National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Improving the design and reporting of animal studies, tools:
ARRIVE Guidelines, EDA and SyRF

Dr Nathalie Percie du Sert

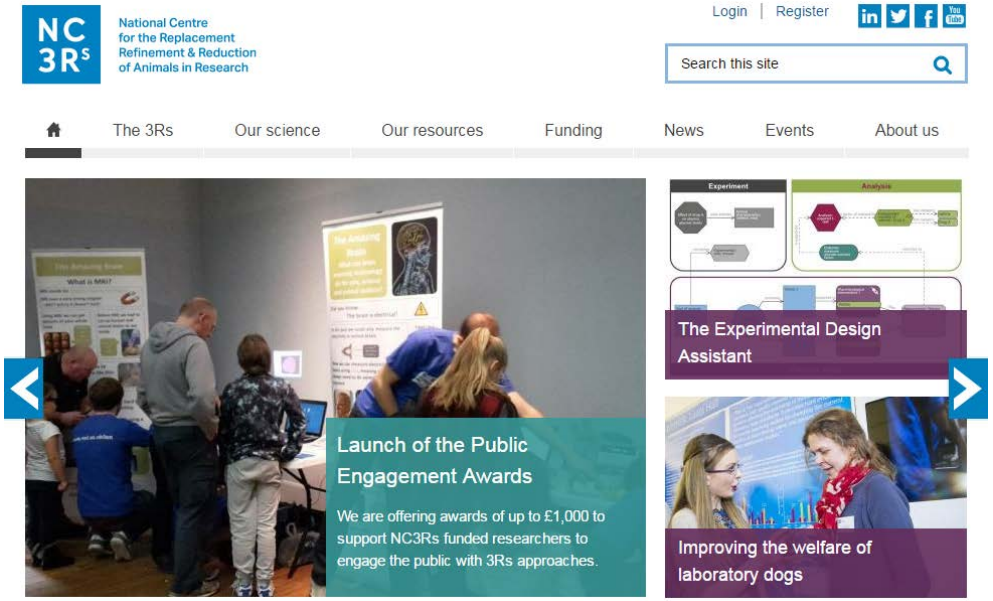
Information Meeting for Grant Applicants on the Call for Proposals
for Confirmatory Preclinical Studies and Systematic Reviews

Berlin, Tuesday 19 February 2019

Pioneering Better Science

The NC3Rs
(UK National Centre for the 3Rs)

- Lead on the discovery and application of new technologies and approaches to replace, reduce and refine the use of animals for scientific purposes (the 3Rs)



Visit our website:
www.nc3rs.org.uk

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National Centre for the 3Rs

- Work with research funders, journals, academia, industry and regulators
- Activities divided between:
  - Research funding
  - Centre-led science programmes

Experimental Design programme

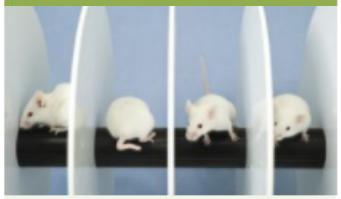
Overview of resources in the experimental design hub

Video presentation describing our resources and the importance of good experimental design.

NC3Rs/NIH OLAW Experimental design and reporting survey

A survey looking at the quality of research using animals, revealing problems in the design, analysis and reporting of animal studies.

Conducting a pilot study



Brief guidance on the importance of carrying out a pilot study.

ARRIVE guidelines

Download our reporting guidelines and find a range of additional resources to support their use and dissemination.

Workshop: Improving peer review of in vivo research proposals

Videos from our May 2018 workshop on experimental design for panel members of the NC3Rs, BBSRC, CRUK, MRC and Wellcome Trust.

Impact of rodent age on study outcome

NC3Rs project to explore and understand the current thinking around the choice of the age of rodents being used by researchers.

The CAMARADES-NC3RS Systematic Review Facility (SyRF)

SYSTEMATIC Review Facility

An online resource to help you perform systematic review and meta-analysis.

