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The 3Rs of Russell and Burch:

Replacement, Reduction & Refinement

Adrian Smith
adrian.smith@norecopa.no
@adrian_3r

These slides are available at norecopa.no/3Rs

version 18.11.22 09.00 CET

With some material from:
Smith AJ & Richmond J (Forthcoming). The Three-Rs.
In: *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals.* 9th edition.
Richardson CA and Golledge HDR (eds).
Oxford: Wiley-Blackwell.

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How it all started

The UK organisation *Universities Federation for Animal Welfare* (UFAW) appointed William (Bill) Russell in July 1954 to

'undertake research into the history and progress of the introduction of humane methods into biological research with a view to encouraging further such progress.'

W.M.S. Russell (1925 - 2006)

en.wikipedia.org/wiki/W._M._S._Russell

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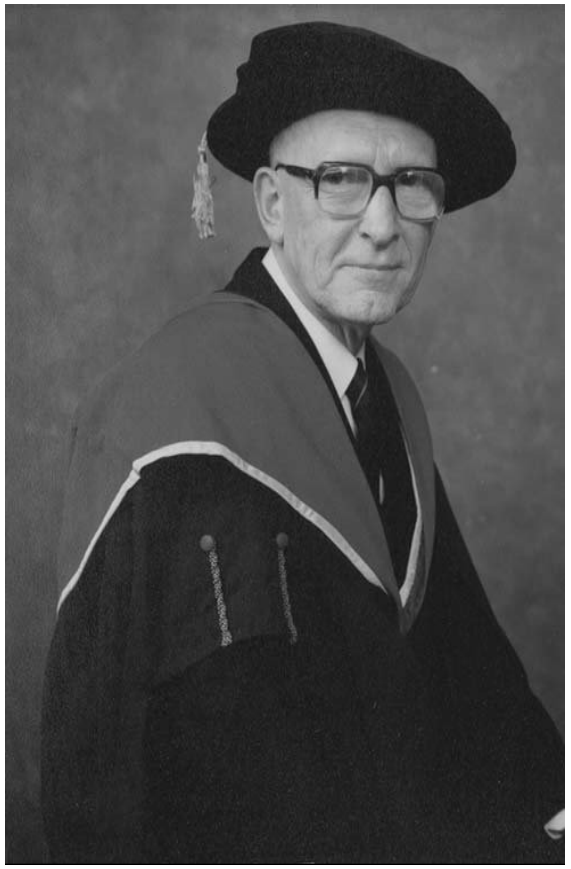
Hubrecht & Carter (2019)

How it all started

UFAW appointed microbiologist Rex Burch to assist Russell by visiting and interviewing research workers on:

- *their attitudes*
- *the techniques that they had adopted to improve the humaneness of their work*
- *the feasibility of replacements to the use of animals*

Their primary task was to find ways of reducing inhumanity in animal experiments - whether it is physical or mental distress



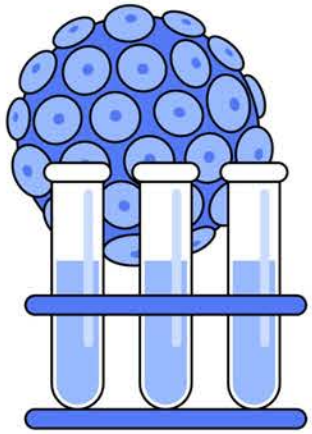
R.L. Burch (1926 - 1996)

from Stephens (2009)

“Alternatives”?

The word ”alternatives” 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’.



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Stephens (2009)

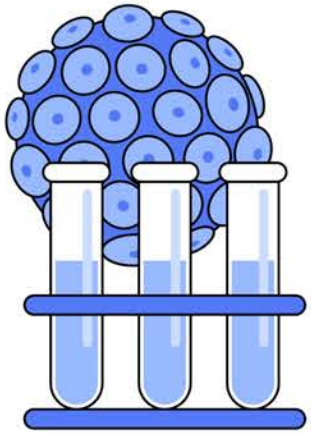
"Alternatives"?

The word was used by Burch, but Russell considered it sounded like *Replacement.* It is not used in their book.

The term was used in a paper by Terence Hegarty (FRAME trustee) in 1971 and (for all the 3Rs) by DH Smyth in his book *Alternatives to animal experiments* (1978).

Some now talk about

- *Replacement alternatives*
- *Reduction alternatives*
- *Refinement alternatives*



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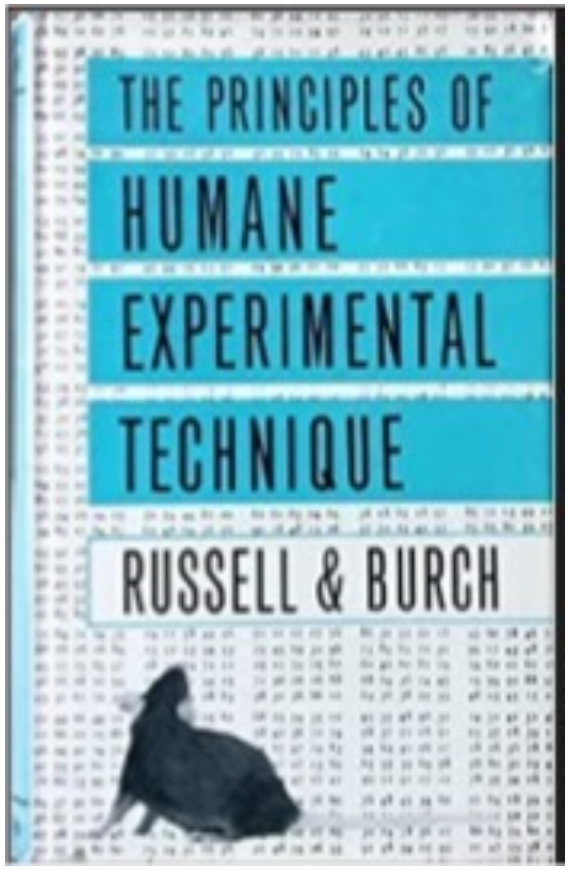
Smyth DH (1978); Russell (2005)

Timeline for the 3Rs

- By 1955, the concept of the 3Rs was essentially present in a paper published by Russell
- The explicit term "The 3Rs" evolved sometime between 1955 and 1957 (Russell, 2005)
- The 3Rs were formally presented at a UFAW Symposium in May 1957 on *Humane Technique in the Laboratory*
- Russell and Burch published ***The Principles of Humane Experimental Technique*** in 1959



Russell WMS & Burch RL (1959)

Russell (2005); Hubrecht & Carter (2019)

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Russell and Burch's original definition of the 3RS:

- **Replacement**: *any scientific method employing non-sentient material which may in the history of animal experimentation replace methods which use conscious living vertebrates*
- **Reduction**: *means of minimising, other than by Replacement, the number of animals used to obtain information of a given amount and precision*
- **Refinement**: *measures leading to a decrease in the incidence or severity of inhumane procedures applied to those animals which have to be used*.

