

Improving experimental design:

Ethical implications and how the Experimental Design Assistant (EDA) can help



National Institutes of Health  
*Office of Laboratory Animal Welfare*

**September 14, 2023**

Esther Pearl, PhD

Programme Manager for Experimental Design,  
National Centre for the Replacement, Refinement, and  
Reduction of Animals in Research (the NC3Rs).



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

# Improving experimental design: Ethical implications and how the Experimental Design Assistant (EDA) can help

**Dr Esther Pearl**

14 September 2023

National Institutes of Health Office of Laboratory Animal Welfare  
Online Seminar



PIONEERING BETTER SCIENCE

# Replacement, Reduction and Refinement

A UK-based scientific organisation dedicated to helping the research community worldwide to identify, develop and use 3Rs technologies and approaches.

[Our mission](#)



# Learning objectives



## By the end of this talk you will:

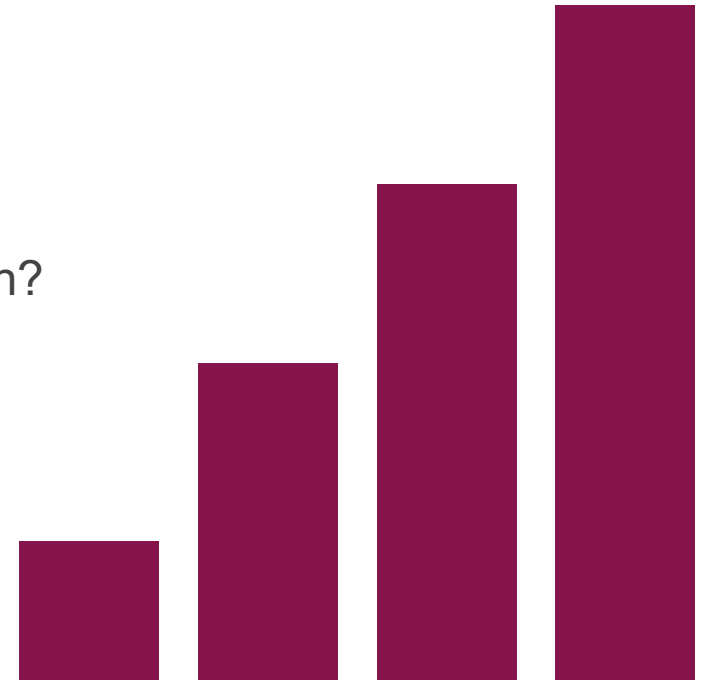
- Understand why we need to improve experimental design
- Identify key ways to improve biomedical research
- Know what the Experimental Design Assistant (EDA) is and its benefits to researchers
- Understand how the EDA can be used as part of the ethical review process

# Poll



## Introductory poll

- Are you involved in designing experiments?
- Have you had any formal training in experimental design?
- Do you have access to statistical support?
- Do you sit on an IACUC?
- Do you conduct your own research?



# Why we need to improve experimental design

# Research waste



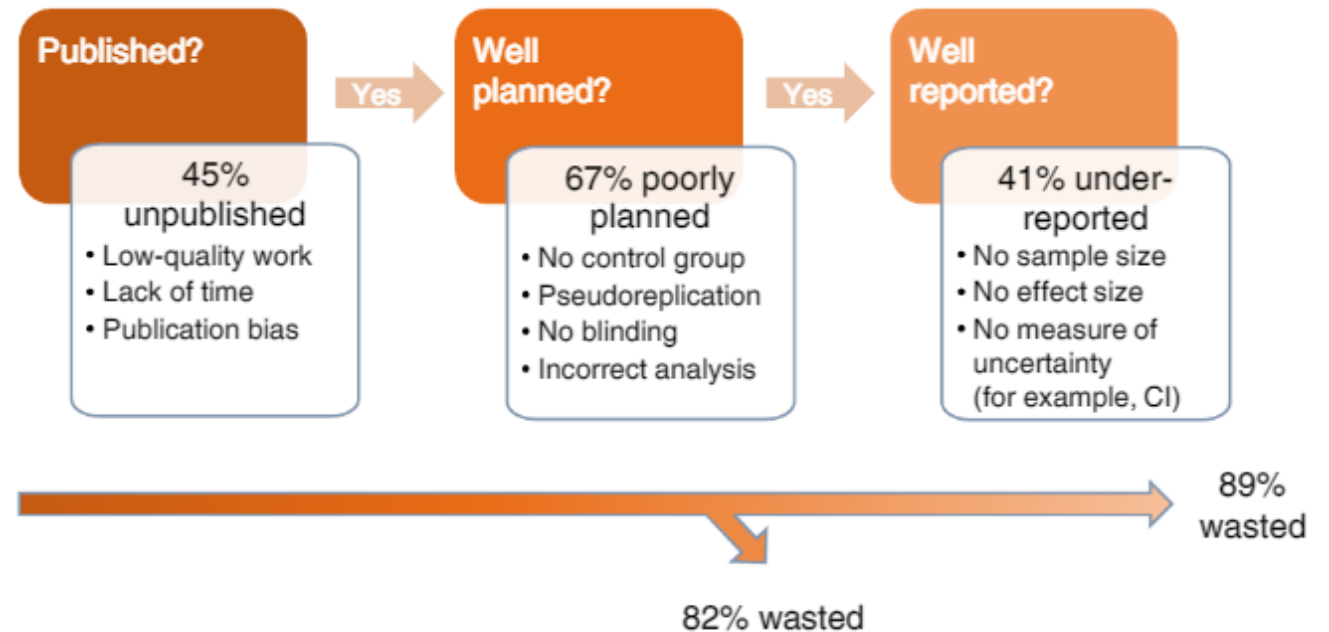
- 33 meta-studies in ecology
- 10,464 studies



## THE LANCET



**85% of investment in biomedical research wasted**



Purgar M, Klanjscek T and Culina A (2022). Quantifying research waste in ecology. *Nat Ecol Evol.* <http://dx.doi.org/10.1038/s41559-022-01820-0>

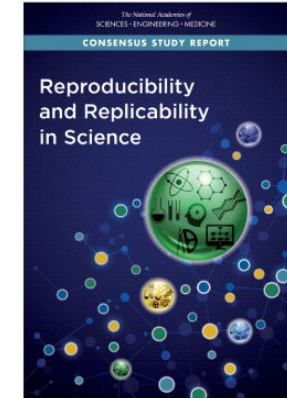
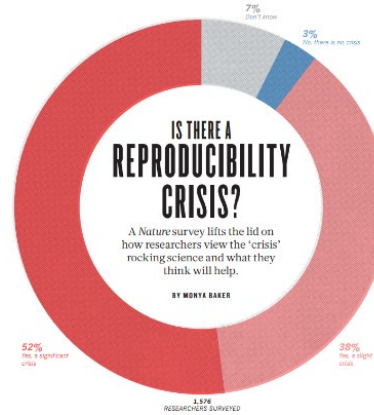


# Research waste in *in vivo* studies

## 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.




# Contemporary definitions of the 3Rs

	Standard	Contemporary
Replacement	Methods which <b>avoid the use of animals</b> (or species 'protected' under law) in areas where they otherwise would have been used	Accelerating the development and use of models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals (i.e. <i>in vitro</i> or <i>in silico</i> )
Reduction	Methods which <b>minimise the number of animals used</b> per experiment (or maximise the information gained from a given number of animals)	Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base
Refinement	Improvements to scientific procedures and husbandry which <b>minimise pain, suffering, distress or lasting harm</b> and/or improve animal welfare	Advancing research animal welfare by exploiting the latest <i>in vivo</i> technologies and by improving understanding of the impact of welfare on scientific outcomes


# Resources from the NC3Rs

U.S. Department of Health & Human Services | National Institutes of Health



## ACD Working Group on Enhancing

U.S. Department of Health & Human Services



National Institutes of Health  
*Turning Discovery Into Health*

Health Information | Grants & Funding | News

**COVID-19 is an emerging, rapidly evolving threat**

- Get the latest public health information from CDC »
- Get the latest information on COVID-19 »



Home » Research & Training » Rigor and Reproducibility

### RIGOR AND REPRODUCIBILITY

Rigor and Reproducibility

- Reporting Guidelines
- Application Instructions
- Training
- Funding Opportunities

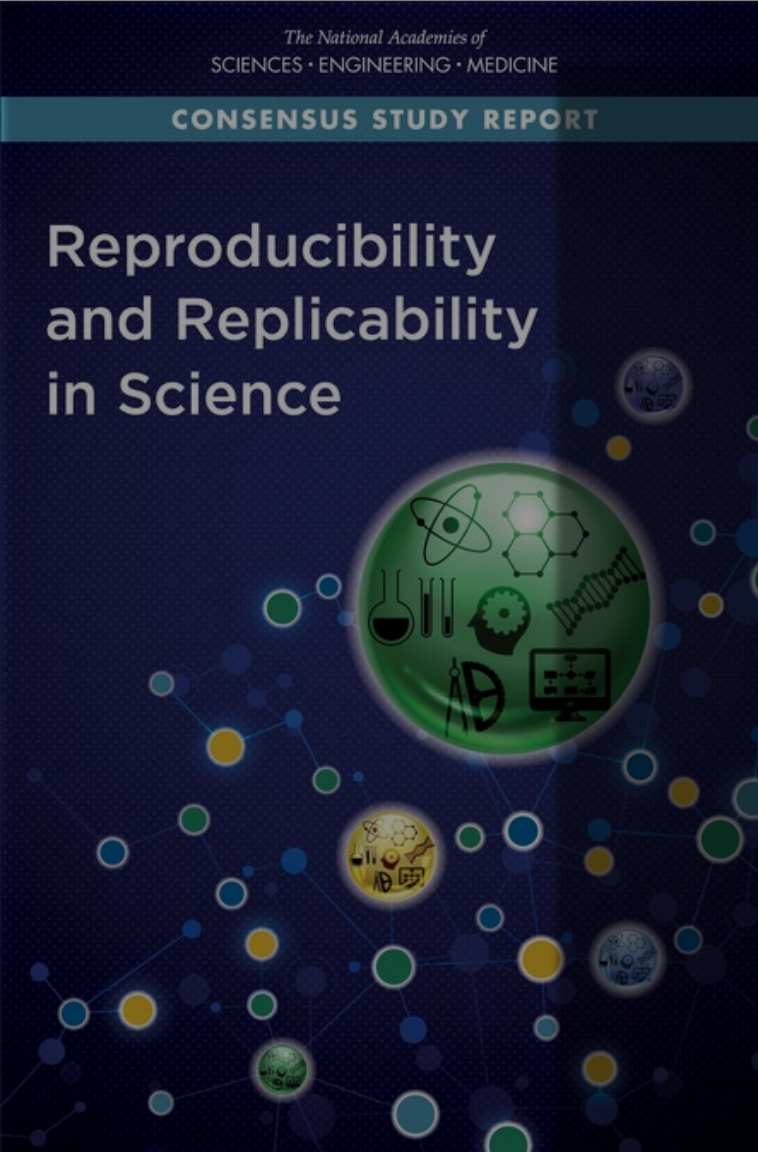
Principles and Best Practices for Preclinical Research



