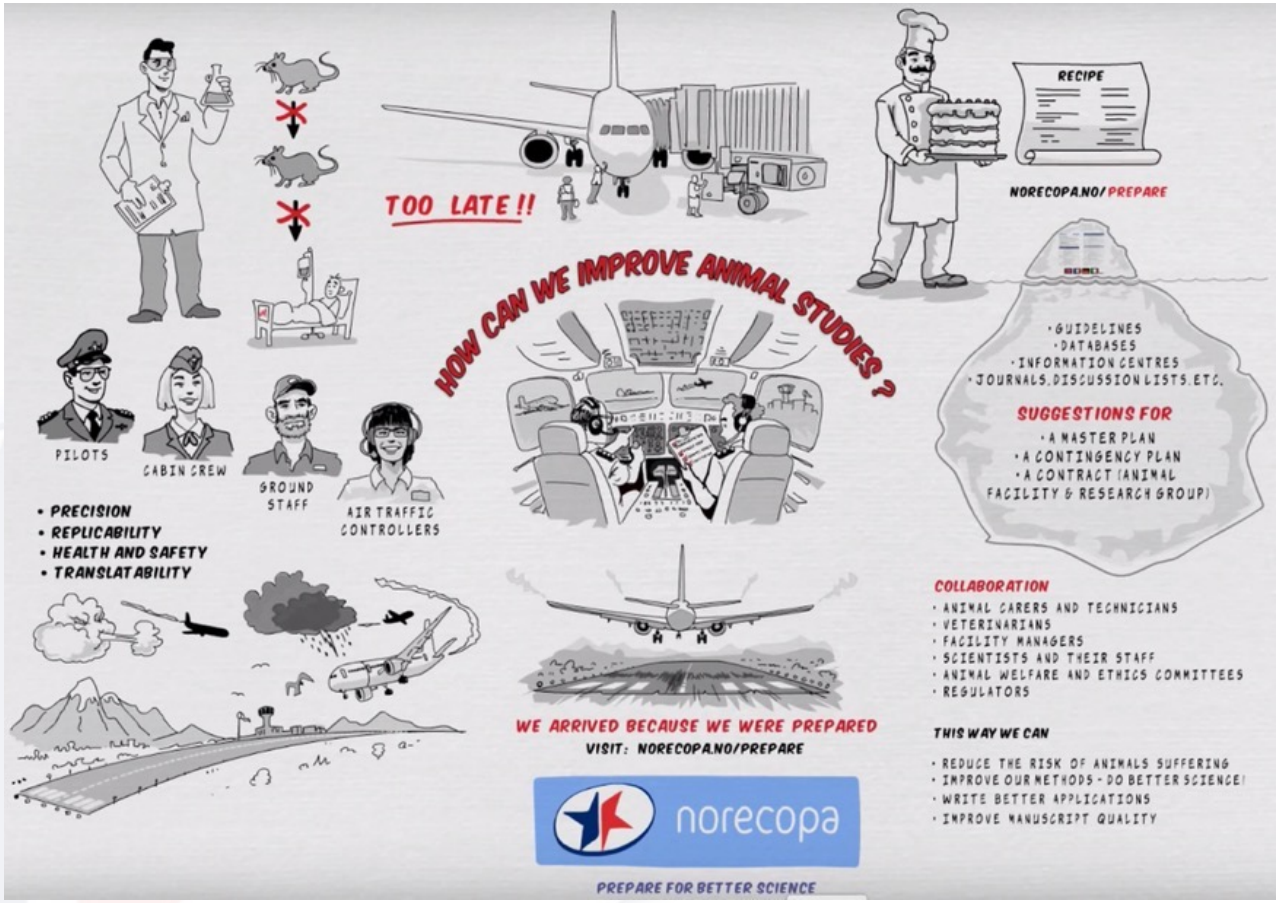
norecopa.no/PREPARE *and* ivd-utrecht.nl/en/news/better-animal-research-through-open-science-1

reddit.com

We cannot improve our research by better reporting alone...

norecopa.no/PREPARE/film

3-minute whiteboard film



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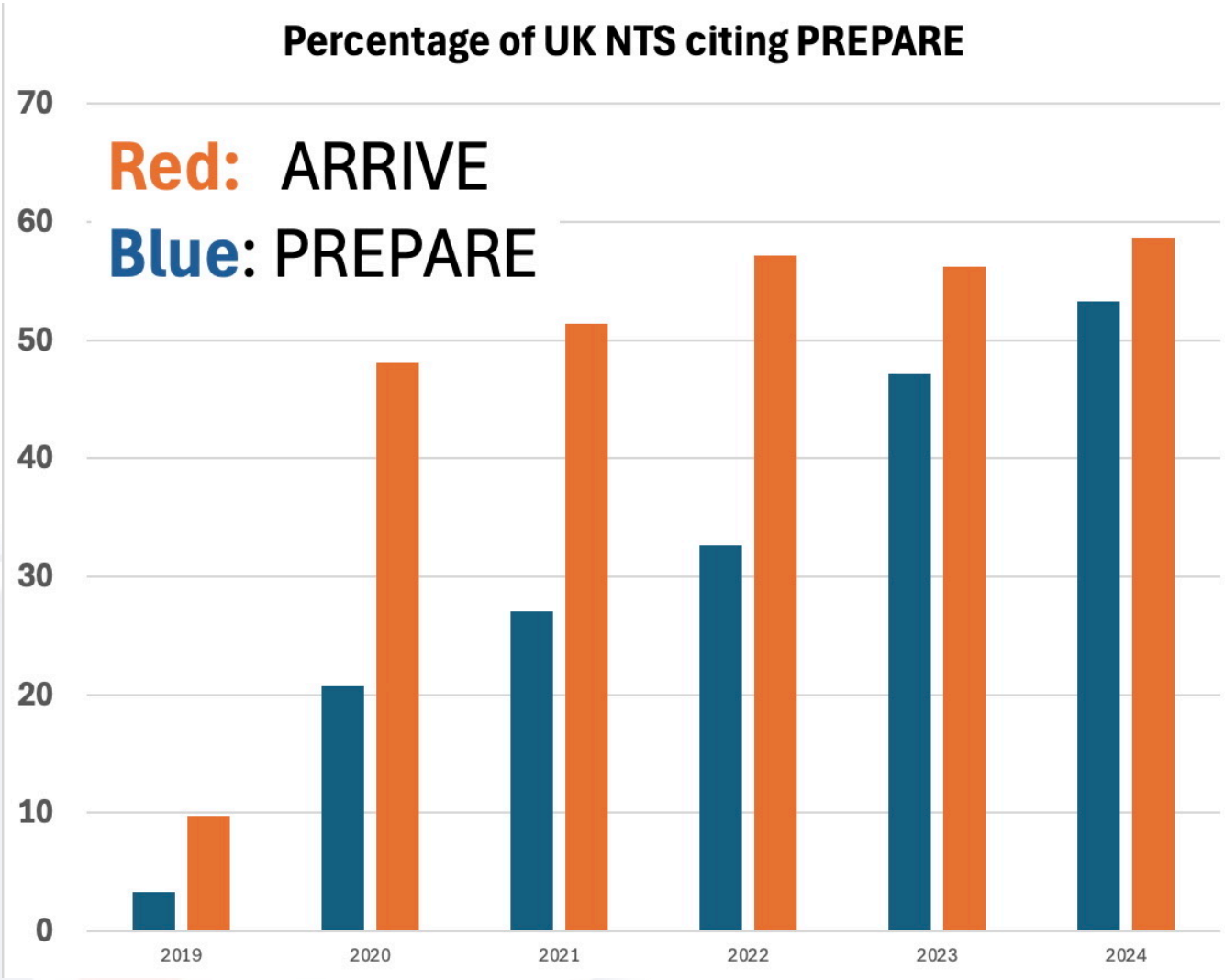

