Working towards the ‘Essential 10’

Nikki Osborne BSc. PhD.

RESPONSIBLE RESEARCH IN PRACTICE

ARRIVE Essential 10		
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g., a single animal, litter, or cage of animals).
Sample size	2	a. 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. b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units, or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g., cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e., the outcome measure that was used to determine the sample size.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done, and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g., mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.

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Step 1: Awareness & Understanding

1. Study design;
2. Sample size;
3. Inclusion & exclusion criteria;
4. Randomisation;
5. Blinding;
6. Outcome measures;
7. Statistical methods;
8. Experimental animals;
9. Experimental procedures;
10. Results

RESPONSIBLE RESEARCH IN PRACTICE

Step 1: Awareness & Understanding

PLOS BIOLOGY

PERSPECTIVE

The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research

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

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Abstract

Reproducible science requires transparent reporting. The ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) were originally developed in 2010 to improve the reporting of animal research. They consist of a checklist of information to include in publications describing in vivo experiments to enable others to scrutinise the work adequately, evaluate its methodological rigour, and reproduce the methods and results. Despite considerable levels of endorsement by funders and journals over the years, adherence to the guidelines has been inconsistent, and the anticipated improvements in the quality of reporting in animal research publications have not been achieved. Here, we introduce ARRIVE 2.0. The guidelines have been updated and information reorganised to facilitate their use in practice. We used a Delphi exercise to prioritise and divide the items of the guidelines into 2 sets, the "ARRIVE Essential 10," which constitutes the minimum requirement, and the "Recommended Set," which describes the research context. This division facilitates improved reporting of animal research by supporting a stepwise approach to implementation. This

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: AA is the editor in chief of the British Journal of Pharmacology. WJB, ICC, and ME are authors of the original ARRIVE guidelines. WJB serves on the Independent Statistical Standing Committee of the funder CHDI foundation. AC is a Senior Editor for PLOS ONE. AC, CJM, MM, and ESS were involved in the

PLOS BIOLOGY

COMMUNITY PAGE

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

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

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Abstract

Improving the reproducibility of biomedical research is a major challenge. Transparent and accurate reporting is vital to this process; it allows readers to assess the reliability of the findings and repeat or build upon the work of other researchers. The ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments) were developed in 2010 to help authors and journals identify the minimum information necessary to report in publications describing in vivo experiments. Despite widespread endorsement by the scientific community, the impact of ARRIVE on the transparency of reporting in animal research publications has been limited. We have revised the ARRIVE guidelines to update them and facilitate their use in practice. The revised guidelines are published alongside this paper. This explanation and elaboration document was developed as part of the revision. It provides further information about each of the 21 items in ARRIVE 2.0, including the rationale and supporting evidence for their inclusion in the guidelines, elaboration of details to report, and examples of good reporting from the published literature. This document also covers advice and best practice

Citation: Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, et al. (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol 18(7): e3000411. https://doi.org/10.1371/journal.pbio.3000411

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Translations

- Chinese
- Dutch
- French
- German
- Italian
- Portuguese
- Spanish
- Turkish
- Japanese
- Korean

https://doi.org/10.1371/journal.pbio.3000410
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RESPONSIBLE RESEARCH IN PRACTICE

https://arriveguidelines.org/translations

Step 1: Awareness & Understanding

ARRIVE

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- ARRIVE guidelines
- Essential 10
  - 1. Study Design
  - 2. Sample size
  - 3. Inclusion and exclusion criteria
  - 4. Randomisation
  - 5. Blinding/Masking
  - 6. Outcome measures
  - 7. Statistical methods
  - 8. Experimental animals
  - 9. Experimental procedures
  - 10. Results
- Recommended Set
- Glossary

The ARRIVE guidelines 2.0

This section of the website provides detailed explanations about each item of the guidelines. Use the left-hand side menu to navigate to each item. The guidelines in their entirety can also be downloaded as a PDF, in English or a variety of translations.

To facilitate a step-wise approach to improving reporting, the guidelines are organised into two prioritised sets:

ARRIVE Essential 10

These ten items are the basic minimum that must be included in any manuscript describing animal research. Without this information readers and reviewers cannot assess the reliability of the findings.

