



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

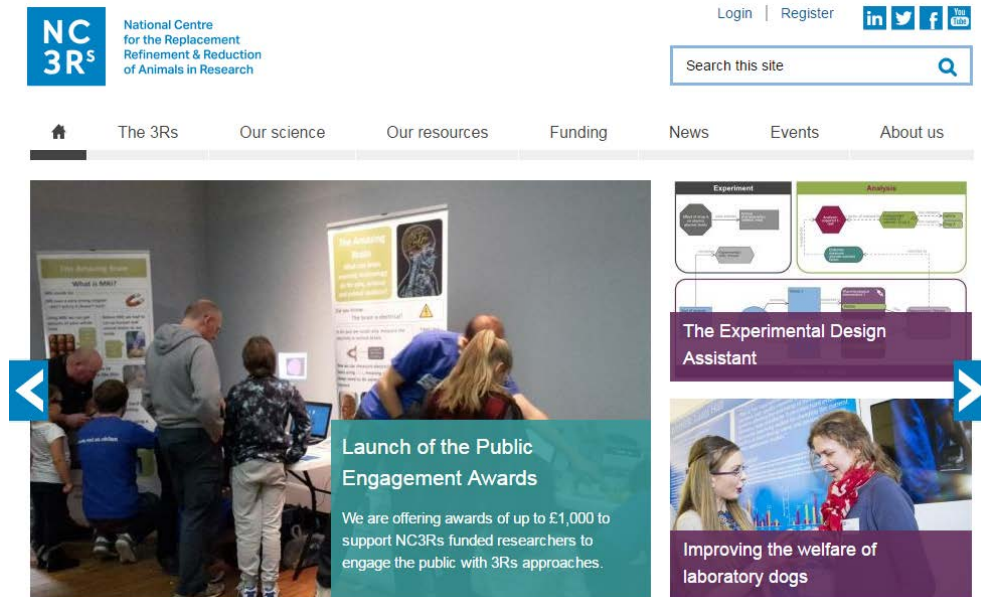
Information Meeting for Grant Applicants on the Call for Proposals
for Confirmatory Preclinical Studies and Systematic Reviews

Berlin, Tuesday 19 February 2019

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

 @NC3Rs

 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



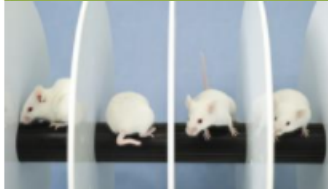
Video presentation describing our resources and the importance of good experimental design.

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)



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



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

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

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

The screenshot shows the title page and a checklist table from the ARRIVE Guidelines document. The title page includes the NC 3R^S logo and the title 'The ARRIVE Guidelines: Animal Research: Reporting of *In Vivo* Experiments'. Below the title, it states that the guidelines were developed as part of a FACTA initiative to improve the design, conduct and reporting of research using animals.

The checklist table is as follows:

Item	Recommendation	Checklist	Score	Comments
Title	Provide an accurate and concise description of the content of the article	Specific and	9	Provide abstract
Abstract	Provide an accurate summary of the background, reason for publication, including the scientific objectives and where appropriate, the study's experimental design and main findings of the study	Specific and	9	4. Missing type of facility, e.g. specific pathogen-free (SPF), type of cage or housing, bedding material, number of cage companions, time of day at material use for study
Introduction	Provide a clear and concise background including relevant references to provide context for the objectives and rationale for the study, and explain the experimental approach and rationale	Specific and	9	5. Randomised controlled trial design, randomised, double-blind, parallel, quality of care for fish, type of food, access to food and water, environmental enrichment
Objectives	Clearly describe the primary and any secondary objectives of the study or the experimental design	Specific and	9	6. Blinded randomised assessments and interventions that were carried out prior to, during, or after the experiment
Methods	Indicate the nature of the study and any permissions, relevant licences (e.g. ARRIVE licence) and approvals for the study, and indicate any institutional guidelines for the use of animals in research	Specific and	9	7. Specify the total number of animals used in each experiment, and the number of animals in each experimental group
Study design	For each experimental group, provide details of the study design including: <ul style="list-style-type: none"> a. The number of experimental and control groups b. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives c. The experimental unit (e.g. single animal, group or cage of animals) d. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	8. Specify the number of independent replications of each assessment, if relevant and appropriate
Experimental animals	For each experimental group, provide details of the animals used, including: <ul style="list-style-type: none"> a. The species, strain, sex, age, and sex of administration, and any other characteristics of the animals b. Whether a humane laboratory source was used c. Whether a humane source was used for specific animals, such as mice d. Whether a humane source was used for specific animals, such as mice e. Whether a humane source was used for specific animals, such as mice 	Specific and	9	9. Describe the number of independent replications of each assessment, if relevant and appropriate
Experimental methods	Provide a clear and concise description of the experimental methods used, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	10. Describe the number of independent replications of each assessment, if relevant and appropriate
Statistical analysis	Provide a clear and concise description of the statistical methods used, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	11. Describe the number of independent replications of each assessment, if relevant and appropriate
Results	Provide a clear and concise description of the results, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	12. Describe the number of independent replications of each assessment, if relevant and appropriate
Conclusions	Provide a clear and concise description of the conclusions, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	13. Describe the number of independent replications of each assessment, if relevant and appropriate
References	Provide a clear and concise description of the references, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	14. Describe the number of independent replications of each assessment, if relevant and appropriate
Supplementary material	Provide a clear and concise description of the supplementary material, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	15. Describe the number of independent replications of each assessment, if relevant and appropriate
ARRIVE checklist	Provide a clear and concise description of the ARRIVE checklist, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	16. Describe the number of independent replications of each assessment, if relevant and appropriate
ARRIVE checklist	Provide a clear and concise description of the ARRIVE checklist, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	17. Describe the number of independent replications of each assessment, if relevant and appropriate
ARRIVE checklist	Provide a clear and concise description of the ARRIVE checklist, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	18. Describe the number of independent replications of each assessment, if relevant and appropriate
ARRIVE checklist	Provide a clear and concise description of the ARRIVE checklist, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	19. Describe the number of independent replications of each assessment, if relevant and appropriate
ARRIVE checklist	Provide a clear and concise description of the ARRIVE checklist, including: <ul style="list-style-type: none"> a. The primary and secondary objectives of the study and other objectives, and how they are related to the primary and secondary objectives b. The experimental unit (e.g. single animal, group or cage of animals) c. A time-log diagram or flow chart to describe the experimental design 	Specific and	9	20. Describe the number of independent replications of each assessment, if relevant and appropriate



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

Endorsement of the ARRIVE guidelines

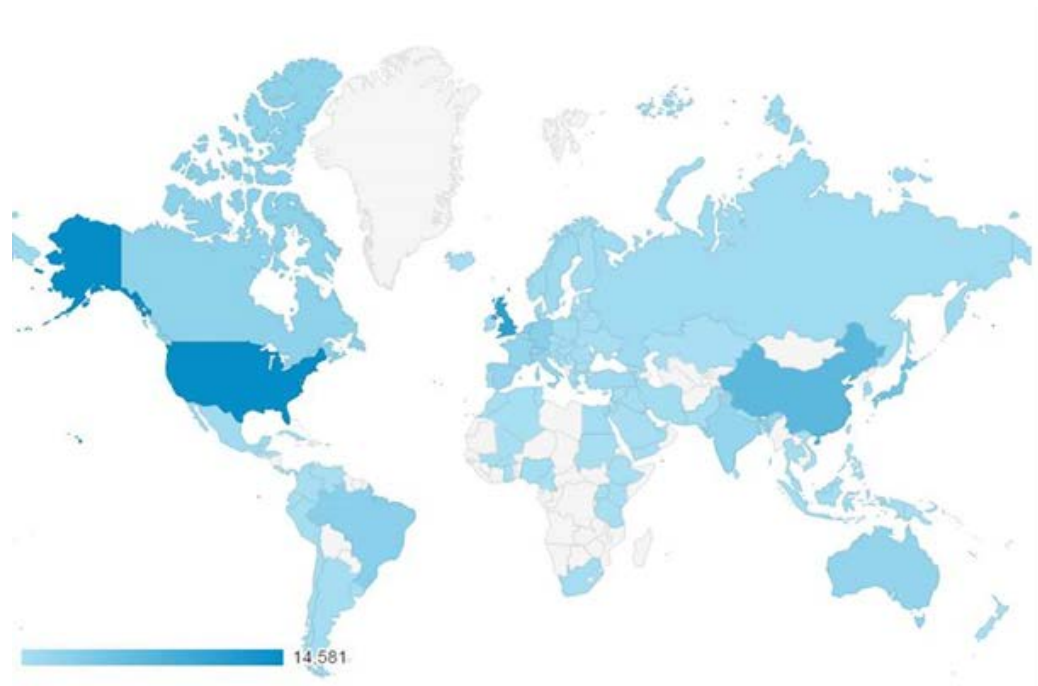
Over **1000** journals and organisations recommend the ARRIVE guidelines