Norecopa: PREPARE for better Science

norecopa.no/PREPARE/prepare-checklist

PREPARE

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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 Brattelid[e]

[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] består av retningslinjer for planlegging av dyreforsøk. Disse komplementerer retningslinjer for rapportering av dyreforsøk, som f.eks. ARRIVE[2]. PREPARE dekker de tre store områdene som bestemmer kvaliteten av arbeidet med å forberede dyreforsøk:

1. **Designet av studiet**
2. **Dialogen mellom forskerne og dyreavdelingen**
3. **Kvalitetskontroll av de ulike komponentene i studiet**

I praksis vil ikke temaene alltid behandles i den rekkefølgen som er presentert her, og enkelte temaer overlapper. PREPARE-sjekklisten kan endres for å ivareta spesielle behov, f.eks. ved feltforsøk. PREPARE inkluderer råd om drift av dyreavdelinger, fordi laboratorieforsøk er helt avhengige av deres kvalitet. Den fulle versjonen av PREPARE er tilgjengelig på Norecopas nettsider, med lenker til globale ressurser, på **https://norecopa.no/PREPARE.**

2. Juridiske spørsmål	☐ Vurdere hvordan forsøket er påvirket av relevant lovgivning for dyreforsøk og andre aktuelle områder som f.eks. dyretransport og helse, miljø og sikkerhet. ☐ Finne relevante veiledningsdokumenter (f.eks. EUs retningslinjer for prosjektevaluering).
3. Etiske spørsmål, kostnad-nytteanalyse og humane endepunkter	☐ Skrive et sammendrag av prosjektet på legmannsspråk. ☐ I dialog med etiske komitéer, vurdere om uttalelser om denne typen forsøk er allerede blitt produsert. ☐ Adressere "de 3 R-ene" (Replacement, Reduction, Refinement) og "de 3 S-ene" (Good Science, Good Sense, Good Sensibilities). ☐ Vurdere forhåndsregistrering av forsøket og publisering av negative resultater. ☐ Foreta en kostnad-nytteanalyse ("Harm-Benefit Assessment") og diskutere eventuelle lidelser som kan oppstå under forsøket. ☐ Diskutere læringsmålene dersom dyrene skal brukes i undervisnings- eller treningsøyemed. ☐ Klassifisere prosjektet etter belastningsgraden. ☐ Definere objektive, lett målbare og utvetydige humane endepunkter. ☐ Diskutere behovet (hvis det er noe) for å bruke død som endepunktet for forsøket.
4. Eksperimentelt design og statistisk analyse	☐ Vurdere pilotforsøk og diskutere statistisk styrke og signifikansnivåer. ☐ Definere den eksperimentelle enheten og bestemme antallet forsøksdyr. ☐ Bestemme metodene for randomisering, forhindre observasjonsskjevheter, og bestemme inklusjons- og eksklusjonskriterier.

Tema	Anbefaling
(B) Dialogen mellom forskerne og dyreavdelingen	
5. Mål og tidshorisont, finansiering og arbeidsfordeling	☐ Arrangere møter med alle relevante personell når tidlige planer for prosjektet foreligger. ☐ Lag en omtrentlig tidsramme for prosjektet, som viser behovene for assistanse med forberedelser, dyrestell, prosedyrer og avfallshåndtering/dekontaminasjon. ☐ Diskutere og legge frem alle forventede og potensielle kostnader. ☐ Lage en detaljert plan for fordelingen av både arbeidsoppgavene og utgiftene, på alle stadiene i forsøket.
6. Evaluering av dyreavdelingen	☐ Foreta en fysisk inspeksjon av fasilitetene, for å evaluere bygningsmassen, standarden på utstyret og spesielle behov. ☐ Diskutere bemanningsbehovet ved perioder med ekstra risiko.
7. Utdanning og trening	☐ Vurdere den nåværende kompetansen hos personalet og evaluere behovet for videreutdanning og trening før forsøket.
8. Helsefarer, avfallshåndtering og dekontaminasjon	☐ I samarbeid med dyreavdelingen, foreta en risikoevaluering som omfatter alle personene og dyrene som er påvirket, direkte eller indirekte, av studiet. ☐ Evaluere, og om nødvendig produsere, spesifikke retningslinjer for alle stadiene av prosjektet. ☐ Diskutere metoder for å ivareta, dekontaminere og avhende alt utstyret som skal brukes i studiet.
12. Oppstalling og stell	☐ Ta hensyn til dyrenes spesifikke instinkter og behov, i samråd med eksperter. ☐ Diskutere akklimatisering, optimale oppstallingsforhold og prosedyrer, miljøfaktorer og eventuelle begrensninger på disse (f.eks. fasting eller oppstalling i enebur).
13. Eksperimentelle prosedyrer	☐ Utvikle optimale metoder for fangst, immobilisering, merking og frisetting eller omplassering. ☐ Utvikle optimale metoder for å gi dyrene behandling, samt for prøvetaking, sedasjon og anestesi, kirurgi og andre inngrep.
14. Human avliving, frisettelse eller omplassering	☐ Konsultere relevant lovgivning og retningslinjer i god tid før studiet. ☐ Definere de primære metodene for avliving, samt metoder som kan brukes i en nødssituasjon. ☐ Evaluere kompetansen til personene som må foreta disse handlingene.
15. Obduksjon	☐ Lage en systematisk plan for alle stadiene i obduksjonen, inkl. hvor den skal foregå, og identifikasjon av alle dyrene og prøvene som tas.