Some contemporary descriptions emphasise **welfare benefit** and **knowledge gain** as well as minimising inhumanity

	Basic	Updated
Replacement	Avoiding or replacing the use of animals in areas where they otherwise would have been used.	Accelerating the development and use of predictive and robust models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals.
Reduction	Minimising the number of animals used consistent with scientific aims.	Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base.
Refinement	Minimising the pain, suffering, distress or lasting harm that research animals might experience.	Advancing research animal welfare by exploiting the latest *in vivo* technologies and by improving understanding of the impact of welfare on scientific outcomes.

nc3rs.org.uk/who-we-are/3rs

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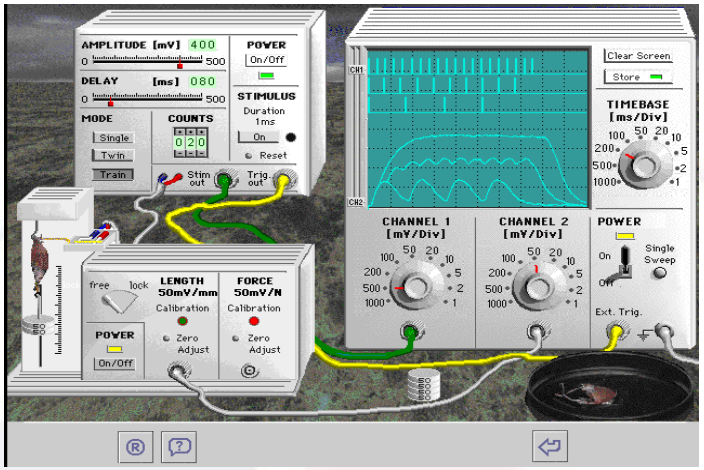
Tannenbaum & Bennett (2015)

Replacement

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

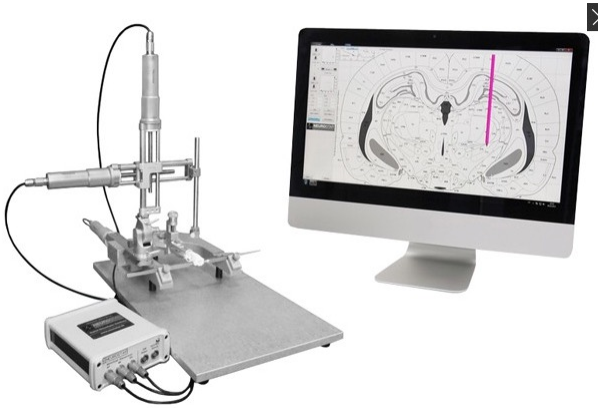
Full/absolute replacement



virtual-physiology.com

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

Partial/relative replacement



agnthos.se/569-stereotaxic-frames

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

Replacement

Examples of replacement methods:

Relative

- animals not currently considered to be sentient*
  e.g. fruit flies, roundworms and very early developmental stages of sentient species
- procedures performed on animals thar are fully anaesthetised before the procedure is started, and which are killed by an anaesthetic overdose before they awake (= non-recovery, terminal, acute experiment)
- cells and tissues from animals
- surplus research animals, clinical veterinary cases or slaughterhouse material
- observation of animals in brief captivity or their natural setting

**not all animals currently believed to be sentient are covered by all legislation e.g. cephalopods and decapods*

Absolute

- Computer simulations
- Films, video, virtual reality
- Models, mannikins, simulators
- QSAR *(Quantitative Analysis of Structure/Activity Relationships)*
- Human cell and tissue cultures
- Organs-on-a-chip and organoids of human origin
- High Throughput Screening (HTS)
- Biochemical & immunological methods (RIA, ELISA)
- Hybrid DNA technique
- Collection of environmental DNA from animals (e.g. hair, faeces, urine)
- Genetically modified microorganisms
- Plants
- Human volunteers
- Synthesis of Evidence from previously published studies, following a Systematic Review of the literature
- Replacement of a practical class with a theoretical session

N.B. *Many non-animal methods are not actually being used to replace animal experiments (e.g. use of the human placenta)*

Replacement: win-win

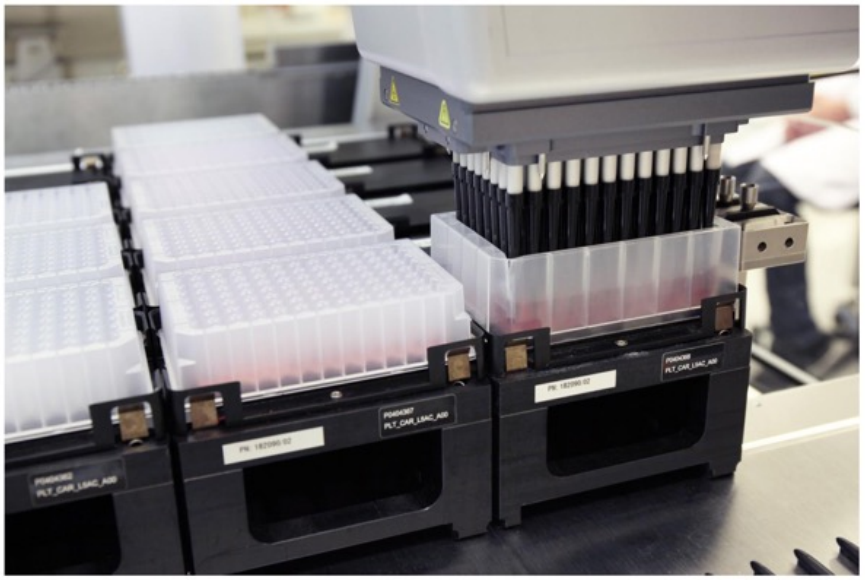
Replacement alternatives are not just substitutes for animal models:

they are often

better science
more powerful
more versatile
faster
cheaper
easier to standardise and replicate

e.g. high-throughput screening of potential novel pharmaceuticals

High Throughput Screening (HTS) laboratory



The High Throughput Screening (HTS) laboratory is set up to produce large amounts of data on large chemical libraries with high precision and speed.

joint-research-centre.ec.europa.eu/laboratories-and-facilities/eurl-ecvams-vitro-laboratory-facility_en#high-throughput-screening-hts-laboratory

Reduction

- Methods that minimise the number of animals, without compromising
  - experimental design
  - statistical analysis
  - validity
  - animal welfare

It's all about ***Optimisation*** of animal numbers:

- fewer animals (if possible)
- more information from the same number of animals
- *more* animals (if the original suggestion was too low to achieve conclusive results)

Too few animals can lead to false conclusions and is a waste of animal lives and human resources.

