The Path to Better Science: Resources from Norecopa

Adrian Smith[1], Elisabeth Pagels[2] & Øyvind Wærenskjold[2]

[1]Norecopa, Ås & [2]Bitfarm, Kristiansand, Norway

adrian.smith@norecopa.no

Slides at: norecopa.no/Path

Disclosures

“Norecopa: A one-stop-shop for global 3R resources”

Manager of the Norecopa website

- The PREPARE guidelines (lead author)
- Refinement Wiki

Member of AAALAC

"...better science?"

- 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)
- Culture of Care at the animal facility
- Communication of best practice to others

colourbox.com

Norecopa: PREPARE for better Science

The Path to Better Science:

norecopa.no/PREPARE *and*
https://riojournal.com/article/105198

Norecopa: PREPARE for better Science

The Path to Better Science:

Norecopa: PREPARE for better Science

https://nrkbeta.no/2010/09/28/mediebransjens-svar-paa-elg-i-solnedgang

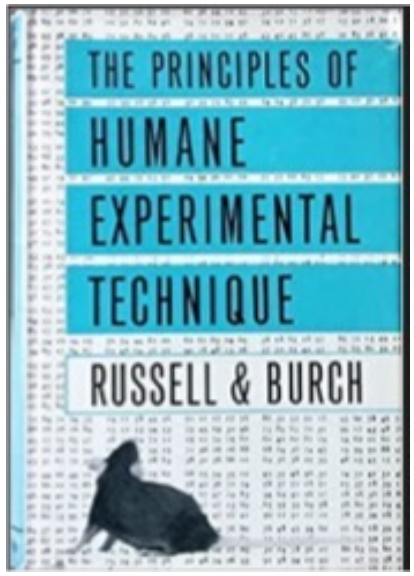
Bad habits have been around for a long time…

Russell & Burch (1959) 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."

…or is it because they didn't do good science?



Russell WMS & Burch RL (1959)

fourwaves.com/blog/how-to-make-a-scientific-poster

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Europhysiology, Copenhagen, 2022

Experiments were performed on spontaneously breathing adult male Wistar rats (anesthetized with sodium thiopentone 100 mg/kg i.p.). Two trephinations were made over the left parieto-occipital cortex, the dura mater was opened, and the exposed brain areas were superfused with regular artificial cerebrospinal fluid (ACSF, warmed to 37 ºC equilibrated with carbogen). DC potentials were recorded at two sites in the cerebral cortex with pairs of glass microelectrodes (tip diameter 5 µm) in cortical layers II and V. The frontal trephination hole was surrounded by a wall of dental acrylic, and there Gal was applied topically to the cortical surface (see Figure). The electrocardiogram and the systemic blood pressure were continuously monitored.

Germany

no mention of analgesia

PCOS for 21 days. Exercise groups were trained for 38 min/day five days a week for 12 weeks. After experimental protocol, thoracotomy was performed under 50 mg/kg sodium thiopental anesthesia. HOMA-IR, FSH, LH, thiol levels were analyzed in blood. Myokines were analyzed in

Turkey

no mention of analgesia

Norecopa: PREPARE for better Science

The Murine Intensive Care Unit: Integrated Cardiovascular Assessment of the Anesthetized Mouse In Vivo

Background
Investigating the cardiovascular system is complex. Many existing methods are often limited to assessment of few cardiovascular variables. We aimed to establish an *in vivo* murine model for comprehensive assessment of cardiovascular variables including blood pressure (BP), total peripheral resistance (TPR), cardiac output (CO), stroke volume (SV), and parameters of the electrocardiogram (ECG) with simultaneous pacing of heart rate.
I hypothesize, that i) *in vivo* in anaesthetized mice, electric cardiac pacing modulates cardiac function without direct vascular effects and, ii) this model can detect the multifarious cardiovascular changes following intervention with α_1-adrenoceptor agonist, phenylephrine (PE), and distinguish between the PE-induced changes to cardiac and vascular functions.

