The Role of the 3Rs

in Planning Animal Experimentation

Replacement, Reduction and Refinement

norecopa.no/Basel

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Accommodation
Sustainability

The 3Rs explained (Reduce, Reuse, Recycle)

By Sophia Rice, 1st March 2023

In this article, Travelife for Accommodation explains what the 3Rs are and the importance of using them when it comes to managing waste.

The 3Rs of waste management is an initiative that was developed in the early 2000s as a method that helps all of us to reduce the amount of waste we send to landfill or incineration, and to reduce the amount of items being produced unnecessarily. The 3Rs stand for Reduce, Reuse, Recycle. These three small words are pivotal to managing waste and helping to combat climate change.

Reduce means to make smaller/less in amount.

Reuse means to use something again, either for its original purpose or repurposed for a different task.

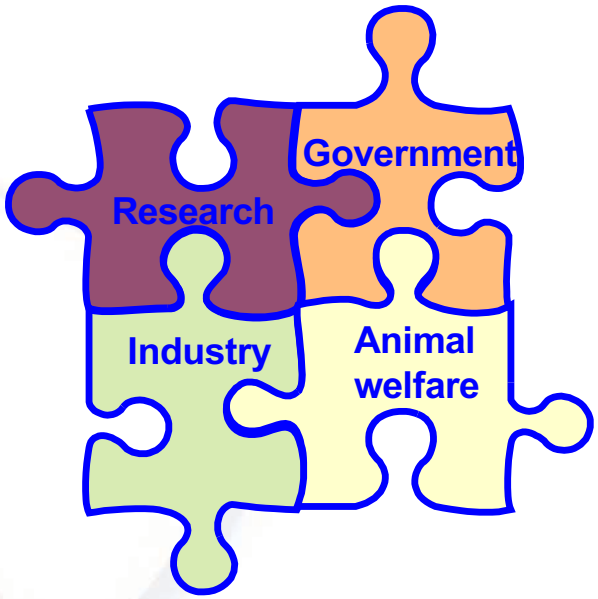
Recycle means to convert waste into material that can be used to remake the item, or to make something else.

https://travelifestaybetter.com/the-3rs-explained-reduce-reuse-recycle

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



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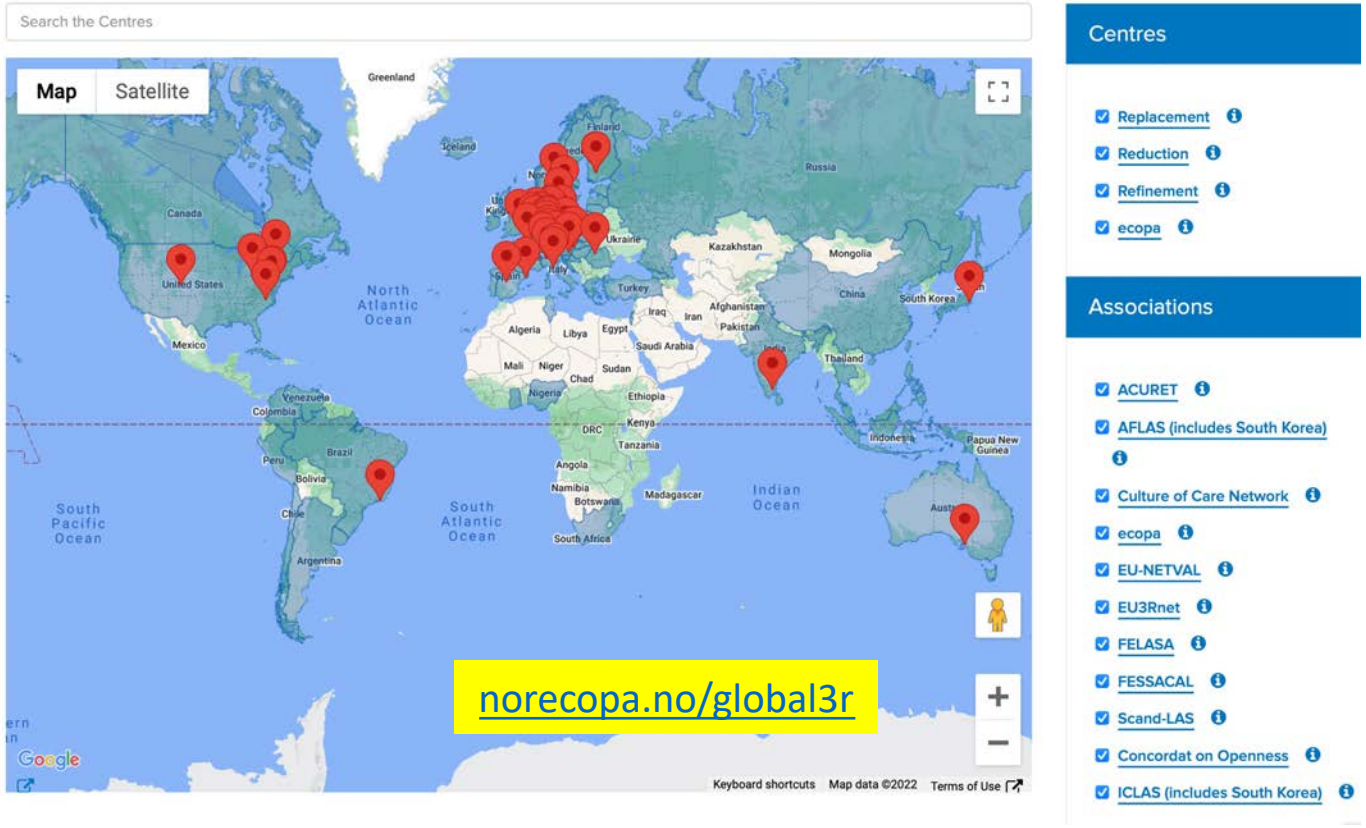
An overview of 3R centres and associations



EU3Rnet, a network of 3R centres: norecopa.no/3r-guide/eu3rnet

ccac.ca

The 3Rs of Russell and Burch:

Replacement, Reduction & Refinement

*A slide deck available at **norecopa.no/3Rs***

With some material from:
Smith AJ & Richmond J (2024). 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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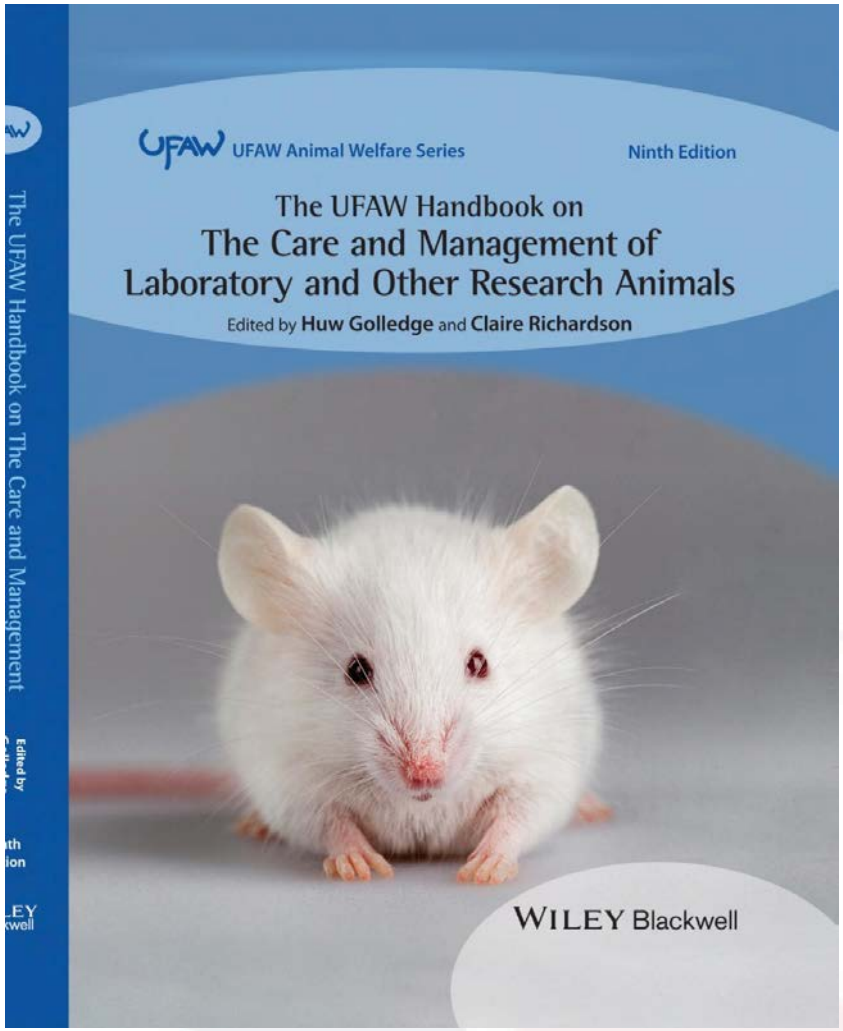

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Due to be published this week.

Google books preview:

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https://norecopa.no/textbase/the-ufaw-handbook-on-the-care-and-management-of-laboratory-and-other-research-animals

Norecopa: *PREPARE for better Science*

Les 3Rs de Russell et Burch:

Remplacement, Réduction & Raffinement

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Ces diapositives sont disponibles sur norecopa.no/3Rs

version 12.03.24

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Las 3Rs de Russell y Burch:

Reemplazo, Reducción y Refinamiento

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Las láminas están disponibles en: norecopa.no/3Rs