The National Academies of  
SCIENCES • ENGINEERING • MEDICINE

## CONSENSUS STUDY REPORT

# Reproducibility and Replicability in Science



## PROCEEDINGS OF A WORKSHOP

# Enhancing Scientific Reproducibility in Medical Research through Transparent Reporting

The National Academies of  
SCIENCES • ENGINEERING • MEDICINE



# Reproducibility and reliability of biomedical research: improving research practice

Symposium report, October 2015



Omitting null results



Data dredging



Weak experimental design



Underpowered study



Errors



Underspecified methods

# Key ways to improve biomedical research

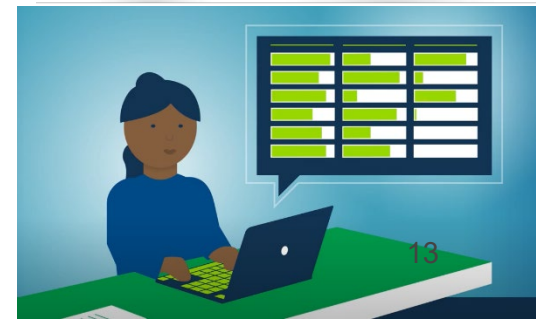


# Key aspects of good experimental design

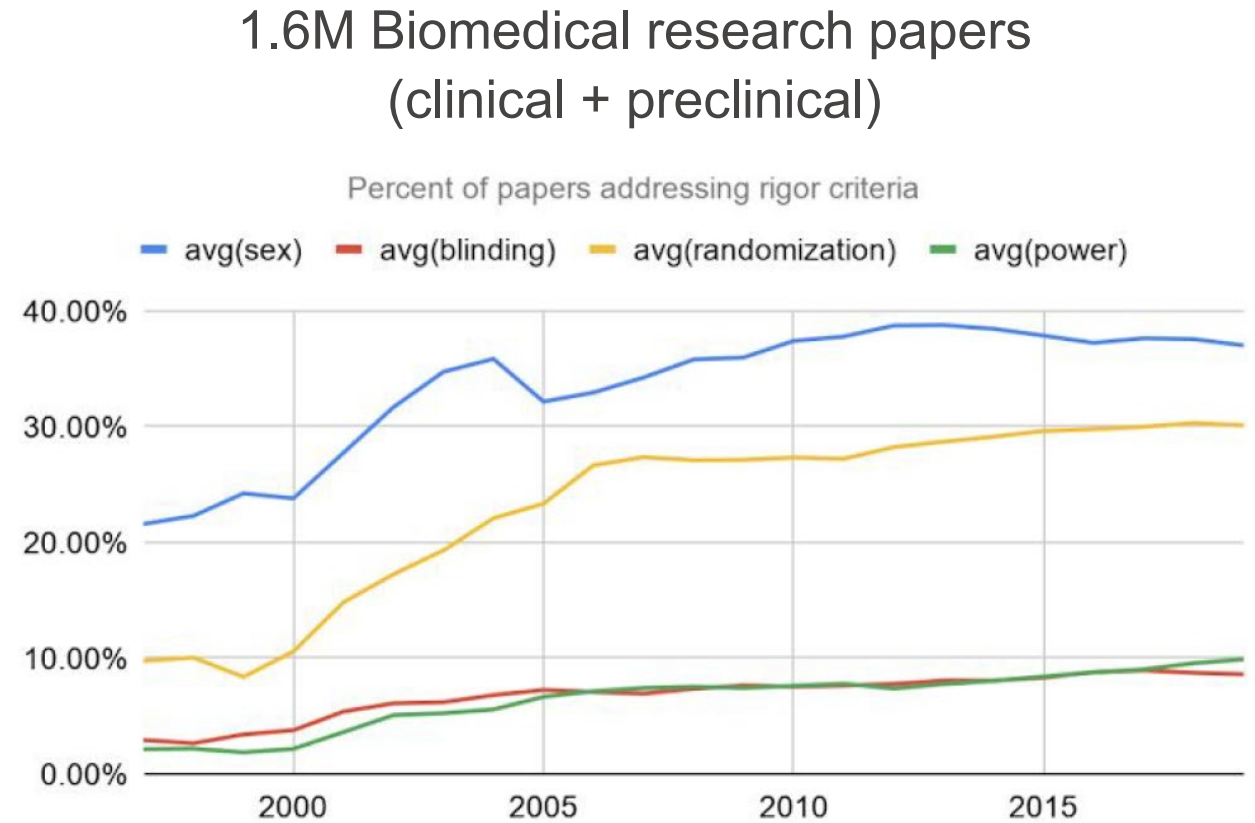
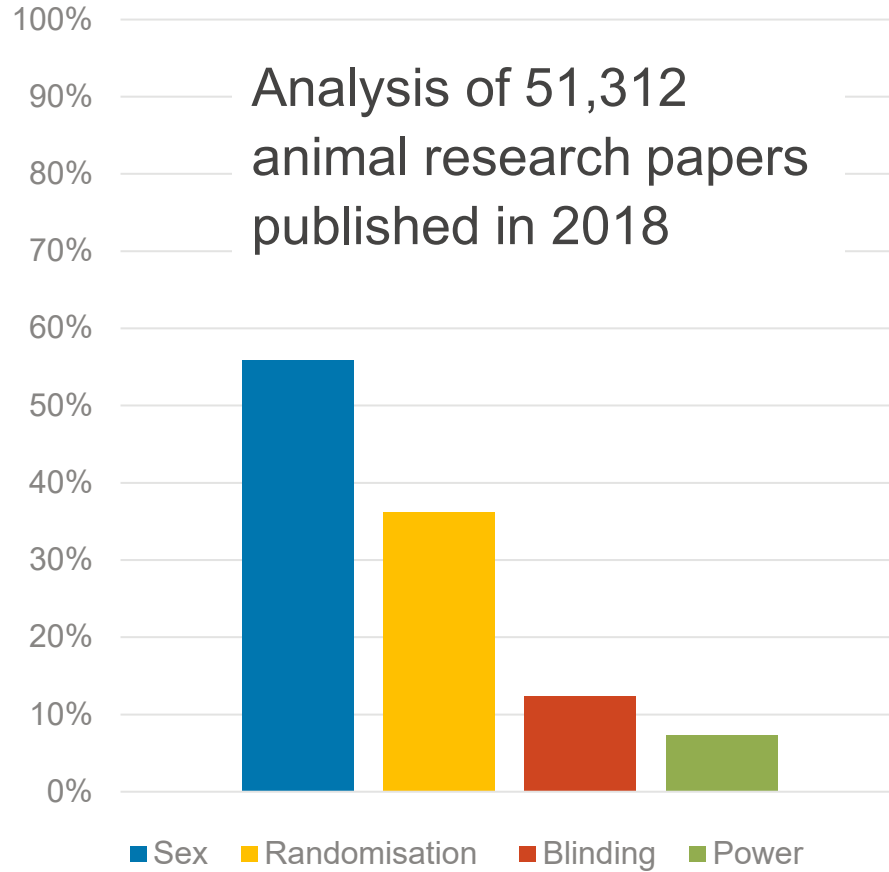
If experiments are not rigorously designed the results are unlikely to be reliable.

Aspects of good experimental design you can look out for:

- Randomisation
- Masking (blinding)
- Using both sexes
- Appropriate sample size
- Pre-planned statistical analysis method



# Reporting of rigour criteria in animal research publications



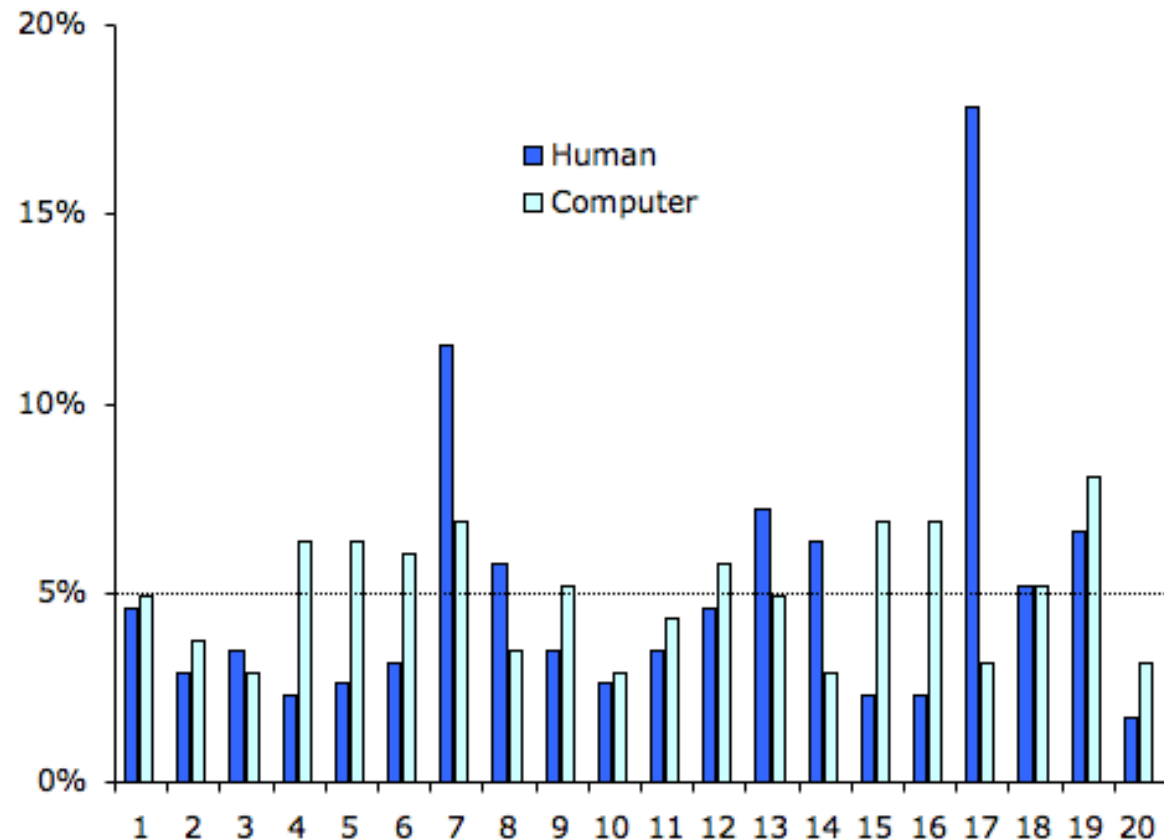


# Randomisation



How the randomisation sequence is generated is important – haphazard is not random. Random sequences can be generated by computers, dice or flipping coins.

Pick a number between 1 and 10



# Randomisation



How the randomisation sequence is generated is important – haphazard is not random. Random sequences can be generated by computers, dice or flipping coins.

## Random allocation to interventions

Randomisation is crucial for two reasons:

1. Minimise selection bias

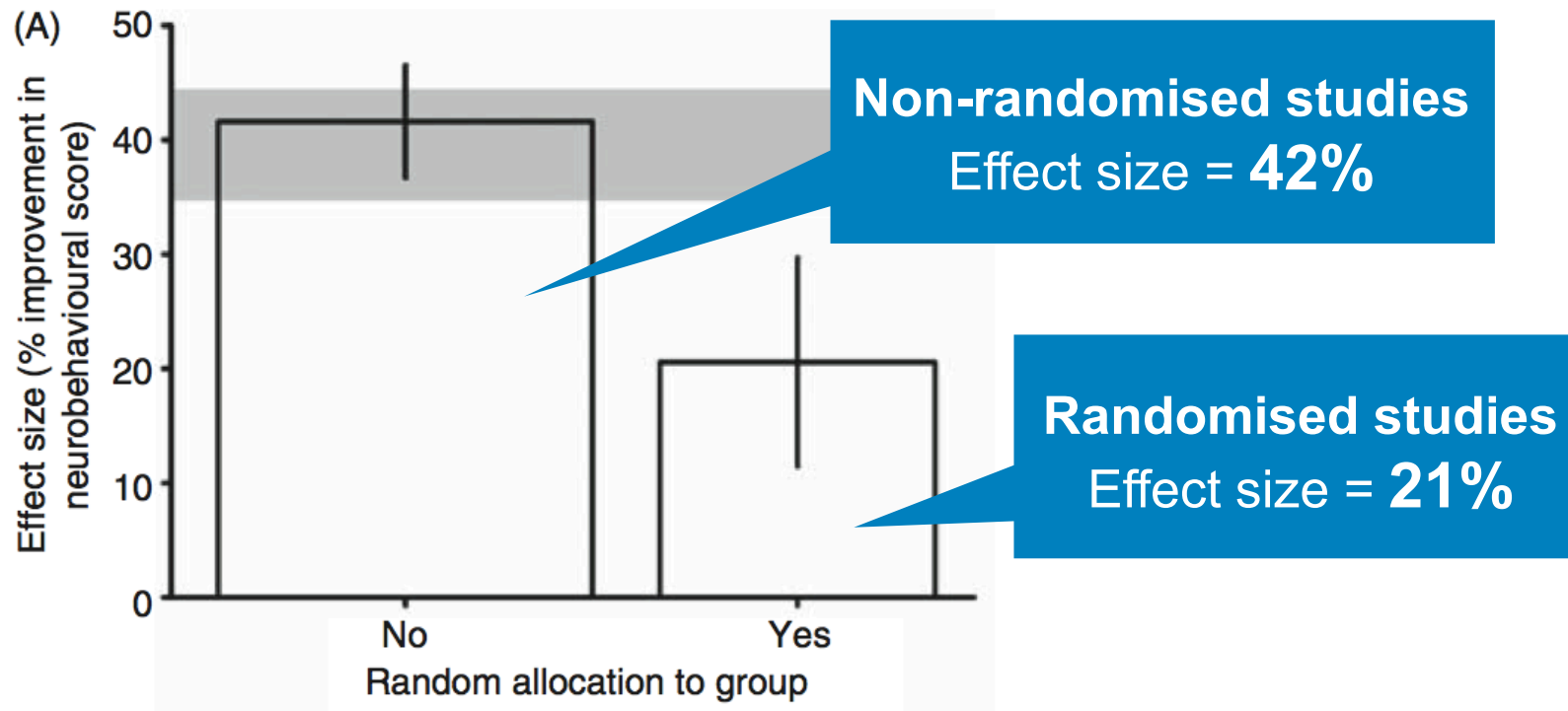
e.g. haphazard selection may result in slowest mice allocated to the same group

2. Key assumption of the statistical analysis

Different groups should be drawn from the same background population using random sampling

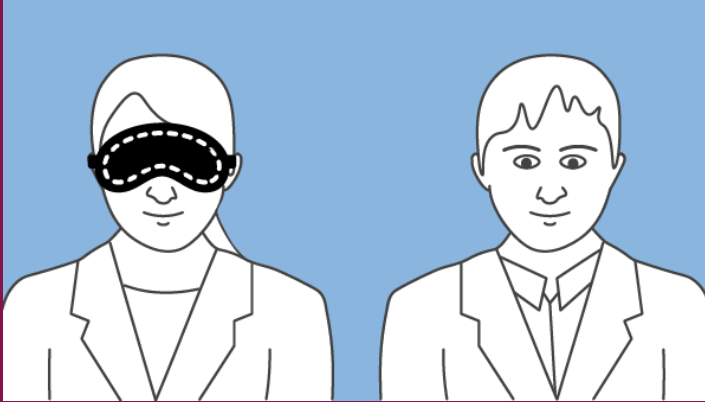
# Randomisation

- Animal models of multiple sclerosis
- Comparison of randomised and non-randomised studies



Studies not randomised **overestimate** treatment efficacy

# Masking



Being unaware of group assignment (e.g. not knowing which intervention any animal has received, how they are grouped or which group data came from).

