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Acronyms

Acronyms	Expanded description
AEC	Animal ethics committee
ARC	Australian Research Council
AWC	Animal Welfare Committee of the National Health and Medical Research Council
CSIRO	Commonwealth Scientific and Industrial Research Organisation
NHMRC	National Health and Medical Research Council
UA	Universities Australia

Key terms

For the purposes of this document, key terms are defined as follows:

Term	Definition
3Rs	Replacement, reduction and refinement of the care and use of animals for scientific purposes: • Replacement: methods that permit a given purpose of an activity or project to be achieved without the use of animals. • Reduction: methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures or for obtaining more information from the same number of animals. • Refinement: methods that alleviate or minimise potential pain and distress, and enhance animal wellbeing.
Animal	As defined in the Code: Any live non-human vertebrate (that is, fish, amphibians, reptiles, birds and mammals, encompassing domestic animals, purpose-bred animals, livestock, wildlife) and cephalopods.
Code	Australian code for the care and use of animals for scientific purposes, 2013 (as updated from time to time).
Code of Conduct	Australian Code for the Responsible Conduct of Research, 2018 (as updated from time to time).
Competent	As defined in the Code: The consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments.
Current best practice	As defined in the Code: A practice, procedure, method or process that has proven to be most effective in supporting and safeguarding animal wellbeing, and that: • takes into consideration the relevant aspects of species-specific biology, physiology and behaviour • is based on the best available scientific evidence (or, in the absence of scientific evidence, accepted practice), which includes the potential adverse impact of conditions and procedures on the wellbeing of the animals • includes strategies to minimise adverse impacts.
Duplication	The repetition of scientific work that provides no advances in knowledge.
Repeatability	Ability of successive measurements, made by the same person or group under the same conditions, of the same measurand to achieve a similar outcome.
Replication	The process of repeating results by an independent researcher using the same or similar methods and analysis, to ensure validity of original results.
Reproducibility	Ability of results to be validated through either replication, or the use of different methods and analysis, that achieve the same outcome/conclusion.

Term	Definition
Research	As defined in the Code of Conduct: The concept of research is broad and includes the creation of new knowledge and/or the use of existing knowledge in a new and creative way so as to generate new concepts, methodologies, inventions and understandings. This could include synthesis and analysis of previous research to the extent that it is new and creative.
Rigour	The strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results.
Robust methodology	Methodology that reveals minimal errors under scrutiny and yields results capable of being replicated.
Robust results	Results that should reveal minimal errors under scrutiny and are capable of being reproduced.
Scientific purposes	As defined in the Code: All activities conducted with the aim of acquiring, developing or demonstrating knowledge or techniques in all areas of science, including teaching, field trials, environmental studies, research (including the creation and breeding of a new animal line where the impact on animal wellbeing is unknown or uncertain), diagnosis, product testing and the production of biological products.
Transparency	Experimental details and resources (e.g. methodology, data, analysis, results) are reported and made available so that others may accurately reproduce and extend the findings.

Introduction

High quality research that is rigorous, transparent and reproducible contributes to scientific progress, fosters translation of outcomes into practical and clinical application, provides value for research investment, and ensures public trust in its findings. The ethical use of animals for scientific purposes and the value of the outcomes of their use are dependent on the studies being rigorous, transparent and reproducible.

Reports in the international literature and in the general and scientific media have outlined the growing concern that published studies in many scientific disciplines — including studies involving the use of animals — are not reproducible.[1-5] This issue does not challenge the validity or legitimacy of the scientific method. Indeed, failure to reproduce a study may prompt examination of the source of the discrepancy and lead to subsequent identification of new knowledge.[6] It is the rigorous, careful application of the scientific method that has translated into significant advances in many areas of science.[3] For example, biomedical research has led to genuine improvements in human health from which all Australians have benefited.

Irreproducibility can happen for many legitimate reasons — for example, natural variability in biological systems or small changes in conditions. Consequently, there is acceptance in the scientific community that some irreproducibility will occur. However, it is the current scale and the implications of irreproducible research that are of concern.[4,6,7]

Research irreproducibility is reportedly attributable to multiple factors. Questionable or unsatisfactory research practices contribute to irreproducibility, and are a more common cause than scientific misconduct such as deliberate fabrication and falsification of data.[6,8]

Regulatory framework

In Australia, the state and territory governments are responsible for the regulation of animal welfare, including the care and use of animals for scientific purposes. All states and territories have incorporated the *Australian code for the care and use of animals for scientific purposes (2013)* (the Code)[9] in legislation.