Experimental design/statistics

Key elements of a well designed experiment.

The Experimental Design Assistant - EDA

EDA

A new online tool to guide researchers through designing experiments involving the use of animals.

How to decide your sample size when the power calculation is not straightforward

Dr Simon Bate, from Statistical Sciences, GSK, covers a few nagging questions about statistics and the 3Rs that he regularly encounters.

Background

Quality of published animal research

Experimental design

Only 12% of publications report randomisation and 14% report blinding

Sample size justification – missing in 100%

Statistical analysis

Only 70% of publications fully described the statistical methods and presented the results with a measure of variability

Reporting of studies

Animal characteristics – missing in 25%

Only 59% stated the study hypothesis, number and characteristics of animals used

Survey reviewed 271 publications and identified key areas for improvement

NC 3Rs

Kilkenny C, Parsons N, Kadyszewski E, Festing MF, Cuthill IC, Fry D, *et al.* (2009). Survey of the quality of experimental design, statistical analysis and reporting of research using animals. *PLoS One* 4(11): e7824.

Reproducibility in preclinical research

The Reproducibility Project: Cancer Biology began in 2013

Set out to replicate experiments from 50 high-impact cancer biology papers

Ten replications published in eLife with mixed results

2015: aim for 37 papers

2017: aim for 29 papers

2018: aim for 18 papers

Main reasons to reduce scope:

- **Lack of methodological details**
- **Materials not available**

DAVIDE BONAZZI

Plan to replicate 50 high-impact cancer papers shrinks to just 18

By Jocelyn Kaiser | Jul. 31, 2018 , 5:45 PM

The ARRIVE guidelines

Animal Research: Reporting of *In Vivo* Experiments

The ARRIVE guidelines were developed to improve the reporting of biomedical research using animals.

- Checklist of 20 items, containing key information necessary to describe a study comprehensively and transparently.
- Consensus between:
  - Scientists
  - Statisticians
  - Journal editors
  - Research funders
- Used to improve reproducibility of animal research

ARRIVE

Internal validity

6. Study design
10. Sample size
11. Allocation to experimental groups
12. Experimental outcomes
13. Statistical methods
15. Number analysed
16. Outcomes and estimation

Reproducibility

7. Experimental procedures
8. Experimental animals
9. Housing and husbandry
14. Baseline data
17. Adverse events

Context/ relevance

1. Title
2. Abstract
3. Background
4. Objectives
5. Ethical statement
18. Interpretation/scientific implications
19. Generalisability/translation
20. Funding sources

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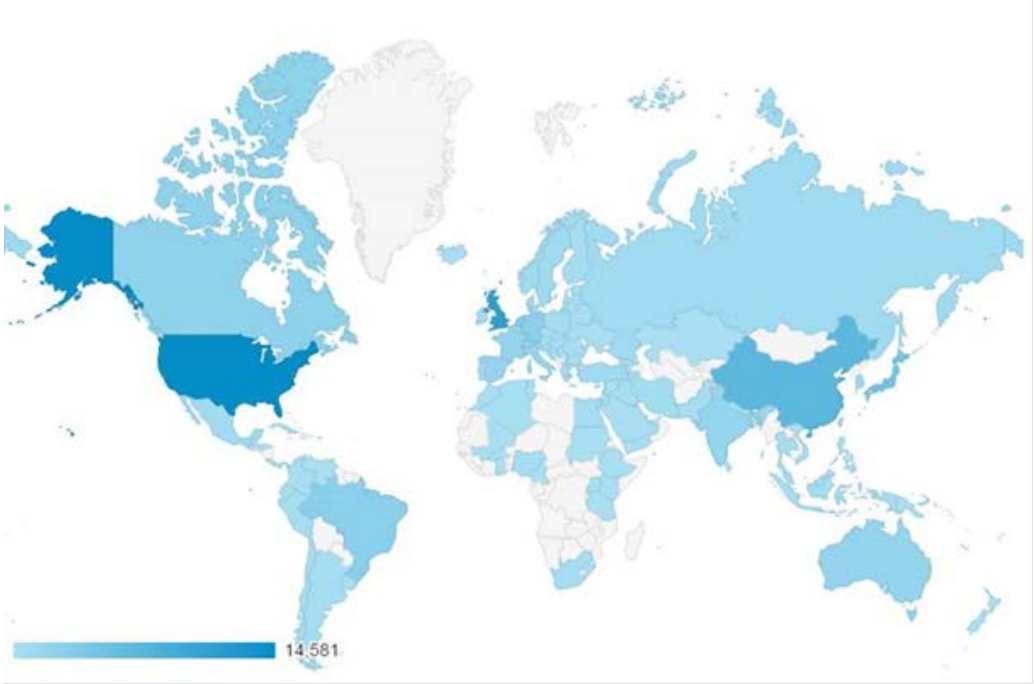
Endorsement of the ARRIVE guidelines