Recommended Set

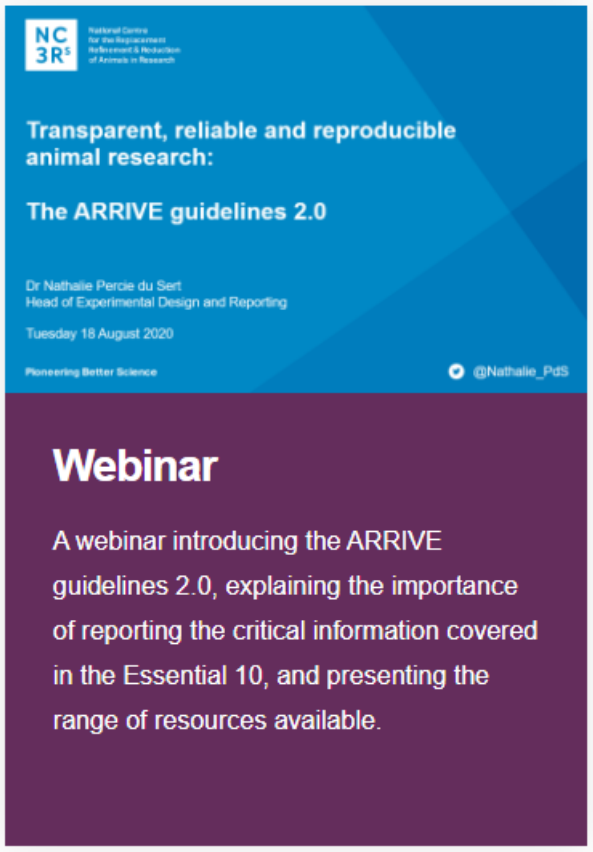
These items complement the Essential 10 set and add important context to the study described. Reporting the items in both sets represents best practice.

Each item of the guidelines includes examples of good reporting from the published literature, extracted from different types of studies, in model organisms ranging from mammals to invertebrates. This battery of examples will be regularly expanded.

Consulting this information during the planning of an animal study ensures that researchers can benefit from the explanations and advice on experimental design, minimisation of bias, sample size and statistical analyses, helping the design of rigorous and reliable *in vivo* experiments.

The Explanation and Elaboration for the ARRIVE guidelines 2.0 were originally published in *PLOS Biology* doi:10.1371/journal.pbio.3000411 under a CC-BY license.

https://arriveguidelines.org/arrive-guidelines



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ESSENTIAL 10

5. Blinding/Masking

5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).

Explanation | Examples

Researchers often expect a particular outcome, and can unintentionally influence the experiment or interpret the data in such a way as to support their preferred hypothesis [1]. Blinding (also known as masking) is a strategy used to minimise these subjective biases.

Whilst there is primary evidence of the impact of blinding in the clinical literature that directly compares blinded vs unblinded assessment of outcomes [2], there is limited empirical evidence in animal research [3,4]. There are, however, compelling data from systematic reviews showing that non-blinded outcome assessment leads to the treatment effects being overestimated, and the lack of bias-reducing measures such as randomisation and blinding can contribute to as much as 30-45% inflation of effect sizes [5-7].

Ideally, investigators should be unaware of the treatment(s) animals have received or will be receiving, from the start of the experiment until the data have been analysed. If this is not possible for every stage of an experiment (see "Blinding during different stages of an experiment" below), it should always be possible to conduct at least some of the stages blind. This has implications for the organisation of the experiment and may require help from additional personnel, for example a surgeon to perform interventions, a technician to code the treatment syringes for each animal, or a colleague to code the treatment groups for the analysis. Online resources are available to facilitate allocation concealment and blinding [8].

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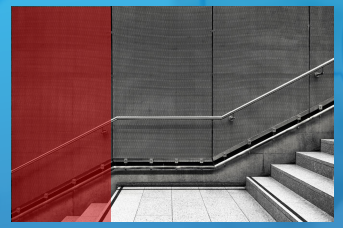
RESPONSIBLE RESEARCH IN PRACTICE



Step 1: Awareness & Understanding

ARRIVE

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ESSENTIAL 10

5. Blinding/Masking

5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).