Reduction and Refinement are therefore inseparable

Refinement

Methods that

- minimise pain, suffering, distress and lasting harm
- maximise animal welfare

All the way from procurement of the animals to humane killing or other outcomes (e.g. re-use, rehoming)

→ An enormous scope for refinement

e.g. better housing, environmental enrichment, handling, dosing, sampling, anaesthesia and analgesia

Refinement: win-win

- Improved animal welfare
- More valid data from animals in harmony with their surroundings
- Easier to detect treatment effects in non-stressed animals
- Less variation between animals
- Possible to use smaller group sizes

colourbox.com

Refinement and Reduction go hand-in-hand

'Happy animals make good science'
(Poole, 1997)

Implementation of Refinement requires knowledge of

- how to observe discomfort (e.g. use of grimace scales and other behavioural indicators of pain)
- how to establish humane endpoints

Replacement → Reduction → Refinement

'Suppose, for a particular purpose, we cannot use replacing techniques. Suppose it is agreed that we shall be using every device of theory and practice to reduce to a minimum the number of animals we have to employ. It is at this point that refinement starts, and its object is simply to reduce to an absolute minimum the amount of distress imposed on those animals that are still used.'

Russell & Burch (1959), Chapter 7

Direct / contingent inhumanity

Russell and Burch distinguished between

- direct inhumanity: the pain or distress of a procedure (even when performed perfectly)
  e.g. pain of injection, immobilisation stress

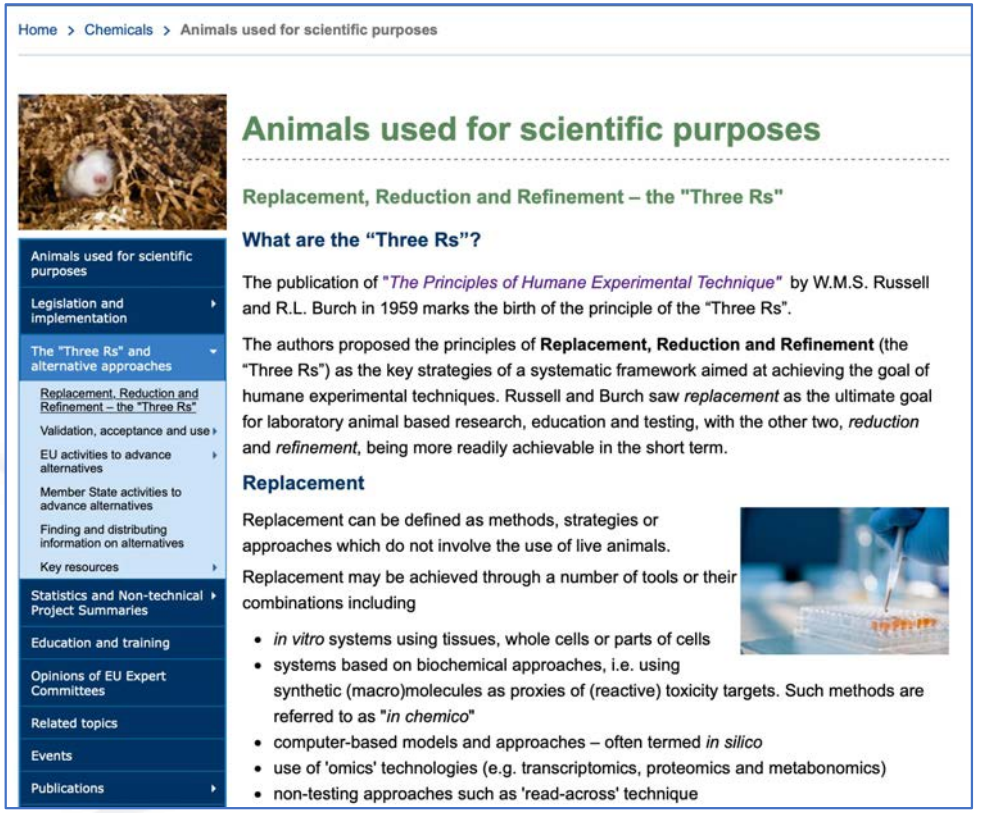
- contingent inhumanity: the side-effects of a procedure that are not necessary for its success
  e.g. poor housing, care, handling, analgesia

colourbox.com

Pain and suffering is experienced at the level of the individual

Is it Replacement or Refinement?

Those working under the EU Directive 2010/63/EU should study the definitions of Replacement, Reduction and Refinement on the EU Commission website



ec.europa.eu/environment/chemicals/lab_animals/3r/alternative_en.htm

Discrimination and fidelity

Russell & Burch warned against the '**high-fidelity fallacy**':

the false assumption that high-fidelity dictates which model is best.

High-fidelity '*ignores all the advantages of **correlation***', whereby '*the responses of two utterly different systems may be correlated with perfect regularity*'

Russell & Burch (1959)

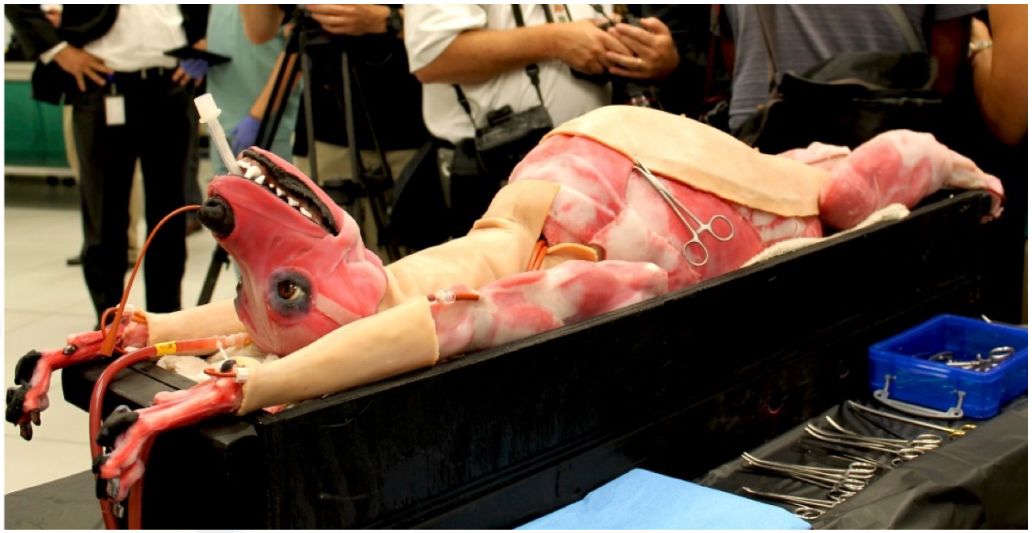
Replacement alternatives do not have to look like an animal!
e.g. cell and tissue cultures, bacterial and chemical assay systems

Discrimination and fidelity

In educational and training aids:

Rikke Langebæk

High discrimination



syndaver.com

High fidelity

norecopa.no/media/8099/langebæk.pdf

Interest in the 3RS

- *A largely unknown concept for the first 20 years*
- *1969: The UK organisation FRAME (Fund for Replacement of Medical Experiments) was established, and also worked (independently of UFAW/Russell & Burch) on alternatives*
- *1991: The HSUS (Humane Society of the United States) instigated a Russell and Burch Award*
- *1995: ECVAM, CAAT and FRAME organised a workshop which Russell and Burch both attended*
- *2000: The European Science Foundation 'strongly endorses the principles of the Three Rs'*

FRAME

Rex Burch & William Russell in Sheringham, UK, in 1995

journals.sagepub.com/doi/abs/10.1177/026119299502300614

Interest in the 3RS

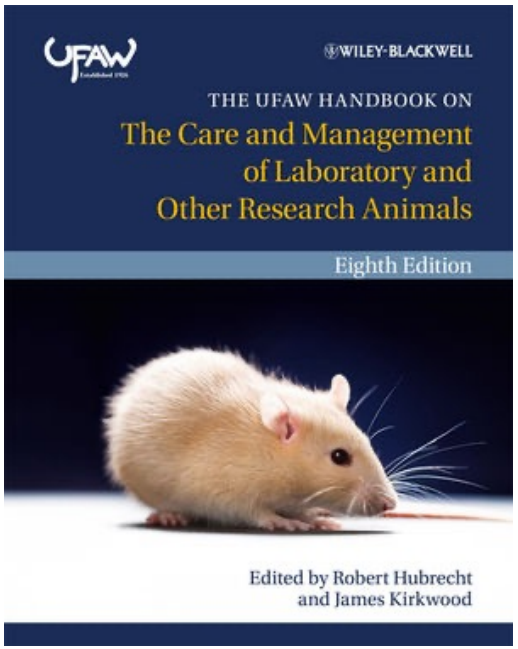
UFAW continued to update its *Handbook on the Care and Management of Laboratory and Other Research animals* (first published in 1947, 9th edition in 2023)

1986: The European Directive 86/609/EEC did not explicitly mention the 3Rs but it required member states to implement national legislation which effectively implemented them

1991: ECVAM (European Centre for the Validation of Alternative Methods) was established

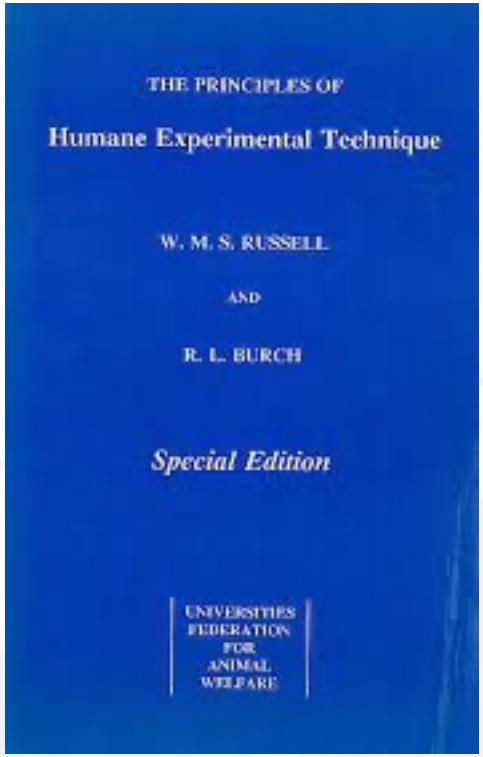
1993: A series of *World Congresses on Alternatives and Animal Use in the Life Sciences* was started in Baltimore

2010: EU legislation mentioned the 3Rs specifically for the first time in Directive 2010/63/EU. ECVAM became EURL-ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing)



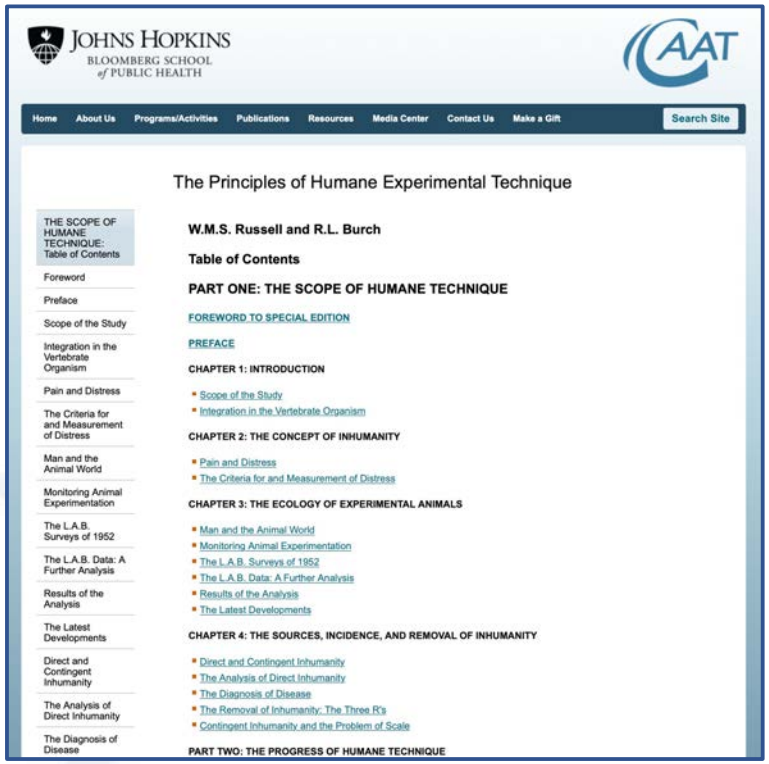
joint-research-centre.ec.europa.eu/eu-reference-laboratory-alternatives-animal-testing-eurl-ecvam/eurl-ecvam-faqs/frequently-asked-questions-general_en

Reprinted by UFAW in 1992



norecopa.no/textbase/the-principles-of-humane-experimental-technique

The text of the book is available online



caat.jhsph.edu/principles/the-principles-of-humane-experimental-technique

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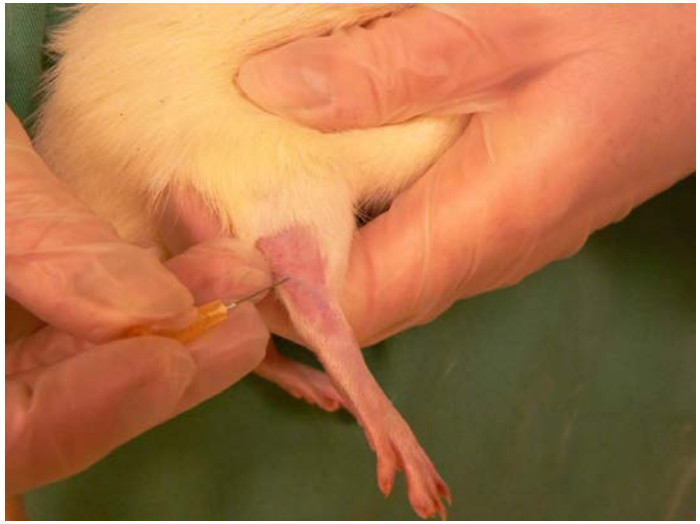
Why are the 3Rs important?

