Evolution of practice in animal research for the development of new therapies

Dr Elliot Lilley
UK National Centre for the 3Rs

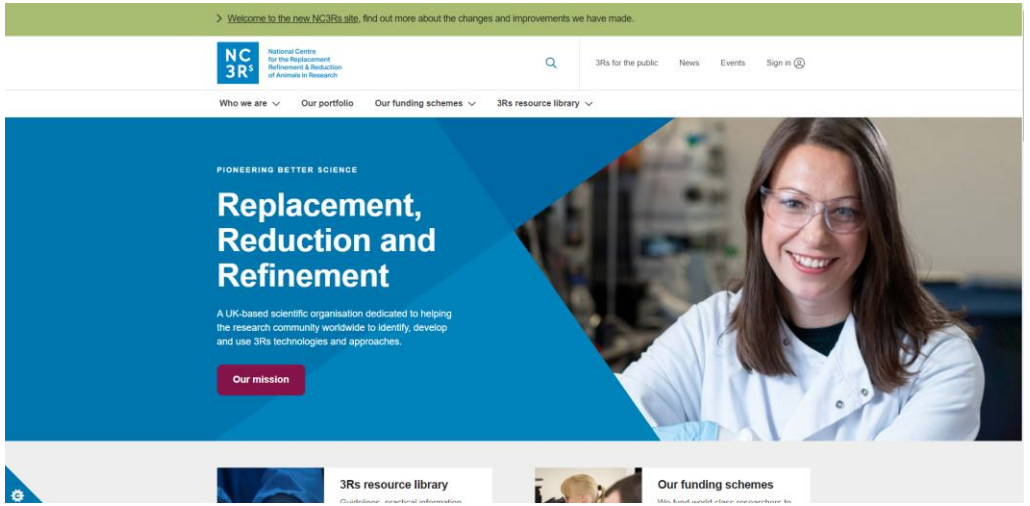
EURORDIS Barcelona June 2024

Learning objectives

- **Understanding the 3Rs**
  - replacement, reduction and refinement
- **Why their application is scientifically important**
  - as well as economically and ethically
- **Understanding how non-animal methods can advance knowledge**
  - how non-animal approaches can help rare disease research
- **Appreciating research and regulatory trends to move away from animal use wherever possible**
  - limitations of animal research and ethical pressures

NC3Rs – the UK's national centre for the 3Rs

- Established in 2004 to accelerate the development & uptake of the 3Rs
- Research funder plus in-house programmes led by NC3Rs staff
- Work with industry, academia, regulators & funders – not just UK, but also with collaborators in Europe, North America & Asia
- Remit includes any area of animal use for research purposes
- Team based in London, plus regional staff
- Budget ~ £10 million p.a.
- Independent Board



3Rs definitions in 1959

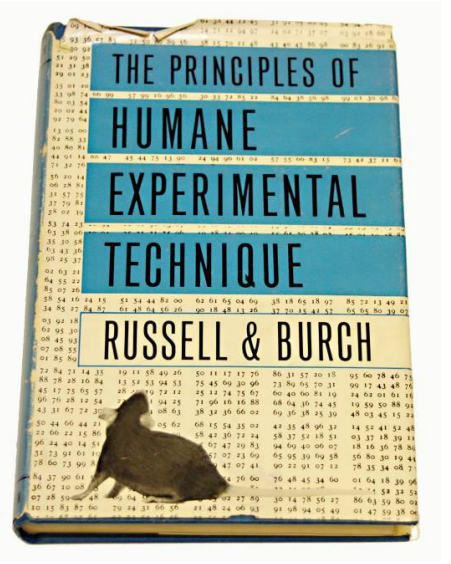
Replacement: Avoiding or replacing the use of animals in experiments where they otherwise would have been used:

Absolute: human tissue, established cell lines, computer models
Relative: invertebrates, early forms of vertebrates, (non-regulated)

Reduction: Minimising the number of animals used in an experiment consistent with scientific aims

Refinement: Minimising the pain, suffering, distress or lasting harm that lab animals might experience e.g. by giving pain relief, providing appropriate caging, training animals to cooperate with procedures.

Rex Burch and Bill Russell



A fresh approach – from ethics to better science

	Standard	Contemporary
Replacement	Non-animal methods	Accelerating the development and use of new models and tools to address important scientific questions without the use of animals
Reduction	Minimum number of animals consistent with scientific aims	Well designed and analysed animal experiments that are reproducible, and truly add to the knowledge base
Refinement	Minimum pain, suffering, distress or lasting harm	Advancing research animal welfare and showing the impact of welfare on scientific outcomes

NC 3Rs

The 3Rs

A continuum

- Ultimate goal is replacement
- Refinement and Reduction should always be considered.
  - Reducing impact on animals on the way towards full replacement
  - Aggregation of marginal gains

Should not be viewed in isolation

- Refinement can lead to reduction
  - Statistical power has an inverse square relationship to noise (variation).
  - Poor welfare can be a source of variation in animal research
  - Improving welfare has greater impact than increasing sample size
- Refinement can lead to replacement.
  - Mechanistic models with biomarker-driven experimental and humane endpoints can reduce suffering and point the way towards cell-based alternatives

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Application of the 3Rs in Europe is not optional

European Directive 2010/63

- Research on any animal should lead to medical, veterinary, scientific or educational benefits

- The research must be ethically justifiable: extreme levels of suffering cannot (normally) be justified on the basis of the importance of the research

- The 3Rs are applied – use alternatives if possible

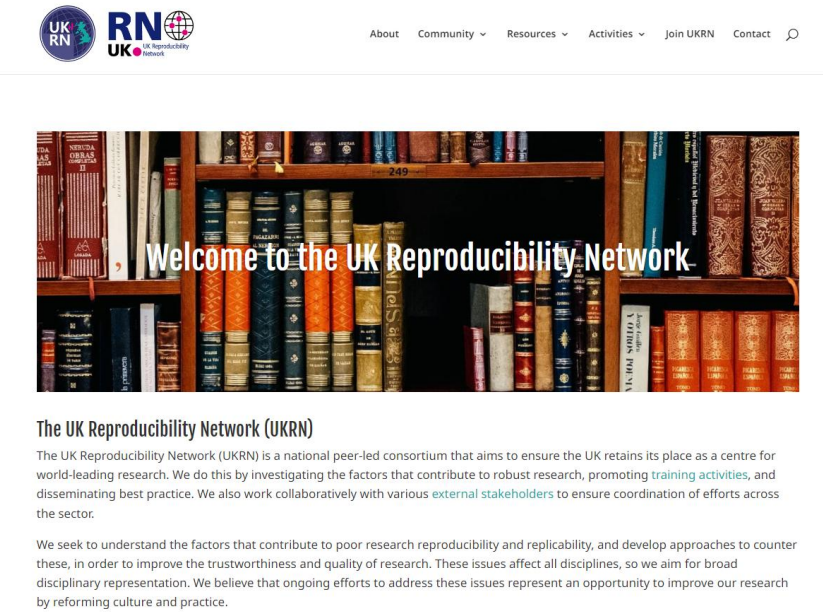
Quality of published animal research

- NC3Rs-funded survey revealed problems in the design, analysis and reporting of animal studies, and identified key areas for improvement