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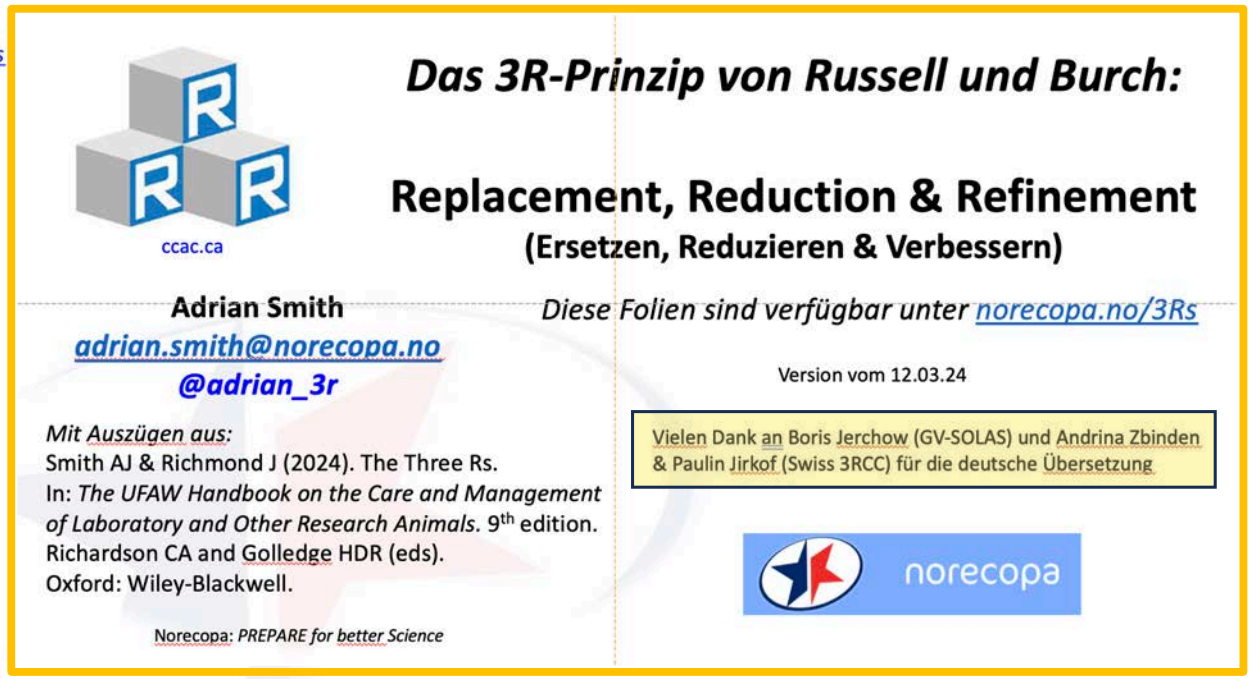

norecopa.no/3Rs

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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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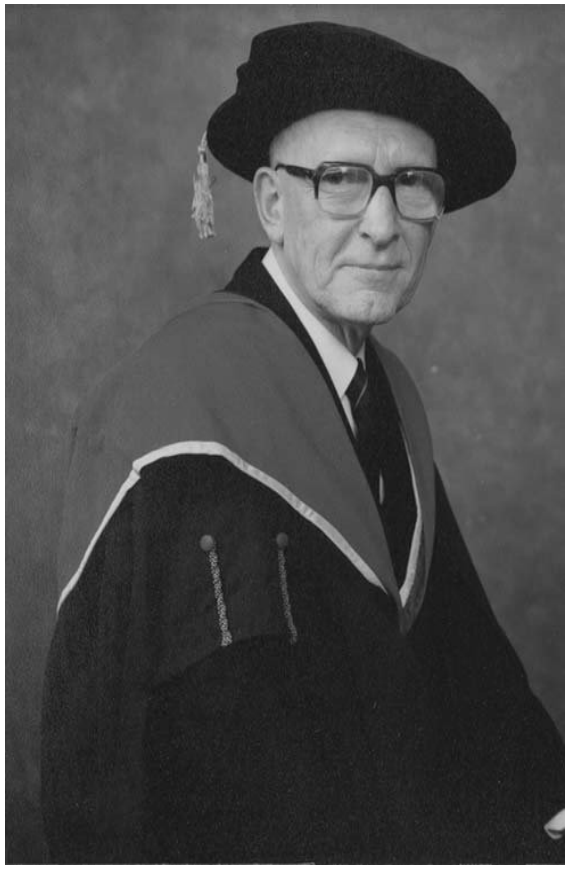
Hubrecht & Carter (2019)

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



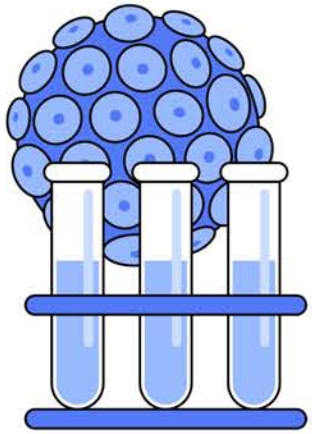
R.L. Burch (1926 - 1996)

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)

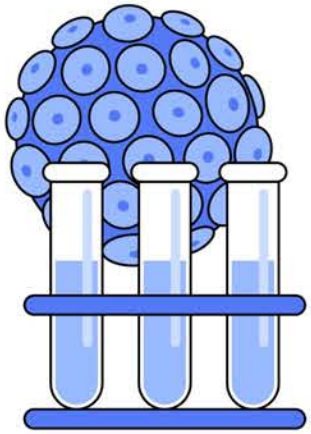
"Alternatives"?

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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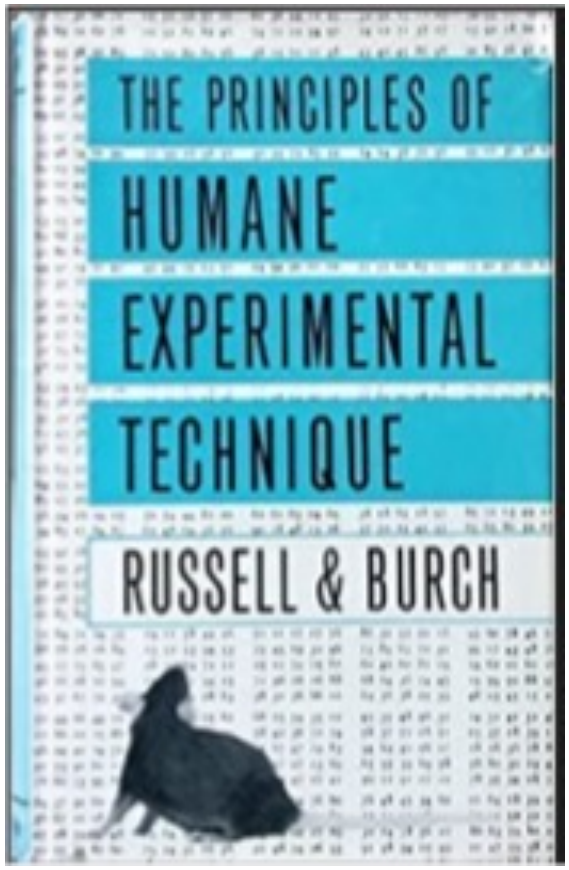
Smyth DH (1978); Russell (2005)

Timeline for the 3Rs

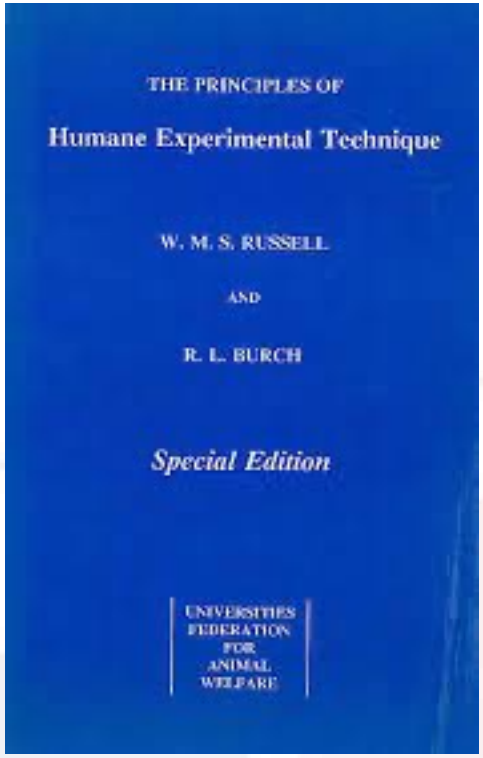
- By 1955, the concept of the 3Rs was essentially present in a paper published by Russell
- The explicit term "The 3Rs" evolved sometime between 1955 and 1957 (Russell, 2005)
- The 3Rs were formally presented at a UFAW Symposium in May 1957 on *Humane Technique in the Laboratory*
- Russell and Burch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



Russell WMS & Burch RL (1959)

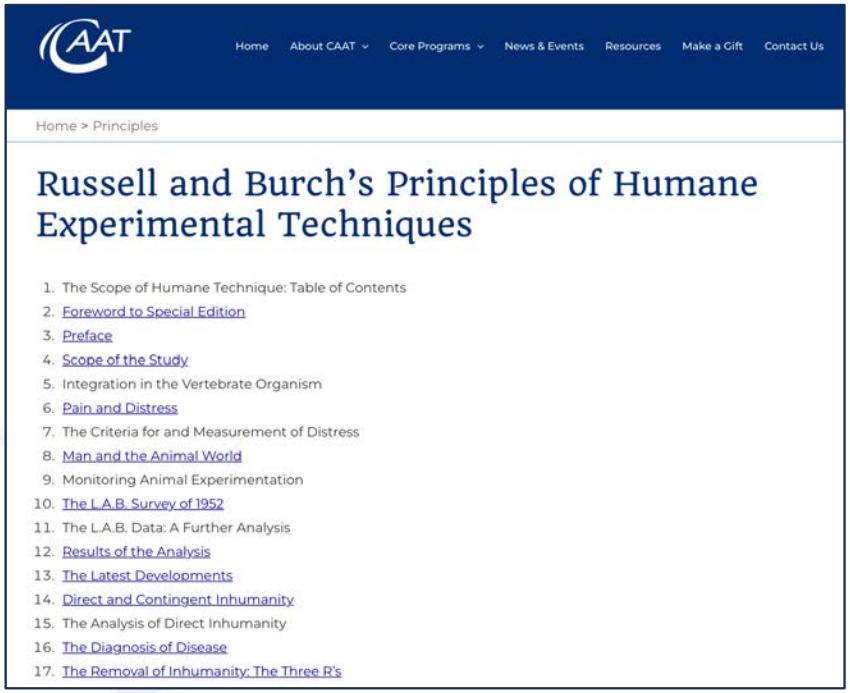
Russell (2005); Hubrecht & Carter (2019)

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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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

The concept actually predates Russell & Burch

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

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.*

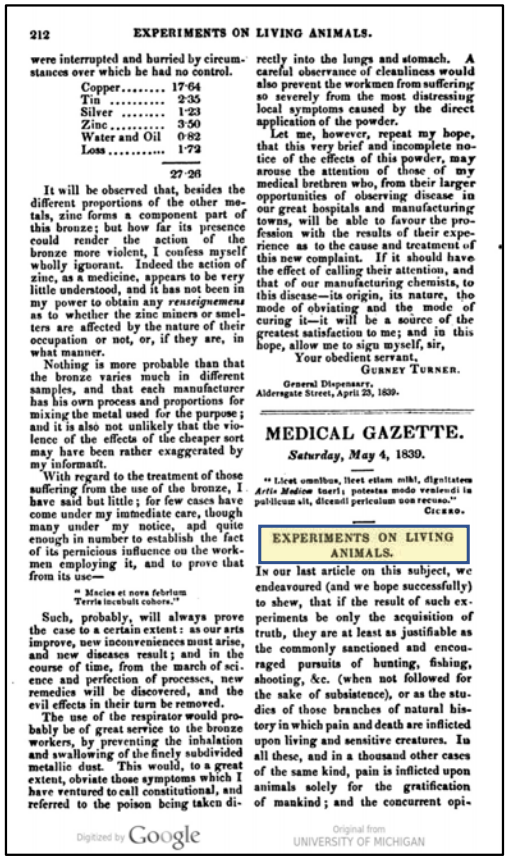
en.wikipedia.org/wiki/Marshall_Hall_(physiologist)