Journals

Funders

Universities

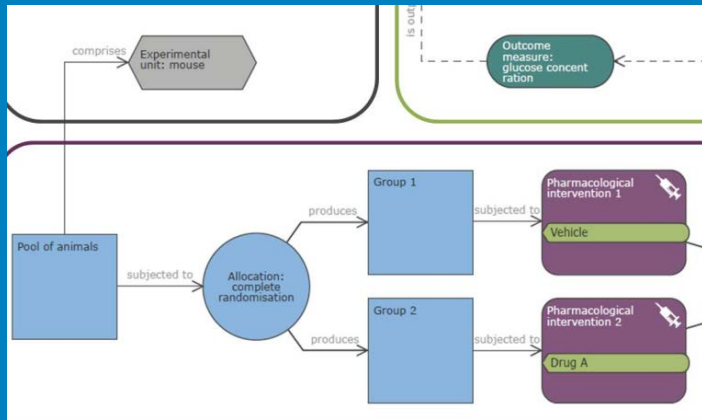
Learned Societies



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/>



The Experimental Design Assistant

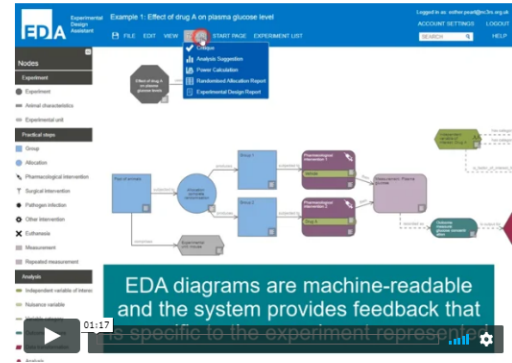
A free resource from the NC3Rs used by over 5,000 researchers worldwide to help design robust experiments more likely to yield reliable and reproducible results.

The EDA helps you build a diagram representing your experimental plan, which can be critiqued by the system to provide bespoke feedback. The EDA also:

- Recommends statistical analysis methods
- Provides support for randomisation and blinding
- Performs sample size calculations

For an overview of how the EDA works, watch our one minute video.

The EDA website also provides information about the different concepts of experimental design, and how to apply these in your experiments.



Step 1

[Login or Register](#)

Start using the EDA application

Step 2

Plan your experiment as a diagram

Check the [examples](#) and the [user guide](#) for more information

Step 3

Critique your design

The critique function enables you to get feedback and advice on your diagram, find more information [here](#)