The Code is published by NHMRC and endorsed by NHMRC, ARC, CSIRO and UA. It sets out the framework for the ethical, humane and responsible care and use of animals for scientific purposes, and provides guidance for institutions, animal ethics committees (AECs) and investigators. The Code applies to the care and use of all live non-human vertebrates and cephalopods.

Some aspects of the care and use of animals for scientific purposes are also subject to Commonwealth legislation — for example, the import and export of animals.

National standards and guidelines

In addition to the Code, NHMRC publishes the *Australian Code for the Responsible Conduct of Research (2018)* (Code of Conduct).[10] The Code of Conduct is a national standard for research integrity and is co-authored by ARC and UA. It articulates the broad principles that characterise an honest, ethical and conscientious research culture. The Code of Conduct establishes a framework for responsible research conduct that provides a foundation for high-quality research, credibility and community trust in the research endeavour. It outlines the expectations for the conduct of research in Australia or research conducted under the auspices of Australian institutions.

Aim of this document

This document is intended to support the implementation of the Code and the Code of Conduct by:

- outlining best practice for the conduct of high quality animal-based studies
- highlighting common flaws in methodologies employed in animal-based studies and issues that impact on their reproducibility
- providing practical strategies for the implementation of best practice methodology, and to address issues related to reproducibility, during the planning, conduct, reporting and review of the use of animals for scientific purposes.

All activities outlined in this document must be conducted in accordance with relevant Commonwealth and state and territory legislation, and the Code.

The issues and recommendations in this document are outlined in broad rather than prescriptive or detailed terms. Key publications and resources providing detailed guidance and information are provided in accompanying references. Users should also be aware that the information in this document was current at the time of its publication, and that the practices they follow must accord with any changes in legislation and current best practice.

Scope

The majority of publications on the reproducibility of animal-based studies focus on biomedical research. However, this document is intended to apply to the use of animals for scientific purposes, to reflect the scope of the Code.

Factors that may affect the conduct of animal-based studies and the reproducibility of results, but are out of scope for this document, include:

- funding and resources, technology and infrastructure available to individual institutions and investigators
- publication policies of individual journals, in particular, policies on acceptance of articles reporting neutral or negative results
- funding application processes and requirements
- culture and environment in which the animal-based studies take place.

Structure

This document provides information in six sections:

- Section 1 describes the framework for best practice methodology in the use of animals for scientific purposes to foster the conduct of ethical and high quality animal-based studies, as reflected in the Code and the Code of Conduct.
- Section 2 outlines the consequences of poor methodology.
- Section 3 provides information on the most commonly reported flaws in animal-based studies.
- Section 4 summarises key guidance and advice that may provide a basis for current best practice for the conduct of ethical and high quality animal-based studies.
- Section 5 provides practical strategies for institutions, investigators and animal ethics committees for the implementation of best practice methodology in animal-based studies, and to address issues related to their reproducibility.

- Section 6 provides information on relevant publications and resources. These are intended to act as a starting point for further reading and detailed advice, rather than an exhaustive list.

References to the Code and the Code of Conduct are included throughout the document to highlight key principles in these documents that provide the basis for the framework for best practice methodology in the use of animals for scientific purposes. However, the requirements of the Code and the Code of Conduct are to be considered in their entirety when applying this document to a specific circumstance.

Intended audience

This document is intended for use by:

- institutions
- investigators, particularly those who are inexperienced with the use of animals for scientific purposes, and those who supervise inexperienced investigators
- members of animal ethics committees
- animal carers.

1. Framework for best practice methodology

The following governing principles in the Code and the Code of Conduct should be noted when considering this section. While key principles in the Code and the Code of Conduct are highlighted, the requirements of the Code and the Code of the Conduct must be considered in their entirety.

The Code:

1.1(v)   Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. This respect is demonstrated by … applying Replacement, Reduction and Refinement (the 3Rs) at all stages of animal care and use.

1.15   Regardless of the potential benefits of a project, the methods used must be scientifically valid, feasible, well designed and carefully conducted so that there is a reasonable expectation that the aims of the project will be achieved. Projects that are not scientifically valid must not be performed, no matter how mild the impact on the wellbeing of the animals.