- in many countries they are now part of the legislation to protect animals and improve science quality
- they encourage discussion while a study which appears to need animals is being planned
- they are a tool to achieve ethically defensible animal studies
- they advance the implementation of replacement techniques
- they increase public understanding of the need for animal research and testing



norecopa.no/norina/blood-collection-in-mice-using-the-saphenous-vein-an-alternative-to-retro-orbital-collection

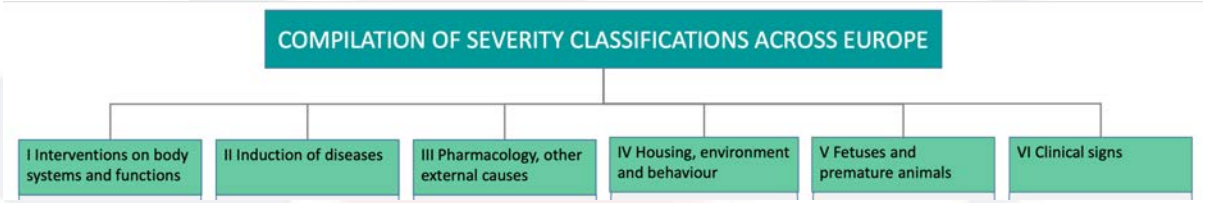
NMBU

Other issues to be aware of

- Re-use of animals in new experiments may be allowed, but their welfare depends upon both their experiences and memories from the first study, and the likely suffering in the second. Cumulative suffering may become excessive:

  e.g. *Mild+Mild+Mild can be Moderate or Severe*

- Guidelines for severity classification of procedures vary, and individuals may differ in their opinions.



norecopa.no/severity

focusonseveresuffering.co.uk

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Summary

Animal experiments must only be performed when

- ✓ the scientific objectives are timely, of sufficient importance, attainable, and maximise scientific and societal benefits;
- ✓ there are no non-sentient replacement alternatives;
- ✓ all relevant and practical Reduction and Refinement strategies have been implemented;
- ✓ the design and conduct of the study minimise the animal welfare cost in terms of the total pain, suffering and distress that may be produced, rather than simply minimising the number of animals used.

Norecopa: *PREPARE for better Science*

Smith & Richmond (forthcoming)

How to promote 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

Memorable quotes

'best welfare is indeed best science'

'aim at well-being rather than at mere absence of distress'

'The greatest scientific experiments have always been the most humane and most aesthetically attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful'

Russell & Burch, 1959

FRAME

The concept actually predates Russell & Burch

Marshal Hall: Seven principles of physiology (1831 & 1847)

en.wikipedia.org/wiki/Marshall_Hall_(physiologist)

1. *We should never have recourse to experiment in cases which observation can afford us the information required.*
2. *No experiment should be performed without a distinct and definite object, and without the persuasion, after the maturest consideration, that that object will be attained by that experiment, in the form of a real and uncomplicated result.*
3. *We should not needlessly repeat experiments which have already been performed by physiologists of reputation.*
4. *After due consideration that a given experiment is, at once, essential and adequate to the discovery of a truth, it should be instituted with the least possible infliction of suffering.*
5. *Every physiological experiment should be performed under such circumstances as will secure due observation and attestation of its results, and so obviate, as much as possible, the necessity for its repetition.*
6. *Facts should be laid before the public in the simplest, plainest terms. If there be a difference of opinion: '...add such views as may seem nearest the truth. These are neither wholly in accord with one opinion nor another, nor exceedingly at variance with both, ... a thing which may be observed in most controversies, when men seek impartially for truth'. (Celsus, translated from Latin)*
7. *In quoting the opinions of other authors, it should always be in their own words.*

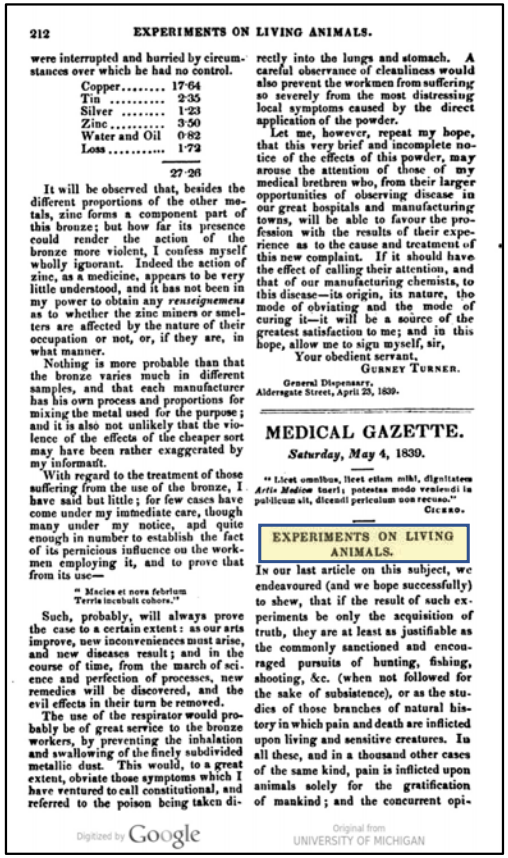
ahajournals.org/doi/epdf/10.1161/01.CIR.48.3.651

The concept actually predates Russell & Burch

Editorial in the London Medical Gazette (1839):

Live animals should not be used

'... till it is sufficiently clear that the fact pursued neither is, nor can be, proved by any other evidence which is within reach, nor by any other more gentle mode of enquiry.'



212 EXPERIMENTS ON LIVING ANIMALS.

were interrupted and hurried by circumstances over which he had no control.

Copper.....	17·64
Tin	2·35
Silver	1·23
Zinc	3·50
Water and Oil	0·82
Loss	1·72
	27·26

It will be observed that, besides the different proportions of the other metals, zinc forms a component part of this bronze; but how far its presence could render the action of the bronze more violent, I confess myself wholly ignorant. Indeed the action of zinc, as a medicine, appears to be very little understood, and it has not been in my power to obtain any *renseignemens* as to whether the zinc miners or smelters are affected by the nature of their occupation or not, or, if they are, in what manner.

Nothing is more probable than that the bronze varies much in different samples, and that each manufacturer has his own process and proportions for mixing the metal used for the purpose; and it is also not unlikely that the violence of the effects of the cheaper sort may have been rather exaggerated by my informant.

With regard to the treatment of those suffering from the use of the bronze, I have said but little; for few cases have come under my immediate care, though many under my notice, and quite enough in number to establish the fact of its pernicious influence on the workmen employing it, and to prove that from its use—

"Mocies et nova febrium
Terris incubuit cohors."