Referanser

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.

Mer informasjon
https://norecopa.no/PREPARE / *post@norecopa.no* / @norecopa

Fillable Word file that can be used to write a **Study Plan**

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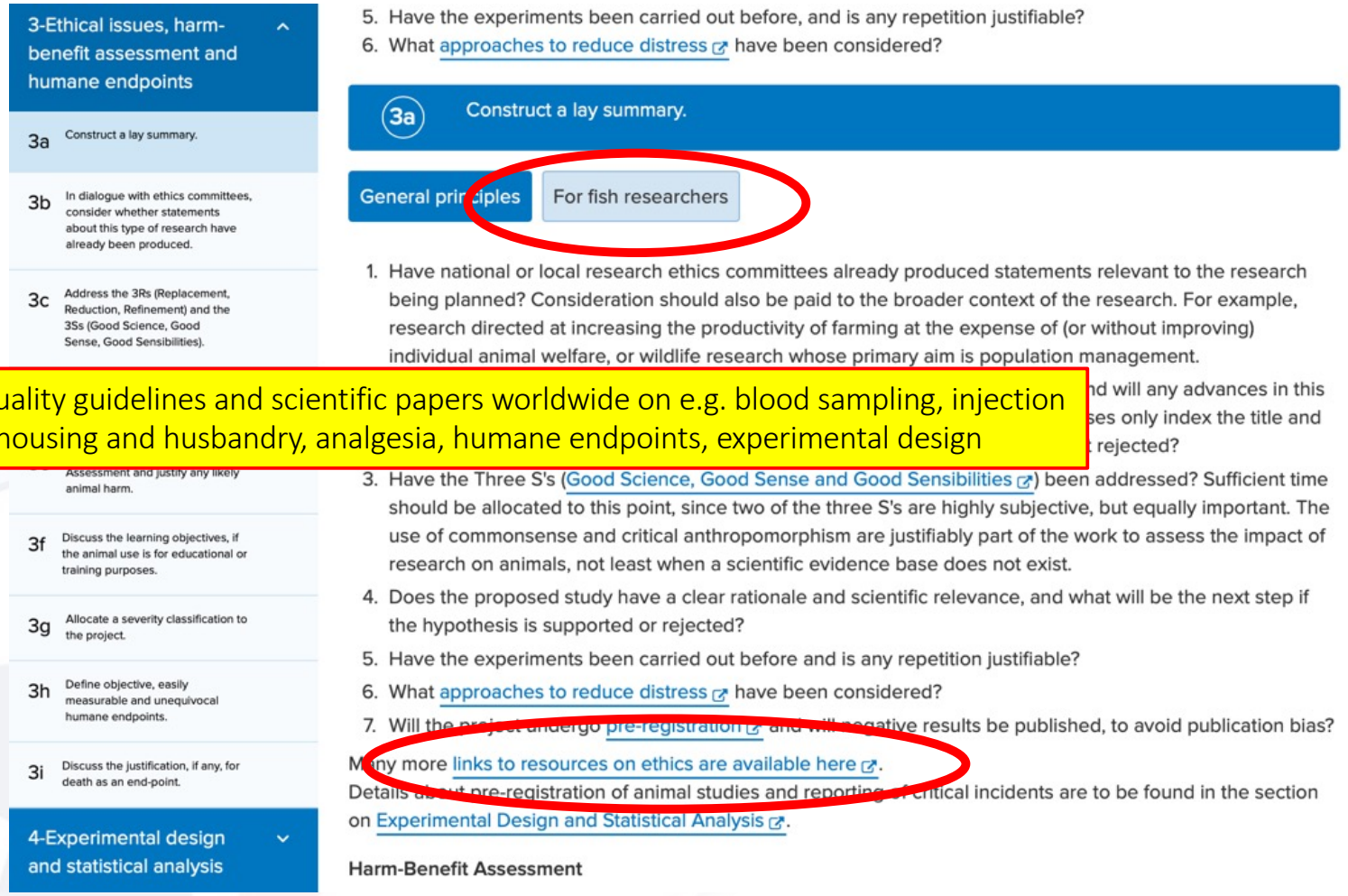
norecopa.no/PREPARE



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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PREPARE encourages scientists to collaborate with animal carers and technicians from Day 1

- they have a right to know and will be more motivated
- they know the possibilities (and limitations) in the animal facility
- they often possess a large range of practical skills and are good at lateral thinking
- they know the animals best
- the animals know them best
- lack of involvement creates anxiety, depression and opposition to animal research, as well as limiting creativity which might improve the experiments


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

Culture of Care

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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

Culture of Care facilitates honest discussion along the path

"***Replacement?***"

"we've always done it that way!»

"there are no alternatives!»

»we only do it as often as necessary"

Closely related to a culture of care is

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

Look for the acceptable, rather than choosing the accepted.

Where do I find information on alternatives?

norecopa

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

These databases are updated regularly. Please give us feedback if you discover errors or omissions.

The Norecopa website also includes five other collections:

- NAL: a collection of literature references relating to the 3Rs from the US National Agricultural Library
- European Commission datasets:
  - 3Rs Knowledge Sources: over 800 resources collected by the Commission in 2016
  - 3Rs Education and Training Resources, over 560 items collected in 2018
  - Non-animal models for respiratory tract diseases, over 280 models identified in a literature review of over 21,000 papers, published in 2020
  - Non-animal models for cardiovascular diseases, citing over 400 models, identified in a literature review of over 14,000 papers, published in 2022