Methods
Isoflurane-anesthetized (2% in 100% O_2) 9-months old male C57BL/6J mice were intubated and mechanically ventilated. Two-lead ECG was recorded. BP was monitored by a solid-state catheter placed in the aortic arc through the common carotid artery. Pancuronium (0.4 mg/kg), an M_2-receptor antagonist, was injected intraperitoneally (i.p.) to immobilize the mice. Thoracotomy was performed for the mounting of i) transit-time flow probe on the ascending aorta for CO measurements and ii) an electrode placed at the right atrium for pacing with increasing pacing frequencies (10-11.3 Hz). TPR was calculated from BP and CO. After the first pacing, mice were randomly allocated to i.p. injections of vehicle control (VC) (9mg/mL, NaCl) or PE (0.3 mg/kg). 10 minutes after injection, the pacing protocol was repeated. Data are presented as mean ± standard error of the mean and compared with two-way ANOVA. Δvalues are calculated in relation to baseline. n = 6.

Results
PE but not vehicle elevated systolic BP (104.6 ± 4.7 mmHg vs. 72.1 ± 5.2 mmHg, $P < 0.0001$) and TPR (Δ: 3.0 ± 1.1 AU. vs. -0.3 ± 0.3 AU., $P = 0.0006$), while heart rate was reduced (Δ: - 45.9 ± 13.3 $beats^{-1}$ vs. 19.3 ± 7.6 $beats^{-1}$, $P < 0.0001$) 18 minutes after PE injection compared to VC. Accordingly, SV was increased after PE injection (Δ: 3.4 ± 1.3 µL vs. 0.2 ± 0.7, $P < 0.0001$).
Pacing successfully modulated cardiac function without vascular effects. SV decreased with increasing pacing frequencies in both VC and PE groups (VC; 14.0 ± 1.6 µL at 10 Hz to 11.9 ± 1.4 µL at 11.3 Hz, and PE; 13.8 ± 1.5 µL at 10 Hz to 11.5 ± 1.2 µL at 11.3 Hz, $P < 0.0001$). However, TPR did not change with increasing pacing frequencies (VC; 6.5 ± 0.6 AU. at 10 Hz to 6.4 ± 0.7 AU. at 11.3 Hz, and PE; 8.9 ± 1.2 AU. at 10 Hz to 9.7 ± 1.3 AU. at 11.3 Hz, $P = 0.2$).

Conclusion
We demonstrated with this model that PE in mice affects the vasculature with secondary cardiac effects mediated by the baroreceptors. The characterized in vivo mouse model allows for simultaneous assessment of distinct cardiac and vascular functions.

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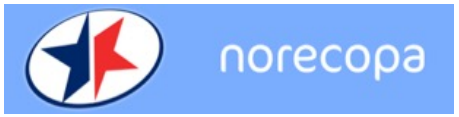
Europhysiology, Copenhagen, 2022

Guidelines for the reporting of anaesthesia and analgesia in poster presentations of surgical research

The two FELASA sessions I'm most looking forward to



Thursday 5 June, 10:35-11:10

P5AM4 It's All About Surgery - Station 4 (Banquet Level, floor -2)

PC015 Bringing Experts Together for Successful Surgery: Working Well as a Team

Poster Presenter: Yao Chen (Lyon, France)

Y. Chen[1], *L. Barrot*[2], *S. Langonnet*[1]

[1]Centre Leon Berard, Lyon, France, [2]INSERM U1032 LabTAU, Lyon, France

If we refer to human surgery, surgical practice is clearly a team effort requiring a combination of diverse skills alongside rigorous organization, planning, and communication to ensure success.

In the context of animal experimentation, although numerous guidelines encourage researchers to surround themselves with the necessary expertise for their projects (e.g., PREPARE Guidelines), a quick assessment reveals that researchers often remain isolated, insufficiently trained, and poorly prepared when they initiate projects involving surgical procedures.

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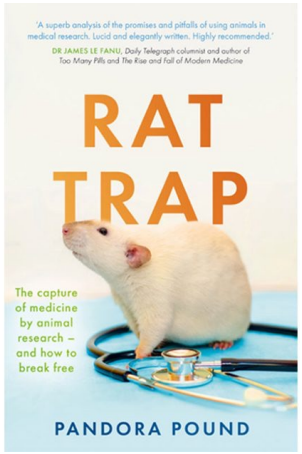
The two FELASA sessions I'm most looking forward to

Monday 2 June, 10:15-11:30

S2E3: You All Shouldn't Be Here! How to Burst the 3R Bubble (Trianti)

This has led to the somewhat frustrating situation that conferences focusing on 3R science may feel like mere echo chambers.