The Code of Conduct:

The principles that are the hallmarks of responsible research conduct:

P1   Honesty in the development, undertaking and reporting of research

• Present information truthfully and accurately in proposing, conducting and reporting research.

P2   Rigour in the development, undertaking and reporting of research

• Underpin research by attention to detail and robust methodology, avoiding or acknowledging biases.

P3   Transparency in declaring interests and reporting research methodology, data and findings

• Share and communicate research methodology, data and findings openly, responsibly and accurately.

• Disclose and manage conflicts of interest.

P5   Respect for research participants, the wider community, animals and the environment

• Ensure that respect underpins all decisions and actions related to the care and use of animals in research.

P7   Accountability for the development, undertaking and reporting of research

• Comply with relevant legislation, policies and guidelines.

• Ensure good stewardship of public resources used to conduct research.

• Consider the consequences and outcomes of research prior to its communication.

P8   Promotion of responsible research practices

• Promote and foster a research culture and environment that supports the responsible conduct of research.

Best practice methodology in the use of animals for scientific purposes aims to foster the conduct of ethical and high quality studies at all stages including planning, designing, conducting, analysing and reporting of animal-based studies. It aims to ensure that the use of animals is necessary and not wasteful, contributes to scientific progress, fosters translation of outcomes into practical and clinical application, and provides value for research investment. The framework for best practice methodology is comprised of the following key elements.

1.1   The application of good scientific method

The application of good scientific method requires the formulation of a clear and valid hypothesis — including determining whether the study is hypothesis-testing or hypothesis-generating — and the use of robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results.[11,12] These practices enable investigators to increase the robustness and validity of their experimental results, and maximise the knowledge gained from each study whilst minimising the number of animals used.

1.2   The application of Replacement, Reduction and Refinement (the 3Rs)

In addition to underpinning the framework for the ethical and humane use of animals, the 3Rs are recognised as providing a structure and a tool for the conduct of high quality animal-based studies and the application of good scientific method.[13-16] The 3Rs must be considered at all stages of animal care and use, including the planning, conduct and review of projects (Code, Clause 1.1 [v]).

Examples of replacement, reduction and refinement of animal care and use are provided in the references in Section 6. The Code (Clauses 1.9 and 1.16) requires methods used to accord with current best practice.

Replacement

Application of the principle of *replacement* involves the use of methods that allow the aims of a project to be achieved without the use of animals in all or part of the study (Code, Clauses 1.18–1.20). Techniques to replace the use of animals include the use of epidemiological data; physical and chemical analysis; computer, mathematical and inanimate synthetic models; simulations; in vitro systems; non-sentient organisms; cadavers; and clinical cases (Code, Clause 1.19).

The planning phase of a study should involve identification of all feasible methods of testing the study's hypotheses including viable non-animal models and the use of less sentient alternatives such as invertebrates. Systematic review of animal-based studies should be considered where appropriate.[17-24] The validity and relevance of a proposed animal model must be assessed. If there is insufficient evidence to the support the validity of an animal model, its use must be rejected.

Reduction

Application of the principle of *reduction* enables the proposed aim(s) of the study to be achieved from fewer animals, whilst ensuring that sufficient numbers of animals are used to satisfy good statistical design (Code, Clauses 1.21–1.27).[25] The use of too few animals may lead to invalid or low-quality results and wastage of animals, and the unnecessary use of animals in future studies

that build on invalid results. The use of too many animals is a potential unnecessary ethical cost to animals and waste of resources. Repetition of experiments to provide assurance about the validity of the observed effect must be essential for the study's statistical design (Code, Clause 1.23).

Application of the principle of *reduction* also enables more information to be obtained from a given number of animals so that fewer animals are used overall. However, *reduction* should not result in greater harm, including pain and distress, to the animals used (Code, Clause 1.24).

Refinement

Application of the principle of *refinement* involves the use of methods that avoid or minimise potential harm, including pain and distress, to the animals and enhance animal wellbeing (Code, Clauses 1.8–1.14 and 1.28–1.30). Animals with compromised wellbeing have disturbed behaviour, physiology and immunology that can lead to unreliable conclusions and/or unwanted variation in scientific output, affecting the reliability and reproducibility of studies.[26] Refinement applies to all aspects of the care and use of animals, including their care and management as well as methods employed during their use.