Over **1000** journals and organisations recommend the ARRIVE guidelines

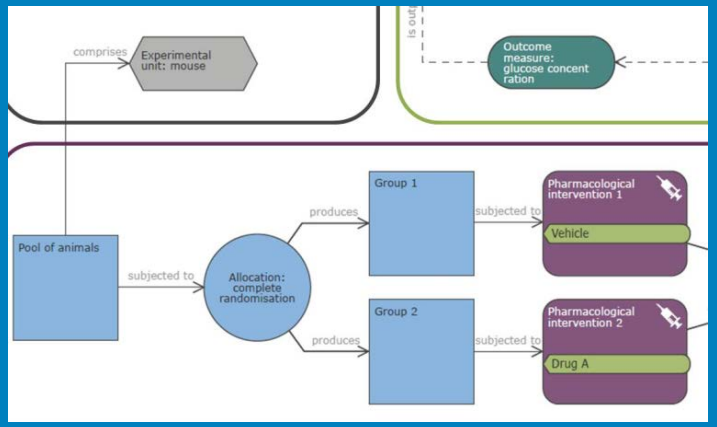
Journals	Funders	Universities	Learned Societies



Over **18,000** copies requested from **50** different countries

Experimental Design Assistant (EDA)

Online tool for researchers to design *in vivo* experiments



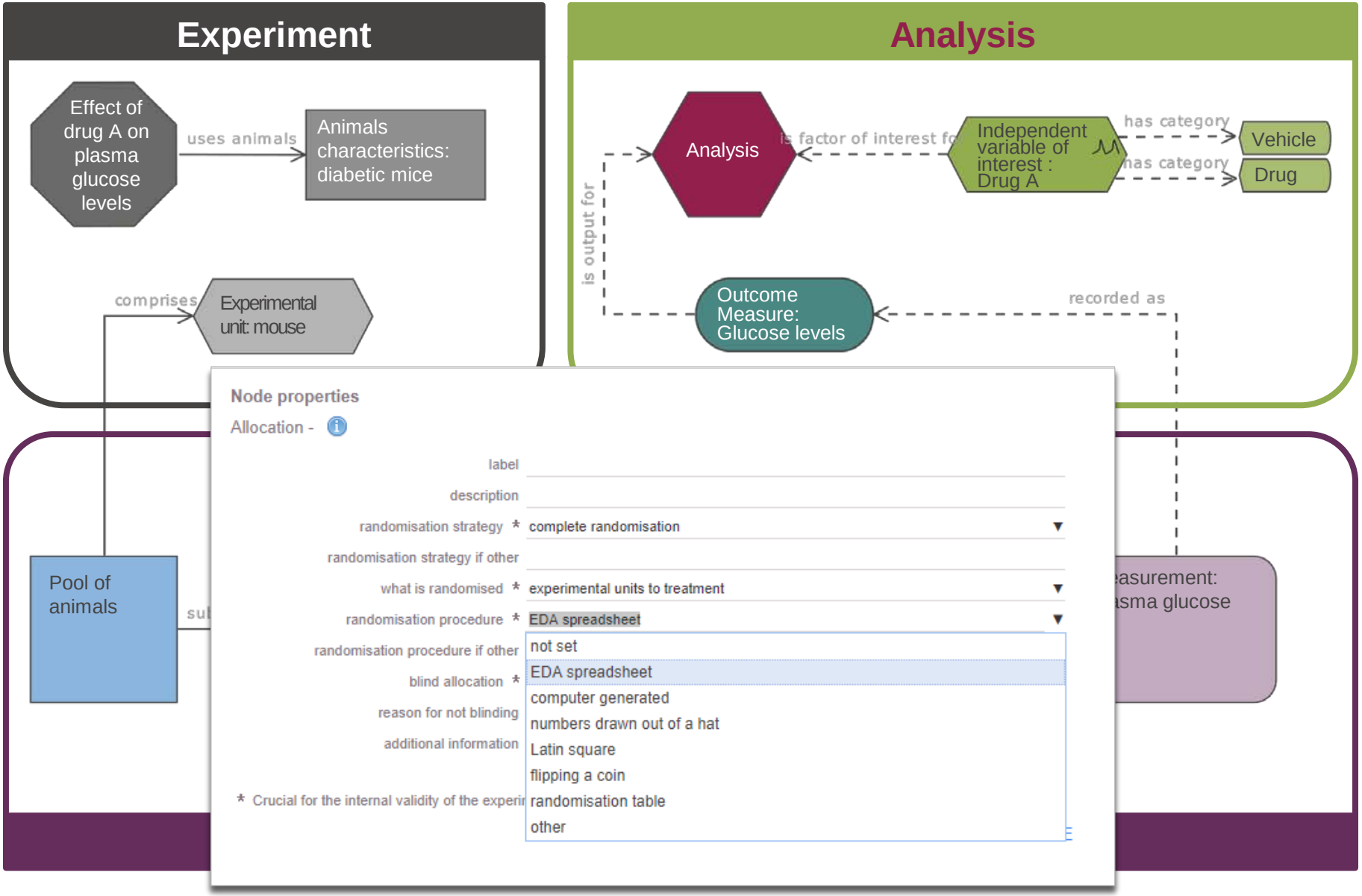
EDA can help to ensure robust study design and reliable and reproducible findings

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

Benefits of the EDA include:

- Advice to improve the experimental plan
- Recommendations for the statistical analysis
- Power calculation
- Randomisation and blinding
- Improved transparency

The EDA diagram



The diagram does not include any nuisance variables | NC3Rs EDA - Google Chrome

https://eda.nc3rs.org.uk/RT0011

The diagram does not include any nuisance variables

In the EDA, nuisance variable nodes
influence the outcome, such as bloc

Having no nuisance variable implies
influence the outcome measure. Thu
by many variables. Identifying these
experiment to detect changes induc

The type of things to consider may i
of the intervention or measurement,
experimenters with different levels o
relevant to a particular experiment, b
to identify new sources of variability.

These should be indicated on the ED
best to account for each of the nuisa
objective of the experiment, there ar

- standardised - for example re
- randomised across - for exan
randomly allocating each cag
- blocked - for example the day
analysis
- nested within another variabl
'mouse' when multiple neuro
- used as a covariate - for exa
- if none of these things are do

Indicate the blinding status during assessment of the outcome | NC3Rs EDA - Google Chrome

https://eda.nc3rs.org.uk/RT0093

Indicate the blinding status during assessment of the outcome

Information crucial to the internal validity of the experiment is missing. In the properties of this node, in the field 'blind measurement' please indicate whether the experimenter will be aware of the group allocation when assessing the results. Note that this only concerns the measurement stage; blinding before, during and after the intervention, and during the data analysis should be indicated in the properties of the allocation and analysis nodes, respectively. Choose from the dropdown menu to indicate how the investigator will be blinded to the group allocation or whether they will be aware of the group allocation during the measurement.