Kilkenny C, Parsons N, Kadyszewski E, et al. (2009). PLoS One 4(11): e7824.

- Poor design – randomisation, blinding…..
- Incorrect statistical analysis – experimental unit, statistical model….
- Incomplete reporting – hypothesis, numbers and type of animals, sources of materials……

- Publication bias
- HARKing (hypothesizing after the results are known)
- *p*-hacking
- Pre-registration



https://www.ukrn.org/

‘Reproducibility crisis’ in science

Research Article

Cancer Biology, Computational and Systems Biology

Investigating the replicability of preclinical cancer biology

Timothy M Errington, Maya Mathur, Courtney K Soderberg, Alexandria Denis, Nicole Perfito, Elizabeth Iorns, Brian A Nosek

Center for Open Science, United States; Quantitative Sciences Unit, Stanford University, United States; Science Exchange, United States; University of Virginia, United States

Dec 7, 2021 · https://doi.org/10.7554/eLife.71601

“A total of 50 experiments from 23 papers were repeated, generating data about the replicability of a total of 158 effects. ……..One (analytical) method compared effect sizes: for positive effects, the median effect size in the replications was 85% smaller than the median effect size in the original experiments, and 92% of replication effect sizes were smaller than the original”

Facts and figures

Rare diseases are not rare

- According to EURORDIS, around 30 million people have rare diseases in Europe (up to 400 million globally)
- Over 6000 clinically defined rare diseases
- 72% have a genetic component and 70% start in childhood
- Less than 10% of RDs have treatment options

Some good news

- Rare disease drug development accounts for nearly one third of all drugs in active R&D
- Currently 150 ongoing gene therapy clinical trials
- Rare diseases account for half of new drug approvals (51% in 2023; FDA; EMA 11 Orphan drugs 2023)
- Approval rates are now approaching those for non-rare diseases

What are the issues for rare disease drug discovery and development?

- **Understanding and (mis)diagnosis**
- **Funding for research for rarer rare diseases**
- **Finding and enrolling enough patients for CTs**
  - 6 times the number of sites for 4 times fewer patients for Phase 1
  - Higher screening and randomisation failures
- **Longer drug development times so higher costs**
  - Less experience with newer modalities (ASO, siRNA, gene and cell therapy)
- **Smaller market opportunities……..**
- **Pricing issues and access to treatment**
  - Orkambi (Vertex) for cystic fibrosis $23,000 per month
  - Spinraza (Biogen) for SMA $4.1m for 10 years
  - Evrysdi (Genentech) for SMA $3.4m for 10 years
  - Zolgensma (Novartis) for SMA $2.1m for one-off treatment
  - Libmeldy (Orchard) for MLD $2.8m for one-off treatment

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Treatments for rare diseases

How to tackle high cost and slow pace of drug development

- Repurpose old drugs
- Reduce attrition in development
  - Better preclinical research
- Reduce time to market
  - Better preclinical research
  - Rethink clinical trial design
  - Involve patient organisations

How to incentivise research

- Expedited regulatory review/lower costs
- Extended market exclusivity for orphan drugs

How to tackle cost of treatment and impact on patient access

- Do all of the above better
- Negotiate prices

NC 3Rs

Rare diseases and the 3Rs

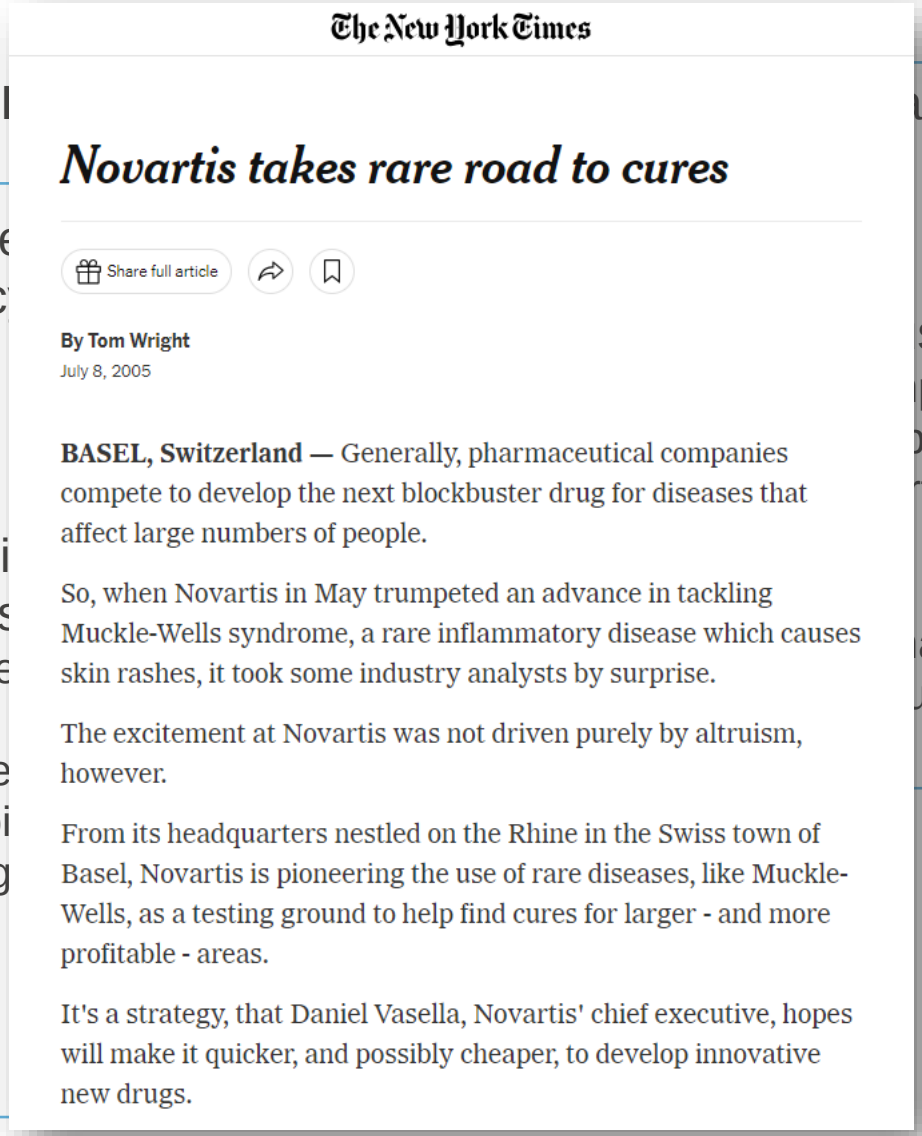
Better preclinical research for rare diseases