## **Masking can be implemented at different steps in an experiment**

### **During allocation and intervention**

Steps where the animals are assigned to experimental groups and steps when they receive the experimental intervention(s).

### **During conduct of the experiment**

Housing and welfare management of the animals during the experiment.

### **When assessing the outcome**

Steps where an outcome is measured, or a sample processed in preparation for a measurement.

### **When assessing results**

Data processing and statistical analysis.

# Reducing bias

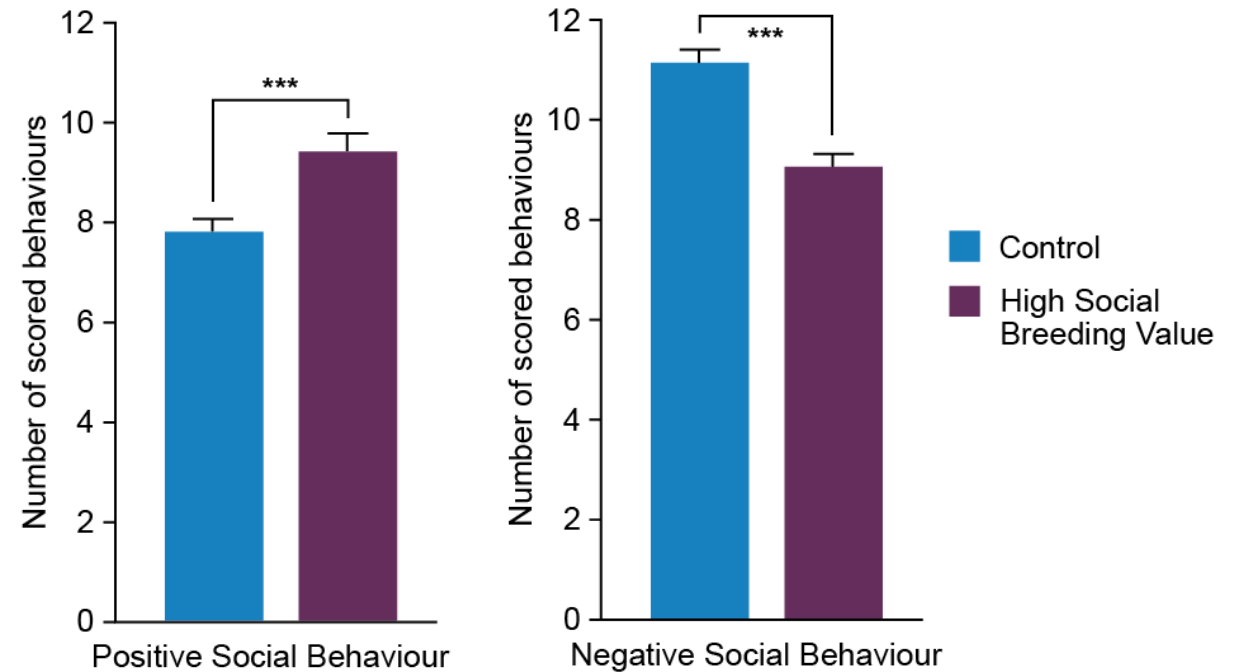
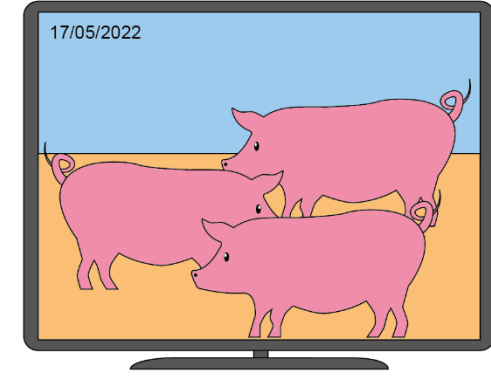
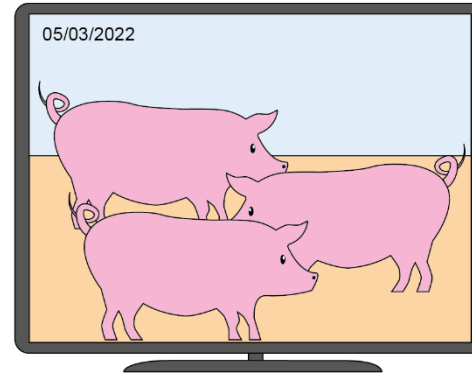
157 veterinary medicine students

Trained to identify positive and negative social interactions in pigs

5-minute video clips

Control pigs, and 'High Social Breeding Value' pigs

## Masking



# Reducing bias

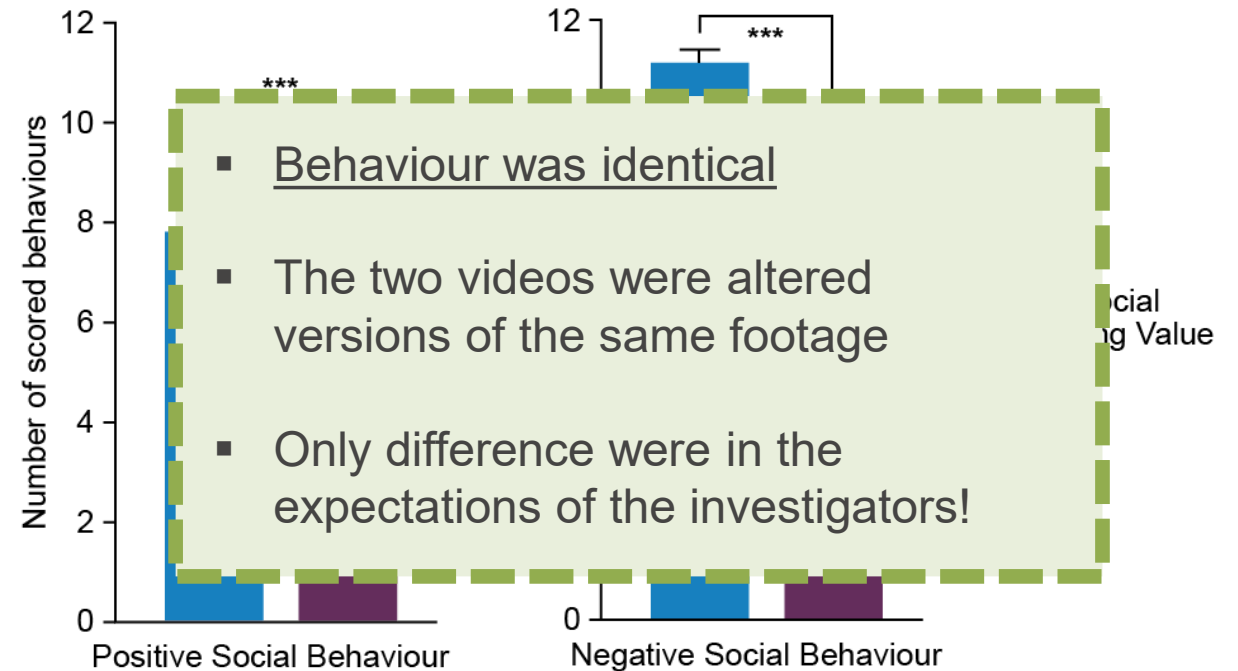
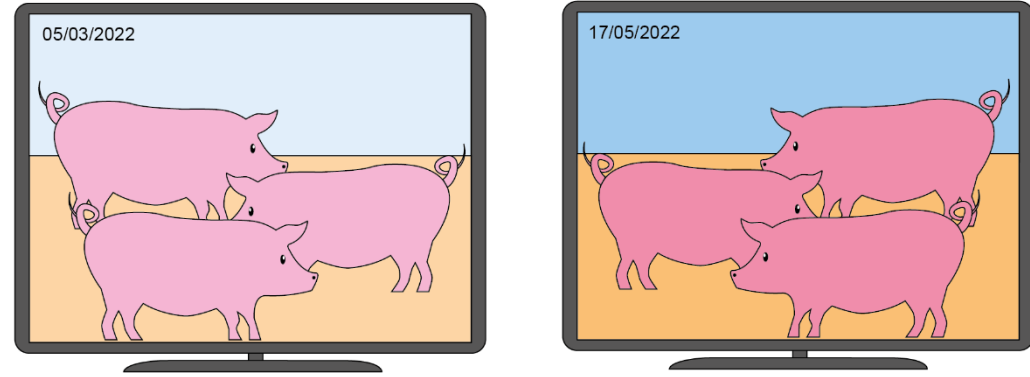
157 veterinary medicine students

Trained to identify positive and negative social interactions in pigs

5-minute video clips

Control pigs, and 'High Social Breeding Value' pigs

# Masking





# Using masking/blinding in in vivo experiments

## On this page

- Why use masking in an animal experiment?
- How to make it work in practice?
- Examples of masking strategies for animal experiments
- References

## Why use masking in an animal experiment?

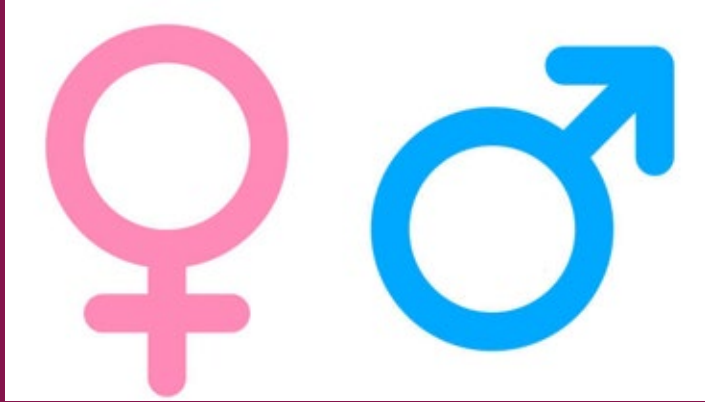
Masking (also known as blinding) is a methodological process where an animal's allocation to a specific experimental group is concealed from the people running the study, caring for the animals or analysing the data.



**Read the full article in PLOS Biology** <sup>Ⓞ</sup>

A qualitative study of the barriers to using blinding in in vivo experiments and suggestions for improvement

# Using both sexes



Most research is conducted on male subjects.