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

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-

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

ccac.ca

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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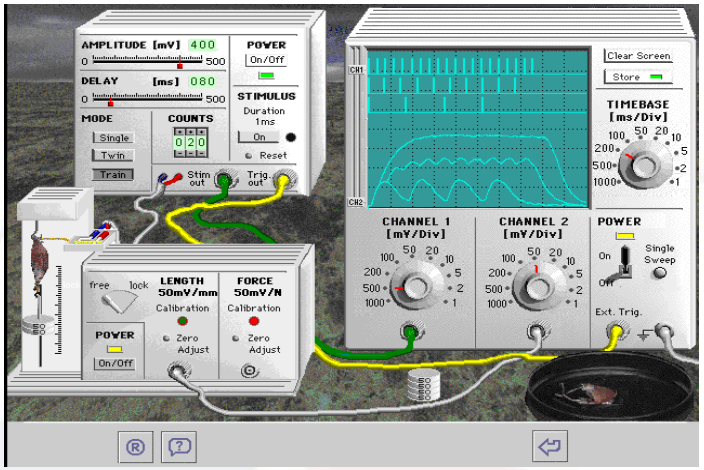
Tannenbaum & Bennett (2015)

Replacement

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

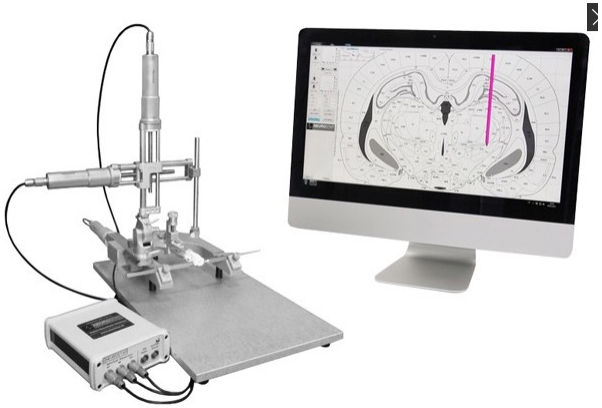
Full/absolute replacement



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

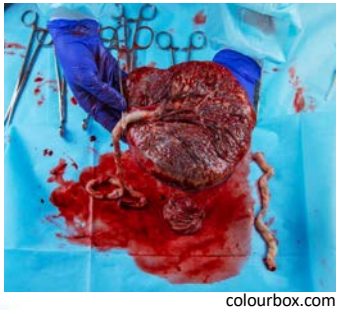
N.B. *Many non-animal methods are not actually being used to replace animal experiments (e.g. use of the human placenta)*

NAMs og NATs

NAMs: New Approach Methodologies (not *Non-Animal Methods!*)

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

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


colourbox.com

	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Structure	xxxxxxxxx	xxxxxxxxx	xxxxxxxxx	xxxxxxxxx
Property 1	● ⇨ ○		● ⇨ ○	
Property 2	● ⇨ ○		○ ⇦ ●	
Property 3	○ ⇦ ●		● ⇨ ○	
Activity 1	● ⇨ ○		● ⇨ ○	
Activity 2	● ⇨ ○		○ ⇦ ●	
Activity 3	○ ⇦ ●		● ⇨ ○	

● Existing data point ○ Missing data point

NATs: Non-Animal Technologies

Alternatives to animal experiments

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

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

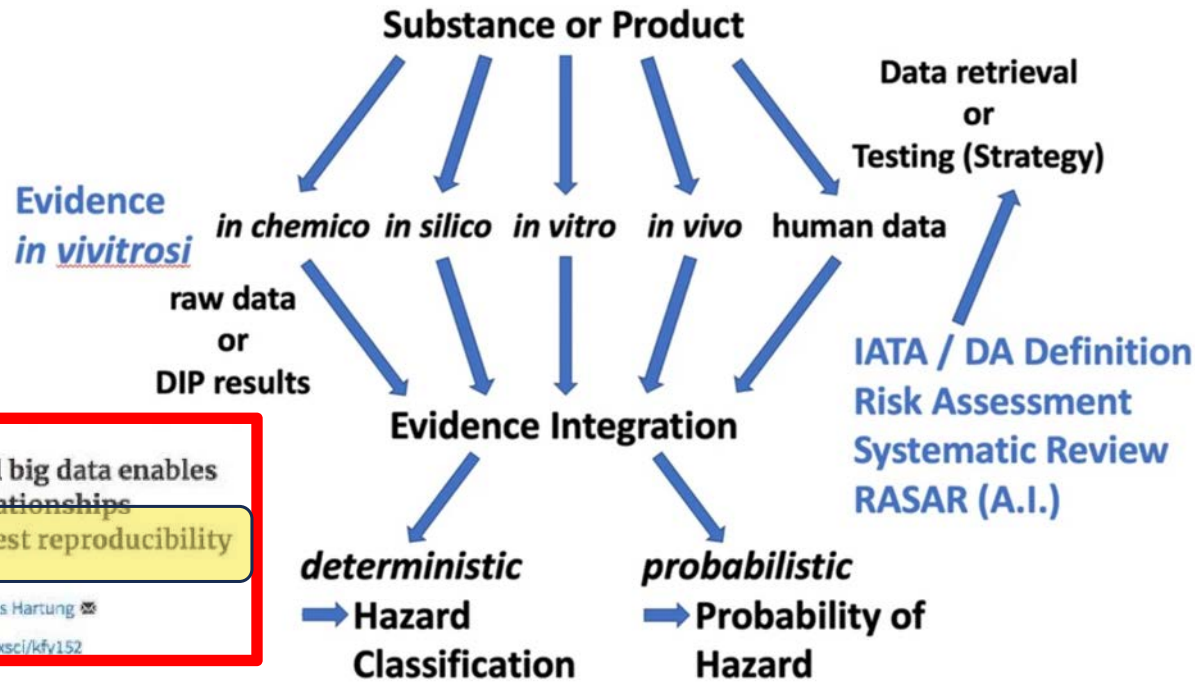
https://nc3rs.org.uk/sites/default/files/documents/NonAnimalTechCO082_RYE_4_nrfinal2.pdf

In vivitrosi

Replacement of animal testing by integrated approaches to testing and assessment (IATA): a call for in vivitrosi

Francesca Caloni[1] · Isabella De Angelis[2] · Thomas Hartung[3,4] Arch Toxicol 2022

Aka Integrated Testing Strategies, IATA, Defined Approaches…



ACCEPTED MANUSCRIPT

Machine learning of toxicological big data enables read-across structure activity relationships (RASAR) outperforming animal test reproducibility

Thomas Luechtefeld, Dan Marsh, Craig Rowlands, Thomas Hartung

Toxicological Sciences, kfy152, https://doi.org/10.1093/toxsci/kfy152

https://link.springer.com/article/10.1007/s00204-022-03299-x
https://webinars.elsevier.com/elsevier/Successful-Alternatives-to-Animal-Testing

Replacement: win-win

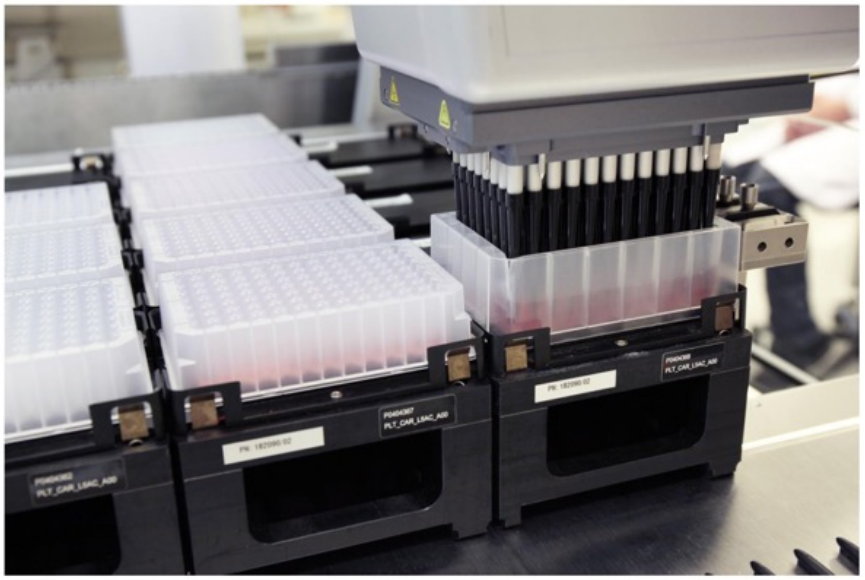
Replacement alternatives are not just substitutes for animal models:

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

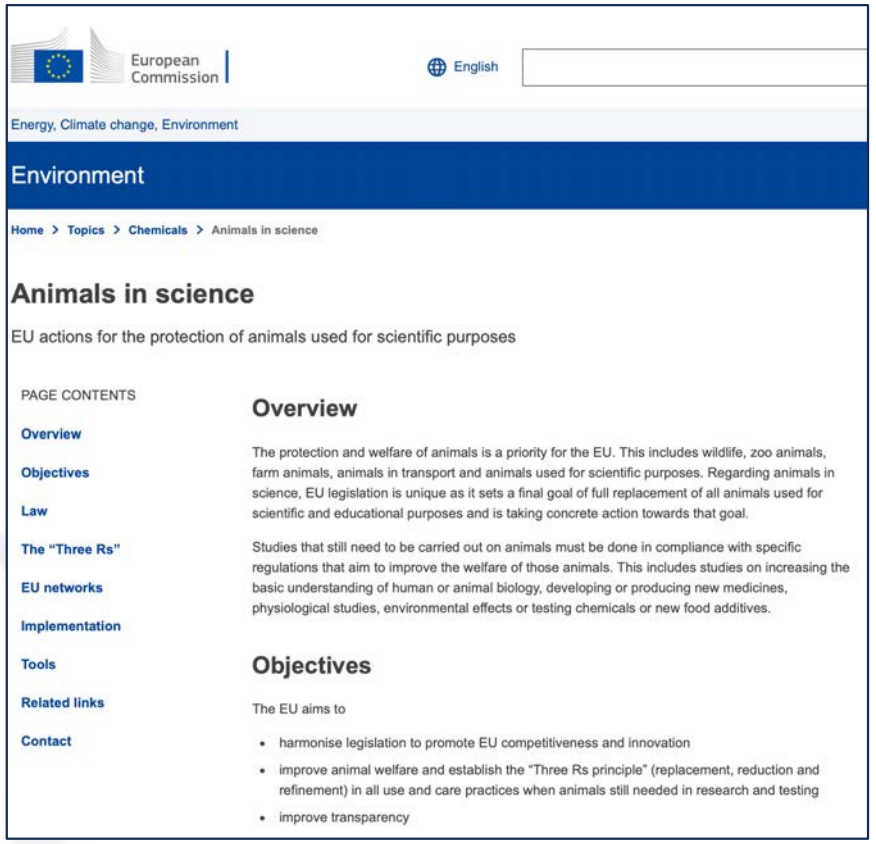
Replacement → Reduction → Refinement

'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

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



environment.ec.europa.eu/topics/chemicals/animals-science_en

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)