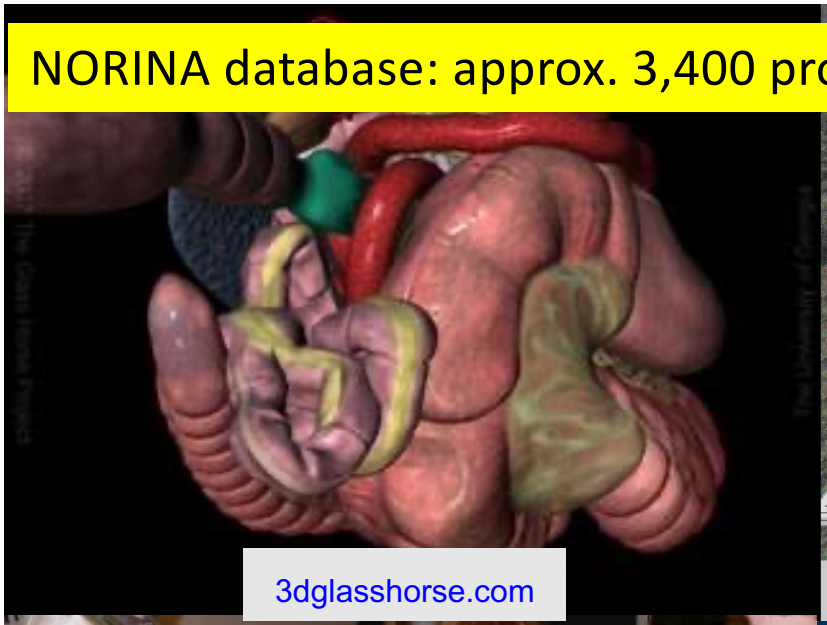
The EU Commission has now published 30 datasets of this type, including reviews of non-animal models within breast cancer, neurodegenerative diseases, immuno-oncology and immunogenicity testing for advanced therapy medicinal products.

Here is an alphabetical global list of all the databases cited on the Norecopa website.

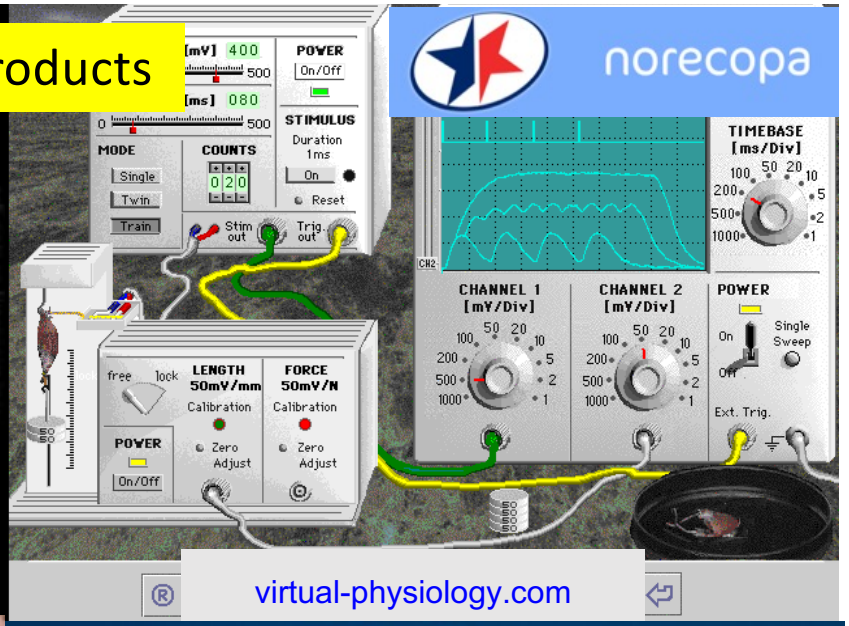
links to over 80 other databases

norecopa.no/databases-guidelines

NORINA database: approx. 3,400 products

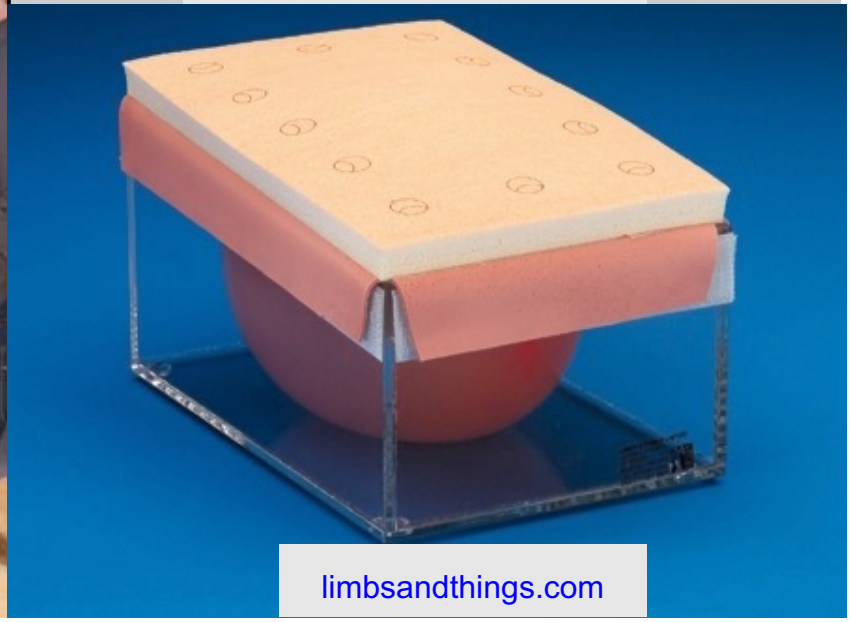


3dglasshorse.com



virtual-physiology.com

rescuecritters.com



limbsandthings.com

TextBase:

1,900 books related to Lab Animal Science, welfare and alternatives:

norecopa.no/textbase

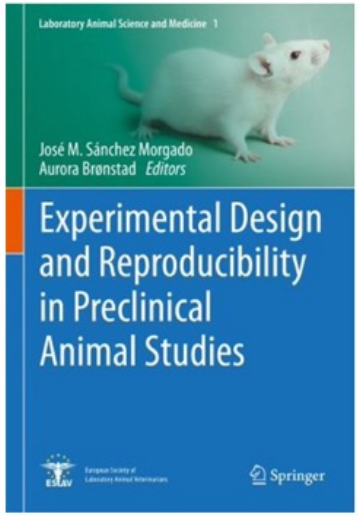
Experimental Design and Reproducibility in Preclinical Animal Studies