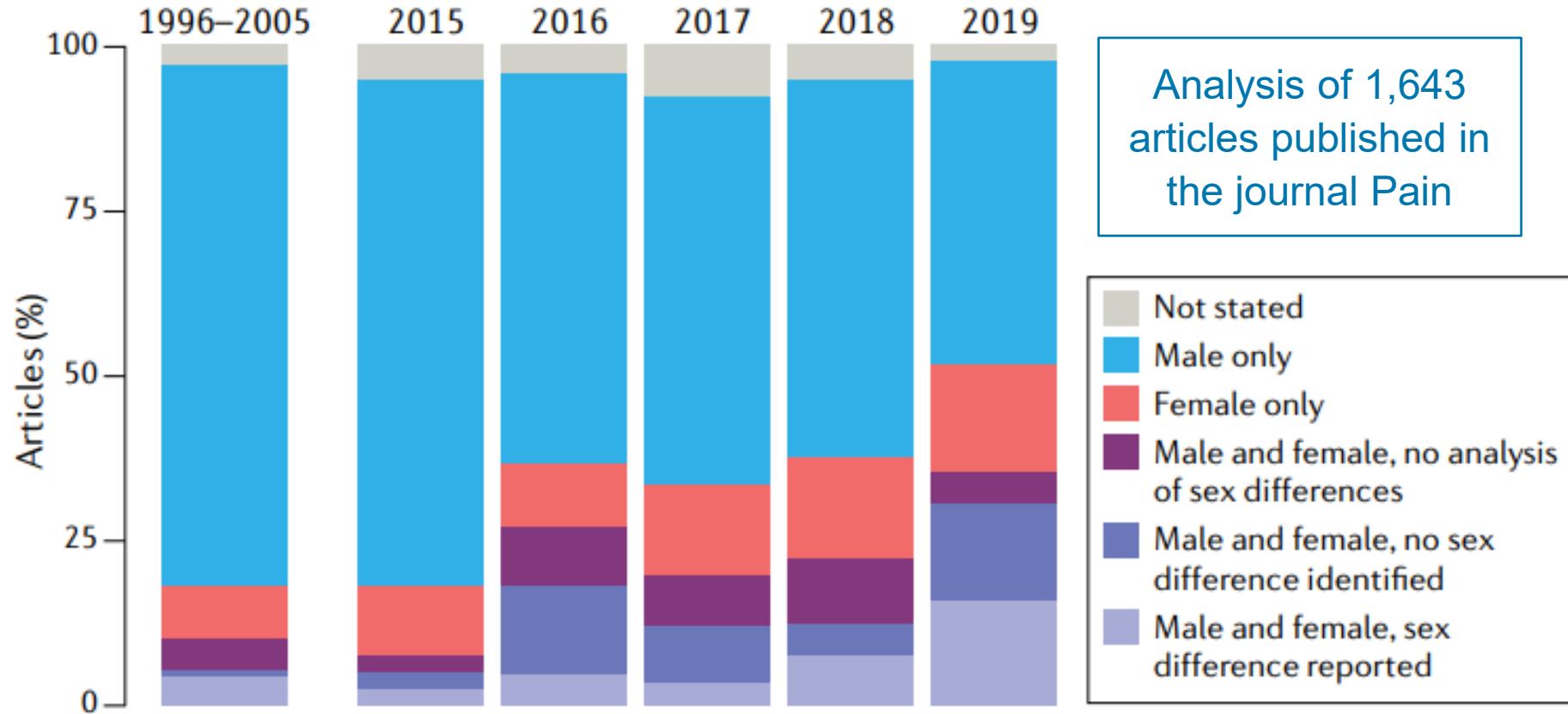
We cannot assume that findings in males translate to females.

## Why it is important to use both sexes in preclinical research

- Issue recognised for clinical trials, which now include women in the research pipeline
- But limited change in preclinical research, despite role in informing clinical trials
- **Example of Thalidomide – teratogenic effects could have been predicted with in vitro testing of female human tissue**
- Funders actively pushing the inclusion of sex as a biological variable (e.g. NIH)

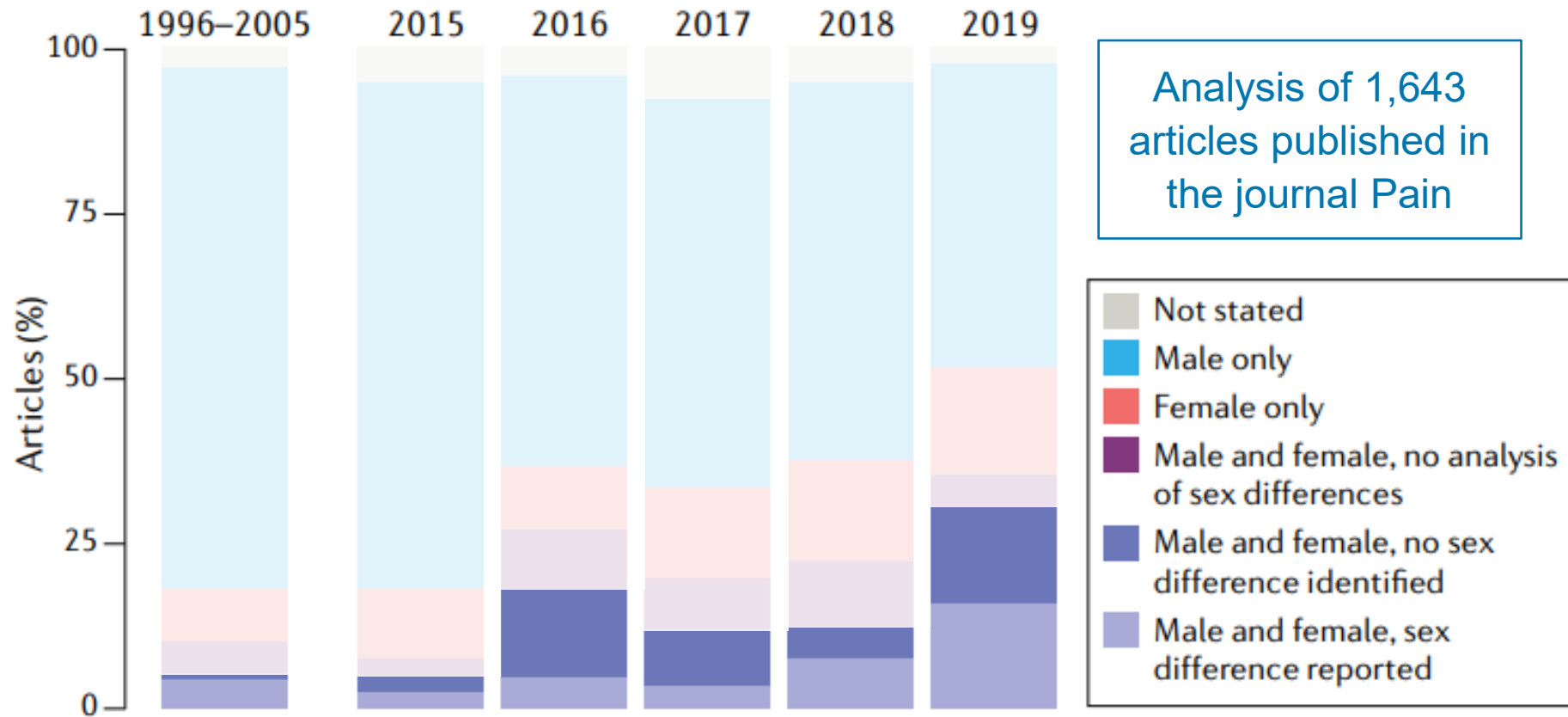
# Sex bias in animal experiments

**b Choice and reporting of animal subjects in the journal *Pain***



# Sex bias in animal experiments

**b Choice and reporting of animal subjects in the journal Pain**



# Using both male and female animals

<https://eda.nc3rs.org.uk/experimental-design-animal-characteristics>

In most cases researchers should include both sexes in their protocol

It should be clear from the protocol what researchers are trying to do

- Are they including both sexes to ensure results are generalisable?
- Are they directly comparing the sexes? Do they have adequate numbers to make this comparison? Will they need to include more animals?
- If the researcher is not directly comparing the sexes they may not need to increase animal numbers
- Suggest the researcher consults a statistician



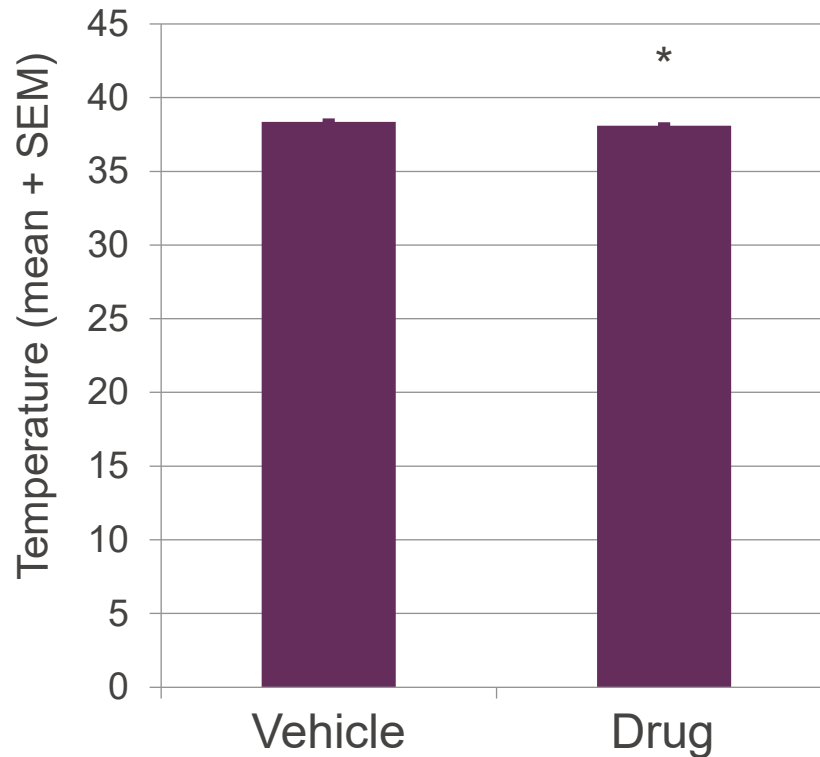
# Sample size



Using an appropriate sample size is essential to the reliability of results.

## The number of animals in an experiment must be justified

If data will be compared with a statistical test (e.g. tests that give a p-value or F statistic) the sample size should be determined with a power calculation.





# Sample size



Using an appropriate sample size is essential to the reliability of results.

## The number of animals in an experiment must be justified

If data will not be compared with statistical tests it is still important to justify the number of animals needed for the study.

For example, if the study aims to establish if a new surgical technique works in mice the number of animals needed depends on how many animals the researcher needs to ascertain if the technique would work and be practical for the future applications (e.g. a further study).

# Analysis plan



**It is important that a statistical analysis plan is made before the study starts**

Analysis plans are an integral part of experimental design.

- Promote careful consideration of variables and outcome measures
- Identify appropriate analysis path (inferential vs descriptive statistics)
- Helps prevent p-hacking
- Identify statistical support needs

# The EDA: benefits for researchers and IACUCs

# Experimental Design Assistant (EDA)

Online tool for researchers to design *in vivo* experiments

Free to use

Secure

Focuses on internal validity

Developed by *in vivo* researchers and statisticians

[eda.nc3rs.org.uk](http://eda.nc3rs.org.uk)



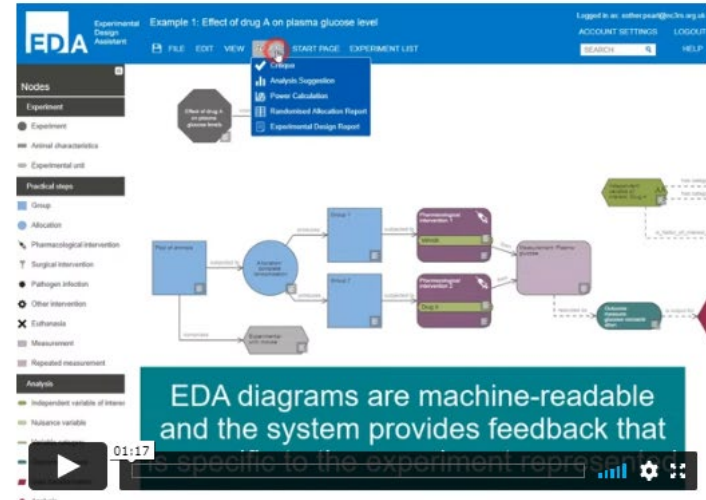
## The Experimental Design Assistant

A free resource from the NC3RS used by over 12,000 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.