1.3   Effective and transparent reporting

Effective and transparent reporting of animal-based studies is essential to inform future scientific studies and policy. Poor reporting makes it difficult for other investigators to reproduce results and to derive the maximum scientific knowledge from studies involving animals, and risks the unnecessary use of additional animals.

Effective and transparent reporting requires the reporting of key information on how studies are designed, conducted and analysed in publications. It also encompasses:

the pre-registration of protocols and plans for analysis[27,28]

- provision of access to data on which findings or conclusions are based
- reporting of negative impacts on animal wellbeing during the conduct of the study
- reporting to the AEC of the outcomes of previous or related work — including adverse outcomes — that are used to justify new and continuing work, particularly when projects continue for many years.

2. Consequences of poor methodology

Scientific practices that do not conform to the framework for best practice methodology outlined in Section 1 can lead to failure to obtain robust and valid results that are reproducible. The ethical costs of poor methodology include the unnecessary negative impacts on the wellbeing of individual animals, the wastage of animals that are used, and the unnecessary use of animals where such use is based on previous studies that are poorly performed or reported.

The consequences of poor methodology include hindering of scientific progress; wastage of valuable resources; and difficulties with the extent to which the outcomes can be generalised to the target population about which inferences are to be made — for example, in biomedical research, poor translation to human studies and human health outcomes.

There are also risks of negative impacts on public perception and support of the use of animals for scientific purposes, with animal-based studies being viewed as wasteful, redundant or uninformative. To maintain community support and the community's good faith, those conducting animal-based studies must be accountable for the ethical and humane use of animals and the responsible spending of public money.

3. Major issues

This section outlines the most commonly reported flaws in animal-based studies. Further details are provided in the references.

While key principles in the Code and the Code of Conduct are highlighted, the requirements of the Code and the Code of the Conduct must be considered in their entirety.

3.1 Quality of experimental design

Relevant governing principles in the Code and the Code of Conduct include:

The Code:

1.1(v) Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. This respect is demonstrated by … applying Replacement, Reduction and Refinement (the 3Rs) at all stages of animal care and use.

1.15 Regardless of the potential benefits of a project, the methods used must be scientifically valid, feasible, well designed and carefully conducted so that there is a reasonable expectation that the aims of the project will be achieved. Projects that are not scientifically valid must not be performed, no matter how mild the impact on the wellbeing of the animals.

1.17 Animals used must be suited to the purpose of the project or activity, taking into account their biological characteristics, including morphology, physiology, behaviour, genetic makeup, temperament and behavioural conditioning, microbiological and nutritional status, and general state of health.

1.25 All possible steps must be taken to reduce factors that are not part of the experimental design of the project and are known to contribute to variability of experimental results, including the use of animals of known genetic, biological and behavioural background. Reduction of experimental variables may result in reduced animal use.

The Code of Conduct:

Researchers will uphold the principles of responsible research conduct in all aspects of their research. To this end, researchers will:

R20 Ensure that the 3Rs (Replacement, Reduction and Refinement) are considered at all stages of research involving animals.

R21 Adopt methods appropriate to the aims of the research and ensure that conclusions are justified by the results.

Flaws include, but are not limited to:

- Failure to use the most appropriate animal model — for example, species, strain, methodology.[27-31]
- Failure to define experimental unit.[32,33]
- Failure to use appropriate control animals.[11,33]
- Failure to use randomisation when selecting animals or allocating animals to treatment groups.[34-39]
- Failure to use blinding when performing an intervention, and when assessing results.[34-36]
- Lack of consideration and control of variables.[11,33,36,39-44] Examples include variables related to:
  - the animal's biological characteristics, including morphology, physiology, behaviour, genetic makeup, temperament and behavioural conditioning, microbiological and nutritional status, microbiome, and general state of health
  - the animal's living conditions including physical, environmental and social conditions
  - the conduct of procedures
  - unnecessary harm to the animal, including pain and distress.
- Failure to consider the use of both sexes in the study.[45,46]
- Failure to consider that findings may not translate to the target species because of inherent species differences.[30,35,37,38,47]
- Lack of consistency in protocols between animal and human studies intending to relate findings from an animal model to humans.[35]

3.2 Quality of experimental statistics

Relevant governing principles in the Code and the Code of Conduct include:

The Code:

1.21   The number of animals used in a project must be the minimum necessary to achieve the proposed aim(s) and to satisfy good statistical design. The use of too few animals may invalidate the experimental result and result in wastage of animals.