Explanation | Examples

Example 1

"For each animal, four different investigators were involved as follows: a first investigator (RB) administered the treatment based on the randomization table. This investigator was the only person aware of the treatment group allocation. A second investigator (SC) was responsible for the anaesthetic procedure, whereas a third investigator (MS, PG, IT) performed the surgical procedure. Finally, a fourth investigator (MAD) (also unaware of treatment) assessed GCPS and NRS, mechanical nociceptive threshold (MNT), and sedation NRS scores." [1]

Example 2

"…due to overt behavioral seizure activity the experimenter could not be blinded to whether the animal was injected with pilocarpine or with saline." [2]

Example 3

"Investigators could not be blinded to the mouse strain due to the difference in coat colors, but the three-chamber sociability test was performed with ANY-maze video tracking software (Stoelting, Wood Dale, IL, USA) using an overhead video camera system to automate behavioral testing and provide unbiased data analyses. The one-chamber social interaction test requires manual scoring and was analyzed by an individual with no knowledge of the questions." [3]

https://arriveguidelines.org/arrive-guidelines

RESPONSIBLE RESEARCH IN PRACTICE

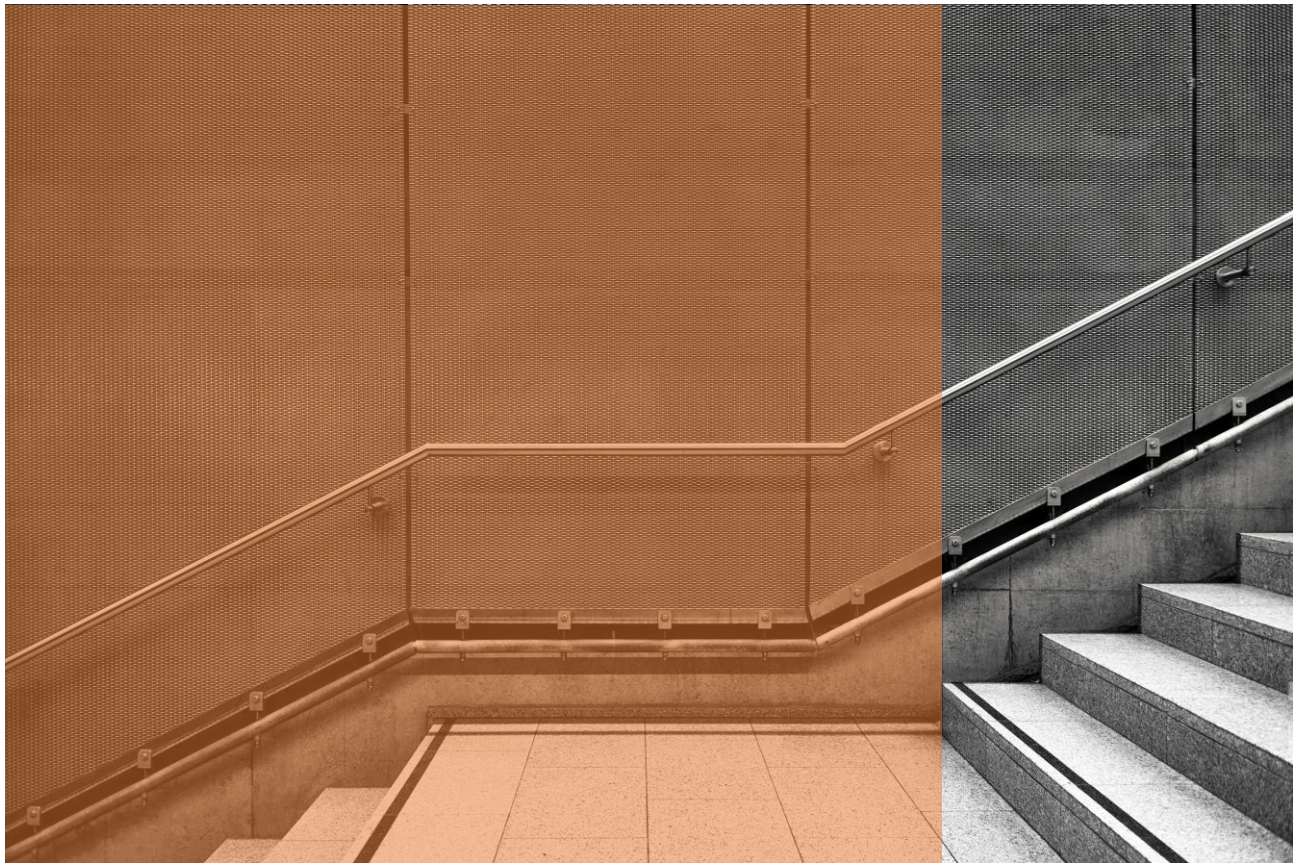
ARRIVE The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