SHI

Increase e
for efficac

More testi
in humans

- Stem ce
- Tissue
  enginee
- Organoi
- Imaging



The New York Times

Novartis takes rare road to cures

Share full article

By Tom Wright
July 8, 2005

BASEL, Switzerland — Generally, pharmaceutical companies compete to develop the next blockbuster drug for diseases that affect large numbers of people.

So, when Novartis in May trumpeted an advance in tackling Muckle-Wells syndrome, a rare inflammatory disease which causes skin rashes, it took some industry analysts by surprise.

The excitement at Novartis was not driven purely by altruism, however.

From its headquarters nestled on the Rhine in the Swiss town of Basel, Novartis is pioneering the use of rare diseases, like Muckle-Wells, as a testing ground to help find cures for larger - and more profitable - areas.

It's a strategy, that Daniel Vasella, Novartis' chief executive, hopes will make it quicker, and possibly cheaper, to develop innovative new drugs.

se concerns about

se of alternatives:
putational methods
le organisms and
tebrates
bacteria, amoebae,
fruit flies, fish embryos
an stem cells
ue engineering, organs
chip

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Better preclinical research for rare diseases

Decrease concerns about safety:

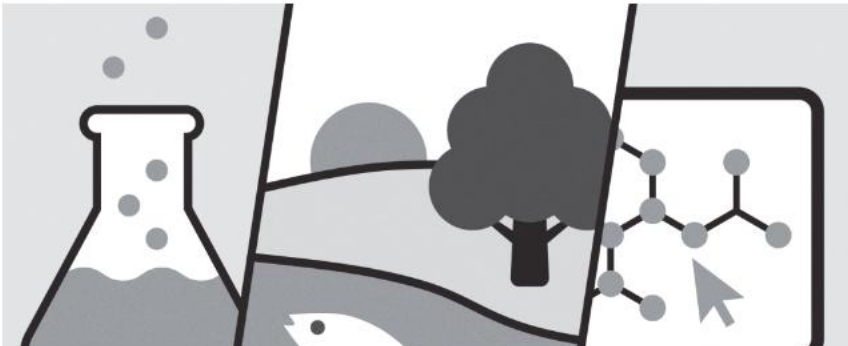
- Research investment in NAMs is underpinning positive statements and policies from regulators
  - European Parliament resolution “Accelerate a Transition to Innovation without the use of Animals in Research, Regulatory Testing and Education” (2021)
  - US FDA: Modernisation Act 2.0 (2022), Alternative Methods Working Group (2019)
  - EMA: 3Rs Working Party (2023), Innovation Task Force (2014)
  - NC3Rs NAMs network (April 2024)
  - UK national non-animal models strategy (in development)

- Part of a larger trend including other sectors
  - Environmental protection: US EPA
  - Food standards: EFSA, UK FSA
  - Chemicals: ECHA

BLOG

NAMs: “Not a matter of if but of when”

10 April 2024



Better preclinical research for rare diseases

Increase evidence for efficacy:

- Testing in animal disease models is limited and unsuited to new modalities
- BUT the closer the model to man the better: genetic alteration of animals is getting easier and can be applied to large animals such as non-human primates
- BUT this is ethically dubious and human-based/derived testing is also advancing rapidly
- BUT aren’t you going to put patients and investment at risk by not proving efficacy in animals?
- Go back to the start!

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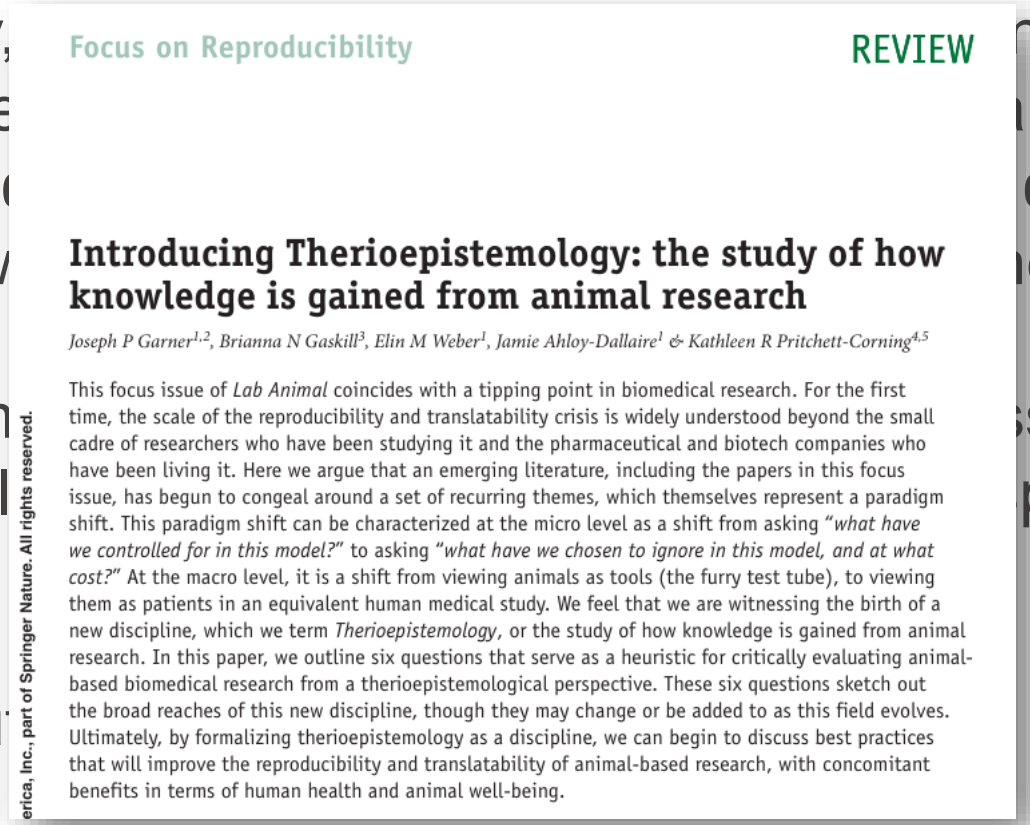
Future of animal and non-animal models

What is a model and what is it for?