In this session we aim to penetrate virtual walls between the "*3R bubble*" and other areas of basic research with a focus on neuroscience and immunology, which together account for more than one fifth of animals used in the EU/UK (2019/2020). The three session talks will discuss mutual benefits, the specific needs and limitations of the selected life science fields regarding 3R methods, and the implementation of new concepts and methods. Finally, together with the speakers and the audience we want to explore ideas on how to improve exchange across different life sciences communities to inform and accelerate the implementation of new 3R advancements.



Norecopa: PREPARE for better Science

The Path to Better Science:

With all due respect to these experts,
Where were those who are used to getting their hands dirty in an animal facility, when the ARRIVE reporting guidelines were compiled?

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen O, Rawle F, Peynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T and Wurbel H (2020).

The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol.* doi: 10.1371/journal.pbio.3000410

The Path to Better Science:

We cannot improve our research by

better reporting alone...

This may not be sufficiently obvious to scientists who are not familiar with the challenges of running an animal facility ... or they assume that we have thought of everything...

The reproducibility/translatability devil is often in the practical details...

reddit.com

Contingency and redundancy

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

Photo: NMBU

Dagbladet.no Publisert fredag 23.04.2010 kl. 18:26

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

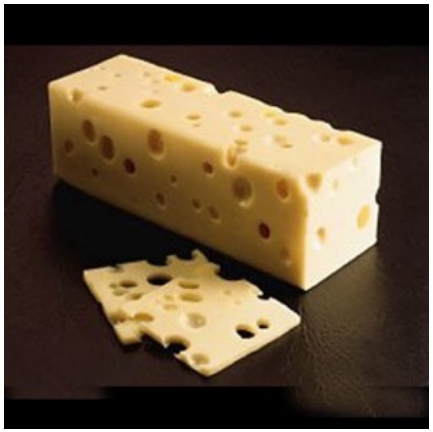
Slår alarm om arbeidsforholdene på Radiumhospitalet. Sykehuset innrømmer rutinesvikt.