Step 4

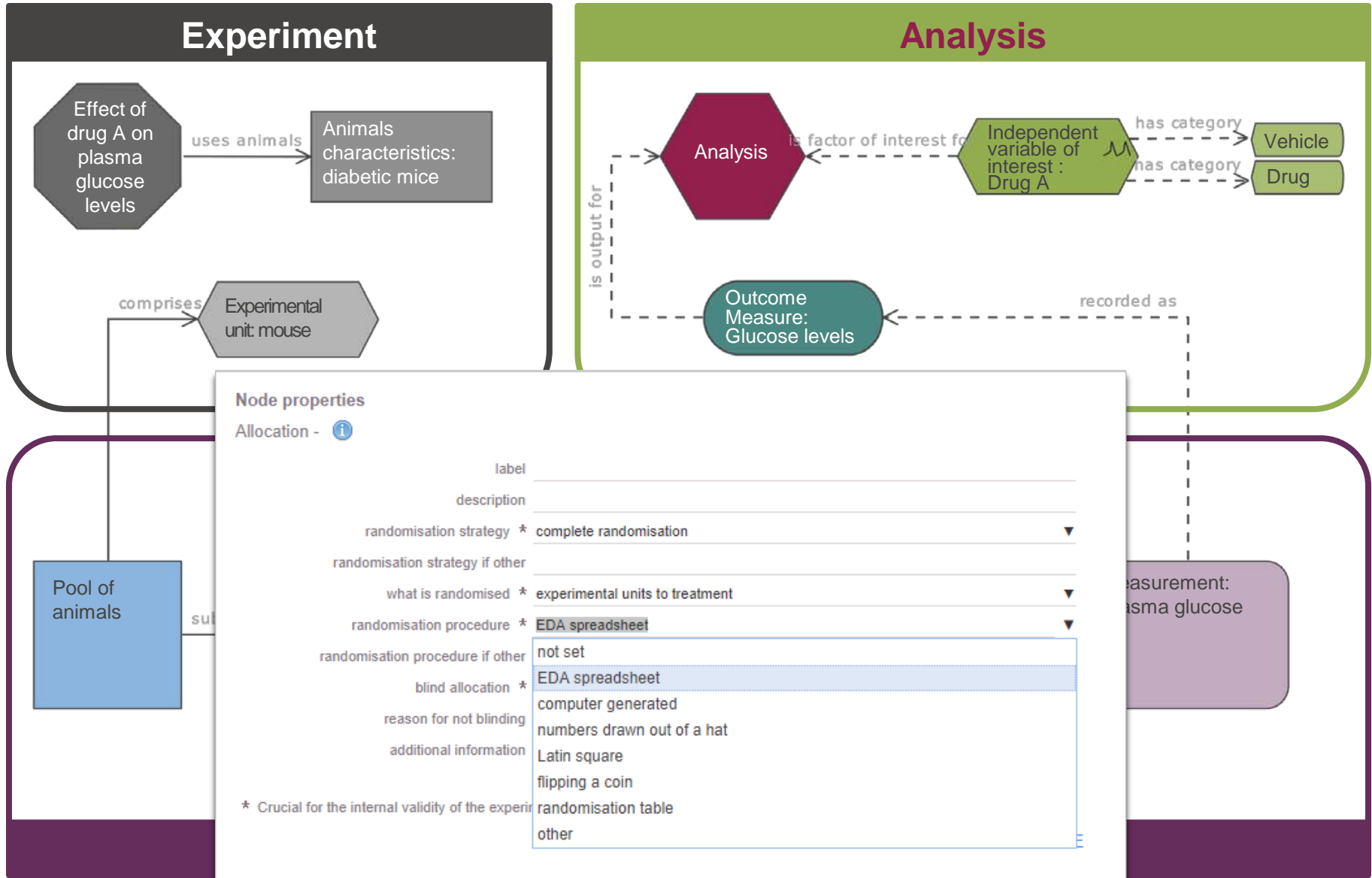
Improve your design

Modify your experimental plan based on feedback from the system

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



NC 3R 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 blocked

Having no nuisance variable implies influence the outcome measure. This is influenced by many variables. Identifying these variables in the experiment to detect changes induced

The type of things to consider may include the intervention or measurement, the experimenter with different levels of blinding relevant to a particular experiment, and the need to identify new sources of variability.

These should be indicated on the EDA. The best way to account for each of the nuisance variables. If the objective of the experiment, there are

- **standardised** - for example randomised
- **randomised across** - for example randomly allocating each cage
- **blocked** - for example the day of analysis
- **nested within another variable** - for example 'mouse' when multiple neurons
- used as a **covariate** - for example
- if none of these things are done

NC 3R 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: Information 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
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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

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

The Experimental Design Assistant

To the Editor: The quality and reliability of much animal research in question. Unreliable or low-quality research represents an unacceptable waste of animals and research resources. In the US alone the cost of irreproducible research has been estimated at \$28 billion annually, and issues with research design and reporting are estimated to account for half of that waste¹. To address these issues, the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) developed the ARRIVE guidelines to improve the reporting of *in vivo* research^{2,3}. We now present the Experimental Design Assistant (EDA; <https://eda.nc3rs.org.uk>), a freely accessible web-based tool, which was launched to help researchers improve their design, conduct, analysis and reporting of animal experiments.

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

OPEN ACCESS

COMMUNITY PAGE

The Experimental Design Assistant

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Version 2

Published: September 28, 2017 • <https://doi.org/10.1371/journal.pbio.2003779>

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Subject Areas

- Experimental design
- Research validity
- Research design

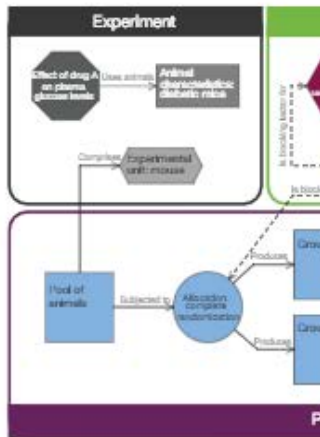


Figure 1 | Example of an EDA diagram. EDA diagram representing a two-grp treatments. Diagrams are composed of nodes and links to represent an entire experiment such as the null and alternative hypotheses, the effect of inter nodes represent the practical steps carried out in the laboratory such as the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