"Too frequently, nvestigator
proposes to cre nd "validate"
that animal mo document
similarities betv e disorder"

"NIMH recomm ssing
neurobiological pecific mental
illnesses"

National Institu



Focus on Reproducibility REVIEW

Introducing Therioepistemology: the study of how knowledge is gained from animal research

Joseph P Garner[1,2], Brianna N Gaskill[3], Elin M Weber[1], Jamie Ahloy-Dallaire[1] & Kathleen R Pritchett-Corning[4,5]

This focus issue of *Lab Animal* coincides with a tipping point in biomedical research. For the first time, the scale of the reproducibility and translatability crisis is widely understood beyond the small cadre of researchers who have been studying it and the pharmaceutical and biotech companies who have been living it. Here we argue that an emerging literature, including the papers in this focus issue, has begun to congeal around a set of recurring themes, which themselves represent a paradigm shift. This paradigm shift can be characterized at the micro level as a shift from asking "*what have we controlled for in this model?*" to asking "*what have we chosen to ignore in this model, and at what cost?*" At the macro level, it is a shift from viewing animals as tools (the furry test tube), to viewing them as patients in an equivalent human medical study. We feel that we are witnessing the birth of a new discipline, which we term *Therioepistemology*, or the study of how knowledge is gained from animal research. In this paper, we outline six questions that serve as a heuristic for critically evaluating animal-based biomedical research from a therioepistemological perspective. These six questions sketch out the broad reaches of this new discipline, though they may change or be added to as this field evolves. Ultimately, by formalizing therioepistemology as a discipline, we can begin to discuss best practices that will improve the reproducibility and translatability of animal-based research, with concomitant benefits in terms of human health and animal well-being.

Where do the 3Rs stand?

The goals:

- the 3Rs are an integral part of mainstream biosciences
- there is active participation from scientists at all levels and in all sectors, because of recognition of the scientific value of the 3Rs
- there is sustained and real progress in 3Rs technologies that is translated into action (policy, practice and regulations)
- there is greater willingness to critique the existing models and approaches

How are we doing?

Where do the 3Rs stand?

The goals:

- the 3Rs are an integral part of mainstream biosciences (7/10)
- there is active participation from scientists at all levels and in all sectors, because of recognition of the scientific value of the 3Rs (5/10)
- there is sustained and real progress in 3Rs technologies (8/10) that is translated into action (policy, practice and regulations) (5/10)
- there is greater willingness to critique the existing models and approaches (5/10)

Could do better

What about the scientific and economic benefits of the 3Rs?

1. Why is replacement important?

- e.g. minimising attrition in drug development

2. Why is reduction important?

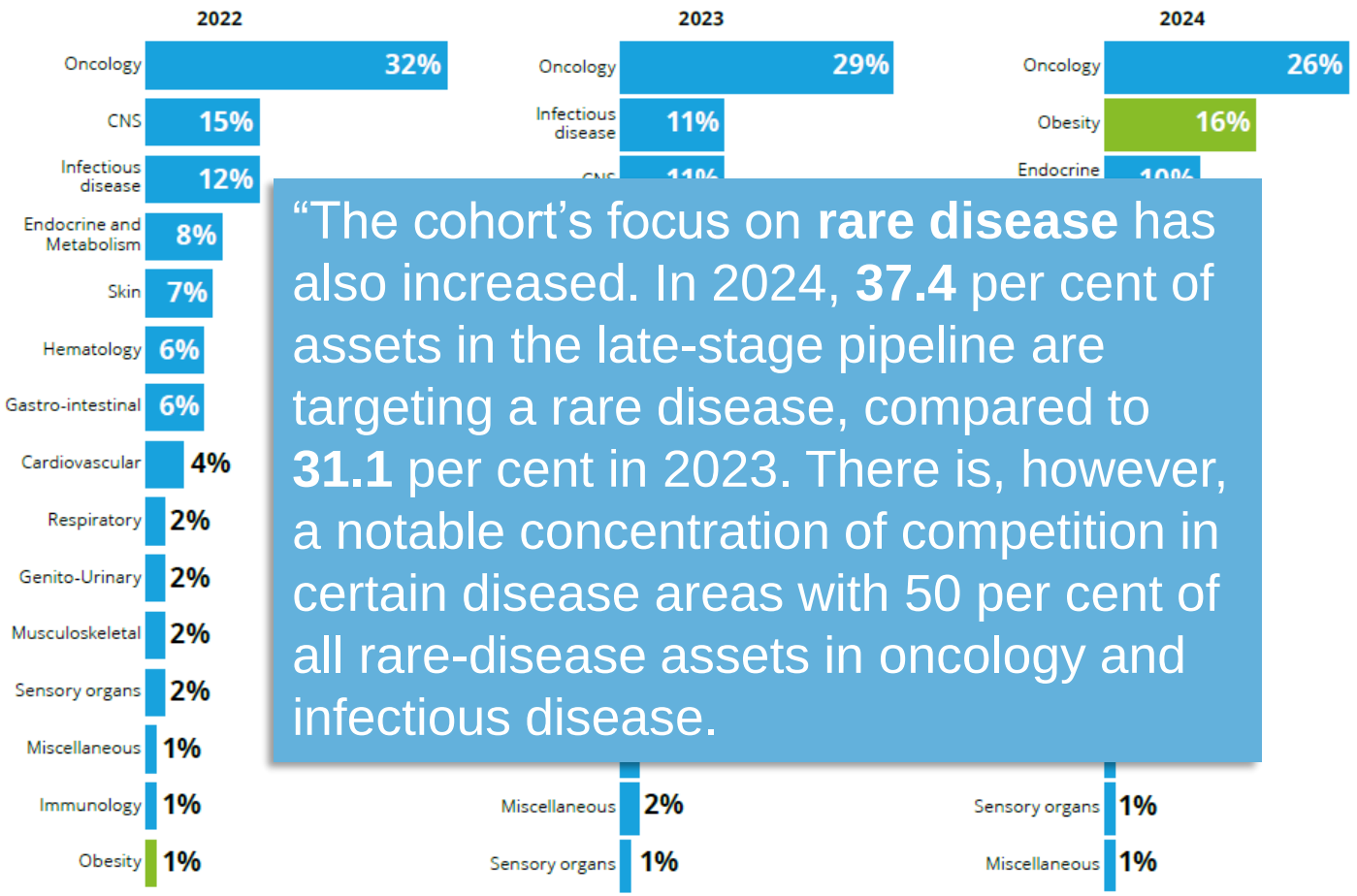
3. Why is refinement important?