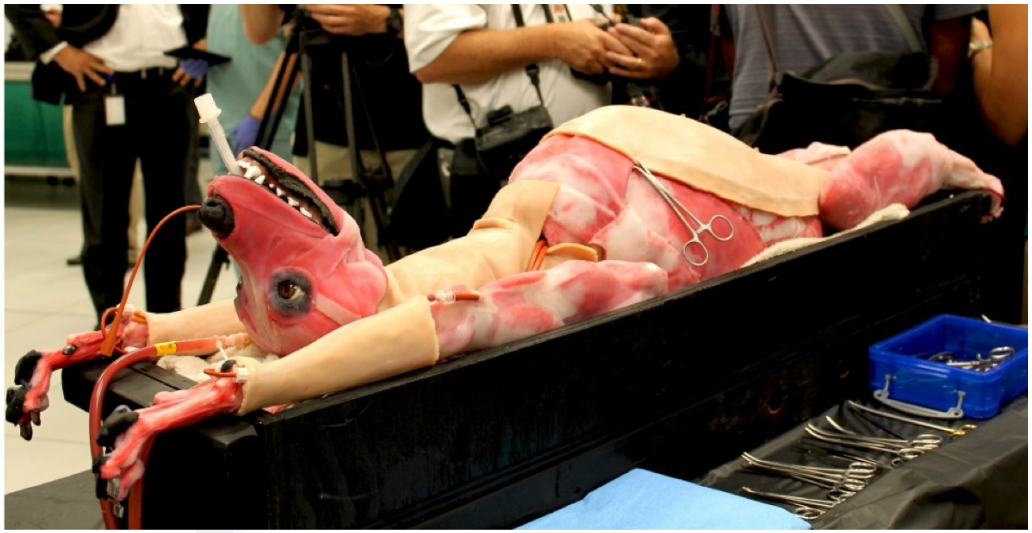
Replacement alternatives do not have to look like an animal!
e.g. cell and tissue cultures, bacterial and chemical assay systems

Discrimination and fidelity

In educational and training aids:

Rikke Langebæk

High discrimination

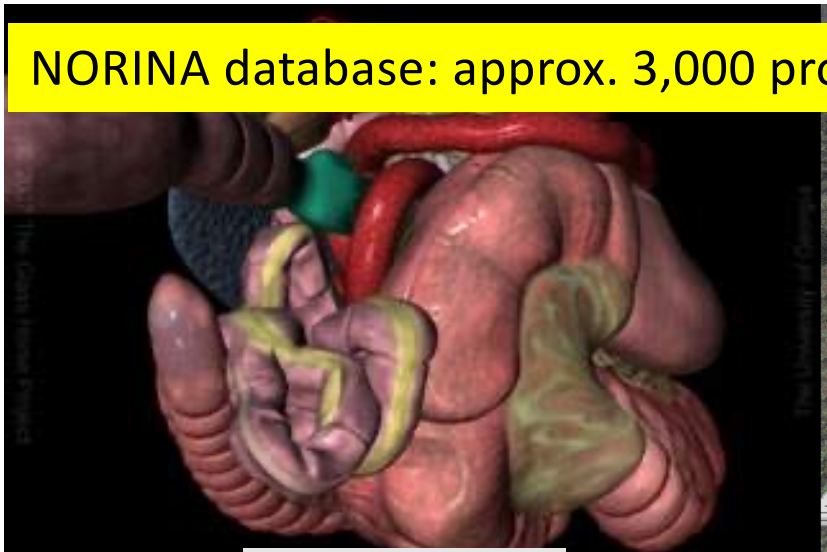


syndaver.com

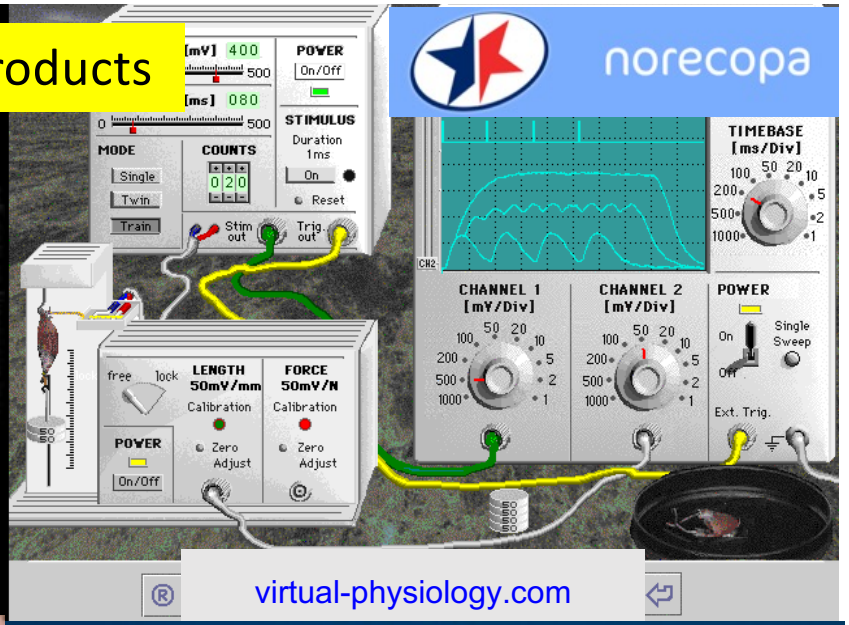
High fidelity

norecopa.no/media/8099/langebæk.pdf

NORINA database: approx. 3,000 products

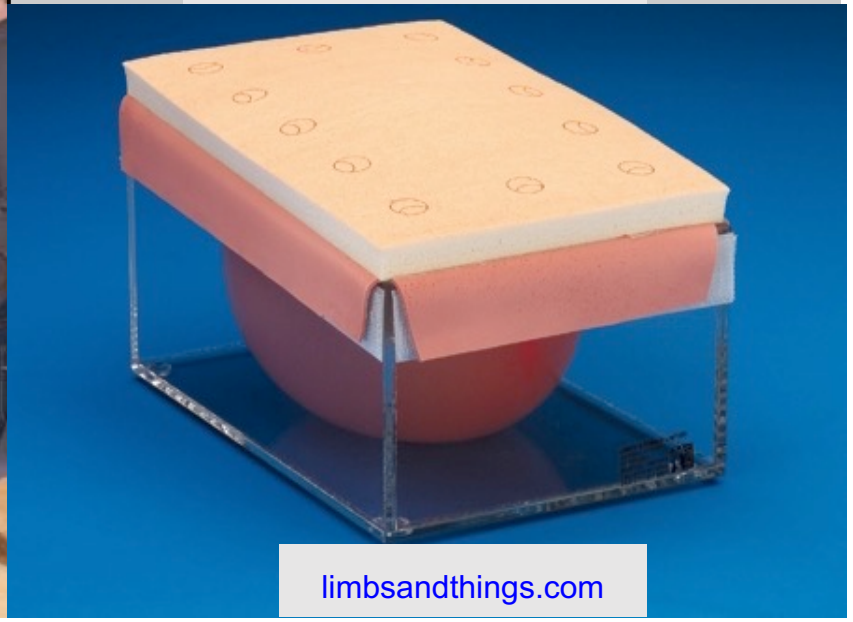


3dglasshorse.com



virtual-physiology.com

rescuecritters.com



limbsandthings.com

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

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 – the next in Rio de Janeiro in August 2025

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

DECLARATION OF BOLOGNA

Reduction, Refinement and Replacement Alternatives in Laboratory Animal Procedures

Adopted by the 3rd World Congress on Alternatives and Animal Use in the Life Sciences – Bologna, Italy – 31 August 1999

The "Three Rs" of Russell and Burch, **reduction, refinement and replacement**, had their origin in a project initiated in 1954 by the Universities Federation for Animal Welfare (UFAW) which led to the publication in 1959 of *The Principles of Humane Experimental Technique*, by W.M.S. Russell and R.L. Burch (1). In 1978, David Smyth used the word **alternatives** to define the "Three Rs" (2).

In their book, Russell and Burch stated that "The greatest scientific achievements have always been the most humane and the most aesthetically attractive, conveying that sense of beauty and elegance which is the essence of science at its most successful." They defined:

Reduction alternatives as methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures, or for obtaining more information from the same number of animals.

Refinement alternatives as methods which alleviate or minimise potential pain, suffering and distress, and which enhance animal well-being.

Replacement alternatives as methods which permit a given purpose to be achieved without conducting experiments or other scientific procedures on animals.

1. Russell, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique*. 238 pp. London: Methuen.
2. Smyth, D. (1978). *Alternatives to Animal Experiments*. 218 pp. London, Scolar Press.

The participants in the 3rd World Congress on Alternatives and Animal Use in the Life Sciences strongly endorse and reaffirm the principles put forward by Russell and Burch in 1959. Humane science is a prerequisite for good science, and is best achieved in relation to laboratory animal procedures by the vigorous promotion and application of the "Three Rs".

The "Three Rs" should serve as a unifying concept, a challenge, and an opportunity for reaping benefits of every kind – scientific, economic and humanitarian.

World Congress on Alternatives, Bologna, 1999

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

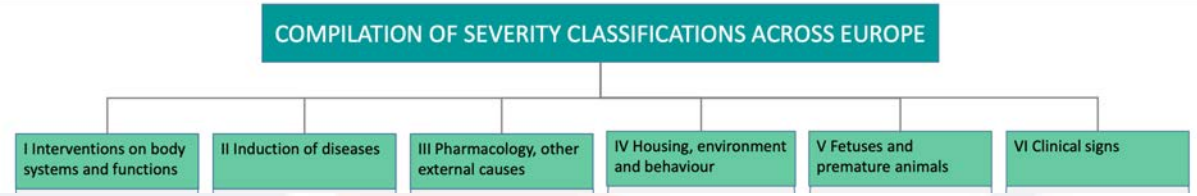
NMBU

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

Anne Zintzsch, Basel

focusonseveresuffering.co.uk

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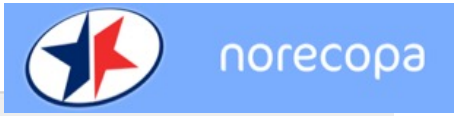
Additional Rs have been proposed...