Such, probably, will always prove the case to a certain extent: as our arts improve, new inconveniences must arise, and new diseases result; and in the course of time, from the march of science and perfection of processes, new remedies will be discovered, and the evil effects in their turn be removed.

The use of the respirator would probably be of great service to the bronze workers, by preventing the inhalation and swallowing of the finely subdivided metallic dust. This would, to a great extent, obviate those symptoms which I have ventured to call constitutional, and referred to the poison being taken directly into the lungs and stomach. A careful observance of cleanliness would also prevent the workmen from suffering so severely from the most distressing local symptoms caused by the direct application of the powder.

Let me, however, repeat my hope, that this very brief and incomplete notice of the effects of this powder, may arouse the attention of those of my medical brethren who, from their larger opportunities of observing disease in our great hospitals and manufacturing towns, will be able to favour the profession with the results of their experience as to the cause and treatment of this new complaint. If it should have the effect of calling their attention, and that of our manufacturing chemists, to this disease—its origin, its nature, the mode of obviating and the mode of curing it—it will be a source of the greatest satisfaction to me; and in this hope, allow me to sign myself, sir,

Your obedient servant,
GURNEY TURNER.

General Dispensary,
Aldersgate Street, April 29, 1839.

MEDICAL GAZETTE.
Saturday, May 4, 1839.

"Licet omnibus, licet etiam mihi, dignitatem *Artis Medicæ* tueri; potestas modo veniendi in publicum sit, dicendi periculum non recuso."
CICERO.

EXPERIMENTS ON LIVING ANIMALS.

In our last article on this subject, we endeavoured (and we hope successfully) to shew, that if the result of such experiments be only the acquisition of truth, they are at least as justifiable as the commonly sanctioned and encouraged pursuits of hunting, fishing, shooting, &c. (when not followed for the sake of subsistence), or as the studies of those branches of natural history in which pain and death are inflicted upon living and sensitive creatures. In all these, and in a thousand other cases of the same kind, pain is inflicted upon animals solely for the gratification of mankind; and the concurrent opi-

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babel.hathitrust.org/cgi/pt?id=mdp.39015031214433&view=1up&seq=268

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Smith & Richmond (forthcoming)

Other events since 2000

- A European umbrella organisation for National Consensus Platforms on Alternatives, ***ecopa***

→ ecopa.eu

- Many national and regional centres for one or more of the 3Rs

→ norecopa.no/global3r

- A European network of 3R centres: **EU3Rnet**

→ norecopa.no/3r-guide/eu3rnet

- An EU website with resources about the use of animals for scientific purposes

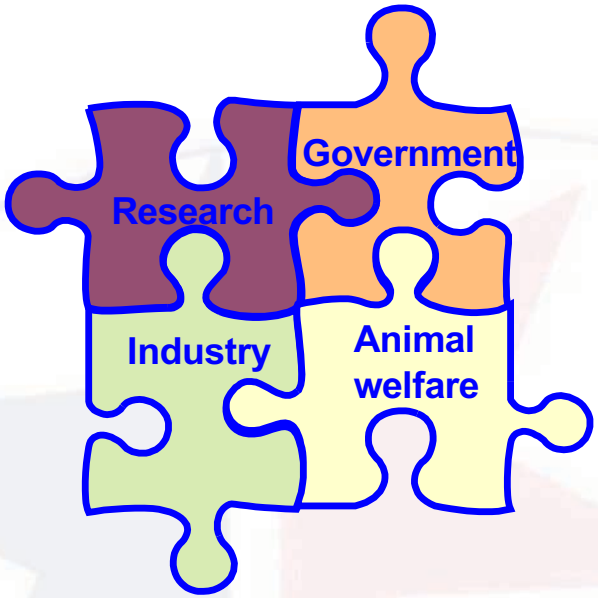
→ European Commission

ec.europa.eu/environment/chemicals/lab_animals/index_en.htm

Neuhaus *et al.* (2022)

ecopa.eu

ecopa recognises 1 National Consensus Platform per country that has representatives of all 4 stakeholders in its governing body:



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norecopa.no/global3r

norecopa.no/3r-guide/ecopa

There are now over 30
3R centres in Europe alone...

norecopa.no/global3r

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*)

**The International Culture of Care Network:* norecopa.no/coc

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

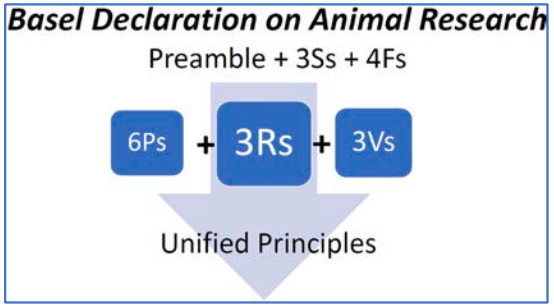
Responsible animal research: a riff of Rs. Rowan A & Goldberg A (1995), *Altern Lab Anim*. 23(3):306-11. doi.org/10.1177/026119299502300307

... and Ss and Vs

- The 3Vs: construct validity, internal validity and external validity (Hanno Würbel)
  Aimed at improving the scientific validity of animal models
  norecopa.no/3V

- The 3Ss: Good Science, Good Sense, Good Sensibilities (Carol Newton)
  Ensuring that common sense and critical anthropomorphism are applied to science
  norecopa.no/3S

  See also Petkov *et al*. (2022) who propose an animal research 'Helsinki Declaration'
  sciencedirect.com/science/article/pii/S2665945X2200033X



The NC3Rs has produced an 18-minute video about the 3Rs

vimeo.com/289645718

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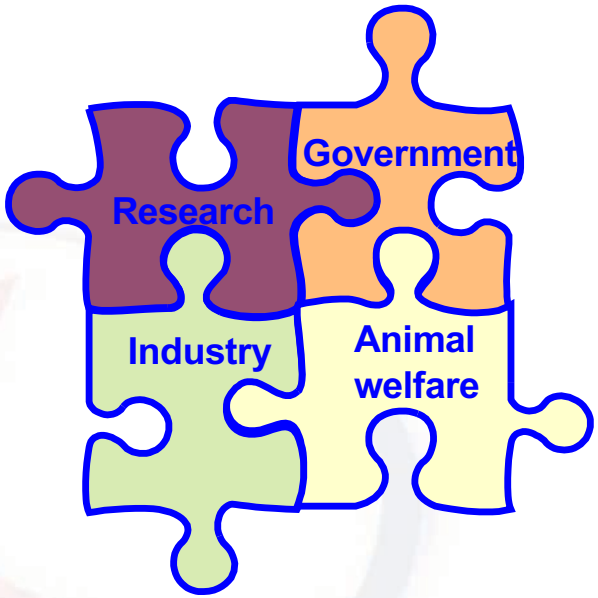
About Norecopa