FTLOSCIENCE		Average Time (Years)	Average Cost 2018 (US$ million)	Average Cost 2022 (US$ million)
Early Drug Discovery		2.5	299	353
Lead Optimization		2	477	562
Pre-Clinical Trials		1	288	340
Clinical Trials	Phase I	1.5	53	63
	Phase II	2.5	101	119
	Phase III	3.5	202	344
FDA Review and Ap				
Total				

Cost of drug discovery

- High and increasing
- Failure in development is a major contributor to cost
- Many approved drugs do not recoup their costs
- Innovative Genomics Institute estimated up to $5b for gene therapies

Figure 7. Forecast revenue for the top 20 cohort by therapy area, 2022-2024



"The cohort's focus on **rare disease** has also increased. In 2024, **37.4** per cent of assets in the late-stage pipeline are targeting a rare disease, compared to **31.1** per cent in 2023. There is, however, a notable concentration of competition in certain disease areas with 50 per cent of all rare-disease assets in oncology and infectious disease.

Source: Deloitte analysis of Evaluate Pharma data, 2025

Roadmap to Reducing Animal Testing in Preclinical Safety Studies

Executive Summary

This roadmap outlines a strategic, stepwise approach for FDA to reduce animal testing in preclinical safety studies with scientifically validated new approach methodologies (NAMs), such as organ-on-a-chip systems, computational modeling, and advanced *in vitro* assays. By partnering with federal agencies like NIH and VA through ICCVAM, FDA can accelerate the validation and adoption of these human-relevant methods, improving predictive accuracy while reducing animal use. This transition will enhance public health by streamlining drug development and ensuring safer therapies reach patients faster, while positioning FDA as a global leader in modern regulatory science and innovation.

EMA guidance on gene therapies

5.2 Animal models
It is acknowledged that appropriate animal models are not always available…..In such cases, an alternative approach is needed to build up the weight of evidence supporting the *safe* clinical use. Such an approach may include in vitro and ex vivo cell and tissue-based models, in silico analyses, literature-based evidence and clinical experience with related products……Where appropriate, animal testing could be replaced by in vitro or ex vivo studies.

5.3 Pharmacology studies
Generally, animal disease models or experimentally induced models mimicking the condition to be treated are considered most relevant for demonstrating the proof of concept. In addition, in vitro and ex vivo cell and tissue-based models can be used to supplement or substitute in vivo animal studies to demonstrate the proof of concept.

What is the future for animal models?

- On scientific and ethical grounds, animal models of disease are falling out of favour
  - Why do you need to make a sick animal and cure it in order to advance your drug to clinical trials?

- EMA guidance on gene therapy products is ambiguous
  - preferably use a disease model, even if you have to use larger animals
  - if for scientific reasons you can't, then an alternative is acceptable
  - always apply the 3Rs

- If you apply the 3Rs, if an alternative is acceptable, it **must** be used in preference

NC 3Rs

CRACK IT Challenges – innovation in the 3Rs

R&D funding competition designed by the NC3Rs to:

- Respond directly to issues facing industry that relate to the use of animals.
- Build on collaborations already established with industry through data sharing activities.
- Tap into the shift in the R&D model towards increased external collaboration and the need for multidisciplinary approaches.
- Develop new opportunities directly for the academic and SME sectors.

CRACK IT Challenges to date

47 Challenges Launched since 2011

51 Sponsors/ Partners

Committed over £34M

27 Challenges Completed

19 Products and services developed

Four new companies formed

defra
Department for Environment Food and Rural Affairs

Alzheimer's Research UK
Defeating Dementia

EPSRC

VERSUS ARTHRITIS

Unilever

Innovate UK

Environment Agency

MRC Medical Research Council

[dstl]

NC 3Rs

Retinal 3D Challenge

Sponsored by:
Merck Healthcare KGAa,
Novartis, Roche

Retinal model for toxicology studies

- Retinal organoids from human iPSCs, containing key retinal cell types, that are physiologically-competent and predictive of human physiology.

- Organoids mimic the physiological features of the retina *in vivo* and can be used for a wide variety of applications, including gene therapy studies, toxicology and disease modelling.

- Reliable alternative to animal models with increased relevance to human health.

Scalable assays up to 96-well plate format.

Recapitulates the architecture of the human retina.

Projects can be carried out at Newcells Biotech state-of-the-art facility.

Regular batch release of organoids allowing on-demand supply shipped at room temperature throughout Europe and the USA.

3Rs

Reduces the need for preclinical in vivo studies.

NEWCELLS
BEST BIOLOGY DRIVING *IN VITRO* INNOVATION

What about the scientific and economic benefits of the 3Rs?

1. Why is replacement important?

- e.g. minimising attrition in drug development

2. Why is reduction important?

3. Why is refinement important?

What about the scientific and economic benefits of the 3Rs?