ASLE HANSEN
ash@dagbladet.no

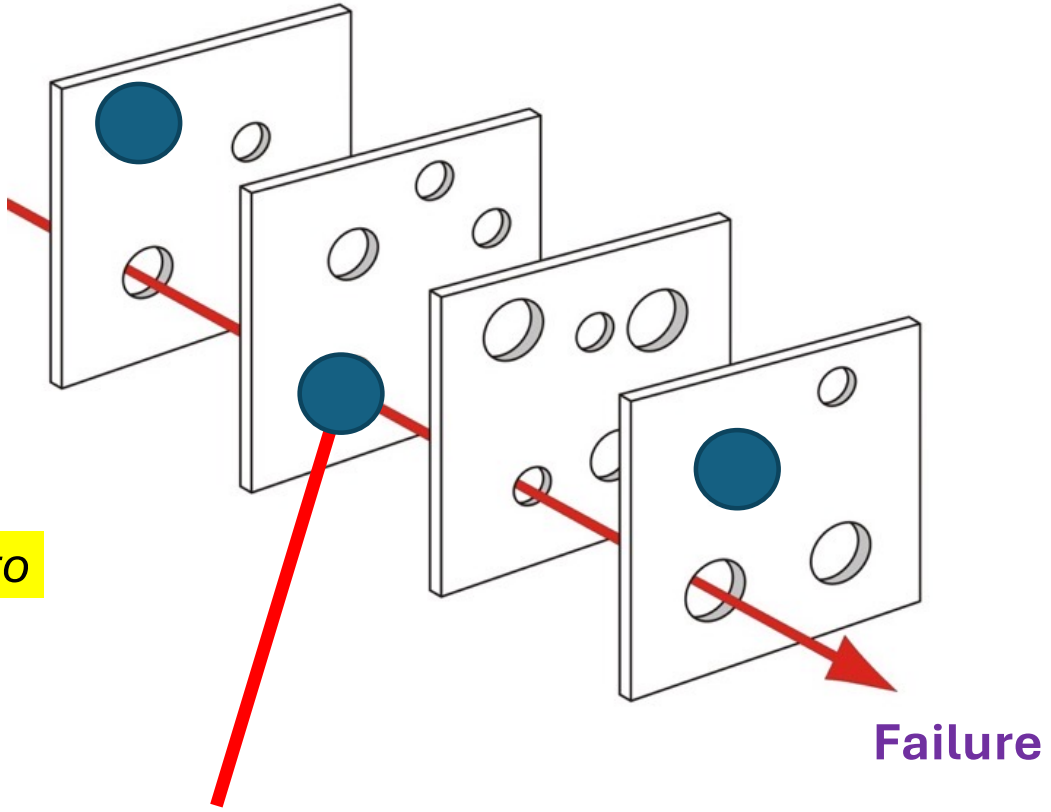

DIANA BADI
dba@dagbladet.no

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

Threat and Error Management



eaugallecheese.com/Swiss-Cheese



wikipedia.org/wiki/Swiss_cheese_model

Embrace these as opportunities to improve the quality of our work!

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**"

Program Description

A. Animal Care and Use Program

B. Animal environment, Housing and Management

C. Veterinary Care

D. Physical plant

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

63 pages

A simple but effective Master Plan

norecopa.no/more-resources/master-plan-and-sop

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
  - 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

Many of these needed revision in the light of Covid-19
norecopa.no/be-prepared

Temporary staff at weekends and holidays

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

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

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PREPARE

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The PREPARE Guidelines Checklist

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

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] consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE[2].

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

Topic	Recommendation
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. ☐ Discuss and disclose all expected and potential costs. ☐ Construct a detailed plan for division of labour and expenses at all stages of the study.
6. Facility evaluation	☐ Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. ☐ Discuss staffing levels at times of extra risk.
7. Education and	☐ Assess the current competence of staff members and the need for further education or training prior
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.
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

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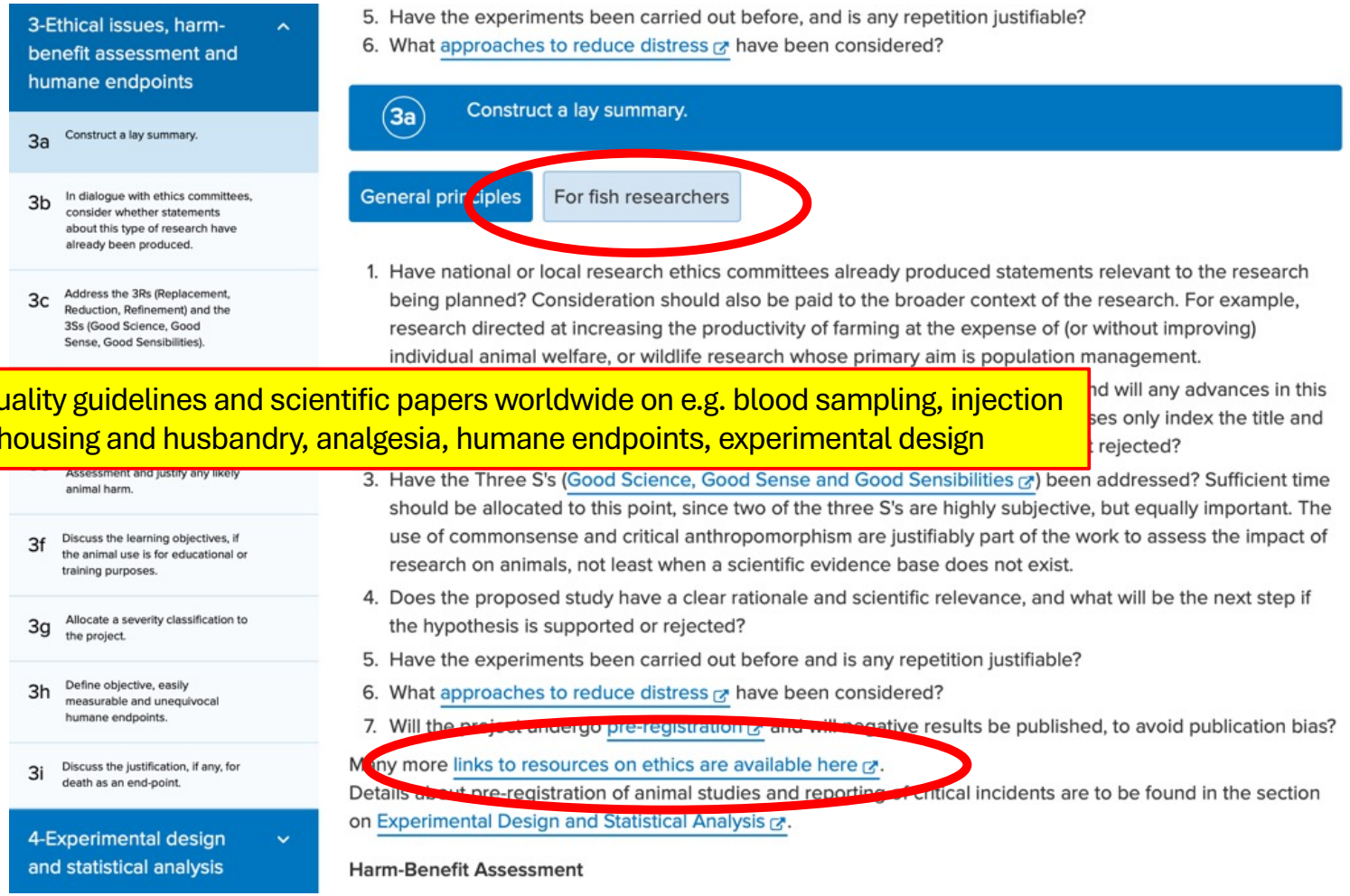
norecopa.no/PREPARE