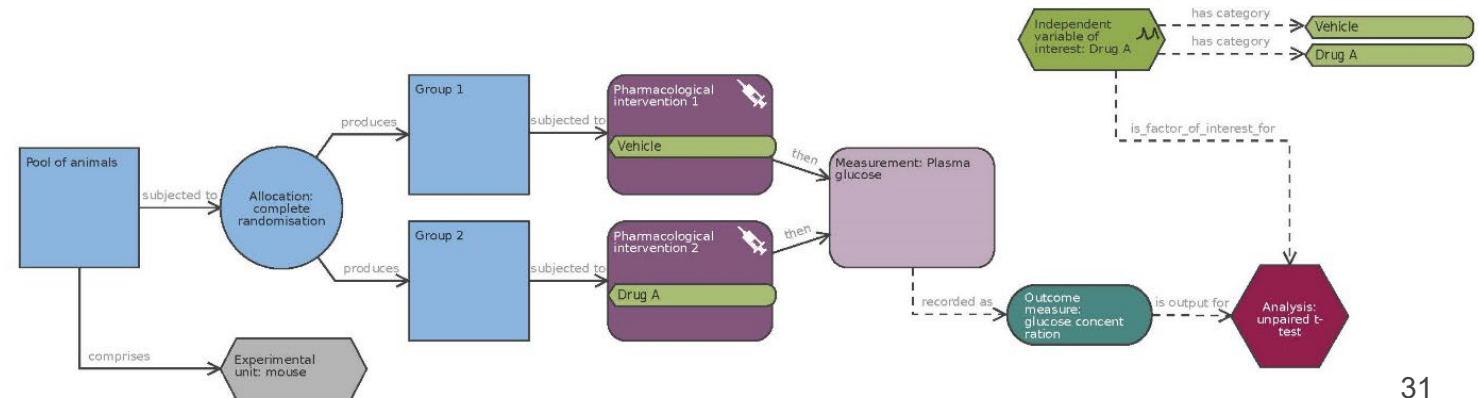
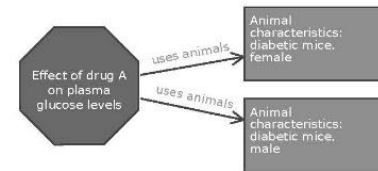


The EDA website also provides information about the different concepts of experimental design, and how to apply these in your experiments.

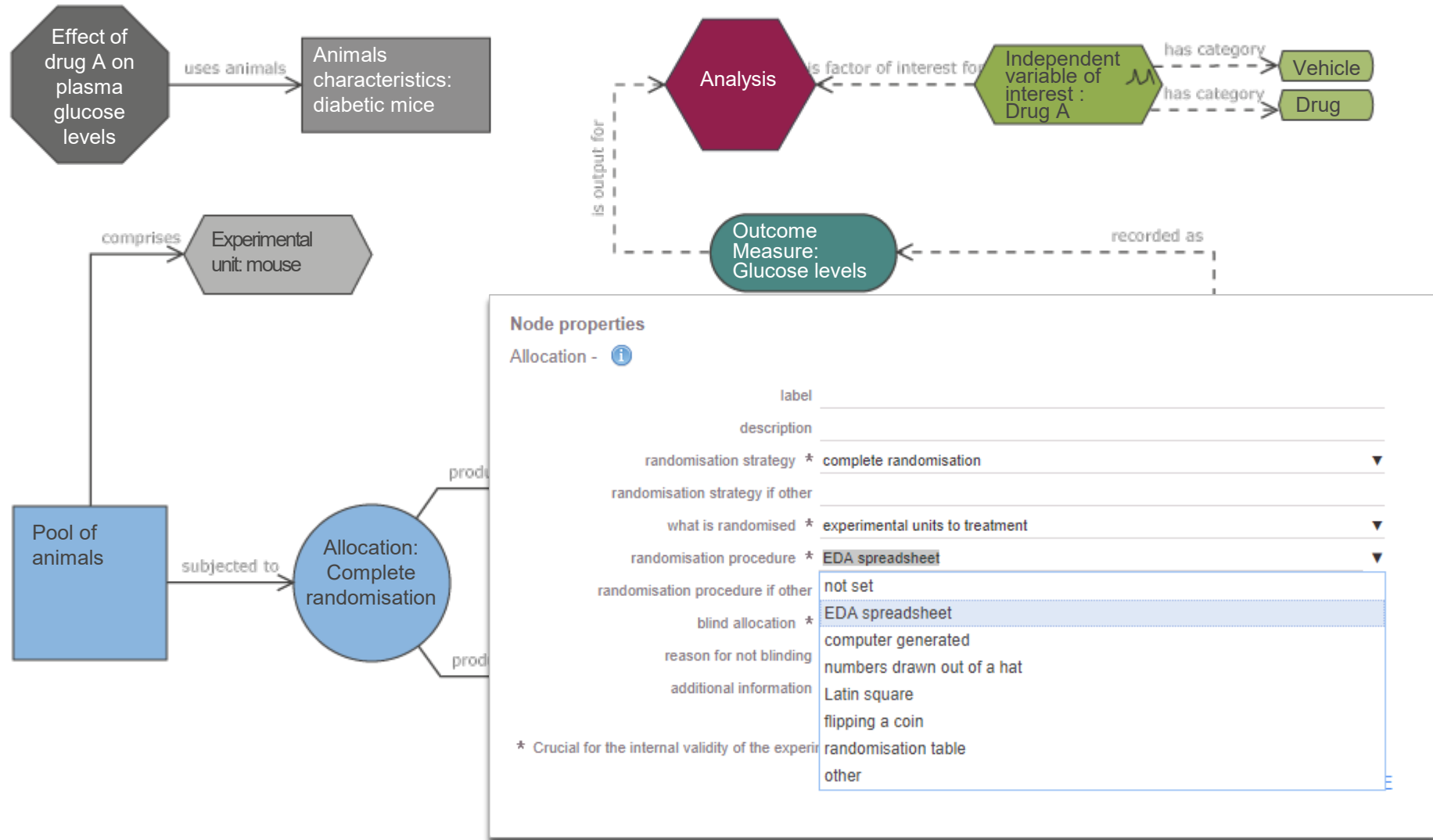


# Features of the EDA

- Advice to improve the experimental plan
- Recommendations for the statistical analysis
- Power calculation
- Randomisation and masking
- Improve transparency
- Teach experimental design



# The EDA diagram





## The diagram does not include any nuisance variables

In the EDA, nuisance variable nodes are used to indicate *other sources of variability or confounding factors that may influence the outcome*, such as blocking factors or covariates.

Having no nuisance variable implies that only the independent variable(s) of interest (e.g. the treatment) influence the outcome measure. Thus it is rarely appropriate as the result of an experiment is influenced by many variables. Identifying these nuisance variables and accounting for them increases the internal validity of the experiment to detect changes induced by the variable(s) of interest.

The type of things to consider may include cages or rooms, if the animals are not all housed in the same way; the experimenter, if the intervention or measurement, or the person doing it if animals are not all processed by the same person; the experimenter's skills. The list could be endless but the important thing is to identify the factors relevant to a particular experiment, based on common sense and past experimental results, and to identify new sources of variability.

These should be indicated on the EDA diagram using nuisance variable nodes; then the user should indicate the best way to account for each of the nuisance variables identified. Depending on the type of nuisance variable and the objective of the experiment, there are different options to account for the variability; the variables are:

- **standardised** - for example record all measurements on the same piece of equipment
- **randomised across** - for example the effect of the location of the cage in the room could be accounted for by randomly allocating each cage to the housing rack
- **blocked** - for example the day of the experiment can be used as a blocking factor in the statistical analysis
- **nested within another variable** - for example individual neurons can be nested within 'mouse' when multiple neurons are recorded for each mouse
- used as a **covariate** - for example baseline locomotor activity could be included as a covariate
- if none of these things are done, then the variable is deemed **uncontrolled**.

## 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

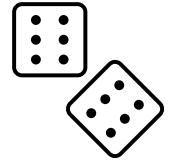
**Investigator aware of the group allocation (not blinded)** – the investigator taking the measurement knows what treatment each animal has received, or what animals are grouped together. Sometimes the person assessing the outcome cannot be blinded to the group allocation, for example if there are obvious 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.

# Key features for rigorous research

## Randomisation sequence

EDA generates sequences for:

- Complete randomisation
- Block randomisation
- Randomisation within factors



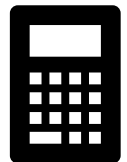
## Workflow for blinding (masking)

Randomisation sequence directly sent to the person helping with blinding so that the researcher remains unaware of the group allocation



## Sample size calculators

- For unpaired t-tests, paired t-tests
- Full guidance to identify parameters for the calculation
- Decision tree to select the correct calculator



# EDA Report

## Key experimental information:

- Objectives and hypotheses
- Animal numbers and justification for sample size

## 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 researcher 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 researcher and the feedback from the EDA.

### Section 1: Summary

Title of EDA diagram	Effect of drug A on plasma glucose level
Date report generated	06/12/2019

### Section 2: Information provided by the researcher

#### 1: Objectives

Null hypothesis	Drug A has no effect on plasma glucose levels in diabetic mice
Alternative hypothesis	Drug A modulates plasma glucose levels in diabetic mice
Effect of interest	Change in plasma glucose level
Effect size	100
Justification for effect size	A difference smaller than 100 mg/dL would not be biologically relevant

#### 2: Groups and sample size

Total number of animals in the experiment	12
Groups included in the primary analysis	2 groups: <ul style="list-style-type: none"><li>• Group 1 role=control/comparator; n=6</li><li>• Group 2 role=test; n=6</li></ul>
Justification for sample size	Power calculation to detect a difference of 100 with 85% power (sig level: 0.05, SD: 50)

# EDA Report

## Key experimental information:

- Steps taken to minimise the effect of bias
- Primary and secondary outcome measures
- Planned statistical analysis

The Experiment in vivo experimental specific protocol

This report is

**Section**

Title of EDA

Date reported

---

**Section 1: Objectives**

Null hypothesis

Alternative hypothesis

Effect of interest

Effect size

Justification

**2: Groups**

Total number of groups

Groups in experiment

- Group
- Group

Justification

**3: Randomisation and blinding**

Experimental unit	animal
-------------------	--------

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

- Allocation: complete randomisation

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

There is one step in this experiment where measurements are taken:

- Measurement: Plasma glucose

Blinding during result assessment	animals individually coded
-----------------------------------	----------------------------

There is one analysis in this experiment:

- Analysis: unpaired t-test

Blinding during analysis of the data	groups coded
--------------------------------------	--------------

**4: Analysis**

Details of the primary analysis (Analysis: unpaired t-test)

Statistical analysis method	unpaired t-test
Factor of interest	Independent variable of interest: Drug A, categorical, with 2 levels (Drug A, Vehicle)
Blocking factor	NONE
Covariate	NONE

Outcome measures

Outcome measures in the primary analysis	Outcome measure: glucose concentration, treated as continuous
Other outcome measures	NONE

# EDA Report

## Key experimental information:

- Characteristics of the animals in the study

The Experiment in vivo experimental specific problem

This report summarises the experimental design and results.

**Section 1: Objectives**

Title of EDA

Date reported

---

**Section 2: Groups**

Total number of groups

Groups included

- Group 1
- Group 2

Justification for the design

**3: Randomisation**

Experimental unit

There is one step

- Allocation: complete

**Randomisation**

Randomisation method

**Allocation concealment**

There is one step

- Measurement: appropriate

**Blinding during randomisation**

There is one analysis

- Analysis: unpaired

**Blinding during analysis**

**4: Analysis**

Details of the primary analysis

**Statistical analysis**

Factor of interest

**Blocking factors**

**Covariate**

Outcome measurement

**Outcome measurement**

**Other outcomes**

**5: Characteristics of animals in this experiment**

Animal characteristics: diabetic mice, female

Species	mouse
Strain	B6.V-Lepab/J
Sex	female
Age	mean=20, range=19-21, unit=week
Weight	mean=25, range=20-30, unit=g

Animal characteristics: diabetic mice, male

Species	mouse
Strain	B6.V-Lepab/J
Sex	male
Age	mean=20, range=19-21, unit=week
Weight	mean=25, range=20-30, unit=g

**Section 3: Summary of the feedback provided by the EDA**

Critique (Table 6) and advice (Table 7) from the EDA is dependent on the quality, including accuracy and completeness, of the information inputted by the researcher. Where the researcher has not addressed issues detected by the EDA, it is important to consider whether this undermines the design of the study.