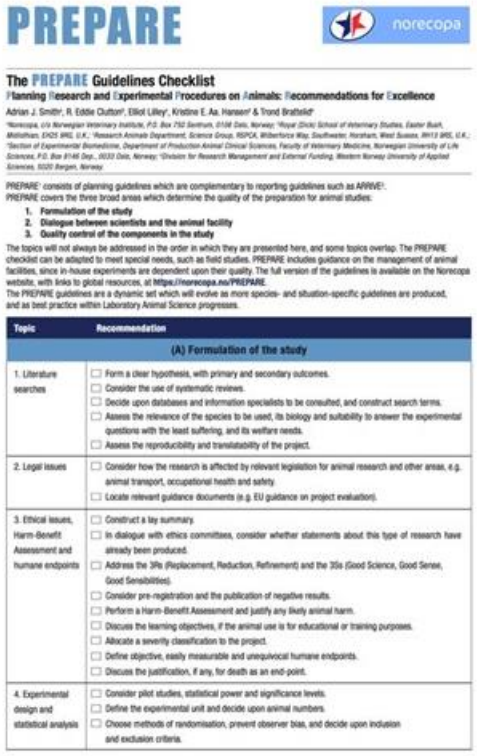
Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated. b. The experimental unit (e.g. a single animal, litter, or cage of animals).	
Sample size	2	a. 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. b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly. b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. c. For each analysis, report the exact value of *n* in each experimental group.	
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used. b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: a. What was done, how it was done and what was used. b. When and how often. c. Where (including detail of any acclimatisation periods). d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report: a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). b. If applicable, the effect size with a confidence interval.	

Step 2: Recognition



RESPONSIBLE RESEARCH IN PRACTICE

Step 2: Recognition



PREPARE

norecopa

The PREPARE Guidelines Checklist
Planning Research and Experimental Procedures on Animals: Recommendations for Excellence

Adrian J. Smith[1], R. Eddie Clutton[2], Elliot Lilley[3], Kristine E. Aa. Hansen[4] & Trond Brattelid[4]

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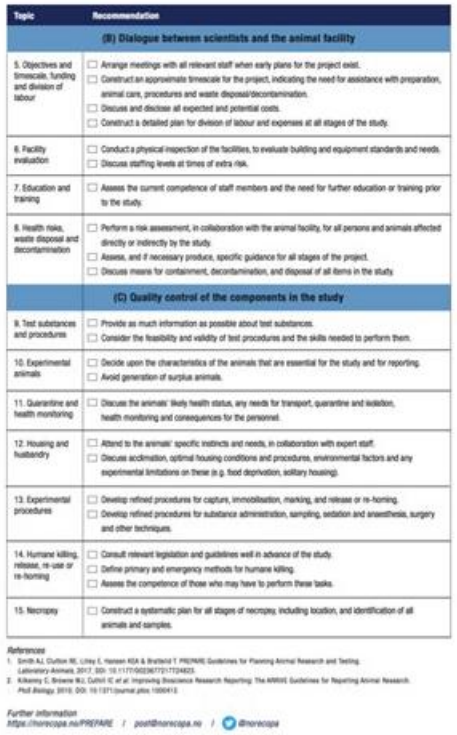
PREPARE[1] consists of planning guidelines which are complementary to reporting guidelines such as ARRIVE[2].
PREPARE covers the three broad areas which determine the quality of the preparation for animal studies:

1. **Formulation of the study**
2. **Dialogue between scientists and the animal facility**
3. **Quality control of the components in the study**

The topics will not always be addressed in the order in which they are presented here, and some topics overlap. The PREPARE checklist can be adapted to meet special needs, such as field studies. PREPARE includes guidance on the management of animal facilities, since in-house experiments are dependent upon their quality. The full version of the guidelines is available on the Norecopa website, with links to global resources, at **https://norecopa.no/PREPARE**.
The PREPARE guidelines are a dynamic set which will evolve as more species- and situation-specific guidelines are produced, and as best practice within Laboratory Animal Science progresses.

Topic	Recommendation
(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.
2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).
3. Ethical issues, Harm-Benefit Assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (Replacement, Reduction, Refinement) and the 3Rs (Good Science, Good Sense, Good Sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a Harm-Benefit Assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.
4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.