1.22   The number of animals used may be reduced by the appropriate reuse of individual animals. The benefits of reusing animals must be balanced against any adverse effects on their wellbeing, taking into account the lifetime experience of the individual animal. Reuse of animals requires particular justification and specific AEC approval.

1.23   Activities involving the use of animals must not be repeated within a project or between projects unless such repetition is essential for the purpose or design of the project (e.g. sound experimental design, statistical analysis, corroboration by the same or another investigator).

1.24   Reducing the number of animals used should not result in greater harm, including pain and distress, to the animals used.

1.25   All possible steps must be taken to reduce factors that are not part of the experimental design of the project and are known to contribute to variability of experimental results, including the use of animals of known genetic, biological and behavioural background. Reduction of experimental variables may result in reduced animal use.

1.26   Where practicable, tissue and other biological material from animals being killed must be shared among investigators or deposited in a tissue bank for subsequent distribution.

The Code of Conduct:

Researchers will uphold the principles of responsible research conduct in all aspects of their research. To this end, researchers will:

R20   Ensure that the 3Rs (Replacement, Reduction and Refinement) are considered at all stages of research involving animals.

Flaws include, but are not limited to:

- Failure to determine statistical power, biologically relevant effect size, the appropriate statistical significance level and appropriate sample size prior to commencing a study.[14,34-36]
- Failure to apply correct statistical tests for analysis of data.[14,35,36,39,48,49]
- Lack of appropriate training in statistics to design meaningful experiments.[14]

3.3 Quality of techniques and procedures

Relevant governing principles in the Code and the Code of Conduct include:

The Code:

1.9   Practices and procedures used for the care and management of animals must be based on current best practice (*see definition*).

1.16   Investigators must use methods that accord with current best practice (*see definition*).

1.28   Steps must be taken at all times to support and safeguard animal wellbeing.
The effectiveness of strategies for supporting and safeguarding animal wellbeing must be kept under review during the lifetime of activities, including projects. Where relevant and applicable, the outcome of this review must be implemented in current activities and taken into account in planning future activities, including projects.

1.29   People who care for and use animals must ensure that procedures are performed competently, and

   i)   be competent for the procedure they perform, or

   ii)   be under the direct supervision of a person who is competent to perform the procedure.

The Code of Conduct:

To foster responsible research conduct, institutions will:

R4   Provide ongoing training and education that promotes and supports responsible research conduct for all researchers and those in other relevant roles.

R5   Ensure supervisors of research trainees have the appropriate skills, qualifications and resources.

Researchers will uphold the principles of responsible research conduct in all aspects of their research. To this end, researchers will:

R16   Undertake and promote education and training in responsible research conduct.

Flaws include, but are not limited to:[50,51]

- Failure to use methods that accord with current best practice.
- Failure to apply refinement to methods and procedures.
- Lack of dissemination and communication of best practices for experimental methods and procedures.
- Inadequate training and competence of those using animals.

3.4 Reporting

Relevant principles in the Code of Conduct include:

To foster responsible research conduct, institutions will:

R7   Support the responsible dissemination of research findings. Where necessary, take action to correct the record in a timely manner.

Researchers will uphold the principles of responsible research conduct in all aspects of their research. To this end, researchers will:

R22   Retain clear, accurate, secure and complete records of all research including research data and primary materials. Where possible and appropriate, allow access and reference to these by interested parties.

R23   Disseminate research findings responsibly, accurately and broadly. Where necessary, take action to correct the record in a timely manner.

Flaws include, but are not limited to:

- Lack of uniform and/or incomplete reporting of animal-based studies in publications, in particular animal characteristics (e.g. sex, strain, age), randomisation and blinding, sample size estimation and data handling, and negative impacts on animal wellbeing.[35,36,38,52]
- Lack of reporting of key methodological parameters that can introduce bias.[37-39]
- Lack of reporting of conflicts of interest that may introduce bias.[6,39,53]
- Lack of reporting of neutral and negative results with the potential consequence of unnecessary duplication of studies.[37-39]
- Lack of reporting of outcomes of previous and related work, including adverse events, to the AEC.