1. Why is replacement important?

- e.g. minimising attrition in drug development

2. Why is optimising experimental design important?

3. Why is refinement important?

‘Reproducibility crisis’ in science

In cancer science, many "discoveries" don't hold up

BY SHARON BEGLEY
NEW YORK | Wed Mar 28, 2012 2:09pm EDT

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Archive > Volume 505 > Issue 7485 > Comment > Article

NATURE | COMMENT

Policy: NIH plans to enhance reproducibility

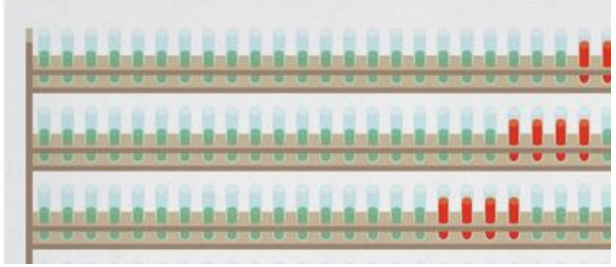
Francis S. Collins & Lawrence A. Tabak

27 January 2014

Francis S. Collins and Lawrence A. Tabak discuss initiatives that the US National of Health is exploring to restore the self-correcting nature of preclinical research

PDF | Rights & Permissions

Subject terms: Biological techniques · Lab life · Peer review · Research managem



Studies show only 10% of published science articles are reproducible. What is happening?

Posted on May 3, 2012 by Moshe Pritsker

Studies show a very low reproducibility for articles published in scientific journals, often as low as 10-30%. Here is a partial list:

News & Comment > News Blog > Post

Previous post
Cool climate paper sinks journal editor

Next post
Illegal drug sales threaten vultures in India

The Economist | World politics | Business & finance | Economics | Science & technology | Culture

Unreliable research

Trouble at the lab

Scientists like to think of science as self-correcting. To an alarming degree, it is not

Oct 19th 2013 | From the print edition | Timekeeper

Reproducibility

ional weekly journal of science

ch | Careers & Jobs | Current Issue | Archive | Audio & Video | For

46 > Editorial > Article

educing our irreproducibility

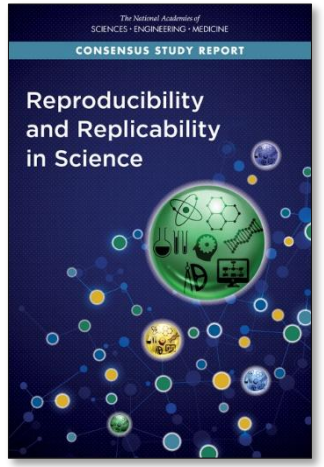
lished a string of articles that highlight failures in the reliability earch (collected and freely available at go.nature.com/huhbyr).

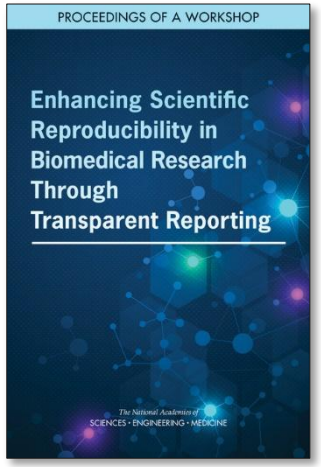
NC 3Rs

Research waste in *in vivo* studies - Ethical implications

If research is not reported in enough detail, or if findings are not reliable, benefits cannot be realised

→ **Research is unethical**



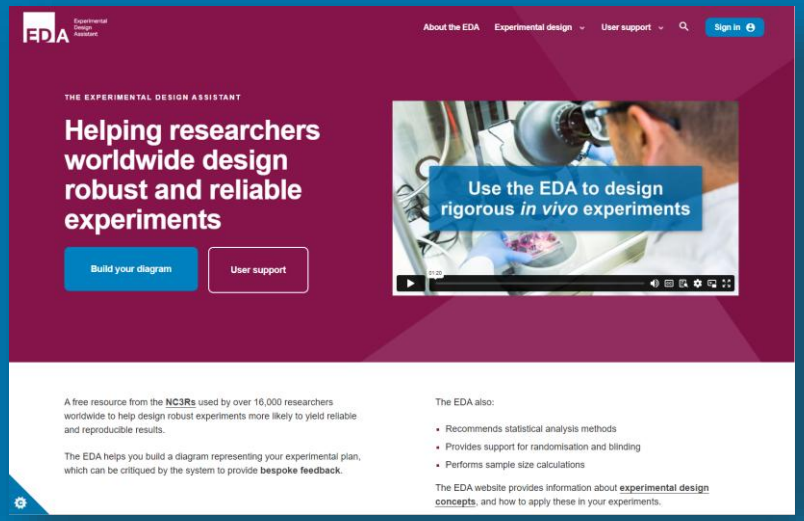


Likely benefits to science and society

- New scientific knowledge
- Improvements in human (or animal) health or safety

Likely harms to the animals involved

- Scientific procedures and their effects
- Contingent suffering due to housing, transport, etc.



The EDA can help to ensure robust study design and reliable and reproducible findings

Output can be used in grant application, ethical approval or publication

NC 3Rs

Experimental Design Assistant (EDA)

Free to use online tool for researchers to design *in vivo* experiments

Computer-based logical reasoning provides:

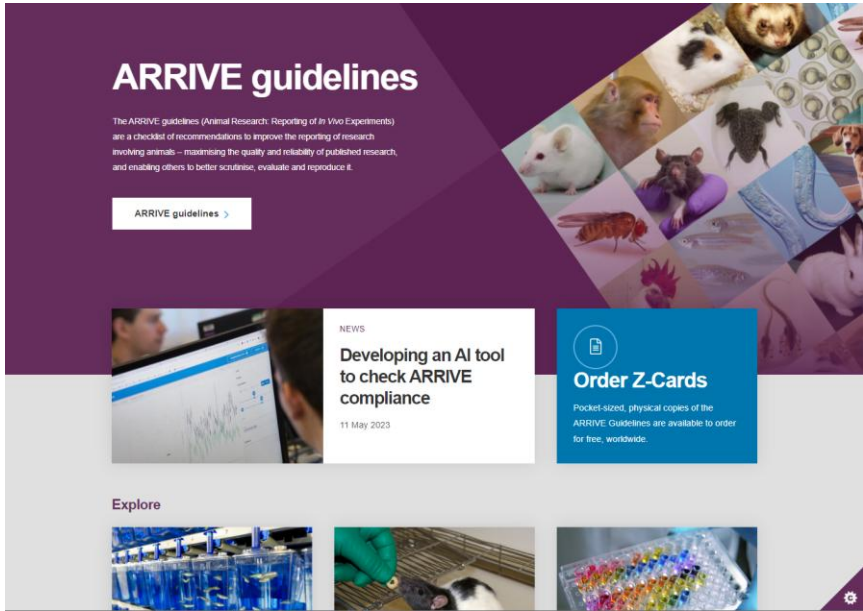
- Advice to improve the experimental plan
- Recommendations for the statistical analysis