Blinding is especially important when it comes to assessment, particularly if there is a subjective element in assessing the outcome of the treatments, for example when assessing behavioural changes or reading histological slides. The person taking care of the animals and the person assessing the outcome should not know which intervention each of the animals received (i.e. the group allocation) and which animals are grouped together. Randomising the order of examination can help with this. For further information about blinding, click here.

Options in the dropdown menu

Investigator aware of the group allocation (not blinded) – the investigator taking the measurement knows what treatment each animal has received, or what animals are grouped together. Sometimes the person assessing the outcome cannot be blinded to the group allocation, for example if there are obvious phenotypic differences between groups of genetically modified animals; this could be mitigated by, for example, taping the behaviours and sending them to a third party, who has no vested interest in the experiment and does not know whether the transgenic should improve or worsen the outcome. Such an approach would at least counter the directional expectation.

If the person measuring the results is aware of the group allocation at this stage, please explain why in the free text

EDA Report

Key information requested by funders:

- Objectives and hypotheses
- Animal numbers and justification for sample size
- Steps taken to minimise the effect of bias
- Primary and secondary outcome measures
- Planned statistical analysis

EDA diagram

EDA Report

The Experimental Design Assistant (https://eda.nc3rs.org.uk) is an online tool which guides researchers through the design and analysis of *in vivo* experiments. Information is provided by the investigator to build an EDA diagram – see Annex. Depending on the information inputted specific prompts are triggered by the EDA which provide tailored advice and feedback on the experimental plan.

This report summarises the information provided by the investigator and the feedback from the EDA.

Section 1: Summary

Title of EDA diagram	Example 5: Effect of THC on body temperature
Date report generated	25/05/2017

Section 2: Infomation provided by the investigator

1: Objectives

Null hypothesis	THC does not have an effect on body temperature
Alternative hypothesis	THC affects body temperature
Effect of interest	Difference in body temperature
Effect size	1 degree
Justification for effect size	biologically relevant, greater than circadian variation

2: Groups and sample size

Total number of animals in the experiment	24
Groups included in the primary analysis	3 groups:
• Group 1	role=control/comparator, n=8
• Group 2	role=test, n=8
• Group 3	role=test, n=8
Justification for sample size	power calculation for unpaired t-test (ES=1, SD=0.55, sig=0.05, power:0.9, 2-sided)

3: Randomisation and blinding

Experimental unit	animal

There is one step in this experiment where experimental units are allocated to groups:

• Allocation: randomisation

Randomisation strategy	complete randomisation
Randomisation procedure	EDA spreadsheet
Allocation concealment	treatments coded for individual animals

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

EDA Experimental Design Assistant

The EDA workflow

Objectives of the EDA

- Improve the reliability of published results
- Promote better understanding of experimental design, raise awareness about issues
- Facilitate peer review/assessment of the experimental plans with an explicit description
    - Transparency
    - Pre-registration
- Promote more careful consideration of the experimental plans
    - Spend time planning
    - Diagram facilitate discussion

nature methods
Techniques for life scientists and chemists

CORRESPONDENCE

EDA

The Experimental Design Assistant

To the Editor: The quality and reliability of much animal research in question. Unreliable or low-quality research represents an una ceptable waste of animals and research resources. In the US alon the cost of irreproducible research has been estimated at $28 billio annually, and issues with research design and reporting are estimate to account for half of that waste[1]. To address these issues, the Nation Centre for the Replacement, Refinement and Reduction of Anima in Research (NC3Rs) developed the ARRIVE guidelines to impro the reporting of *in vivo* research[2,3]. We now present the Experiment Design Assistant (EDA; https://eda.nc3rs.org.uk), a freely accessib web-based tool, which was launched to help researchers improve t design, conduct, analysis and reporting of animal experiments.