4. International guidance

Guidelines and advice issued in response to concerns regarding research reproducibility, and to assist in determining appropriate parameters for meaningful animal-based studies, include:

- The ARRIVE Guidelines for reporting animal research (2010).[54] The ARRIVE Guidelines Checklist is provided in Appendix 1.

- Guidance for the description of animal research in scientific publications (2011). National Research Council (US).[55]

- Reproducibility and reliability of biomedical research: improving research practice (2015). The Academy of Medical Sciences, Biotechnology and Biological Sciences Research Council, Medical Research Council and Wellcome Trust.[56]

- Principles and guidelines for reporting preclinical research (2016). US National Institutes of Health.[57,58]

- Fostering integrity in research (2017). The National Academies of Sciences, Engineering, and Medicine.[59]

- Experimental Design Assistant. National Centre for the Replacement, Refinement and Reduction of Animals in Research.[60]

- The PREPARE Guidelines for planning animal research and testing (2017).[61] The PREPARE Guidelines Checklist is provided in Appendix 2.

Publications and resources providing additional guidance are included in Section 6.

5. Practical strategies

Poor methodology and its consequences are risks those involved with the use of animals for scientific purposes need to be aware of and seek to address. This section outlines some practical strategies for institutions, investigators and animal ethics committees for the implementation of best practice methodology in animal-based studies, and to address issues that affect their reproducibility. Proposed strategies are based on the requirements in the Code and the Code of Conduct, and current best practice reported in the international literature. The strategies are provided in broad rather than prescriptive or detailed terms to facilitate their application to any specific circumstance. Publications and resources providing detailed guidance are included in Section 6.

5.1 Institutions

5.1.1 Develop guidelines for investigators on how to achieve the following during the planning, designing, conducting, analysing and reporting of animal-based studies:

- the application of good scientific method

- the application of the 3Rs at all stages of animal care and use

- effective and transparent reporting.

The guidelines may be in the form of detailed guidance, a statement of expectations with reference to external guidance, or another form that is considered appropriate by the institution. The guidelines should make reference to, or require the adoption of, international standards and current best practice (see Section 4). The guidelines may include advice on, or Standard Operating Procedures for, current best practice for specific procedures and methods.

The guidelines must be developed in consultation with and approved by the AEC (Code, Clause 2.1.5 [v]), and promoted and consistently implemented within the institution.

5.1.2 Provide support to investigators to facilitate their adoption of current best practice for planning, designing, conducting, analysing and reporting animal-based studies. Strategies may include:

- provision of access to relevant expert advice — for example, statistician; other investigators and research groups with experience in working with animals and the relevant animal model; veterinarians; animal care staff; specialists in laboratory animals, livestock or wildlife; experts in the 3Rs; Category B members of the AEC

- implementing systems for pre-review of applications prior to their submission to the AEC

- fostering systems and formal networks to facilitate sharing of tissues and other biological material from animals being killed

- support for investigators wishing to publish negative results.

5.1.3 Ensure appropriate and ongoing education and training for investigators in the use of the 3Rs, experimental design, choice of animal model, use of experimental statistics, technical expertise, and reporting of outcomes — in accordance with current best practice.

5.1.4 Ensure the development of guidelines on how competence of investigators will be assessed and ensured (Code, Clauses 1.29, 2.1.5 [v] [a] and 2.1.8 [ii]).

5.1.5 In consultation with the AEC, ensure that the AEC application form is designed and regularly reviewed so that all relevant matters highlighted in Sections 1 and 3 are addressed by the applicant. Section 2.7 of the Code outlines information to be provided in an application to the AEC. The inclusion of the following specific information should also be considered:

- reference to the ARRIVE Guidelines[54] or the ARRIVE Guidelines checklist (Appendix 1), and the PREPARE Guidelines[61] or the PREPARE Guidelines Checklist (Appendix 2)

- reporting of outcomes of previous or related work — including adverse outcomes — that are used to justify new and continuing work, particularly when projects continue for many years.

5.1.6 Ensure procedures are in place so that the AEC is provided with adequate information about the merit of a project described in an application, and the robustness of its experimental design, statistics and methodology — for example, appropriate peer review of the project by a funding body or an independent expert/s.