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

Record number: 8619d

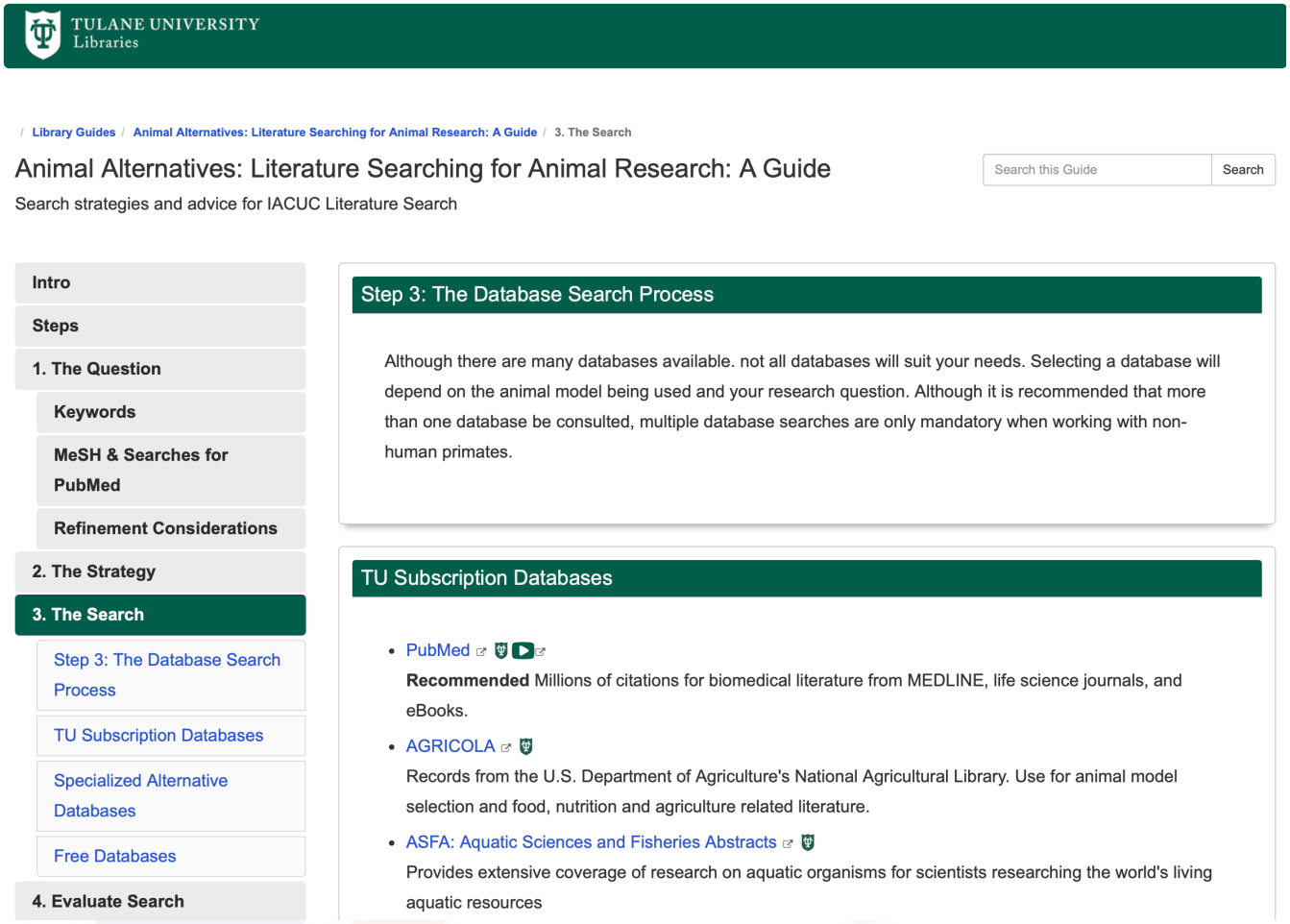
This book provides grounds on how to plan and conduct animal experiments that can be reproduced by others. It touches on factors that may impact the reproducibility of animal studies including: the animal genetic background, the animal microbial flora, environmental and physiological variables affecting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

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



norecopa.no/textbase/experimental-design-and-reproducibility-in-preclinical-animal-studies

Many scientific institutions (should) have their own collection of search guides and alternatives databases



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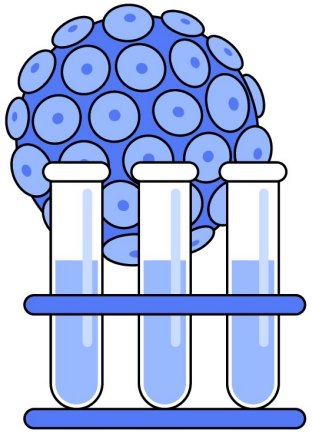
https://libguides.tulane.edu/iacuc/search

Some thoughts on Replacement Alternatives .....

When Russell & Burch started their work, the word ”**alternatives**”, suggested by Rex Burch, was deliberately not used in the invitations to interviews, to avoid the risk of researchers declining to participate.

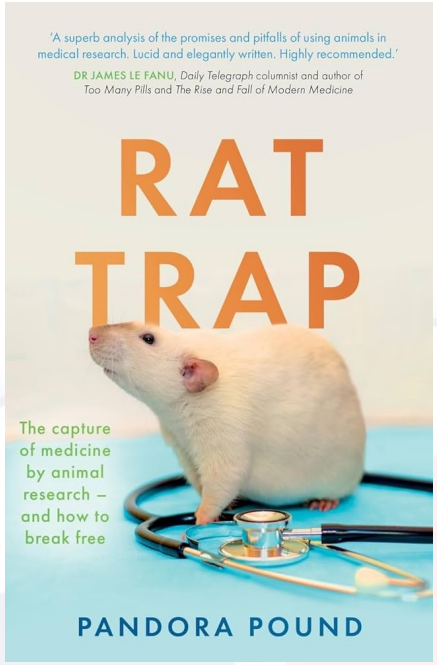
Instead, they wrote:
‘a review of progress in the development of humane techniques’.



colourbox.com

Slide from https://norecopa.no/3Rs

“Respectful dialogue”?