The system was developed by progressive interaction betwe an expert group experienced in providing advice on experiment design to researchers and a software development team. It includ a computer-aided design tool through which the user develops diagram that embodies the experimental plan. The diagram offe a new standard notation for describing experiments in whic methodological details and analysis plans are explicit (**Fig. 1**). Th facilitates communication between collaborators, funding bodie ethical review committees, journal editors and peer reviewers;

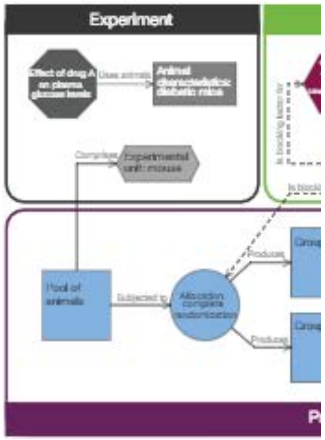


Figure 1 | Example of an EDA diagram. EDA diagram representing a two-gro treatments. Diagrams are composed of nodes and links to represent an enti experiment such as the null and alternative hypotheses, the effect of inter nodes represent the practical steps carried out in the laboratory such as th and the measurements taken. The green and red nodes represent the analys variables (e.g., blocking factors). For more details, see https://eda.nc3rs.o

PLOS BIOLOGY
FIFTEENTH ANNIVERSARY

COMMUNITY PAGE

The Experimental Design Assistant

Nathalie Percie du Sert, Ian Bamsey, Simon T. Bate, Manuel Berdoy, Robin A. Clark, Innes Cuthill, Derek Fry, Natasha A. Karp, Malcolm Macleod, Lawrence Moon, S. Clare Stanford, Brian Lings

Version 2 ▾ Published: September 28, 2017 • https://doi.org/10.1371/journal.pbio.2003779

Abstract

Addressing the common problems that researchers encounter when designing and analysing animal experiments will improve the reliability of in vivo research. In this article, the Experimental Design Assistant (EDA) is introduced. The EDA is a web-based tool that guides the in vivo researcher through the experimental design and analysis process, providing automated feedback on the proposed design and generating a graphical summary that aids communication with colleagues, funders, regulatory authorities, and the wider scientific community. It will have an important role in addressing causes of irreproducibility.

Figures

Included in the Following Collections

Meta-Research: Methods
Meta-Research: Tools for Improving Biomedical Research
Meta-Research: Reproducibility

Subject Areas

Experimental design
Research validity
Research design

The SyRF web app

SYSTEMATIC Review Facility

- Fully integrated online platform
- Free to use
- Secure screening database, data repository and analysis applications
- Educational resources for systematic review and meta-analysis
- Guidance on any aspect of preclinical systematic review and meta-analysis
- Flexible for individual projects
- Tools to support living systematic reviews

Formulate research question
↓
Protocol development and registration
↓
Systematic search
↓
Screen to include relevant studies
↓
Assess study quality
↓
Extract outcome data
↓
Analysis
↓
Publication / dissemination

NC 3Rs

Collaborative Approach to Meta Analysis and ·C·A·M·A·R·A·D·E·S· Review of Animal Data from Experimental Studies