5.1.7 Ensure appropriate education and training for AEC members at the time of their appointment and on an ongoing basis. Such education and training may include support for attendance at relevant workshops, conferences and seminars, including those organised by relevant state and territory government departments.

5.2 Investigators

5.2.1 Be aware of and comply with institutional and AEC guidelines, and international standards and current best practice for the planning, design, conduct, analysis and reporting of animal-based studies, and ensure that all relevant issues outlined in Section 3 are addressed.

5.2.2 When planning and designing studies (Code, Clauses 2.4.6–2.4.9), ensure that the studies are designed in accordance with institutional and AEC guidelines (see Part 5.1), and international guidance and current best practice (see Section 4). The ARRIVE Guidelines checklist (Appendix 1) and the PREPARE Guidelines checklist (Appendix 2) can serve as additional useful guides during the planning stage.

5.2.3 Seek advice from relevant experts (Code, Clause 2.4.3) — for example, statistician; other investigators and research groups with experience in working with animals and the relevant animal model; veterinarians; animal care staff; specialists in laboratory animals, livestock or wildlife; experts in the 3Rs; Category B members of the AEC.

5.2.4 Access systems for sharing of tissues and other biological material from animals being killed.

5.2.5 Ensure that all relevant matters outlined in Sections 1 and 3 are addressed in the application to the AEC, and any funding application.[62] Advice to the AEC should include justification for the animal model to be used, any issues associated with the proposed animal model, and any evidence of issues of translation of the animal model to the target species — including humans — because of inherent species differences.

5.2.6 Attend relevant training programs.

5.2.7 When conducting projects, ensure:

- the continuing consideration of the 3Rs and improved methodologies throughout the lifetime of the project, with prior approval from the AEC for any necessary amendments to the protocol
- the competence or appropriate supervision of all persons involved with animals on the project as required by the Code
- the control of variables related to the wellbeing of the animal — for example, maintenance of a health status of the animals that safeguards their wellbeing and meets the requirements of their proposed use; avoidance and alleviation of harm including pain and distress.

5.2.8 Disseminate a full account of the work as broadly as possible, including:

- complete reporting of the work in publications in accordance with international standards and current best practice
- reporting of neutral and negative findings and results (e.g. Journal of Negative Results in Biomedicine, PLoS journals, Physiological Reports)
- pre-registration of protocols and plans for analysis, where applicable
- provision of access to data on which findings or conclusions are based
- reporting to the AEC of the outcomes of previous or related work — including adverse outcomes — that are used to justify new and continuing work.

5.2.9 Ensure ongoing discussion of best practice methodology outlined in Section 1 amongst members of the research team to embed good research practices in the workplace culture.

5.3 Animal ethics committees

5.3.1 Ensure that all relevant matters highlighted in Sections 1 and 3 have been addressed by the applicant before making a judgement about the ethical acceptability of the proposed use of animals as required by Clause 2.3.4 of the Code. Procedures must be in place to provide members with assurance regarding the merit of a proposed project and the robustness of its experimental design, statistics and methodology (see Section 5.1.6).

5.3.2 Raise with the institution any recommendations or concerns regarding training and competence of investigators and compliance with institution and AEC guidelines on best practice methodology.

6. References and resources

The information provided in this section is intended to act as a starting point for further reading and detailed advice, rather than as an exhaustive list.

1. Ioannidis JPA. (2005) Why most published research findings are false. PLoS Med. 2(8): e124. doi:10.1371/journal.pmed.0020124

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Additional publications and resources

• Fund for the Replacement of Animals in Medical Experiments (FRAME). Strategic planning for research programs. Accessed from: http://www.frame.org.uk/planning/ and http://www.frame.org.uk/wp-content/uploads/2015/03/FRAME-POSTER-ENGLISH.pdf

• US National Library of Medicine. Research reporting guidelines and initiatives: By organization. https://www.nlm.nih.gov/services/research_report_guide.html

Appendix 1: ARRIVE Guidelines Checklist

The ARRIVE Guidelines Checklist
Animal Research: Reporting In Vivo Experiments

Carol Kilkenny[1], William J Browne[2], Innes C Cuthill[3], Michael Emerson[4] and Douglas G Altman[5]

[1]The National Centre for the Replacement, Refinement and Reduction of Animals in Research, London, UK, [2]School of Veterinary Science, University of Bristol, Bristol, UK, [3]School of Biological Sciences, University of Bristol, Bristol, UK, [4]National Heart and Lung Institute, Imperial College London, UK, [5]Centre for Statistics in Medicine, University of Oxford, Oxford, UK.