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

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

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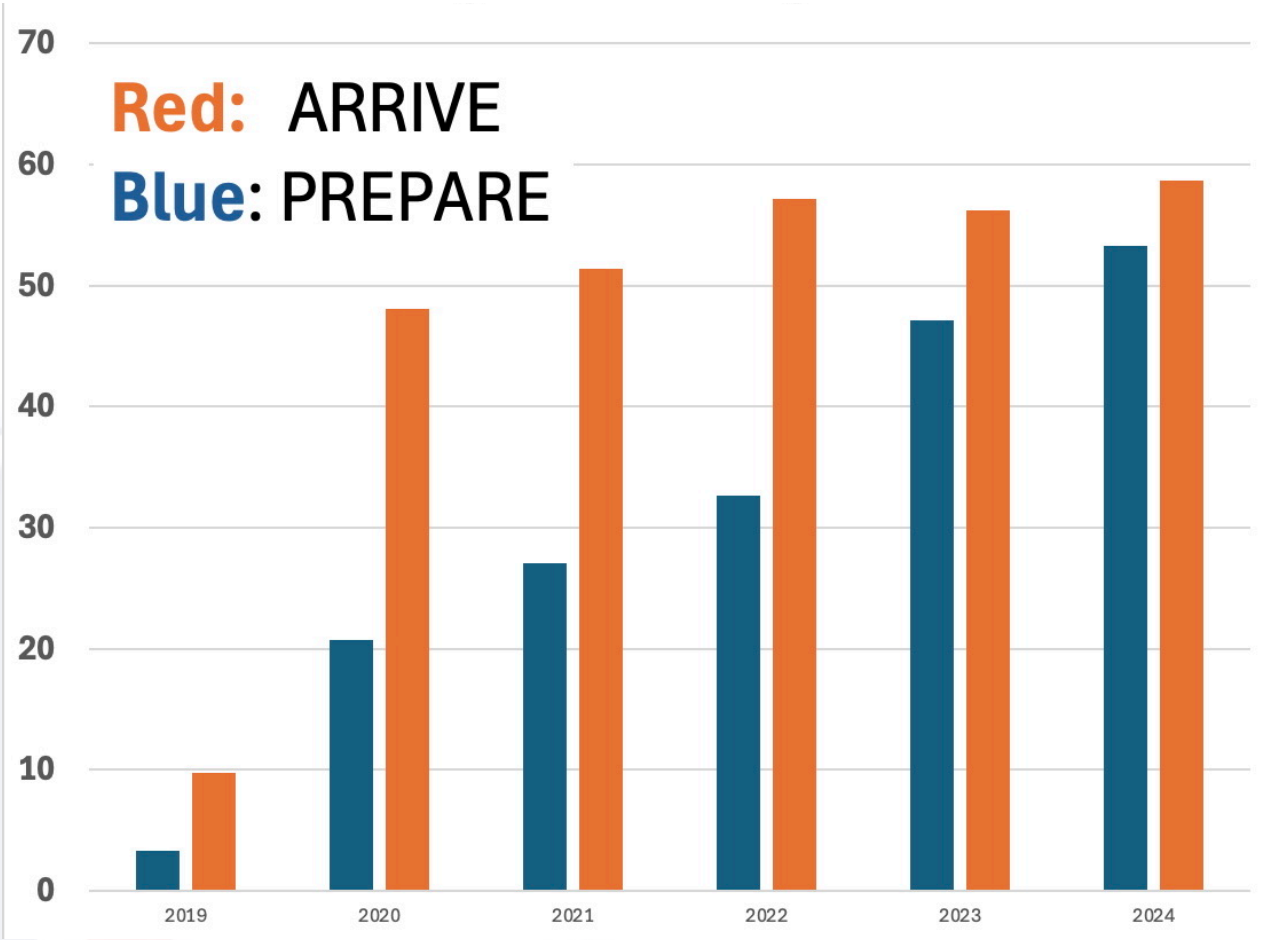
Percentage of projects approved in the UK that use ARRIVE & PREPARE



