Guidance for the Description of Animal Research in Scientific Publications

Institute for Laboratory Animal Research

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

THE NATIONAL ACADEMIES PRESS
Washington DC
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This study was supported by core funding for the Institute for Laboratory Animal Research from the National Science Foundation under contract DBI-0838150; the US Department of the Army under contract W81XWH-09-1-0664; the US Department of the Navy under contract N00014-05-G-0288, Task Order #21; and the Department of Health and Human Services/National Institutes of Health under contract N01-OD-4-2139, Task Order #221. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the organizations or agencies that provided support for the project.

International Standard Book Number-13: 978-0-309-21951-8
International Standard Book Number-10: 0-309-21951-5

Additional copies of this report are available from the National Academies Press, 500 Fifth Street NW, Lockbox 285, Washington DC 20055; (800) 624-6242 or (202) 334-3313 (in the Washington metropolitan area); Internet, http://www.nap.edu

Printed in the United States of America

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The draft of this report was reviewed by individuals chosen for their diverse perspectives and expertise, in accordance with procedures approved by the Report Review Committee of the National Research Council. The purpose of this independent review is to provide candid and critical comments that will assist the committee in making its published report as sound as possible, and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberation process. The committee thanks the following individuals for their review of the draft report:

Floyd Bloom, Scripps Research Institute
Cory Brayton, Johns Hopkins University
Patricia A. Brown, National Institutes of Health
Hershel Raff, Medical College of Wisconsin
Randy W. Schekman, University of California, Berkeley

Although the reviewers listed above provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by John Vandenbergh, North Carolina State University. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Guidance for the Description of Animal Research in Scientific Publications

1 Overview

The publication of research articles involving animal studies is central to many disciplines in science and biomedicine. Effective descriptions in such publications enable researchers to interpret the data, evaluate and replicate findings, and move the science forward.

To promote the inclusion of sufficient information in publications on animal studies,[1] the National Research Council's Institute for Laboratory Animal Research (ILAR) appointed a committee of experts in laboratory animal research and scientific publishing to provide guidance for journal editors, authors, and reviewers. Supported by private funding and other grants, the committee was charged as follows:

> [To] prepare a short report aimed at editors of journals that publish animal studies. The report will outline the information that should be included in scientific papers regarding the animal studies to ensure that the study can be replicated. The extent of the needed information will be determined by the committee, but will include for example, conditions of housing and husbandry, genetic nomenclature, microbial status, detailed experimental manipulations and handling and use of pharmaceuticals. Evidence-based rationale for the need to include this information will be presented.

To complete its task, the committee conducted an extensive literature search about the impacts of various aspects of research animals and their environment. This report is the outcome of the committee's work.

The committee believes that journal editors have a role to play in promoting the proper use of animals in research through the publication of adequate descriptions. The committee urges journal editors to actively promote effective and ethical research[2] by encouraging the provision of sufficient information to enable assessment and interpretation of research findings and advancement of knowledge based on reproducible results.

This report provides journal editors, authors, and reviewers with guidance (and supporting references) for effective reporting of animal research in published articles based on adequate descriptions of

- the research animal (section 3), with detailed information about the animals'
  - — age, sex, weight, and life stage (3.1),
  - — source (3.2),
  - — genetic nomenclature (3.3),
  - — microbial/pathogen status (3.4), and
  - — preparation and assignment (including control groups) (3.5);
- the research animal environment (sections 4 and, for aquatic animals, 6), with detailed information about
  - — the micro- and macroenvironment (4.4 and 6.1),

[1] Including studies that use cells and tissues derived from animals for ex vivo and in vitro research.

[2] The guiding principles for ethical animal research are the "three Rs"—reduction in the number of animals used, refinement of procedures to reduce animal stress and pain, and replacement of animals when possible (Russell and Burch 1959).

    - diet (4.1 and 6.2),
    - water (4.2), and
    - housing (4.3 and 6.3);
- basic animal methodology, including aspects of animal care and use that can affect research outcomes (section 5), with detailed information about
    - experimental effects (5.1),
    - administration of substances (5.2),
    - use of infectious agents (5.3),
    - sample acquisition (5.4), and
    - euthanasia (5.5).