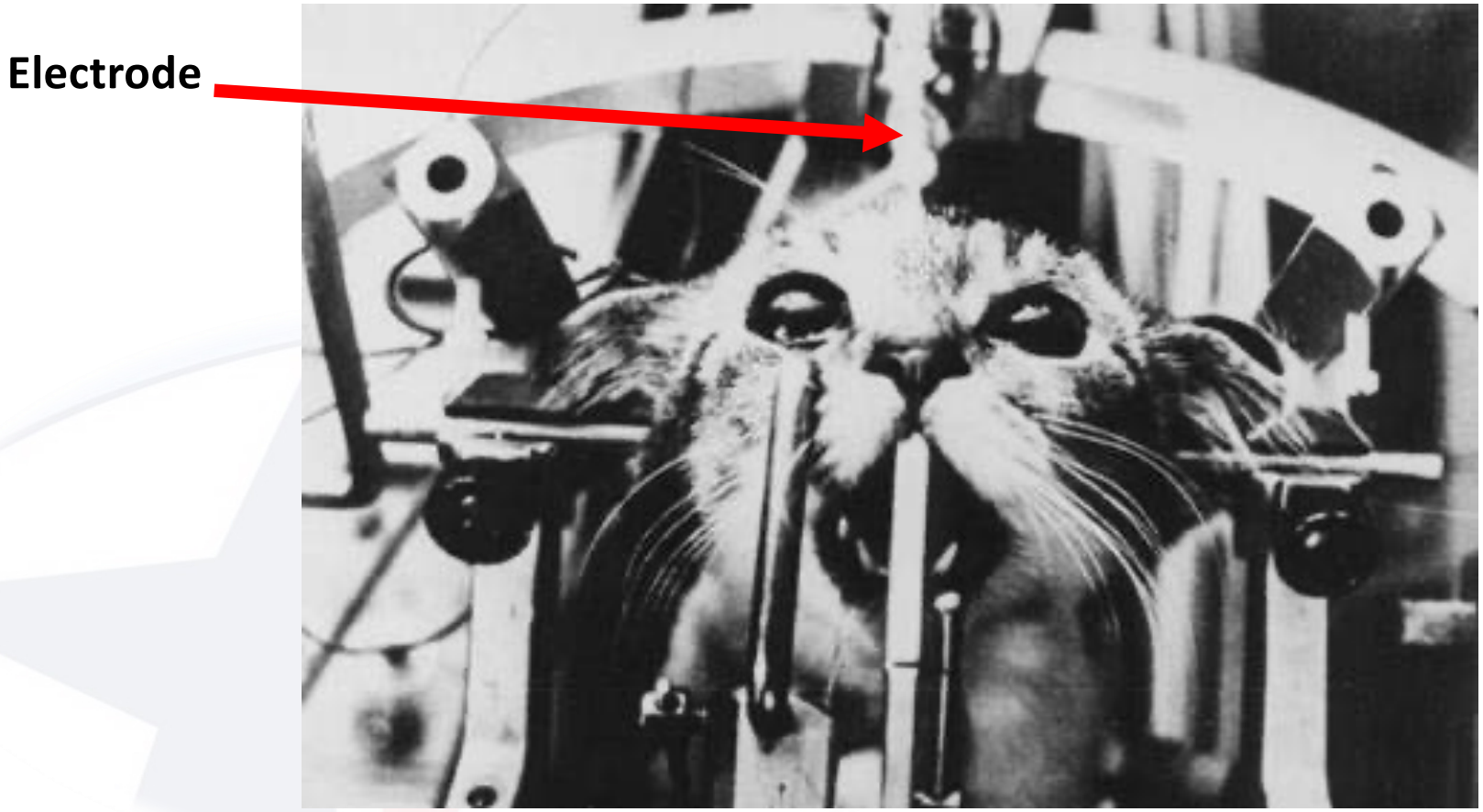
Scientists who have built their career on an animal model

- refined
- humanised (genetically altered)
- validated
- where previous data is available

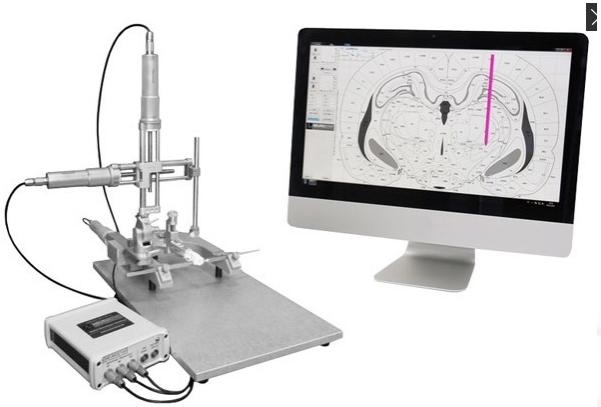
“Animal testing” and “animal research” are often used indiscriminatively, even though the potential for animal replacement is very different

Replacement

Methods that replace or avoid the use of ***sentient*** animals entirely



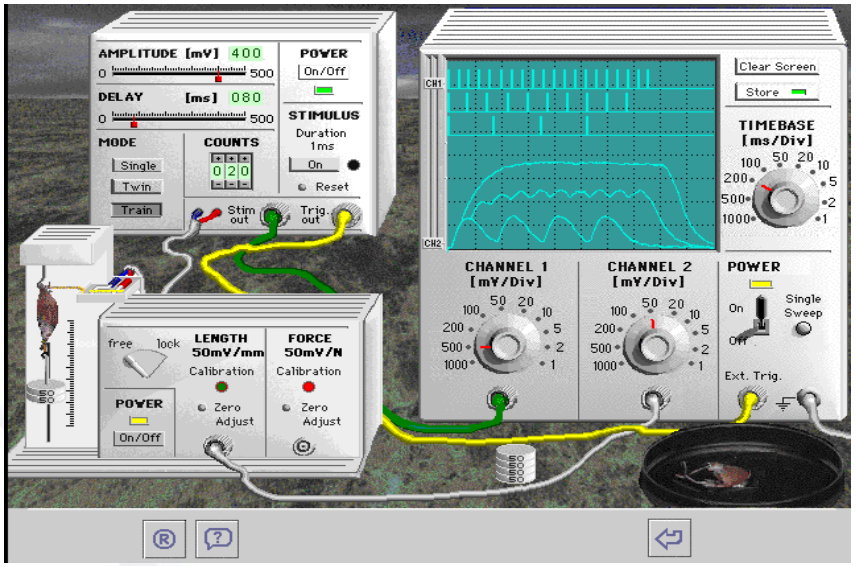
Partial/relative replacement



agnthos.se/569-stereotaxic-frames

Experiments under full anaesthesia from which the animal does not wake up (non-recovery / terminal studies)

Full/absolute replacement



virtual-physiology.com

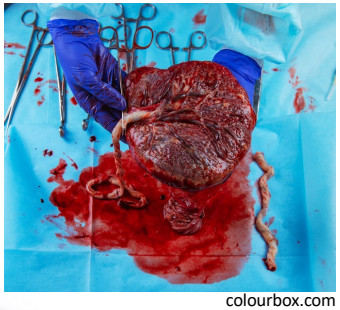
A simulation of an experiment on a frog nerve-muscle preparation