**6: Critique**

Total number of issues	1
Issues related to the diagram structure, which might compromise the accuracy of this report	0
Issues related to internal consistency	0
Issues related to missing information	0
Issues suggesting improvements to the design	1

- Other sources of variability are not accounted for in the design of this experiment

**7: Advice for the primary analysis**

Suggestion for a method of analysis appropriate for the design	Unpaired t-test or Mann-Whitney test
--	--------------------------------------

# EDA Report

## Information about feedback:

- Summary of the EDA feedback that has not been addressed
- Statistical analysis method recommended by the EDA

The Experiment in vivo experimental specific problem

This report summarises the feedback provided by the EDA

**Section 3: Randomisation**

**Experimental use**

There is one step

- Allocation: complete

**Randomisation**

**Randomisation**

**Allocation complete**

There is one step

- Measurement: none

**Blinding during**

There is one analysis

- Analysis: unpaired

**Blinding during**

**4: Analysis**

Details of the primary analysis

**Statistical analysis**

**Factor of interest**

**Blocking factors**

**Covariate**

Outcome measurement

**Outcome measurement**

**Other outcome measurement**

**5: Characteristics of animals in this experiment**

Animal characteristics: diabetic mice, female

Species	mouse
Strain	B6.V-Lepab/J
Sex	female
Age	mean=20, range=19-21, unit=week
Weight	mean=25, range=20-30, unit=g

Animal characteristics: diabetic mice, male

Species	mouse
Strain	B6.V-Lepab/J
Sex	male
Age	mean=20, range=19-21, unit=week
Weight	mean=25, range=20-30, unit=g

**Section 3: Summary of the feedback provided by the EDA**

Critique (Table 6) and advice (Table 7) from the EDA is dependent on the quality, including accuracy and completeness, of the information inputted by the researcher. Where the researcher has not addressed issues detected by the EDA, it is important to consider whether this undermines the design of the study.

**6: Critique**

Total number of issues	1
Issues related to the diagram structure, which might compromise the accuracy of this report	0
Issues related to internal consistency	0
Issues related to missing information	0
Issues suggesting improvements to the design	1

- Other sources of variability are not accounted for in the design of this experiment

**7: Advice for the primary analysis**

Suggestion for a method of analysis appropriate for the design	Unpaired t-test or Mann-Whitney test
--	--------------------------------------




# EDA Report

EDA diagram provides a visual overview to easily see:

- How many groups are being compared
- What variables have been included
- If any variability is being taken into account as a blocking factors or covariates





The Experiment: in vivo experimental specific problem

This report is about:

**Section 3: Randomisation**

Experimental unit: mouse

There is one step:

- Allocation: complete randomisation

**Section 4: Analysis**

There is one analysis:

- Measurement: unpaired t-test

**Section 5: Characteristics of animals in this experiment**

Animal characteristics: diabetic mice, female

Species	mouse
Strain	B6.V-Lepab/J
Sex	female
Age	mean=20, range=19-21, unit=week
Weight	mean=25, range=20-30, unit=g

Animal characteristics: diabetic mice, male

Species	mouse
Strain	B6.V-Lepab/J
Sex	male
Age	mean=20, range=19-21, unit=week
Weight	mean=25, range=20-30, unit=g

**Section 2: Groups**

Total number of groups in experiment: 2

Groups in experiment:

- Group 1: Vehicle
- Group 2: Drug A

Justification: randomisation

**4: Analysis**

Details:

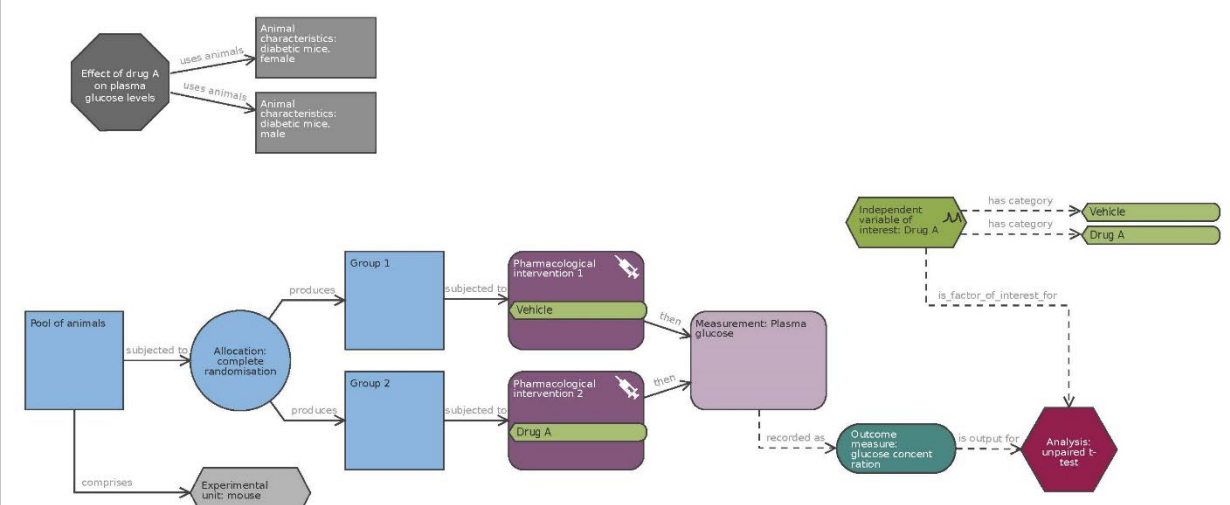
Statistical factor: unpaired t-test

Blocking factor: Allocation: complete randomisation

Covariate: Animal characteristics: diabetic mice, female; Animal characteristics: diabetic mice, male

Outcome: Measurement: Plasma glucose

Other: Experimental unit: mouse



Page 3 of 4

39

# Read-only EDA diagram

eda.nc3rs.org.uk/eda/modelPublicExport/index/D465A36951173BAE0BE404B82F5A7E41



<b>Title of Experiment</b>	Example 1: Effect of drug A on plasma glucose level
<b>Date report generated</b>	22/03/2023

## Key Experimental Design Features

### Section 1: Information provided by the researcher

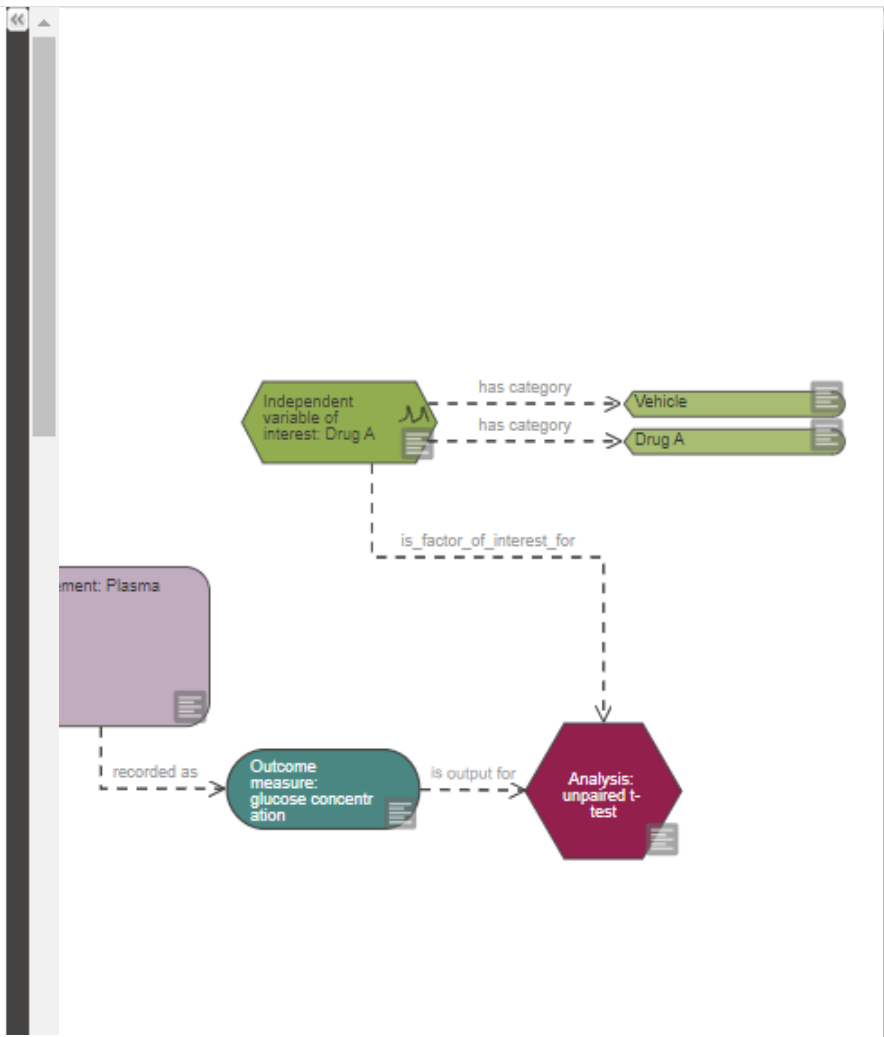
#### 1: Objectives

<b>Null hypothesis</b>	Drug A has no effect on plasma glucose levels in diabetic mice
<b>Alternative hypothesis</b>	Drug A modulates plasma glucose levels in diabetic mice
<b>Effect of interest</b>	Change in plasma glucose level
<b>Effect size</b>	100
<b>Justification for effect size</b>	A difference smaller than 100 mg/dL would not be biologically relevant

#### 2: Groups and sample size

<b>Total number of animals in the experiment</b>	12
<b>Groups included in the primary analysis</b>	2 groups:
• Group 1	role=control/comparator; n=6
• Group 2	role=test; n=6
<b>Justification for sample size</b>	Power calculation to detect a difference of 100 with 85% power (sig level: 0.05, SD: 50)

#### 3: Randomisation and blinding



### Legend

Click on this icon on the diagram for more information about a specific step in the experiment.

#### What each shape represents

**Experiment**

- Experiment
- Animal characteristics
- Experimental unit

**Practical steps**

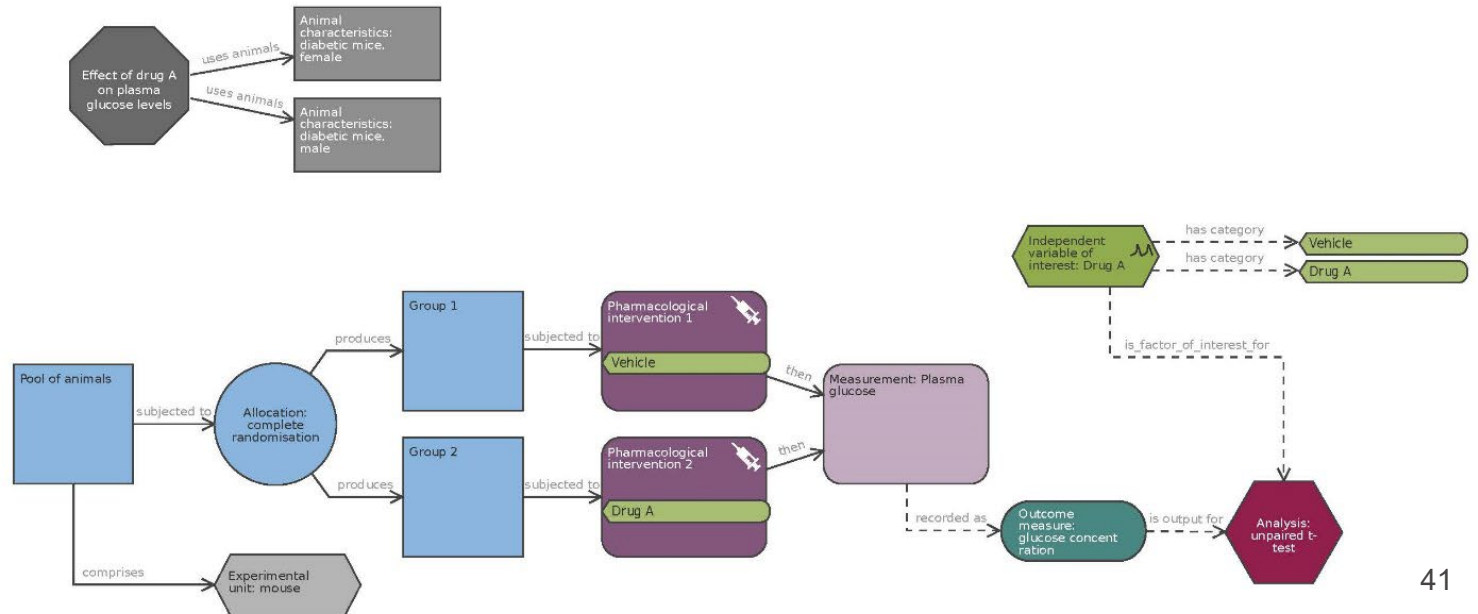
- Group
- Allocation
- 🔪 Pharmacological intervention
- 🔪 Surgical intervention
- ☀️ Pathogen infection
- ⚙️ Other intervention
- ✖️ Euthanasia
- Measurement
- Repeated measurement