... 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)



MAX-PLANCK-GESELLSCHAFT

DEUTSCH Search

ABOUT US | RESEARCH | NEWSROOM | CAREER | INTERNATIONAL

Homepage > Viewpoints > Animal Studies > Responsibility > 4R-Principle

Reduce, refine, replace – responsibility

The scientists at the Max Planck Society are committed to keeping the number of animal experiments as well as the strain on the animals as low as possible in the individual experiments. They apply the so-called 3R principle when planning and carrying out the experiments. '3R' stands for "Reduce, Refine, Replace": the number of animals per experiment is reduced to the absolute minimum ('Reduction'); the performance of the experiments and the keeping of the animals optimized in such a way that the burden on the animals is as small as possible ('Refinement'); and animal experiments are replaced by alternative methods, whenever this is possible ('Replacement').

In addition, the researchers of the Max Planck Society commit themselves to a fourth 'R' for "responsibility". They want to use their knowledge in life sciences and humanities to promote animal welfare in their institutes.

To fulfill the fourth R, the MPG commits itself

- to improve the social life of the experimental animals
- develop further the scientific basis for the objective determination of sentience, experience of pain, consciousness and intelligence in the animal kingdom
- proactively engage in professionalizing the public discourse on questions of animal ethics

www.mpg.de/10973438/4rs

REPRODUCIBILITY – IS IT A FOURTH R?

CCAC's Views on Reproducibility, Considerations of Translatability of Animal-Based Science, Maximizing Data Obtained from Animal Experiments, and Reducing Animal Use

DATE OF PUBLICATION: March 2019

ccac.ca/Documents/Publications/CCAC_Reproducibility-Is-it-a-Fourth-R.pdf

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The Three Rs of Animal Research: What they Mean for the Institutional Animal Care and Use Committee and Why

Original Paper | Published: 31 May 2015
Volume 22, pages 549–565, (2016) Cite this article

Howard J. Curzer ✉, Gad Perry, Mark C. Wallace & Dan Perry

To do this, we analyze the underlying logic of the Three Rs and conclude that the Three Rs accord animals moral standing, though not necessarily "rights" in the philosophical sense. We suggest that the Rs are hierarchical, such that Replacement, which can totally eliminate harm, should be considered prior to Reduction, which decreases the number of animals harmed, with Refinement being considered last. We also identify the need for a hitherto implicit fourth R: Reject, which allows the IACUC to refuse permission for a project which does not promise sufficient benefit to offset the pain and distress likely to be caused by the proposed research.

https://link.springer.com/article/10.1007/s11948-015-9659-8

The Universal Ethical Code for Scientists

"Our social licence to operate as scientists needs to be founded on a continually renewed relationship of trust between scientists and society. The code has been developed in my Office to help us meet this challenge."

Sir David King, Government Chief Scientific Adviser and Head of the Government Office for Science

Rigour

Rigour, honesty and integrity

- Act with skill and care in all scientific work. Maintain up to date skills and assist their development in others.
- Take steps to prevent corrupt practices and professional misconduct. Declare conflicts of interest.
- Be alert to the ways in which research derives from and affects the work of other people, and respect the rights and reputations of others.

Respect

Respect for life, the law and the public good

- Ensure that your work is lawful and justified.
- Minimise and justify any adverse effect your work may have on people, animals and the natural environment.

Responsibility

Responsible communication: listening and informing

- Seek to discuss the issues that science raises for society. Listen to the aspirations and concerns of others.
- Do not knowingly mislead, or allow others to be misled, about scientific matters. Present and review scientific evidence, theory or interpretation honestly and accurately.

https://assets.publishing.service.gov.uk/media/5a7cbbffe5274a2f304efc16/universal-ethical-code-scientists.pdf

The Three R's and The 4th One: Reality!

- Regulations and principles often difficult to apply on wild animals
- Studies on free-ranging wildlife are challenging
- Difficult to get a decent sample size of animals with the right sex and age
- Recaptures often not possible
- You never has a real 'control group' (which is the animals you don't capture)
- Your study animals get shot (legally), poached, disperse or 'disappear' (various reasons)
- A very high rate of problems with GPS (coverage, battery life, technical failures, software bugs etc)
- 'End-point' challenge: Are you able to recapture the animals to remove tags and do you have funding for it? Are you allowed to shoot the animals? Do you have collars with a drop-off function?

https://norecopa.no/media/3yyjbo3u/jon-arnemo-260521.pdf

Aryelle Canedo Pereira *et al*. (2022)

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

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

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

ATLA 23, 306–311, 1995 306

Responsible Animal Research: A Riff of Rs

Andrew Rowan[1] and Alan Goldberg[2]

[1]*Tufts School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536, USA;* [2]*Johns Hopkins School of Hygiene and Public Health, 111 Market Place, Baltimore, MD 21202, USA*

Summary — The Three Rs, reduction, refinement and replacement, were introduced in 1959 by Russell & Burch in their important publication *The Principles of Humane Experimental Technique*. These Three Rs are now collectively referred to as alternatives. Using the letter R, we have developed an expanded list of Rs not only to stimulate our own thinking, but to encourage others to examine the issue of alternatives in animal research.

Key words: reduction, refinement, replacement, alternatives.

Introduction

In the last 25 years, public concern regarding the merits of animal use in research and testing has grown, with concurrent support for animal protection organisations. However, the debate and the arguments used by both sides have changed very little from those used 100 years ago. Some animal protection organisations still state that animal use is irrelevant to human medicine. Most state that researchers do not pay sufficient attention to the alleviation of animal suffering, and that animal use desensitises scientists. For their part, scientists counter that their critics are uninformed, naive, sentimental, unrealistic and possibly even misanthropic. However, there is one significant change from a century ago. Today, the debate includes the notion that scientists might be able to continue to do their research without harming or using animals. This concept is identified by the term "alternatives", and has provided animal protection groups with a message to the public that one may well be able to have the best of both worlds, i.e. modern advances without using animals. Researchers have not been quite so enthusiastic, correctly pointing out that alternatives cannot replace all animal use and that biomedical advance would, at the very minimum, be drastically slowed, if animal research were stopped.

Two British scientists, Russell & Burch, are credited with launching the concept of alternatives in 1959, with their book, *The Principles of Humane Experimental Technique* (1), in which they describe the Three Rs of *replacement*, *reduction* and *refinement*, which have come to be identified as the modern concept of alternatives.

A number of authors have suggested that we should not limit ourselves to only three Rs. Harry Rowsell of the Canadian Council for Animal Care (CCAC) has suggested adding three more — *responsibility* and use of the *right animal* for the *right reason* (2). Alison Lukasko, of Bristol-Myers Squibb (Hillside, NJ, USA), suggests that *respect* also be added, and David Morton, at the University of Birmingham (UK), has developed a list of 11 Rs:

reduction of number of animals used
refinement of endpoints
replacement by *in vitro, ex vivo*
recognition of adverse effects
relief with analgesics and anxiolytics
respect for all animals regardless of species
reward for animals wherever possible
refusal to carry out some procedures

This paper is a modification of an article previously published in the Journal of Social Biology and Human Affairs (57, 55–62, 1992).

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Rowan A & Goldberg A (1995)
Altern Lab Anim. 23(3):306-11
doi.org/10.1177/026119299502300307

David Morton

Recognition
Respect
Relief
Respect
Reward
Refusal
Reconsideration
Reflection
Read

Re-education
Resolve
Review

vimeo.com/289645718

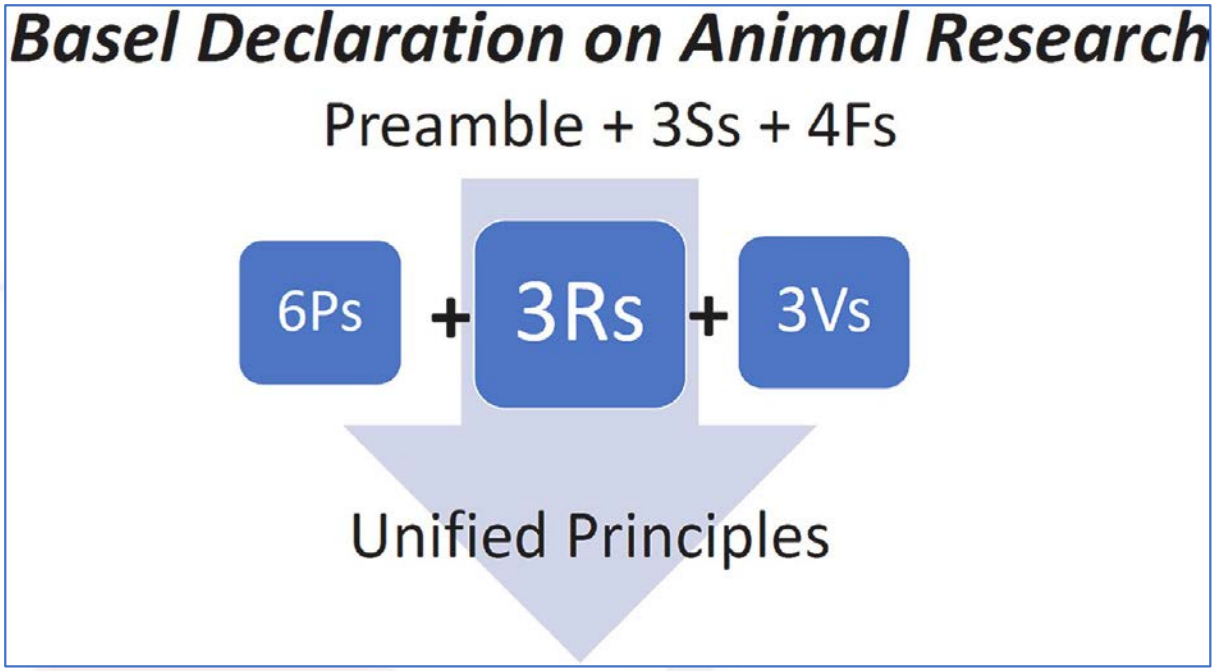
Rowsell H.C. and McWilliam A.A. (1978)

The **r**ight animal for the **r**ight **r**eason

Proceedings of the Canadian Association for Laboratory Animal Science, 1978–1979 Conventions, pp. 211–220.

Toronto, Canada: Canadian Association for Laboratory Animal Science.