Website contains wealth of trusted information on experimental design

Support for:

- Randomisation
- Blinding
- Power calculation

https://eda.nc3rs.org.uk/



MetaArXiv Preprints

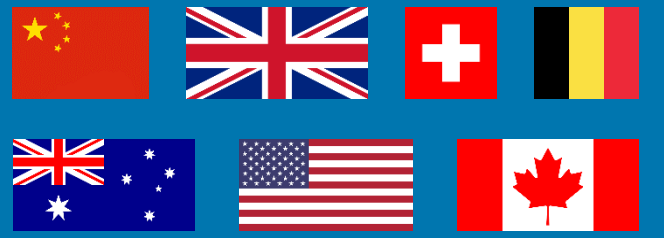
The RIVER recommendations

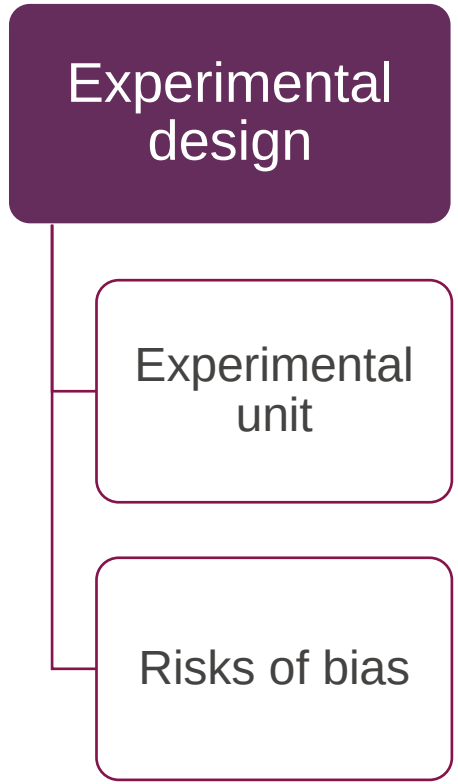
Reporting *In Vitro* Experiments Responsibly

Six recommendations to increase the methodological rigour and reliability of *in vitro* studies

Developed by an international working group including:

- Funders
- Journal editors / publishers
- Methodologists & statisticians
- *In vitro* researchers in industry, academia & government





The RIVER working group (2023). *MetaArxiv*. doi: 10.31222/osf.io/x6aut

What about the scientific and economic benefits of the 3Rs?

1. Why is replacement important?

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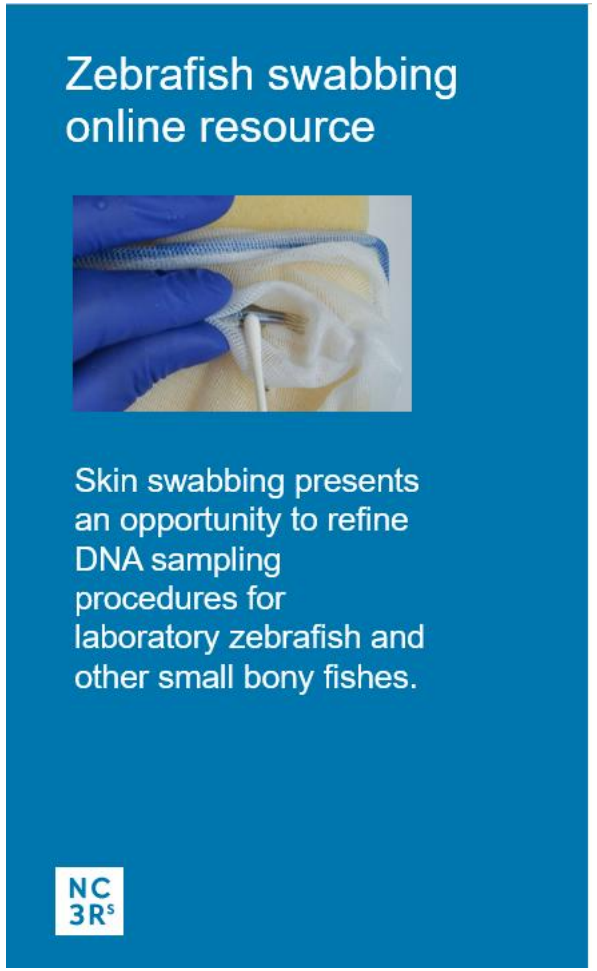
Animal welfare and quality of science are linked

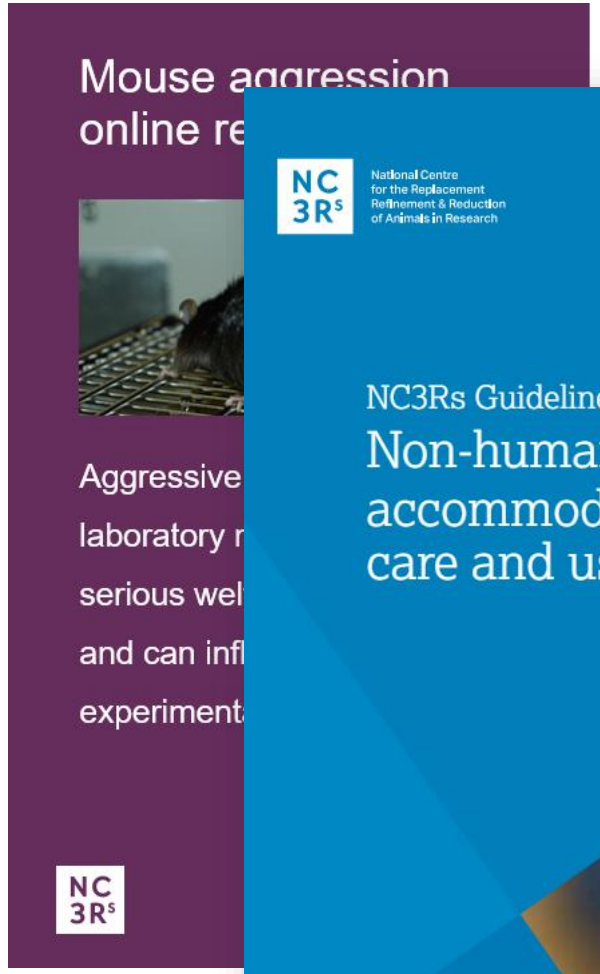
Poor welfare is at odds with good science