The ability to interpret, evaluate, and reproduce biomedical and other types of laboratory animal research and testing is a reasonable minimum standard for the assessment of effective reporting in research articles. Journal editors can substantially contribute to the achievement of this standard through the articulation of clear policies and criteria for their authors and reviewers. This report complements existing checklists and resources by providing guidance and scientific evidence for the specific types of information that should be included in research publications to promote the advancement of science involving animal studies. It also describes approaches to facilitate the provision of such information.

1.1 The Need for Guidance

Analyses of published studies with research animals have demonstrated numerous deficiencies in the reporting of details in research methods for animal studies (Kilkenny 2009; Vesterinen et al. 2011). Despite multiple publications over the past 25 years calling attention to the critical factors and information necessary to enhance such reporting, most scientific journals provide relatively little specific guidance for authors and reviewers and there has been limited effort until quite recently (see next section) to address this systemic problem (Alfaro 2005; Ellery 1985; Öbrink and Rehbinder 2000; Smith et al. 1997). Most biomedical journal policies simply refer to regulatory requirements for animal use, without referring to critically important experimental design information.

Lack of sufficient experimental procedural detail about animal studies in the research literature has both scientific and ethical implications:

- It limits the ability to confirm and build on research findings.
- It can lead to the unnecessary use of animals in studies that fail to reproduce the reported results.
- It may mask problems in the quality of the design and conduct of animal studies (Dirnagl and Macleod 2009; Festing 2003; Festing and Altman 2002; Macleod et al. 2009; Rice et al. 2008).
- It limits the ability to perform systematic reviews (Hooijmans et al. 2010; Peters et al. 2006; Ranstam 2010; Roberts et al. 2002).
- The foregoing impacts may give rise to questions about experimental methods and the overall quality of the studies and thus erode support for the utility—and necessity—of laboratory animal research for informing human health treatments (Perel et al. 2007; Pound et al. 2004; van der Worp et al. 2010).

The articulation of clear guidelines by journals for the reporting of animal-related studies will help to address many of these concerns. Useful journal policies will define requirements for accurate descriptions of the research animal as an experimental test system, the critical elements of the research animal environment, and animal care and use practices that affect research results (Atlas 2003; Osborne et al. 2009).

1.2 Related Guidelines

In recent years there have been growing, but incomplete, efforts to enhance the reporting of animal-related research:

- The ARRIVE Guidelines (Animal Research: Reporting In Vivo Studies, www.nc3rs.org.uk/ARRIVE; Kilkenny 2010) from the UK National Centre for the 3Rs have been endorsed by a variety of journals and funding sources (Danos et al. 2010; Drummond et al. 2010; McGrath et al. 2010). A "Gold Standard Publication Checklist" (Hooijmans et al. 2010, 2011a,b) is also available for the reporting of animal studies.
- Efforts to improve the reporting of biological and biomedical investigations resulted in the MIBBI project (Minimum Information for Biological and Biomedical Investigations, mibbi.org; Taylor et al. 2008).
- The international EQUATOR Network (www.equator-network.org) was established to improve the reliability and value of medical research literature by promoting transparent and accurate reporting of research studies (Altman and Simera 2010; Simera et al. 2009, 2010).
- Some journals (e.g., the *British Journal of Cancer*; Workman et al. 2010) and professional groups have adopted their own guidance, incorporating important aspects of animal care and use relevant to their fields of research (Auer et al. 2007; Ayala et al. 2010; Idris et al. 1996; Portaluppi et al. 2008; Touitou et al. 2006).

In addition to these resources, guidance is available in occasional articles about animal-related information to include in reporting (e.g., genetic and environmental description of important factors that can result in study variability). Such guidance is the result of interest in harmonizing standard operating procedures and methods to facilitate the comparison of animal studies across laboratories (Ayala et al. 2010) and to allow the sharing of animal phenotyping data, particularly for genetically modified mice (Gates et al. 2011; Mandillo et al. 2008; McGuinness et al. 2009; Würbel 2002).