	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	

The ARRIVE guidelines. Originally published in *PLoS Biology*, June 2010[1]

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.

RESULTS

Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.

DISCUSSION

Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
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Appendix 2: PREPARE Guidelines Checklist

PREPARE

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

Adrian J. Smith[a], R. Eddie Clutton[b], Elliot Lilley[c], Kristine E. Aa. Hansen[d] & Trond Brattelid[e]

[a]Norecopa, c/o Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, Norway; [b]Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, EH25 9RG, U.K.; [c]Research Animals Department, Science Group, RSPCA, Wilberforce Way, Southwater, Horsham, West Sussex, RH13 9RS, U.K.; [d]Section of Experimental Biomedicine, Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, P.O. Box 8146 Dep., 0033 Oslo, Norway; [e]Division for Research Management and External Funding, Western Norway University of Applied Sciences, 5020 Bergen, Norway.

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 3Ss (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
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   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
https://norecopa.no/PREPARE   /   post@norecopa.no   /   🐦 @norecopa

Appendix 3: Process report

The development of the *Best practice methodology in the use of animals for scientific purposes* (the Guidance) was overseen by NHMRC's Animal Welfare Committee (AWC). The AWC is established as a working committee under Section 39 of the *National Health and Medical Research Council Act 1992* (NHMRC Act) to advise NHMRC on issues pertaining to the conduct and ethics of using animals in biomedical research.

Animal Welfare Committee

Members	Expertise	Period
Professor Edna Hardeman	Chair	2012–2016
	Person with expertise in the use of animals for health and medical research	2016–2018
Dr Simon Bain	Person with expertise in veterinary science and the care and use of animals for scientific purposes	2012–2016
		2016–2018
A/Professor Thomas Burne	Person with expertise in the use of animals for health and medical research	2016–2018
Professor Neil Dear	Person with expertise in the use of animals for health and medical research	2012–2016
Professor Andy Giraud	Person with expertise in the use of animals for health and medical research	2012–2016
Ms Anna Hall	Person with experience in furthering the welfare of animals	2016–2018
Dr Bidda Jones	Person with experience in furthering the welfare of animals	2012–2015
Dr Mark Lawrie	Person with expertise in veterinary science and the care and use of animals for scientific purposes	2012–2016
		2016–2018
Ms Robin Matthews	Person with an understanding of community attitudes to the care and use of animals for scientific purposes	2012–2016
		2016–2018
Mr Paul Power	Person with an understanding of community attitudes to the care and use of animals for scientific purposes	2012–2014
Dr Carole Webb	Person with experience in furthering the welfare of animals	2012–2016
		2016–2018

Disclosure of interests and management of conflicts of interests

Throughout the development of the Guidance, disclosure of interests and management of conflicts of interest was undertaken in accordance with the requirements of the NHMRC Act and NHMRC's *Policy on the disclosure of interest requirements for prospective and appointed NHMRC committee members*. A record of interests was maintained by NHMRC, and relevant information was made publicly available on the NHMRC website to ensure transparency.

Other contributors

NHMRC Project team

Jillian Barr
Mary Bate
Tim Vines (2018)

Development of the Guidance

- Development of the draft Guidance overseen by NHMRC's AWC.

- Targeted consultation on the draft Guidance (18 August 2016 to 30 September 2016), with 33 submissions received from a range of individuals and organisations. The aim of this consultation was to seek feedback on the advice provided in the draft Guidance, in particular:

  - the accuracy and relevance of the issues discussed

  - the practicality of the proposed strategies to address the issues.

- Consideration of the submissions received to the targeted consultation and revision of the draft Guidance by the AWC. The AWC gave due regard to all submissions, with a consensus reached in each case on incorporating the suggestions made.

- Consideration of the revised draft Guidance by NHMRC's Research Committee on 22 September 2017 and Council of NHMRC on 12 October 2017.

- In May 2018, the Guidance was updated to accord with the guidance provided in the *Australian Code for the Responsible Conduct of Research, 2018* and to add two references (27 and 28).