**Analysis**



# The EDA: Benefits for researchers

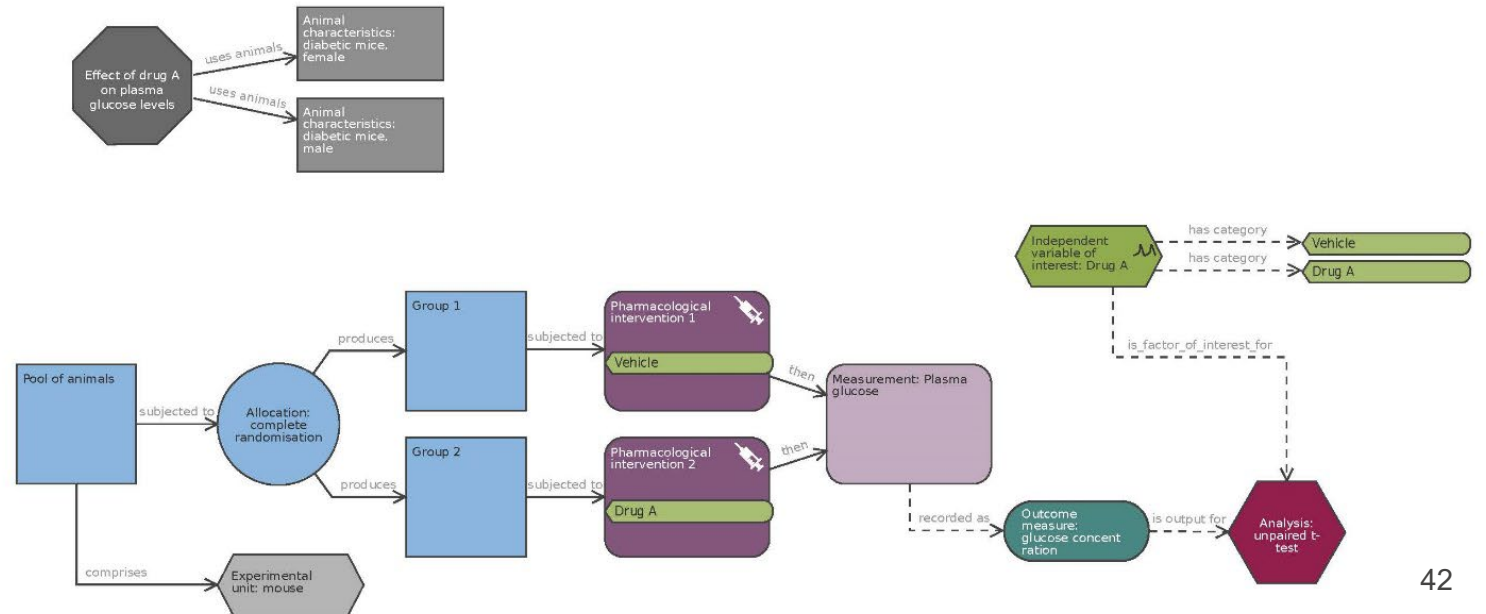
- Bespoke advice on experimental plans, leading to better experimental design
- Analysis recommendations for the appropriate statistical analysis method
- Help determining the appropriate sample size
- Support for randomisation and masking



# The EDA: Benefits for IACUCs

You could request an EDA report, or read-only diagram, as part of an IACUC application.

- The report and read-only diagram highlight missing information.
- The EDA identifies issues with experimental design.
- You can refer researchers to the EDA application and supporting website for experimental design guidance.



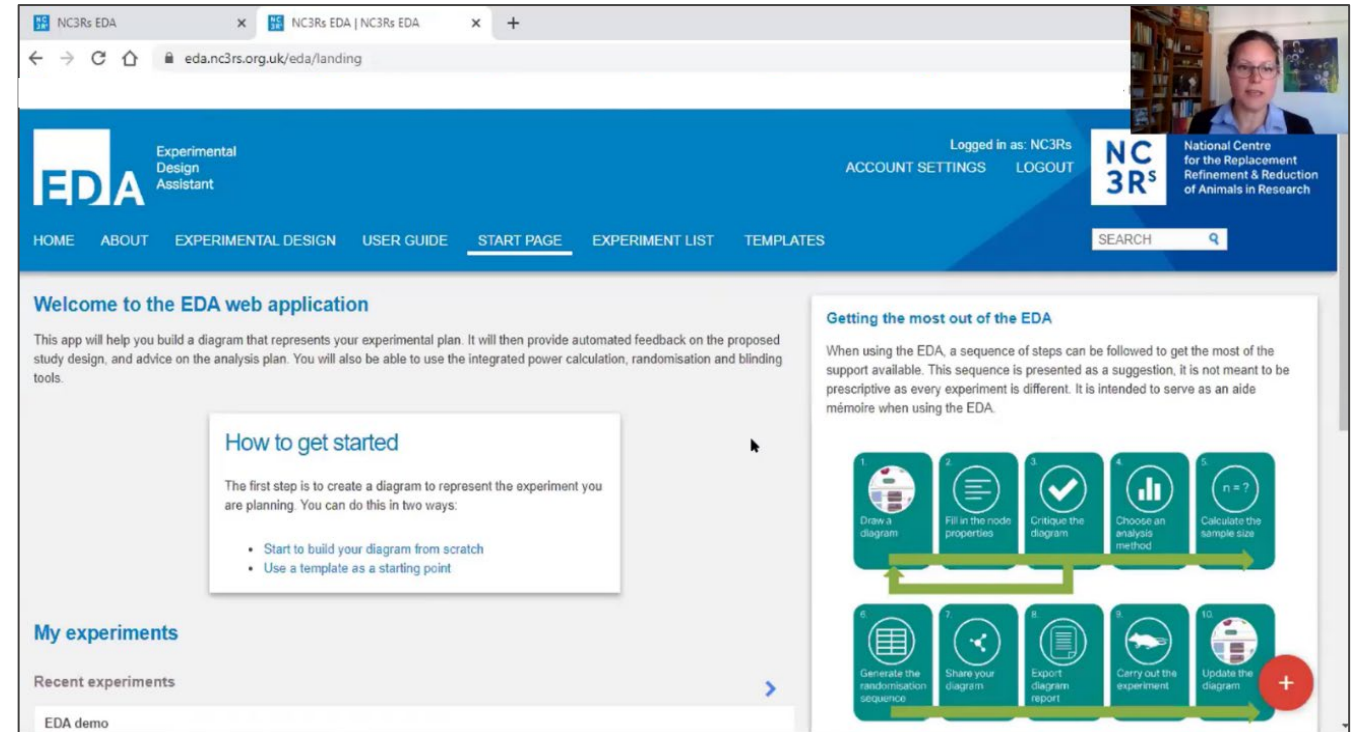
# Live: inside the EDA

# EDA demonstration

[www.nc3rs.org.uk/EDAdemos](http://www.nc3rs.org.uk/EDAdemos)

## Regular live demonstrations

- Building an experiment diagram
- Getting feedback
- Generating a randomisation sequence
- Generating a pdf report
- Generating the read-only diagram
- Finding the built-in help



<https://eda.nc3rs.org.uk/overview-demonstration>

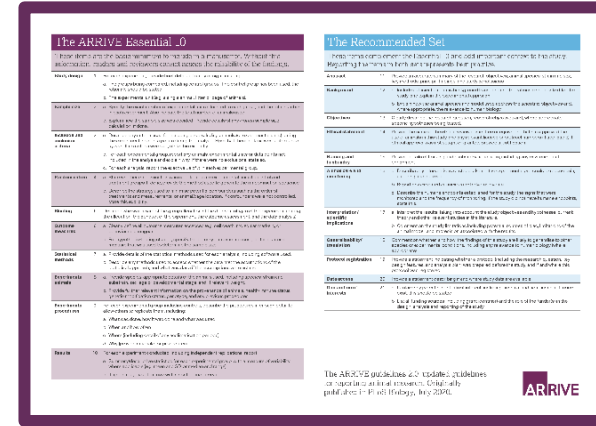
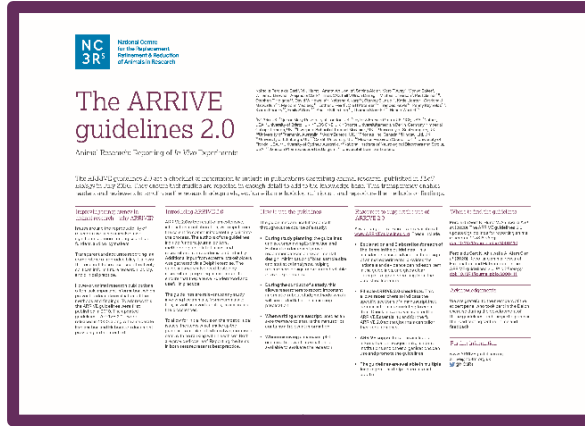
# ARRIVE guidelines

Guidelines to improve the reporting of animal research – maximising information published and minimising unnecessary studies.



Original guidelines published in 2010. Revised in 2020: ARRIVE 2.0. Supporting resources on the website:

[www.ARRIVEguidelines.org](http://www.ARRIVEguidelines.org)

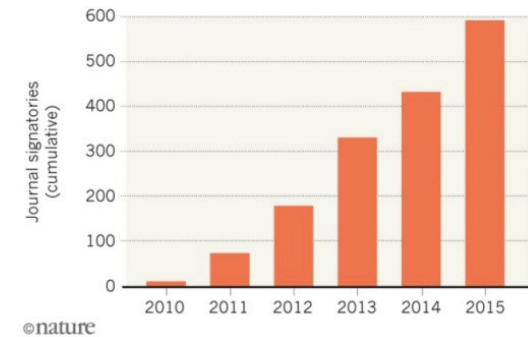


Checklist containing key information necessary to describe a study comprehensively and transparently. Can be used as a framework for planning research studies.



### SURGE IN SUPPORT FOR STUDY GUIDELINES

In 2015, more than 150 journals signed up to the ARRIVE checklist for animal studies — the highest number of signatories in a single year since it was released.



Endorsed internationally by over 1,000 journals, major research funders, universities, learned societies and scientific organisations.

ARRIVE guidelines &gt;

Essential 10 ^

1. Study design &gt;

2. Sample size

3. Inclusion and exclusion criteria &gt;

4. Randomisation &gt;

5. Blinding &gt;

6. Outcome measures &gt;

7. Statistical methods &gt;

8. Experimental animals &gt;

9. Experimental procedures &gt;

10. Results &gt;

Recommended Set v

Glossary &gt;

ESSENTIAL 10

## 2. Sample size

2a

Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.

2b

Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.

Explanation

Examples

For any type of experiment, it is crucial to explain how the sample size was determined. For hypothesis-testing experiments, where inferential statistics are used to estimate the size of the effect and to determine the weight of evidence against the null hypothesis, the sample size needs to be justified to ensure experiments are of an optimal size to test the research question [1,2] (see [item 13 – Objectives](#)). Sample sizes that are too small (i.e. underpowered studies) produce inconclusive results, whereas sample sizes that are too large (i.e. overpowered studies) raise ethical issues over unnecessary use of animals and may produce trivial findings that are statistically significant but not biologically relevant [3]. Low power has three effects: first, within the experiment, real effects are more likely to be missed; second, where an effect is detected, there is an over-estimation of the true effect size [4]; and finally, when low power is combined with publication bias, it leads to the false positive rate in the published literature [5]. Consequently, low powered studies contribute to the waste of research and risk wasting animals used in inconclusive research [6].

Study design can influence the statistical power of an experiment and the power calculation used needs to be consistent with the design implemented. Statistical programs to help perform *a priori* sample size calculations exist for a range of designs and statistical analyses, both freeware (web based applets and functions in R) and commercial. The appropriate calculator or algorithm to use depends on the type of outcome measures and independence of groups. Consultation with a statistician is recommended, especially when the experimental design is unusual.