Notwithstanding the examples cited above, there is no consensus or consistency among scientific publications about the basis for inclusion of adequate procedural detail in the reporting of animal research. The purpose of this report is to provide a firm basis for building such consistency. To the extent that a checklist approach is convenient, the committee cites as an example the ARRIVE Guidelines (Appendix A) and encourages journal editors and authors to use such a resource in conjunction with this report in determining the specific information to include in study reports for their publications.

1.3 Organization and Content of This Report

This report is organized in general sections that align with the types of information to be considered for inclusion in the materials and methods section of a scientific manuscript, with discussion of particular aspects and variables that can influence outcomes:

- the research animal (including source, genetics, microbial/pathogen status, preparation and study assignment, and monitoring during the study);
- the research animal environment (including diet, water, housing, and micro- and macroenvironment); and
- basic animal methodology (including administration of anesthetics, analgesics, and other substances; tissue and fluid sampling; and euthanasia).

These sections present criteria to consider and the rationales behind them, together with supporting references that provide scientific evidence of the potential impacts discussed.

The committee acknowledges that this relatively concise guidance document cannot specifically address the array of animals and animal models used in biomedical research. Furthermore, because the vast majority are laboratory rodents,[3] many of the references in this report pertain to these species, although they generally hold true for other animals used in research. In light of rapidly increasing research interest in zebrafish and other aquatic species, there is a separate section on aquatic systems.

Finally, in light of space limitations especially in print publications, the report presents possible methods to facilitate the provision of appropriate procedural details and data.

2 Defining an Optimal Description of an Animal Study

The definition of each journal's policy will entail editors' determination of the specific information to be included in descriptions of materials and methods, taking into account the field of endeavor, the intended audience of the publication, the type of study, the species and nature of the animal model, and the aims and objectives of the particular study being described.

Complementing the criteria and checklists available, and in the absence of best practice standards, the following paragraphs present factors for journal editors to consider in determining their policy and for authors and reviewers to bear in mind in their approach to manuscript preparation and review.

2.1 How Much Detail Is Necessary?

In descriptions of the materials and methods used in studies with animals, authors frequently simply state that the work was approved by the institutional animal care and use committee (IACUC) and/or conducted in an accredited facility[4] without providing details of the conditions of the animal environment. IACUC approval and animal facility accreditation are general indications of program quality but in no way obviate the need for proper description of the test system and conditions of an experiment.

As an example, multiple characteristics of a single environmental factor in an animal facility—lighting—affect behavioral and physiological processes and can thus influence a research endpoint (Bellhorn 1980; Dauchy et al. 2011). The description should therefore consider including type of lighting (natural vs. artificial), method of provision (fluorescent vs. LED), intensity, spectral qualities, duration, timing of light:dark cycles, control of method of onset, and methods used in reversal of circadian cycles. The amount of detail will depend on the type of study, the type of endpoint, and how light might affect the research—a study of phototoxic retinopathy in albino rodents or a breeding study in cats might require a very different description of lighting than the study of a surgical procedure in dogs.

This report elucidates specific factors to consider when determining the details necessary for descriptions of research animal environment and husbandry, with selected references that provide information about species and types of models as well as factors known to induce variability in research outcomes. It is important to bear in mind that at the time of reporting the impacts of some factors may not be known; it is therefore better to err on the side of providing more rather than less detail.

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[3] In 2007 it was estimated that 60% of National Institutes of Health extramural funding supported research using animals, and that more than 80% of this research involved the use of mice (Valli et al. 2007).

[4] Animal facilities are accredited internationally by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International and in accordance with European Union and national directives and legislation.

2.2 A Values-Based Approach

The following values-based approach is provided to assist editors, reviewers, and investigators in assessing descriptions of the research animal and its care and use. Ideally, the information will be detailed enough to:

(1) enable the reader to effectively interpret and evaluate the work;
(2) ensure that others can replicate the experiments described; and
(3) clearly convey refinement and reduction measures both to ensure transparency about effects on animals and to prevent unnecessary animal use and/or harm in efforts to replicate studies.