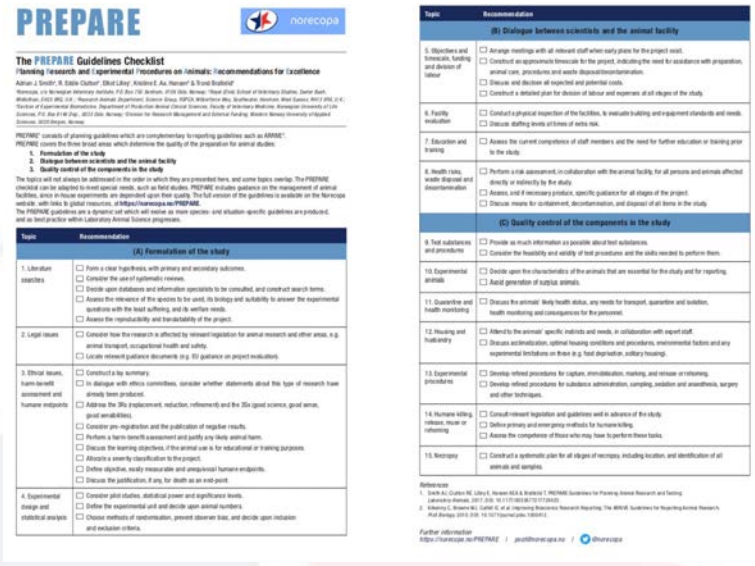
Norecopa is Norway's National Consensus Platform for Replacement, Reduction and Refinement of animal experiments.
Norecopa is an independent member organisation with representatives of 4 major stakeholders in its governing body:



Norecopa maintains a free website with global 3R resources: norecopa.no

PREPARE for animal research

Norecopa's website includes the PREPARE guidelines for planning experiments which may involve the use of animals. PREPARE consists of a checklist (in over 30 languages) and a website with more information about each topic on the checklist.


PREPARE

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The PREPARE Guidelines Checklist

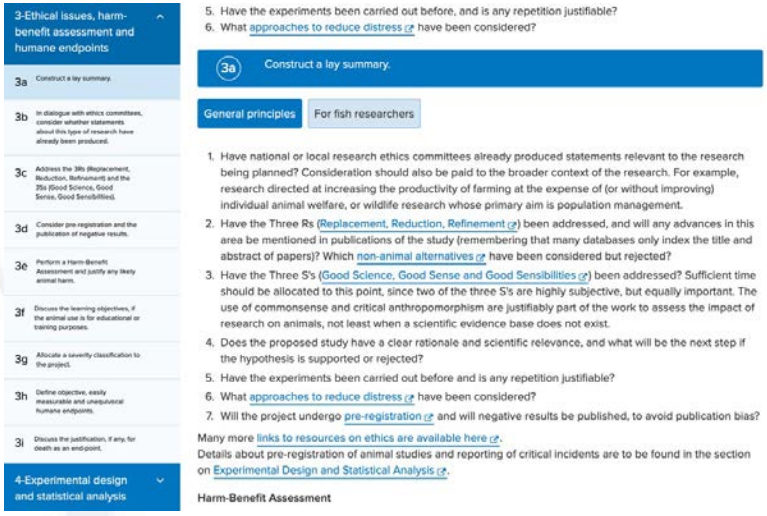
Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

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 3Rs (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.

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 of 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 training	☐ Assess the current competence of staff members and the need for further education or training prior to the study.
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, immobilization, marking, and release or rehoming. ☐ Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, rescue 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

Further information


3-Ethical issues, harm-benefit assessment and humane endpoints

3a Construct a lay summary.

3b In dialogue with ethics committees, consider whether statements about this type of research have already been produced.

3c Address the 3Rs (Replacement, Reduction, Refinement) and the 3Rs (Good Science, Good Sense, Good Sensibilities).

3d Consider pre-registration and the publication of negative results.

3e Perform a Harm-Benefit Assessment and justify any likely animal harm.

3f Discuss the learning objectives, if the animal use is for educational or training purposes.

3g Allocate a severity classification to the project.

3h Define objective, easily measurable and unequivocal humane endpoints.

3i Discuss the justification, if any, for death as an end-point.

4-Experimental design and statistical analysis

5. Have the experiments been carried out before, and is any repetition justifiable?
6. What approaches to reduce distress have been considered?

3a Construct a lay summary.

General principles | For fish researchers

1. Have national or local research ethics committees already produced statements relevant to the research being planned? Consideration should also be paid to the broader context of the research. For example, research directed at increasing the productivity of farming at the expense of (or without improving) individual animal welfare, or wildlife research whose primary aim is population management.
2. Have the Three Rs (Replacement, Reduction, Refinement) been addressed, and will any advances in this area be mentioned in publications of the study (remembering that many databases only index the title and abstract of papers)? Which non-animal alternatives have been considered but rejected?
3. Have the Three S's (Good Science, Good Sense and Good Sensibilities) been addressed? Sufficient time should be allocated to this point, since two of the three S's are highly subjective, but equally important. The use of commonsense and critical anthropomorphism are justifiably part of the work to assess the impact of research on animals, not least when a scientific evidence base does not exist.
4. Does the proposed study have a clear rationale and scientific relevance, and what will be the next step if the hypothesis is supported or rejected?
5. Have the experiments been carried out before and is any repetition justifiable?
6. What approaches to reduce distress have been considered?
7. Will the project undergo pre-registration and will negative results be published, to avoid publication bias?

Many more links to resources on ethics are available here.
Details about pre-registration of animal studies and reporting of critical incidents are to be found in the section on Experimental Design and Statistical Analysis.

Harm-Benefit Assessment

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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 Bratteli[e]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter ... Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, ... [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, ... Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for ... Sciences, 5020 Bergen, Norway.

PRE...
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The to... ... the order in which they are presented here, and some topics overlap. The PREPARE checkl... ... adapted to meet special needs, such as field studies. PREPARE includes guidance on the management of animal facilities, since in-house experiments are dependent upon their quality. The full version of the guidelines is available on the Norecopa website, with links to global resources, at **https://norecopa.no/PREPARE**.