Where the experiment tests the effect of an intervention on the mean of a continuous outcome measure, the sample size is calculated *a priori*, based on a mathematical relationship between the predefined, biologically relevant effect size, variability estimated from prior data, chosen significance level, power and sample size (See "Information used in a power

[www.arriveguidelines.org](http://www.arriveguidelines.org)

### PLOS BIOLOGY

COMMUNITY PAGE



Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0

Nathalie Percie du Sert<sup>1\*</sup>, Amrita Ahluwalia<sup>2,3</sup>, Sabina Alam<sup>4</sup>, Marc T. Avey<sup>5</sup>, Monya Baker<sup>6</sup>, William J. Browne<sup>7</sup>, Alejandra Clark<sup>8</sup>, Innes C. Cuthill<sup>9</sup>, Ulrich Dirnagl<sup>10</sup>, Michael Emerson<sup>11</sup>, Paul Garner<sup>12</sup>, Stephen T. Holgate<sup>13</sup>, David W. Howells<sup>14</sup>, Viki Hurst<sup>1</sup>, Natasha A. Karp<sup>15</sup>, Stanley E. Lazic<sup>16</sup>, Katie Lidster<sup>1</sup>, Catriona J. MacCallum<sup>17</sup>, Malcolm Macleod<sup>18</sup>, Esther J. Pearl<sup>1</sup>, Ole H. Petersen<sup>19</sup>, Frances Rawle<sup>20</sup>, Penny Reynolds<sup>21</sup>, Kieron Rooney<sup>22</sup>, Emily S. Sena<sup>18</sup>, Shai D. Silberberg<sup>23</sup>, Thomas Steckler<sup>24</sup>, Hanno Würbel<sup>25</sup>





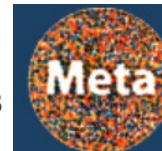
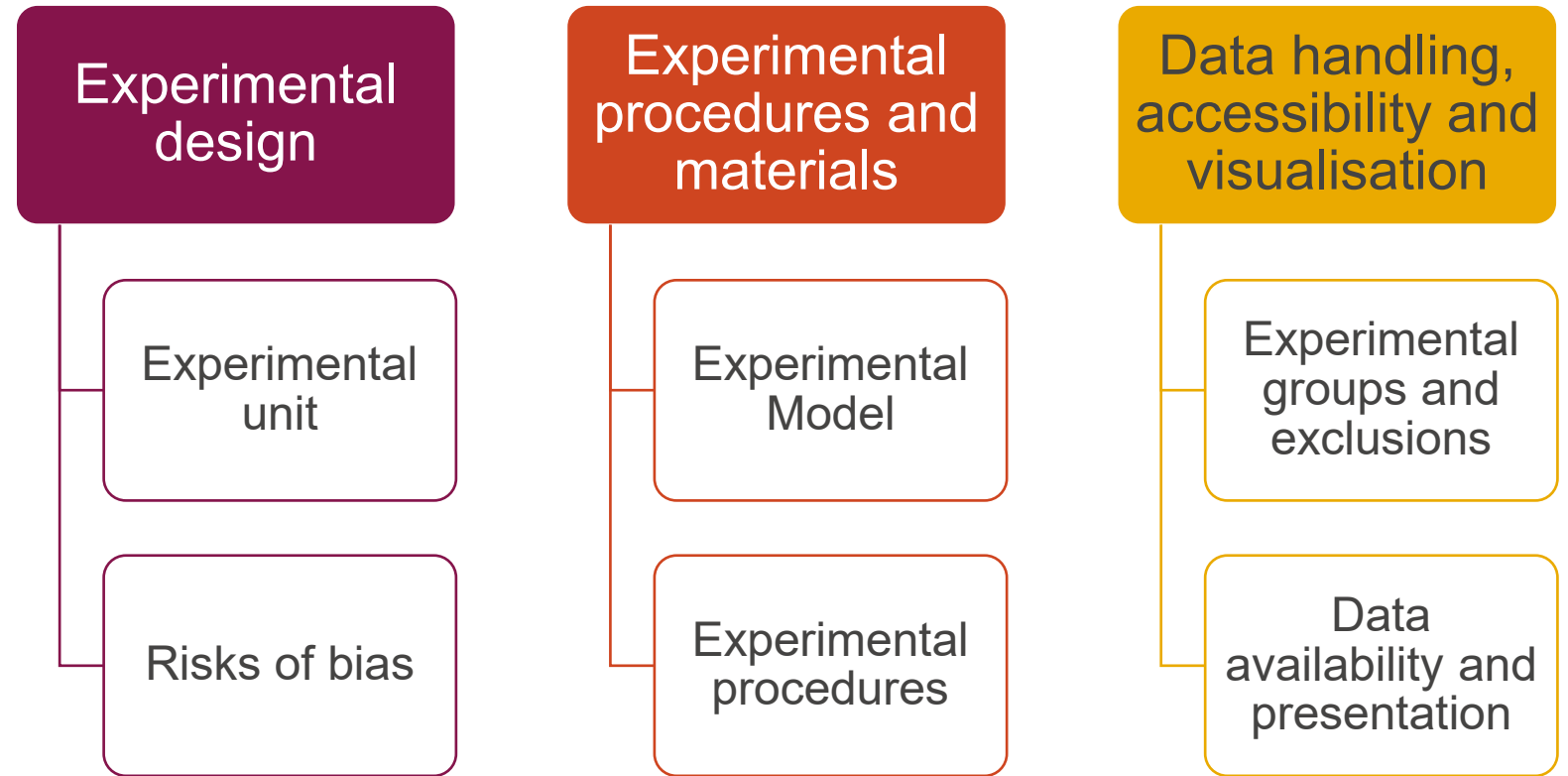
Six recommendations to increase the methodological rigour and reliability of *in vitro* studies

Developed by an international working group including:

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

# The RIVER recommendations

## Reporting *In Vitro* Experiments Responsibly



# Experimental design resource links

Resource	link
Experimental Design Assistant	<a href="https://eda.nc3rs.org.uk/">https://eda.nc3rs.org.uk/</a>
Experimental Design Assistant demonstrations	Register for a live demonstration - <a href="https://nc3rs.org.uk/EDAdemos">https://nc3rs.org.uk/EDAdemos</a> Recorded demonstration - <a href="https://eda.nc3rs.org.uk/index.php/overview-demonstration">https://eda.nc3rs.org.uk/index.php/overview-demonstration</a>
ARRIVE guidelines	<a href="https://arriveguidelines.org/">https://arriveguidelines.org/</a>
RIVER recommendations	<a href="https://doi.org/10.31222/osf.io/x6aut">https://doi.org/10.31222/osf.io/x6aut</a>
Webinar: Best practices in experimental design	<a href="https://vimeo.com/442640803/09faa99012">https://vimeo.com/442640803/09faa99012</a>
MRC/NC3Rs Webinar: Using both sexes in animal research	<a href="https://nc3rs.org.uk/3rs-resources/mrc-nc3rs-webinar-using-both-sexes-animal-experiments">https://nc3rs.org.uk/3rs-resources/mrc-nc3rs-webinar-using-both-sexes-animal-experiments</a>
NC3Rs experimental design and reporting resources	<a href="https://nc3rs.org.uk/3rs-resources/search?topic[]=497">https://nc3rs.org.uk/3rs-resources/search?topic[]=497</a>
NC3Rs resources for masking	<a href="https://www.nc3rs.org.uk/3rs-resources/using-maskingblinding-vivo-experiments">https://www.nc3rs.org.uk/3rs-resources/using-maskingblinding-vivo-experiments</a>
British Pharmacological Society animation – blinding	<a href="https://www.youtube.com/watch?v=hbU5kHC9yH0">https://www.youtube.com/watch?v=hbU5kHC9yH0</a>
British Pharmacological Society animation – experimental unit	<a href="https://www.youtube.com/watch?v=WQSWJLDcy5M">https://www.youtube.com/watch?v=WQSWJLDcy5M</a>
British Pharmacological Society eLearning resources	<a href="https://www.bps.ac.uk/education-engagement/research-animals/blinding-elearning-resource">https://www.bps.ac.uk/education-engagement/research-animals/blinding-elearning-resource</a>



# Thank you!

## For more information

✉ [esther.pearl@nc3rs.org.uk](mailto:esther.pearl@nc3rs.org.uk)

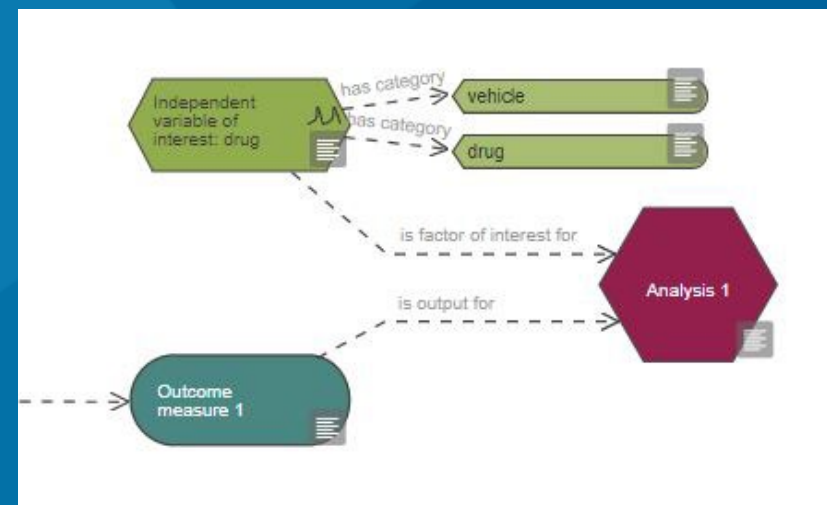
🏠 [www.nc3rs.org.uk](http://www.nc3rs.org.uk)

🐦 @NC3Rs

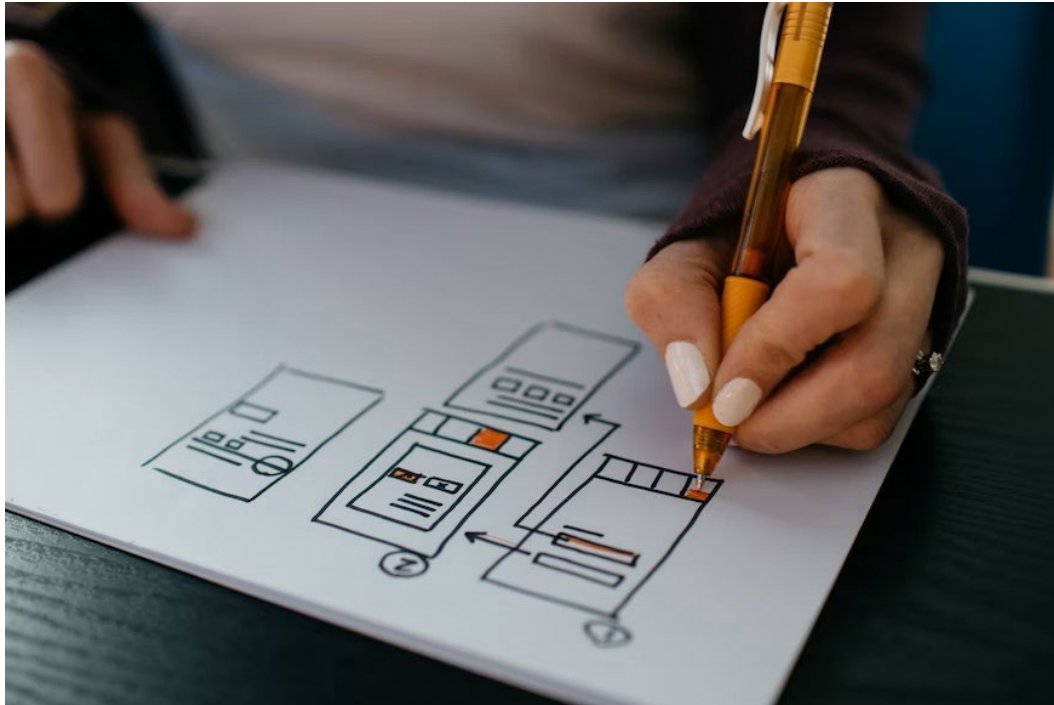
🐦 @DrEJPearl

## Keep in touch

Our monthly newsletter provides the latest updates from the NC3Rs, including funding calls and events [www.nc3rs.org.uk/register](http://www.nc3rs.org.uk/register)



# In case you missed it!



OLAW Online Seminar: March 9, 2023  
Foundations for Evaluating Study Design  
and Statistical Approaches for the IACUC

Recording and materials at:

<https://olaw.nih.gov/education/educational-resources/webinar-2023-03-09.htm>



OLAW Online Seminar: June 15, 2023  
The ARRIVE 2.0 Essential 10:  
Guidance for NIH-sponsored Research

Recording and materials at:

<https://olaw.nih.gov/education/educational-resources/webinar-2023-06-15.htm>

# Next Webinar: Winter 2023

## Topic TBD



**National Institutes of Health**  
*Office of Laboratory Animal Welfare*