Petkov *et al*. (2022) propose an animal research ‘Helsinki Declaration’
sciencedirect.com/science/article/pii/S2665945X2200033X



Basel Declaration for Animal Research (2010) - Fundamental Principles

Fundamental principles; We, the undersigned, shall:

- 1. Respect and protect the animals entrusted to us and not inflict unnecessary pain, suffering, or harm to them by adhering to highest standards of experimental design and animal care.
- 2. Consider carefully whether research involving animals addresses questions of importance that cannot be answered using alternative methods.
- 3. Strive to minimize the number of animals used for research and use the most suitable species to achieve the intended gain of knowledge.
- 4. Encourage collaboration to avoid repetition of animal experiments.
- 5. Implement the highest standards for protection of environment and public health.
- 6. Balance the interests of patients and society with our responsibility towards the animals when developing genetically modified animals.
- 7. Implement the highest standards of education and training for all persons who work with animals and monitor their compliance with standards on a regular basis.
- 8. Adequately recognize the important engagement of scientists in their efforts to promote the public understanding of science.
- 9. Promote the dialogue concerning animal welfare in research by transparent and fact-based communications to the public.
- 10. Provide advice based on scientific knowledge and expertise to political decision makers and government authorities on issues of research involving animals and their welfare.

We, the undersigned,

- 1. Stress that biomedical research cannot be separated into 'basic' and 'applied' research; it is rather a continuum stretching from studies of fundamental physiological processes to an understanding of the principles of disease and the development of therapies.
- 2. Encourage free and transparent communication to avoid unnecessary duplication of research.
- 3. Insist that necessary research involving animals, including non-human primates, be allowed now and in the future.
- 4. Ask that new laws and regulations only be introduced when they are the result of an objective, democratic discourse that is based on facts.
- 5. Request that society and lawmakers condemn the acts of radical groups that resort to unlawful means or violence against the research community under the pretense of animal protection.
- 6. Invite representatives of animal welfare organizations to discuss openly all important issues with the research community.
- 7. Encourage efforts to increase science education in public schools.
- 8. Ask opinion makers, the media and teachers to discuss sensitive issues concerning research involving animal research in an impartial manner and to promote a balanced dialogue with researchers.

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Animal activists last summer set fire to the alpine holiday home of Daniel Vasella, then chief executive of pharmaceutical giant Novartis of Basel, Switzerland, in one of relatively few violent attacks on scientists working with animals in German-speaking countries.

nature.com/articles/468742a

Basel Declaration Society,
now called
Animal Research Tomorrow

animalresearchtomorrow.org

4Fs (Fundamental principles)	*1. Biomedical research principle - statement on the need for biomedical research*	Tannenbaum, 2017
	2. Animal research principle - statement on the requirement for the animal research	
	3. Medical research with human subjects - statement on the limitations of human research	
	4. Animal research aims - minimize pain or distress to the animals	
6Ps (Principles)	*Principle 1 - no alternative method*	Beauchamp and DeGrazia, 2019
	Principle 2 - expected net benefit	
	Principle 3 - sufficient value to justify harm	
	Principle 4 - no unnecessary harm	
	Principle 5 - animal basic needs	
	Principle 6 - upper limits to harm	

PREPARE and ARRIVE guidelines: One area that may be lacking with the current sets of principles is that they do not have explicit principles or guidance on how animal research should be prepared and reported. Such tenets that directly address the likelihood of reproducibility of scientific research are necessary. The issue of how animal research can be planned, which includes online data analysis tools, is addressed in the PREPARE guidelines (Smith et al., 2018) and guidance is provided in the NC3Rs Experimental Design Assistant (https://www.nc3rs.org.uk/our-portfolio/experimental-design-assistant-eda#publications) (Laber et al., 2016). Guidance on how to consistently report research with nonhuman animals in publications can be found in the revised ARRIVE 2.0 principles (Percie du Sert et al., 2020). Also see Table 3 and the AAALAC reproducibility guidance (https://www.aaalac.org/accreditation-program/faqs/#Reproducibility).

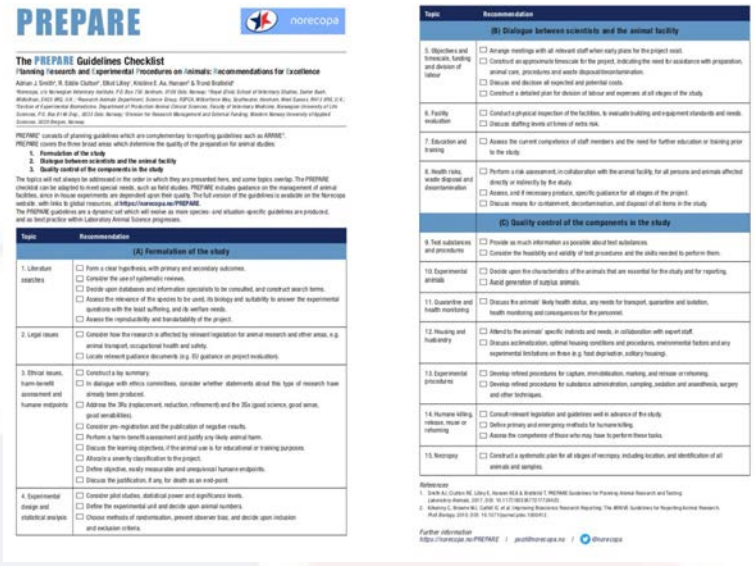
The pathway to better science...

Norecopa: PREPARE for better Science

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

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

norecopa

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.

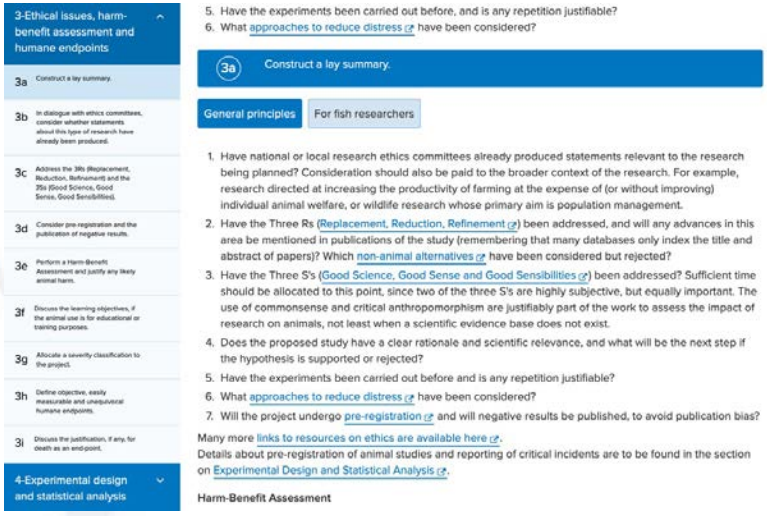
Topic	Recommendation
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance 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

https://norecopa.no/PREPARE / pet@norecopa.no / @norecopa

norecopa.no/PREPARE/prepare-checklist


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

norecopa.no/PREPARE

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norecopa.no/PREPARE/prepare-checklist

norecopa

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]

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PREPARE[1] består av retningslinjer for planlegging av dyreforsøk. Disse
som f.eks. ARRIVE[2]. PREPARE dekker de tre store områdene som beste

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 pr
PREPARE-sjekklisten kan endres for å ivareta spesielle behov, f.eks. ve
dyreavdelinger, fordi laboratorieforsøk er helt avhengige av deres kvalif
Norecopas nettsider, med lenker til globale ressurser, på **https://norec**
PREPARE-retningslinjene er et dynamisk sett som vil videreutvikles ette
produseres, og etterhvert som "best praksis" innenfor forsøksdyrmiljøet forbedres.

Tema	Anbefaling
(A) Designet av studiet	
1. Litteratursøk	☐ Formulere en klar hypotese, med primære og sekundære mål. ☐ Vurdere å foreta en systematisk undersøkelse av litteraturen (Systematic Review). ☐ Bestemme hvilke databaser og informasjonsspesialister som skal brukes, og konstruere søkebegrep. ☐ Vurdere relevansen av dyrearten som skal brukes, dens biologi og egnethet til å svare på de eksperimentelle spørsmålene med minst mulig lidelse, og artens velferdsbehov. ☐ Evaluere prosjektets reproduserbarhet og overførbarhet.
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.

+ 2 online versions
35 languages

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.
	fasilitetene, for å evaluere bygningsmassen, standarden på utstyret og
	ved perioder med ekstra risiko.
	tansen hos personalet og evaluere behovet for videreutdanning og
	en, foreta en risikoevaluering som omfatter alle personene og dyrene direkte, av studiet. dusere, spesifikke retningslinjer for alle stadiene av prosjektet. , dekontaminere og avhende alt utstyret som skal brukes i studiet.
(C) Kvalitetskontroll av de ulike komponentene i studiet	
9. Testsubstanser og -prosedyrer	☐ Oppgi så mye informasjon som mulig om testsubstansene. ☐ Evaluere gjennomførbarheten og validiteten av testprosedyrene, og de praktiske ferdighetene som er nødvendige for å gjennomføre dem.
10. Forsøksdyr	☐ Bestemme egenskapene til dyrene som er essensielle for studiet og som må rapporteres. ☐ Unngå produksjon av overskuddsdyr.
11. Karantene og helsemonitorering	☐ Diskutere dyrenes sannsynlige helsestatus, og eventuelle behov for transport, karantene og isolasjon, samt helsemonitorering og konsekvensene for personalet.
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