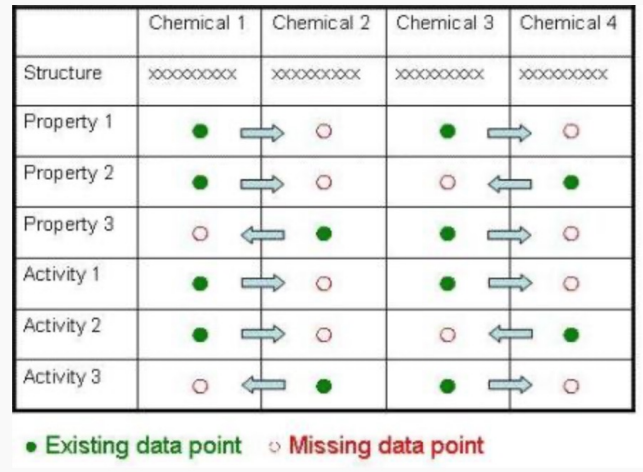
NAMs og NATs

NAMs: New Approach Methodologies

Avoidance (methods which don't directly replace animal experiments)

e.g. studies on the human placenta
"Read-Across"


colourbox.com



NATs: Non-Animal Technologies

Alternatives to animal experiments

e.g. organoids (mini-organs)
organs-on-chips
experiments on fruit flies

norecopa.no/alternatives/nams-and-nats

NB. Those who work with NAMs may not even be aware that they use a method that can reduce animal use.
It is therefore important to build bridges between the lab animal community and the NAMs/NATs-communities !

https://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm

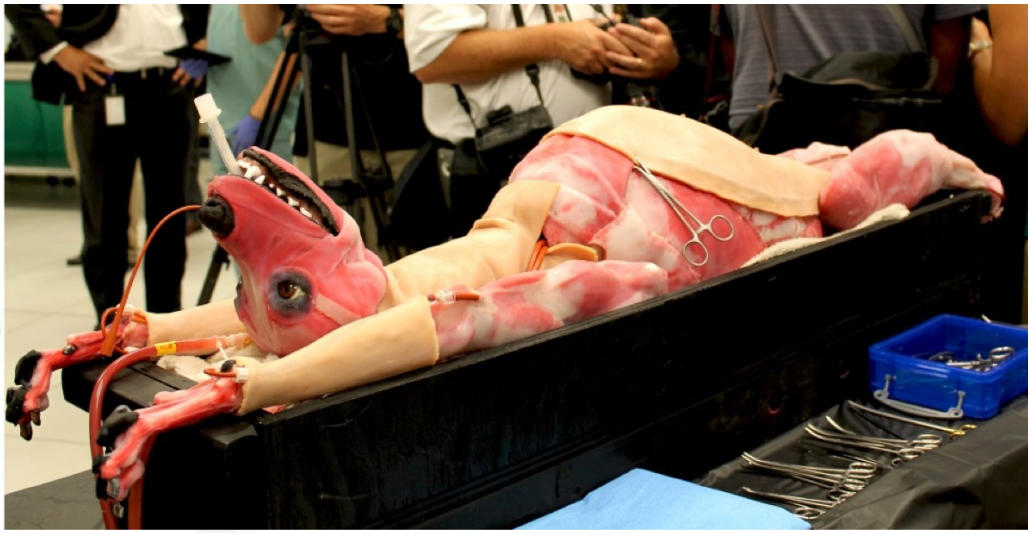
https://nc3rs.org.uk/sites/default/files/documents/NonAnimalTechCO082_RYE_4_nrfinal2.pdf

Discrimination and fidelity

e.g. in educational and training aids:

Rikke Langebæk

High discrimination,
low fidelity



syndaver.com

High fidelity,
no discrimination

norecopa.no/media/8099/langebæk.pdf

Scientists need to become better at promoting the 3Rs:

Replacement

- Highlight alternative methods, even if they are within *in vivo* studies (e.g. antibody production)

Reduction

- Share data, protocols and (if practical and ethically acceptable) animals/tissue
- Publish negative or inconclusive findings

Refinement

- Publish better techniques, preferably as separate methodology papers for high visibility

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

Aaberge I.S. *et al*., Infection & Immunity, 1992, 60 (10): 4146-4153

https://journals.asm.org/doi/epdf/10.1128/iai.60.10.4146-4153.1992

More than 3Rs

The 3 Rs to minimise the harm:

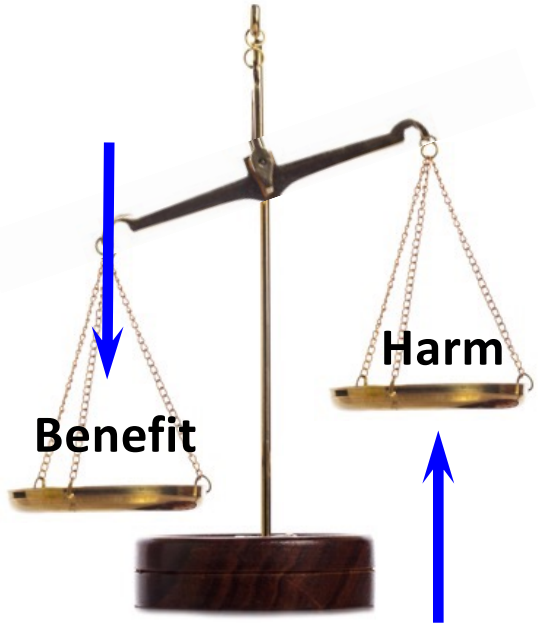
- *Replace the unnecessary experiments*
- *Reduce the number of animals used*
- *Refine the conditions for the animals*

The 3 Ss - use 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?)*

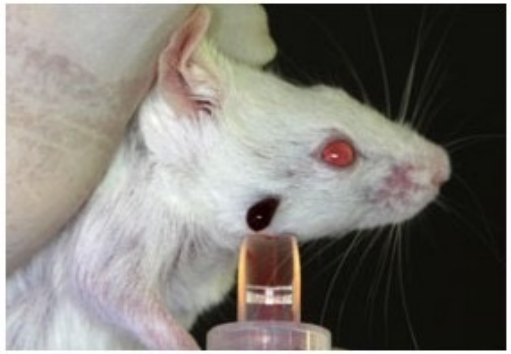


norecopa.no/3R
norecopa.no/3S
norecopa.no/3V

The Three Ss in practice

At the doctor's surgery:

- I think I'll take a blood sample from you today, just to rule out the possibility that you have an infection.
- By the way, I take my blood samples by sticking a knife into your neck without anaesthesia –
- - but don't worry, I'll inject 2 litres of fluid into your stomach first, so you don't die from loss of blood.