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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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Thanks to Norecopa's sponsors

Standing Committee on Business Affairs, Norwegian Parliament
Norwegian Ministries of Agriculture and Fisheries
Research Council of Norway

Aivero
Architect Finn Rahn's Legacy
Laboratory Animals Ltd.
Nordic Society Against Painful Experiments (NSMSD)
Norwegian Society for Animal Protection (Dyrebeskyttelsen Norge)
Norwegian Animal Protection Alliance (Dyrevernalliansen)

Novo Nordisk
PHARMAQ
Royal Society for the Prevention of Cruelty to Animals (RSPCA)
Sanofi
Scand-LAS
Scottish Accreditation Board (SAB)
Stiansen Foundation
Universities Federation for Animal Welfare (UFAW)
US Department of Agriculture (USDA)

norecopa.no/ScandLASposter

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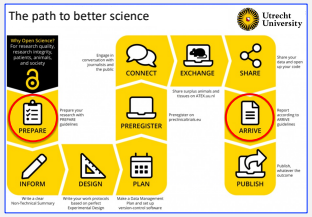
Adrian Smith, Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 64, 1431 Ås, Norway
adrian.smith@norecopa.no

What's the problem?

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

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



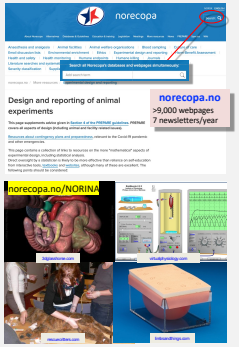
norecopa.no/PREPARE

What can Norecopa offer?

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

- over 9,000 searchable webpages of quality 3R resources, with filters to facilitate searching
- the PREPARE guidelines for planning animal experiments, with a checklist in over 30 languages
- links to recordings of webinars covering all aspects of animal research
- an International Webinars & Meetings Calendar
- a collection of over 400 guidelines for planning and conducting animal research
- an English-language newsletter with the latest developments within the 3Rs
- the NORINA database of alternatives to animal use in education and training
- a slide set describing the 3R concept in detail: norecopa.no/3Rs
- a Refinement Wiki

Examples of Norecopa's resources:



PREPARE covers:

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

norecopa.no/PREPARE

The Refinement Wiki
wiki.norecopa.no

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

Toolbox graphic: colourbox.com

norecopa.no/Path

English-language newsletters

Thank you for listening!

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