Topic	Recommendation
(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. ☐ Discuss and disclose all expected and potential costs. ☐ Construct a detailed plan for division of labour and expenses at all stages of the study.
6. Facility evaluation	☐ Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. ☐ Discuss staffing levels at times of extra risk.
7. Education and training	☐ Assess the current competence of staff members and the need for further education or training prior to the study.
8. Health risks, waste disposal and decontamination	☐ Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study. ☐ Assess, and if necessary produce, specific guidance for all stages of the project. ☐ Discuss means for containment, decontamination, and disposal of all items in the study.
(C) Quality control of the components in the study	
9. Test substances and procedures	☐ Provide as much information as possible about test substances. ☐ Consider the feasibility and validity of test procedures and the skills needed to perform them.
10. Experimental animals	☐ Decide upon the characteristics of the animals that are essential for the study and for reporting. ☐ Avoid generation of surplus animals.
11. Quarantine and health monitoring	☐ Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.
12. Housing and husbandry	☐ Attend to the animals' specific instincts and needs, in collaboration with expert staff. ☐ Discuss acclimation, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing).
13. Experimental procedures	☐ Develop refined procedures for capture, immobilisation, marking, and release or re-homing. ☐ Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, re-use or re-homing	☐ Consult relevant legislation and guidelines well in advance of the study. ☐ Define primary and emergency methods for humane killing. ☐ Assess the competence of those who may have to perform these tasks.
15. Necropsy	☐ Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

References
1. Smith AJ, Clutton RE, Lilley E, Hansen KEA & Brattelid T. PREPARE: Guidelines for Planning Animal Research and Testing. Laboratory Animals. 2017; DOI: 10.1177/0023677217724823.
2. Kilkenny C, Browne WJ, Cuthill IC *et al.* Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biology. 2010; DOI: 10.1371/journal.pbio.1000412.

Further information
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https://www.responsibleresearchinpractice.co.uk/prepare-for-better-science-training-course/

Step 2: Recognition

EDA Experimental Design Assistant

NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

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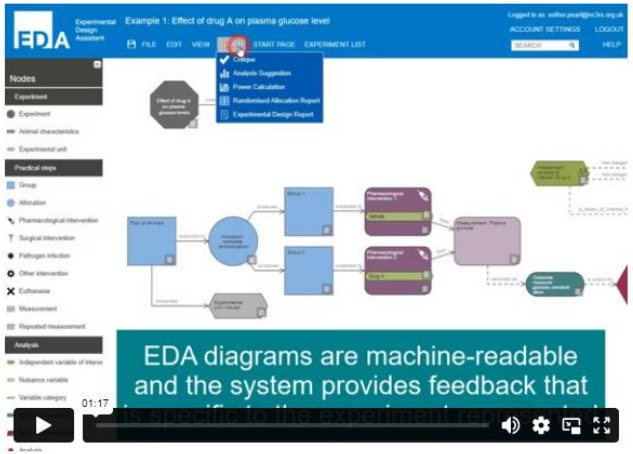
The Experimental Design Assistant

A free resource from the NC3Rs used by over 15,000 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:

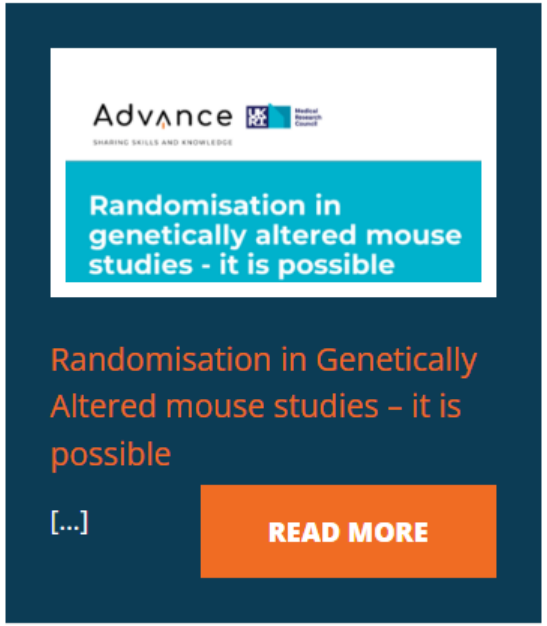
- Recommends statistical analysis methods
- Provides support for randomisation and blinding
- Performs sample size calculations

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.

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



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Step 3: Application

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Step 3: Application

https://www.responsibleresearchinpractice.co.uk/responsible-animal-research-training/

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Mindset Practice LICENSED PRACTITIONER

ATLA Alternatives To Laboratory Animals

UKRIO UK Research Integrity Office

UKRN

ISTT www.transtechsociety.org International Society for Transgenic Technologies

lasa Laboratory Animal Science Association

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