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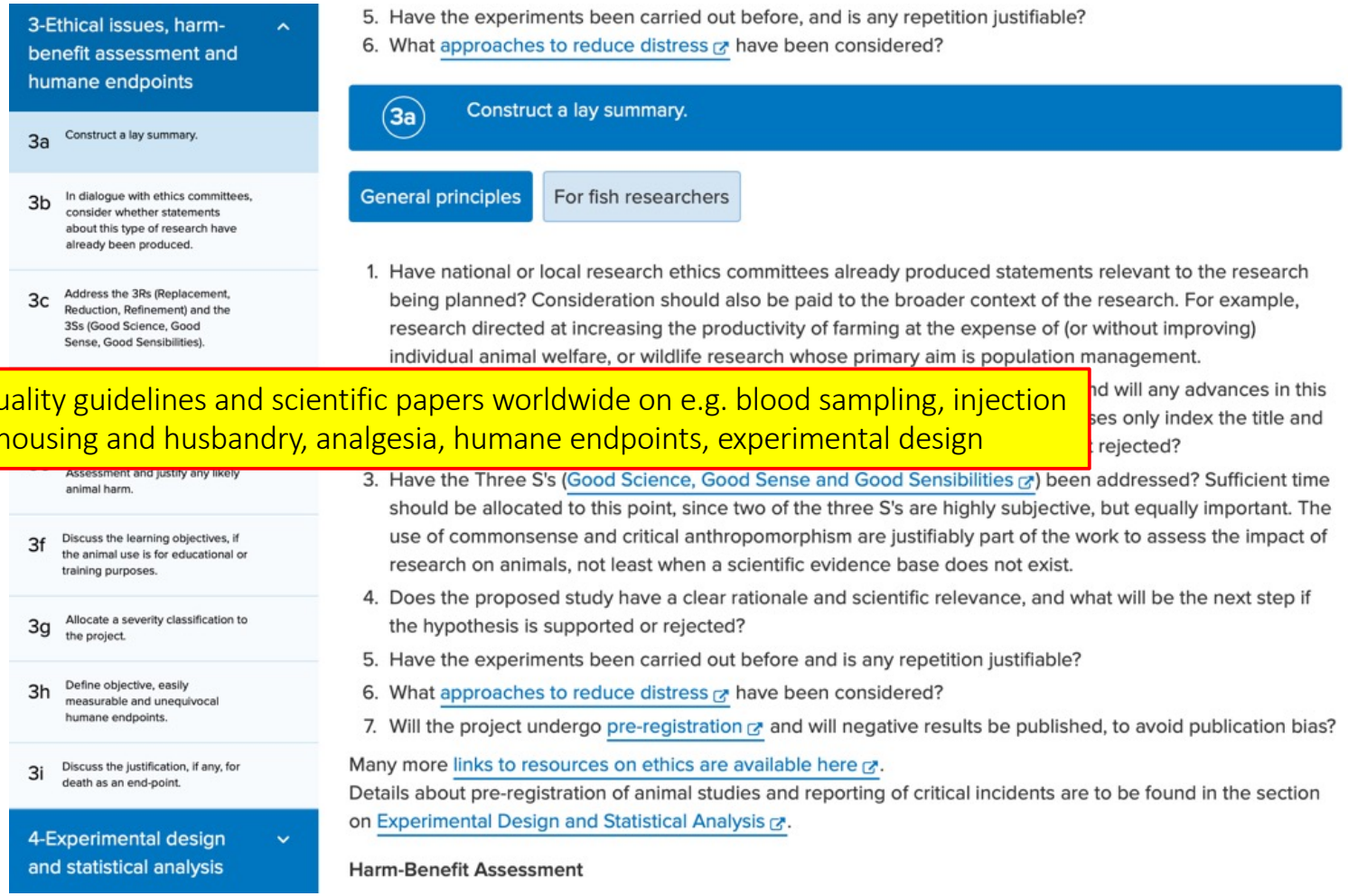

Links to quality guidelines and scientific papers worldwide on e.g. blood sampling, injection volumes, housing and husbandry, analgesia, humane endpoints, experimental design

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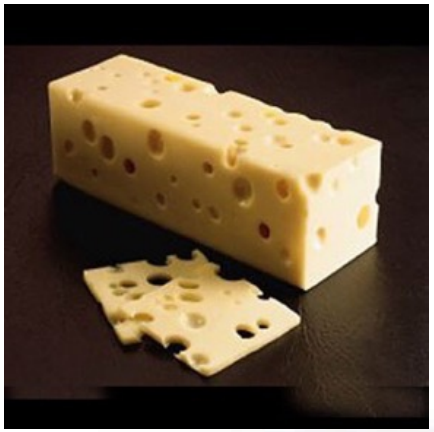
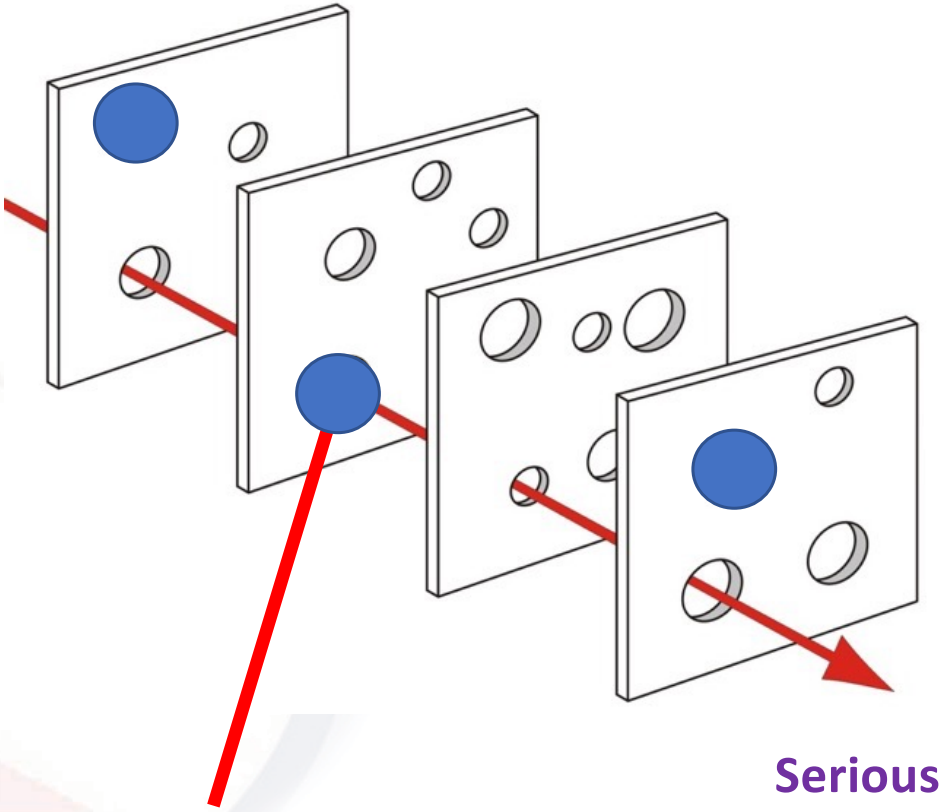
“Threat and Error Management”



eaugallecheese.com/Swiss-Cheese

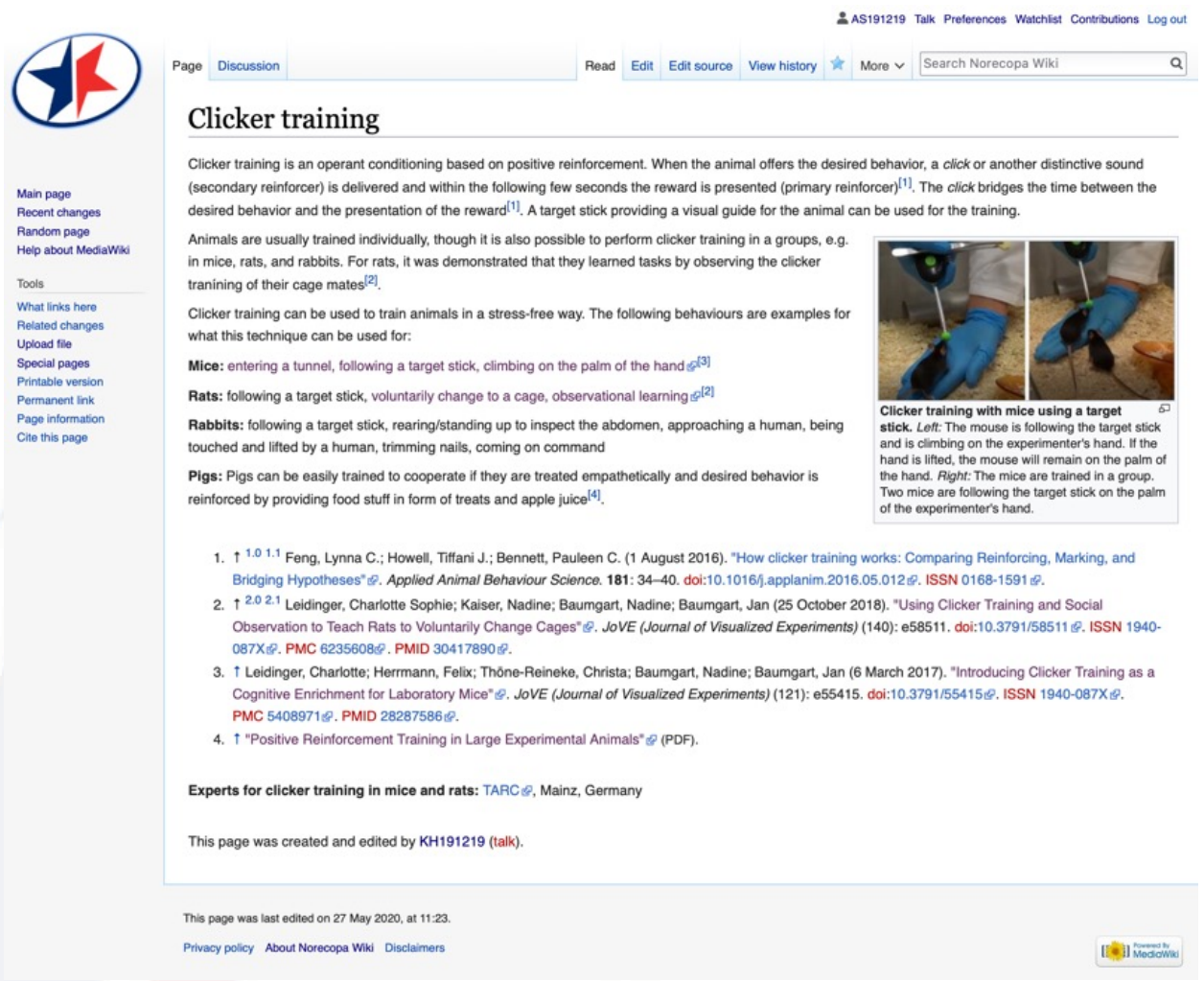


Weaknesses / dangers

Serious incidents

wikipedia.org/wiki/Swiss_cheese_model

Refinement Wiki: wiki.norecopa.no



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Clicker training

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Clicker training is an operant conditioning based on positive reinforcement. When the animal offers the desired behavior, a *click* or another distinctive sound (secondary reinforcer) is delivered and within the following few seconds the reward is presented (primary reinforcer)[1]. The *click* bridges the time between the desired behavior and the presentation of the reward[1]. A target stick providing a visual guide for the animal can be used for the training.

Animals are usually trained individually, though it is also possible to perform clicker training in a groups, e.g. in mice, rats, and rabbits. For rats, it was demonstrated that they learned tasks by observing the clicker tranining of their cage mates[2].

Clicker training can be used to train animals in a stress-free way. The following behaviours are examples for what this technique can be used for:

Mice: entering a tunnel, following a target stick, climbing on the palm of the hand[3]

Rats: following a target stick, voluntarily change to a cage, observational learning[2]

Rabbits: following a target stick, rearing/standing up to inspect the abdomen, approaching a human, being touched and lifted by a human, trimming nails, coming on command

Pigs: Pigs can be easily trained to cooperate if they are treated empathetically and desired behavior is reinforced by providing food stuff in form of treats and apple juice[4].