Abstract

- Introduction
- Conclusion
- Supporting information
- References

- Reader Comments (0)
- Media Coverage (1)
- Figures

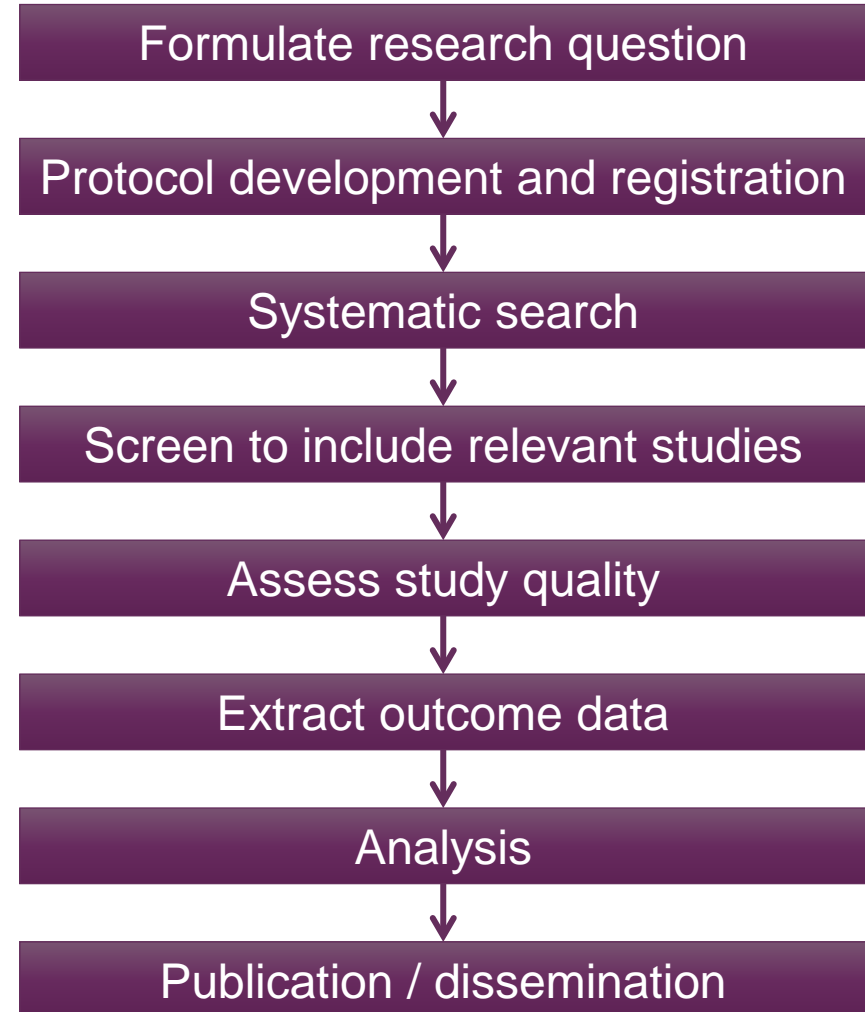
Abstract

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

The SyRF web app

- 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



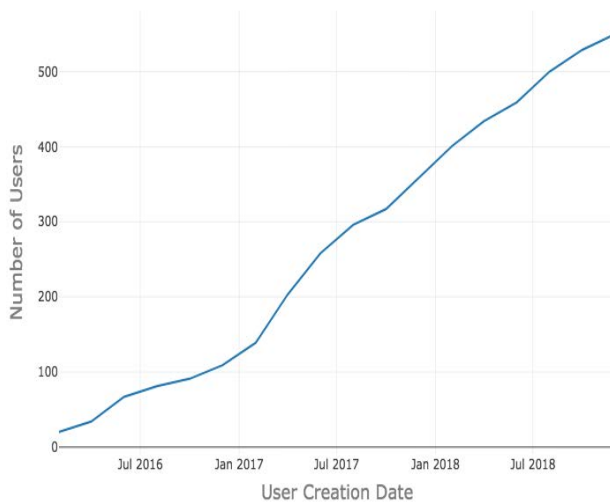


The SyRF web app

Launched in 2017

Over 500 users – 300 projects on platform

Worldwide usage



Further information

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

www.nc3rs.org.uk/ARRIVE

<http://syrf.org.uk/>

www.nc3rs.org.uk

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