The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.
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Animal welfare and the 3Rs

Topic	Recommendation
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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
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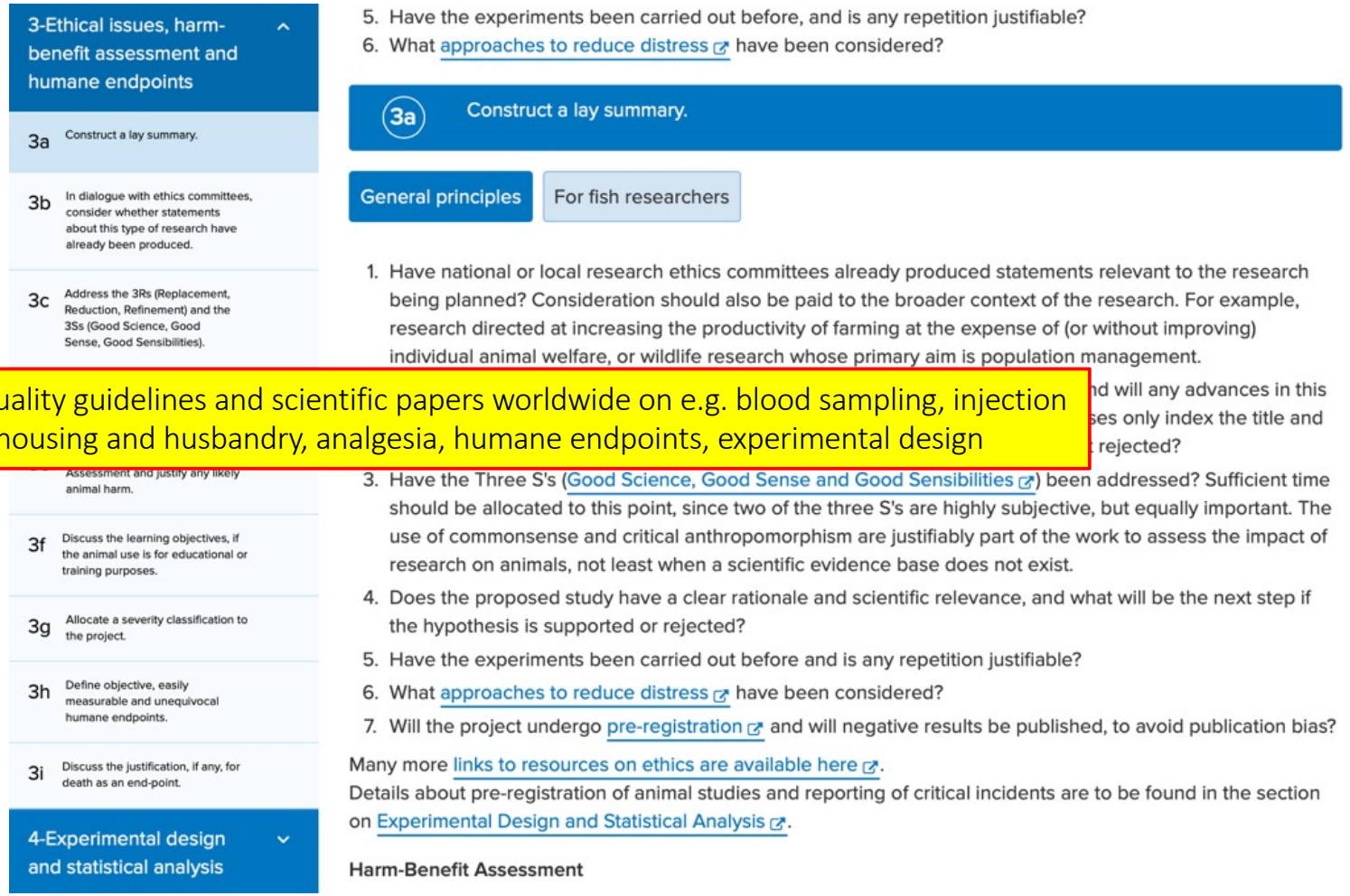
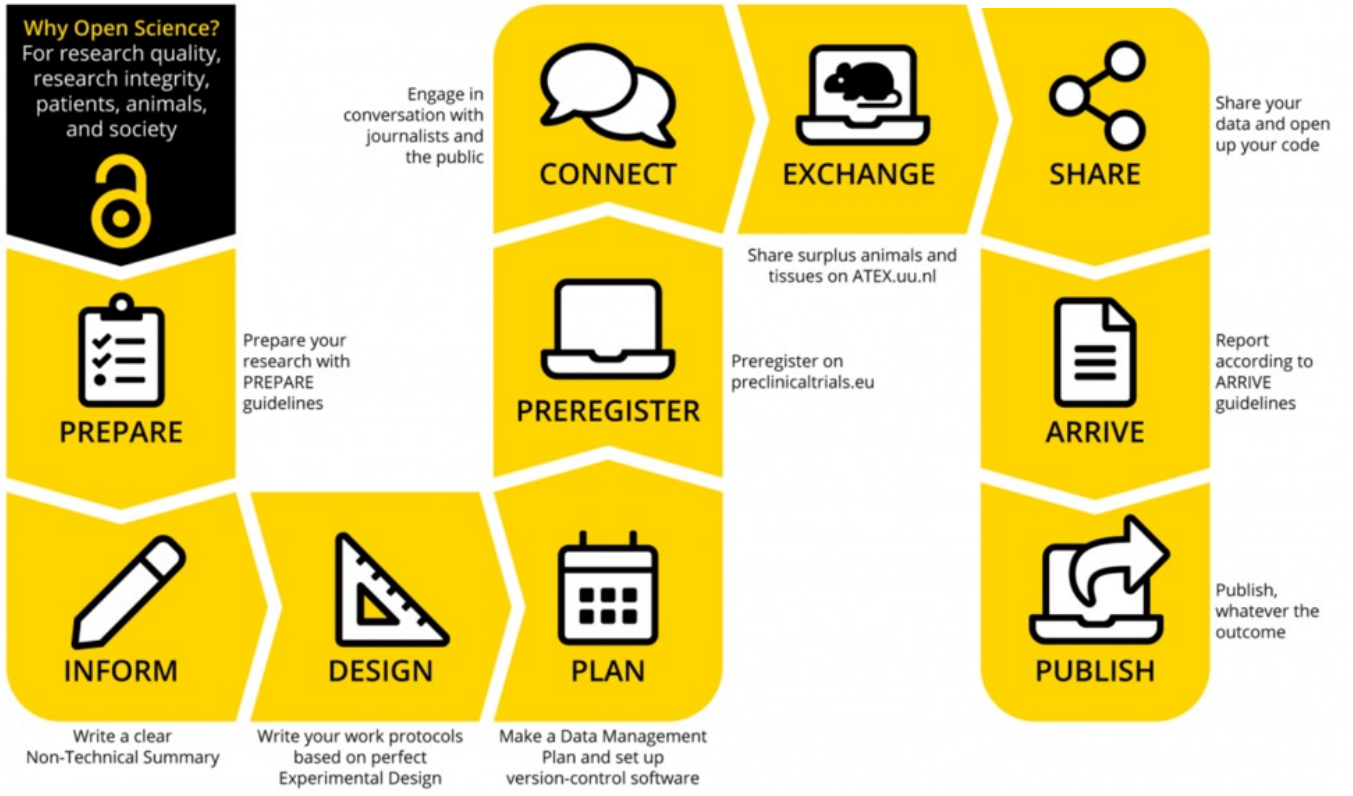


Links to quality guidelines and scientific papers worldwide on e.g. blood sampling, injection volumes, housing and husbandry, analgesia, humane endpoints, experimental design

Norecopa: PREPARE for better Science

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

An overview of 3R centres and associations

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Novo Nordisk
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Sanofi
Scottish Accreditation Board (SAB)
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