Retro-orbital puncture

'critical anthropomorphism' (Smith & Hawkins, 2016)

https://www.mdpi.com/2076-2615/6/11/70

Alternatives to Laboratory Animals

Impact Factor: **2.5**
5-Year Impact Factor: **2.5**

Free access | Review article | First published online March 1, 2025

Reviewing Current Guidance for the 'R' of Replacement and Rethinking it with the 'Replacement Checklist'

Juliet P. Dukes, Amy Beale, and Celean Camp View all authors and affiliations

Volume 53, Issue 2 | https://doi.org/10.1177/02611929251319265 | View article versions

Replacement Checklist

When looking for non-animal technologies, or alternative approaches to the use of animals in research, there are steps to take in order to thoroughly explore existing opportunities and new approaches to answering a research question.

Similarly, for those reviewing proposals, whether an application for funding or for a licence to use animals in research, there are questions that do not require subject-specialist knowledge that can be asked to provide reassurance that the potential to replace animals has been fully explored.

This checklist aims to:

- Support researchers to identify and assess whether scientifically satisfactory replacement approaches exist in line with current legislation.
- Encourage researchers to plan their research projects from a starting point of avoiding animal use.
- Support members of funding review panels, and Animal Welfare and Ethical Review Bodies (AWERBs) to scrutinise applications and applicants on alternative approaches and how these have been explored.
- Assist AWERBs and institutions to disseminate information on how to more thoroughly explore alternatives at an earlier stage to avoid and replace animal use.
- Help those making decisions about funding animal research to ensure they are supporting research activity that demonstrates full compliance with the 3Rs including the use of alternative methodologies and the avoidance of animal use wherever possible.
- Support editors and reviewers of manuscripts to evaluate compliance of submissions with 3Rs policy of scientific journals.

In the simplest terms, asking the **What**, **Where**, **When**, **Who**, **How** and **Why** questions should provide information to guide both researchers and reviewers of proposals and applications to ensure a thorough exploration of opportunities to avoid animal use has been performed.

https://journals.sagepub.com/eprint/H3YHXAH7IJCIHC2YRJFA/full#bibr5-02611929251319265

Norecopa: PREPARE for better Science

What subject area(s) did the search(es) cover?

- **Are the search terms and variants used provided?**
  Searching for potential animal replacements within any given field requires a combination of search terms: subject-specific terms, and keywords focusing on techniques avoiding animal use. Many non-animal approaches with the potential to provide useful data and replace animal use will not necessarily be tagged in literature with '3Rs', 'replacement', or 'alternative' so it is helpful to use terms implying non-animal methods e.g. in vitro, microphysiological, model, assay etc.
- **Are the search terms relevant to the field of study?**
  What subject-specific terms were used to try and identify alternative approaches appropriate to the field? Were any variants of keywords included? (Please note that some databases automatically generate variants of search terms)
- **Is there anything missing from search methodology?**

Where was information obtained?

- **Which databases were searched?**
  Has a list of the databases or other sources of information been provided? Were multiple sources of information explored?
- **Which websites were searched?**
  Have specific (and relevant) websites been included in the report of the search for alternatives?
- **Was any other 'grey literature' included?**
  Has any 'grey literature' been mentioned? Did the search include pre-registered protocols, pre-prints of papers or information produced outside of traditional publishing and distribution channels, including reports, policy literature, newsletters, government documents, white papers or similar?

When were the articles published, and the search(es) completed?

- **What publication years were included?**
  Science and technology can progress rapidly, did the search involve the most up to date publications? What years of publication or release were included in the search?
- **When was the search conducted?**
  How long prior to the application being completed and submitted was the search conducted?
- **Was it repeated?**
  Was the search repeated at multiple time points, or was it carried out only once?

https://journals.sagepub.com/eprint/H3YHXAH7IJCIHC2YRJFA/full#bibr5-02611929251319265

Who was approached for advice?

- ☐ **Which networks, communities or individuals?**
  Were any peers, or subject specific experts consulted? Or any of the expected beneficiaries of the research?
- ☐ **Were any 3Rs or 1R organisations approached for expert advice?**
  Several organisations exist to progress and promote the 3Rs, some of which focus solely on replacement. Where any of these organisations approached for advice?

How was the search conducted?

- ☐ **What combination of operators and search terms was used?**
  Were search terms combined appropriately?
- ☐ **Were search string(s) constructed?**
  Were the combined search terms and operators recorded?
- ☐ **Were different types of searches used for different sources of information?**

Why were the results of the search(es) rejected?

- ☐ **Were the results of the search provided?**
  Have any references (papers, technical information) about potential techniques, or combinations of techniques, reported?
- ☐ **Were the results relevant to the field?**
- ☐ **Could any identified protocols be adapted to suit?**
  What changes would need to be made to existing techniques in order to achieve research goals? Are any in development?
- ☐ **If results were rejected, was it justifiable to do so? Was the output thoroughly evaluated?**
  Has evidence of assessment been provided? Were any approaches found to be relevant to the research? What were the limitations of the approaches found? Would there be an opportunity to replace part of the overall programme of work?

It is important to remember that if a reported search for replacement techniques or alternative methods that avoid animal use falls short of being able to answer these questions, then it is worth asking what kind of support and training with respect to Replacement the researcher has received. If there is a gap in training, then researchers can be signposted to suitable courses or resources (e.g. EU-52: Searching for (existing) non-animal alternatives. Education and Training Platform in Laboratory Animal Science (ETPLAS), Cerdanyola del Valles, Spain. https://learn.etplas.eu/courses/eu-52/.

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https://journals.sagepub.com/eprint/H3YHXAH7IJCIHC2YRJFA/full#bibr5-02611929251319265

Don’t forget that it will always be the scientists who have the best grasp of the literature and (if they’re honest) the potential for replacement in their field, i.e.:

- ***they*** should be given the onus of declaring potential alternatives and providing evidence in their applications that they have looked for this – not just “there are no alternatives”
- it’s quite acceptable for an AWERB to ask the “stupid” questions about the Emperor’s new clothes
- ***your*** time is just as valuable as theirs

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Thank you for listening!

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