- It alters behavioural and physiological parameters, introducing unwanted variation into experimental outcomes
- It may confound the ability to quantify changes in the biological parameters under investigation (e.g. floor/ceiling effects)

These problems can arise from the stress response due to poor handling



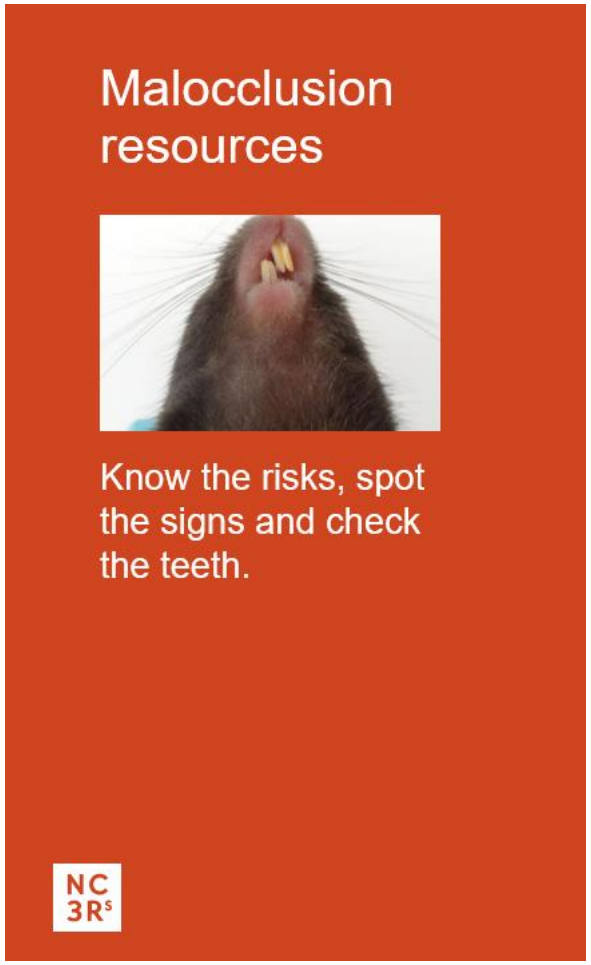
www.nc3rs.org.uk/zebrafish-swabbing



www.nc3rs

aggression

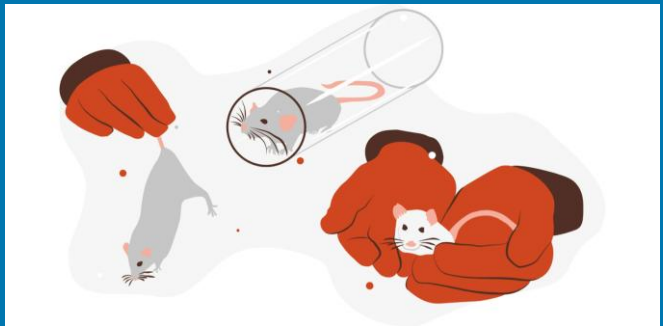
.uk/rat-



www.nc3rs.org.uk/malocclusion-mice

NC 3Rs

Refined handling eLearning course



Learn about the background and practical applications of using refined methods to pick up mice.

NC 3Rs

Refining how we pick up mice

This free online course is aimed at anyone who works with mice in research. The eLearning course should take no longer than 45 minutes and covers:

- The background and evidence behind refined mouse handling.
- How refined handling is better for mice, people and science.
- How to pick up mice using a tunnel and cupped hands.
- How using refined methods to pick up mice is compatible with restraint, procedures, efficient day-to-day operation and maintaining biosecurity.

www.mousehandling.org

Cancer guidelines

Working with cancer research experts to refine animal use in oncology studies.

Revision of the guidelines on animal use and welfare in cancer research

The aims of the working group are to:

- Review changes in oncology science and research practices using animals.
- Update the existing guidelines to reflect the latest developments in the field and address best practice in the selection and use of animal models.
- Publish the new guidelines in a peer-reviewed paper.
- Promote the new guidelines within the cancer research community.

Take home messages

- Incorporate 3Rs thinking into your planning
  - Scientifically and economically advantageous
  - Ethical imperative
  - **All** 3Rs are important

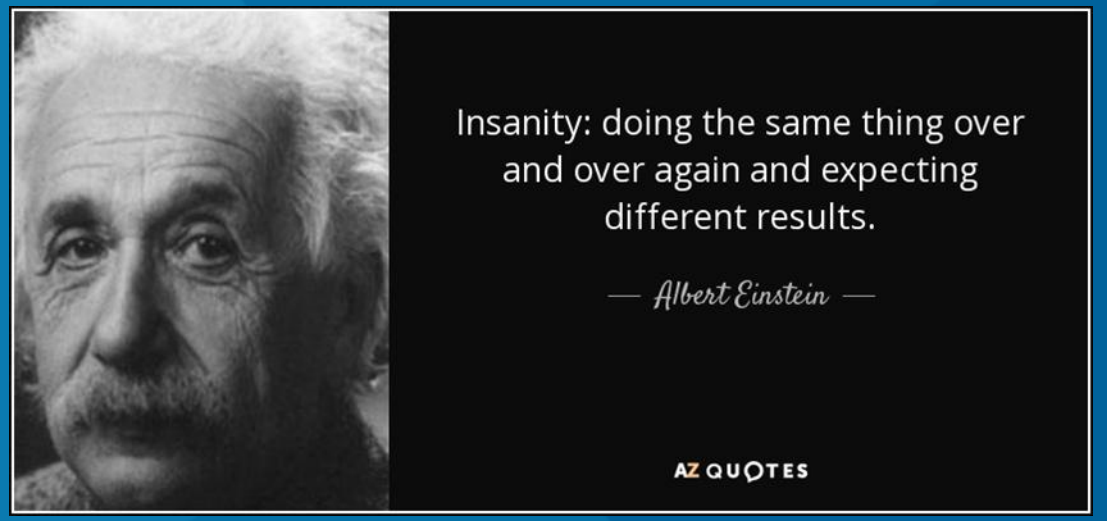
- Critically examine all options – animal and non-animal – that might advance the development and approval of treatments
  - Discuss with more than just academic collaborators even at the start of a project
  - Consider what might be the most cost-effective actions to stimulate further research interest and investment
  - Be aware of public, political and regulatory trends as well as scientific advances

NC 3Rs

NC 3Rs 20 YEARS

Pioneering Better Science

Thank you!



For more information

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