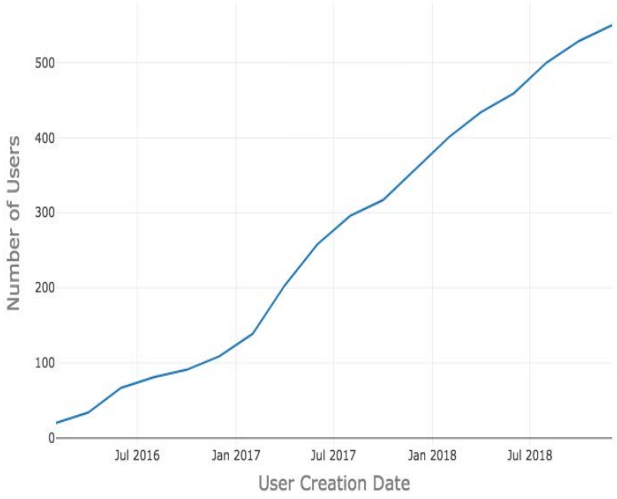
The SyRF web app

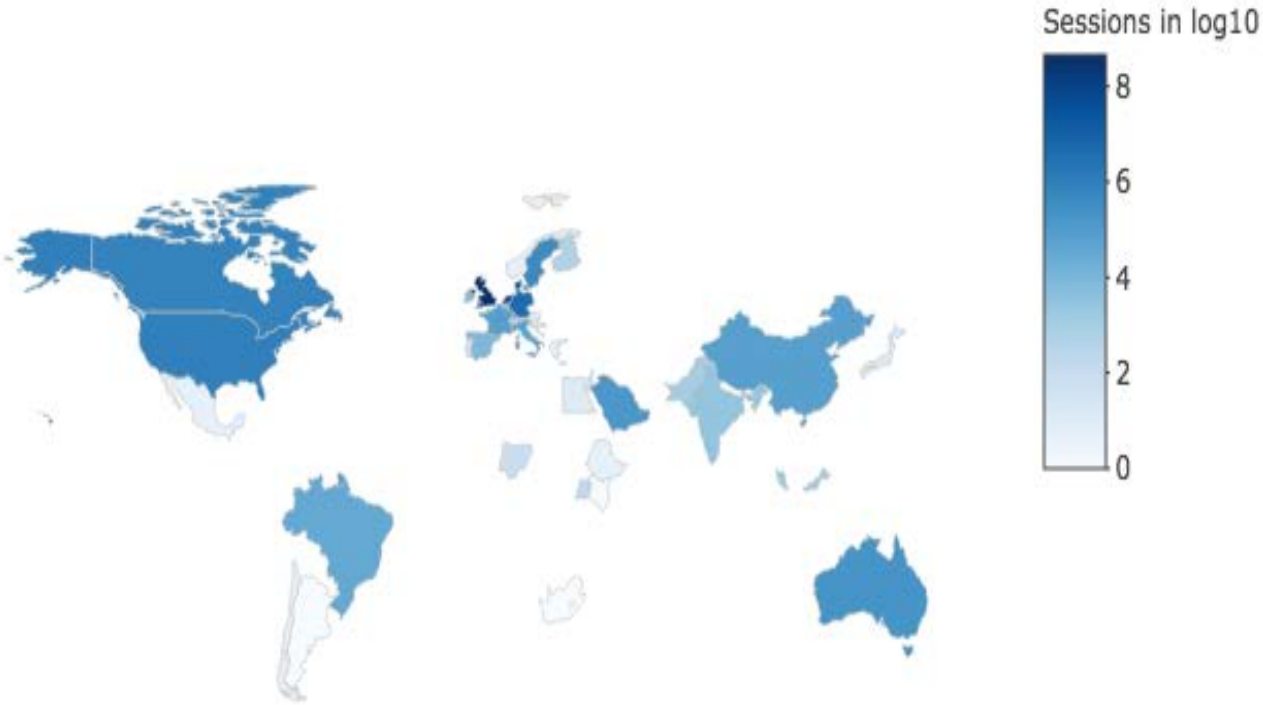
SYSTEMATIC Review Facility

Launched in 2017

Over 500 users – 300 projects on platform

Worldwide usage





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Collaborative Approach to Meta Analysis and ·C·A·M·A·R·A·D·E·S· Review of Animal Data from Experimental Studies

www.syrf.org.uk

Further information

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

www.nc3rs.org.uk/ARRIVE

http://syrf.org.uk/

www.nc3rs.org.uk

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