Clicker training with mice using a target stick. *Left:* The mouse is following the target stick and is climbing on the experimenter's hand. If the hand is lifted, the mouse will remain on the palm of the hand. *Right:* The mice are trained in a group. Two mice are following the target stick on the palm of the experimenter's hand.

1. ↑ 1.0 1.1 Feng, Lynna C.; Howell, Tiffani J.; Bennett, Pauleen C. (1 August 2016). "How clicker training works: Comparing Reinforcing, Marking, and Bridging Hypotheses". *Applied Animal Behaviour Science*. **181**: 34–40. doi:10.1016/j.applanim.2016.05.012. ISSN 0168-1591.
2. ↑ 2.0 2.1 Leidinger, Charlotte Sophie; Kaiser, Nadine; Baumgart, Nadine; Baumgart, Jan (25 October 2018). "Using Clicker Training and Social Observation to Teach Rats to Voluntarily Change Cages". *JoVE (Journal of Visualized Experiments)* (140): e58511. doi:10.3791/58511. ISSN 1940-087X. PMC 6235608. PMID 30417890.
3. ↑ Leidinger, Charlotte; Herrmann, Felix; Thöne-Reineke, Christa; Baumgart, Nadine; Baumgart, Jan (6 March 2017). "Introducing Clicker Training as a Cognitive Enrichment for Laboratory Mice". *JoVE (Journal of Visualized Experiments)* (121): e55415. doi:10.3791/55415. ISSN 1940-087X. PMC 5408971. PMID 28287586.
4. ↑ "Positive Reinforcement Training in Large Experimental Animals" (PDF).

Experts for clicker training in mice and rats: TARC, Mainz, Germany

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Norecopa: *PREPARE for better Science*

Norecopa: *PREPARE for better Science*

My easy summary

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

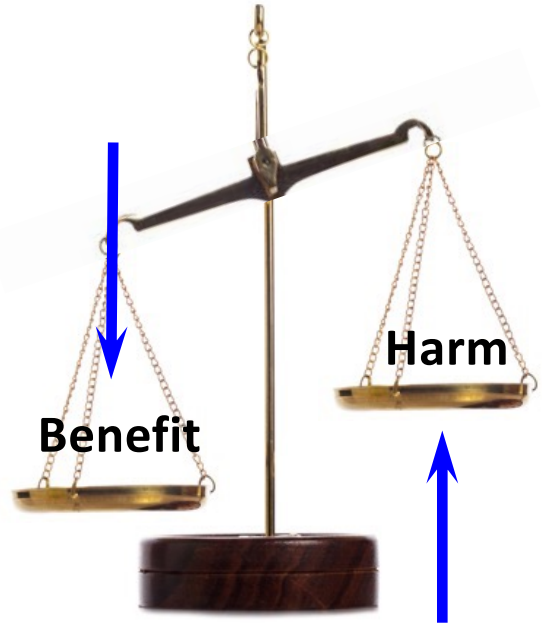
- *Replace the unnecessary experiments*
- *Reduce the number of animals used*
- *Refine the conditions for the animals*

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

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

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

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



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

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

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.

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Smith & Richmond (forthcoming)

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

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1. Hubrecht RC & Carter E (2019): The 3Rs and Humane Experimental Technique: Implementing Change. *Animals* 30;9(10):754. doi: 10.3390/ani9100754.
2. Neuhaus W *et al.* (2022): The Rise of Three Rs Centres and Platforms in Europe. *ATLA - Alternatives to Laboratory Animals.* 50(2):90-120. doi:10.1177/02611929221099165.
3. Poole T (1997): Happy animals make good science. *Laboratory Animals*. 1997;31(2):116-124.
4. Russell WMS & Burch RL (1959): The Principles of Humane Experimental Technique. London: Methuen & Co. Ltd.
5. Russell WMS (2005): The Three Rs: past, present and future. *Animal Welfare* 14: 279-286
6. Smith AJ & Richmond J (2024): 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.
7. Smyth DH (1978): *Alternatives to animal experiments*. 218 pp. London: Scolar Press for the Research Defence Society.
8. Stephens M (2009): Personal reflections on Russell and Burch, FRAME, and the HSUS. *ATLA - Alternatives to laboratory animals* 37 Suppl 2. 29-33.
9. Tannenbaum J & Bennett, BT (2015): Russell and Burch's 3Rs then and now: The need for clarity in definition and purpose. *Journal of the American Association for Laboratory Animal Science*, **54**, 120–132.

Further reading

1. Balls M (2010) The principles of humane experimental technique: timeless insights and unheeded warnings. *ALTEX - Alternatives to animal experimentation*, **27**, pp. 144–148.
2. Karp NA & Fry D (2021): What is the optimum design for my animal experiment? *BMJ Open Sci.* 15;5(1):e100126. doi: 10.1136/bmjos-2020-100126.
3. Sánchez Morgado JM & Brønstad A (Eds.) (2020): *Experimental Design and Reproducibility in Preclinical Animal Studies*. 277pp. Springer.
4. Mogil JS, Pang DSJ, Silva Dutra GG & Chambers CT (2020): The development and use of facial grimace scales for pain measurement in animals. *Neurosci. Biobehav. Rev.* 116:480-493. doi: 10.1016/j.neubiorev.2020.07.013.
5. Smith AJ, Clutton RE, Lilley E, Hansen KEA & Brattelid T (2018): PREPARE: guidelines for planning animal research and testing. *Lab Anim*. 52(2):135-141. doi: 10.1177/0023677217724823.
6. Smith D, Anderson D, Degryse AD, Bol C, Criado A, Ferrara A, Franco NH, Gyertyan I, Orellana JM, Ostergaard G, Varga O & Voipio HM (2018): Classification and reporting of severity experienced by animals used in scientific procedures: FELASA/ECLAM/ESLAV Working Group report. *Lab Anim.* 52(1_suppl):5-57. doi: 10.1177/0023677217744587.

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

vimeo.com/289645718

”PREPARE for 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)
- a culture of care at the animal facility
- communication of best practice to others

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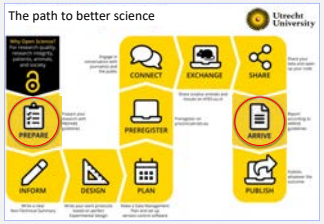
What's the problem?

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.



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What can Norecopa offer?

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

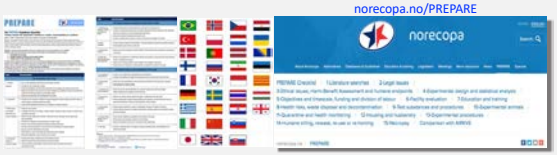
- over 9,000 searchable webpages of quality 3R resources, with filters to facilitate searching
- the PREPARE guidelines for planning animal experiments, with a checklist in over 30 languages
- links to recordings of webinars covering all aspects of animal research
- an International Webinars & Meetings 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

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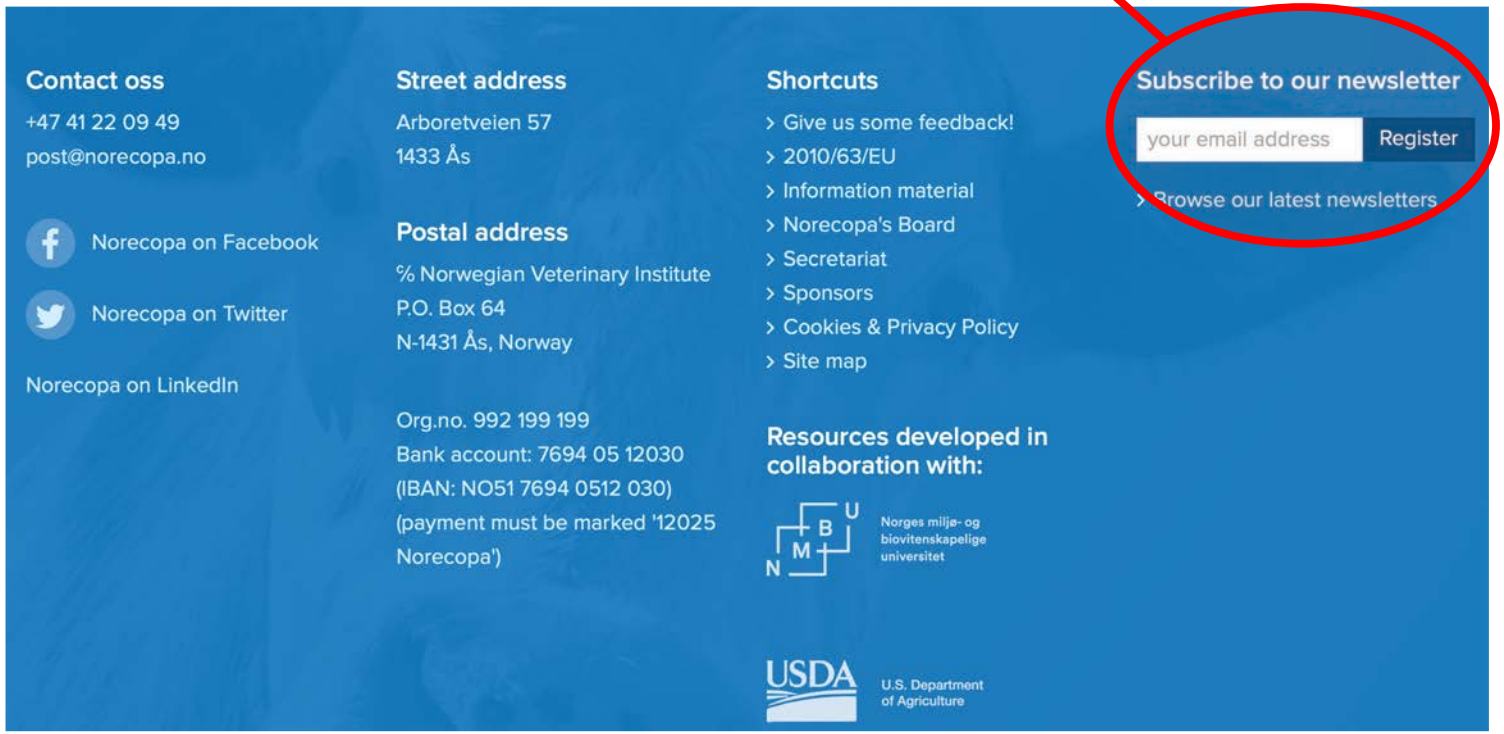
English-language newsletters



Thank you for listening!