These three points are important and fall directly within the role and responsibilities of journals and editors in supporting reproducibility as a means to ensure both effective science and ethical animal use. The committee therefore strongly encourages journals to provide clear, customized guidance for their authors and reviewers about the information to be included in descriptions of the research animal, the research animal environment, and animal care and use methods.

3 The Research Animal

3.1 General

The following information is appropriate to include in the research animal description: genus and species (with the proper Latin designation), sex, internationally accepted genetic nomenclature, age, weight, and source of the animals used. The provision of specific procedural detail for these basic variables is a starting point for enabling replication.

Sex influences numerous biological outcomes (Holdcroft 2007). For studies with mixed sex groups (e.g., with difficult to produce genetically modified rodents), an explanation of the composition and numbers and of how subjects are assigned to the groups is useful. In addition, the physiologic (e.g., pregnant, castrated) and/or pathologic status of the animal is also appropriate to include (more on pathologic status below).

Both age and body weight (with ranges) are critical parameters to provide for all animal studies. The use of terms such as “weanling,” “fry,” “fingerling,” “aged animal,” and “retired breeder” for research animal description in the materials section of manuscripts is not sufficient or clear for describing life stage or physiologic status. Age is a function not only of time but also of species, genetic, and environmental factors (including husbandry) (Deerberg 1991). Age alters many biological outcomes (Deerberg 1991; Huang et al. 2007) and affects lesions, disease course, physiologic state, and response to experimental variables.

Many publications, especially those that involve rat and mouse models, indicate body weight instead of age, and some investigators believe that the approximate age of rodents can be determined from charts of body weight curves available from commercial suppliers. However, body weight is not identical to age; the correlation is highly dependent on the animal’s life stage, stock, and strain. In addition, numerous husbandry, nutritional, and environmental factors strongly influence body weight, often through incompletely understood interactions (Haseman et al. 1997, 2003; Keenan et al. 1999; Laroque et al. 1997). Because weight correlates with many biological outcomes (Gaines Das 2002) it is important to include it in the animal description together with age (Klimentidis et al. 2010).

When life stage factors (e.g., age at weaning, parity status, breeding history) are relevant to a study, they should be described in detail. For studies with pregnant animals, appropriate details include whether the animals were procured from external breeding sources or bred internally. Breeding conditions and gestational age at shipment before experimental use may also be important information to provide, as well as details about litters culled to common size groups. Experimental results can be strongly influenced by breeding and parity status, especially for endpoints and physiologic states that are dependent on endocrine factors (Walker et al. 2001). There are also potentially profound differences between aged animal cohorts and those that are "retired breeders" as husbandry conditions and other aspects of the animal environment often differ between these populations; differences may include the type of housing, method of housing with conspecifics, and type and composition of diet. Retired breeding stock are therefore not to be considered synonymous with nonbreeding aged animals.

3.2 Source

Many study reports do not specify the source of the animals used and instead indicate only stock/strain and/or breeder. But differences in environmental and microbial conditions between commercial breeders and between production facilities within a commercial breeding operation can be substantial and may affect study outcomes depending on the types of study endpoints (Wahlsten et al. 2003, 2006), so information about source colonies or origin (i.e., location) is usually relevant for all animals used.[5]

Different colonies—even from the same commercial vendor—may have been raised under differing husbandry and environmental influences, resulting in differing incidences of lesions (Engelhardt et al. 1993). Rodent colonies also exhibit differences in gastrointestinal microflora, which are dependent on both genetic and environmental factors (Hufeldt et al. 2010); this particular parameter is believed to be important for certain endpoints such as effects of gut flora on xenobiotic metabolism (Levin and Dent 1982). In outbred large animal stocks such as nonhuman primates, studies have shown that the origin of the animals can affect the outcome of an experiment or the development of background or induced lesions (Burwitz et al. 2009; Menninger et al. 2002; Vidal et al. 2010).

Editors and reviewers can help to reduce the risk of inconsistent research outcomes from these variables by ensuring that the animal source and origin are specified by the author and carefully considered in the experimental design so that animals are not assigned to study groups with bias.

Additional relevant information in some studies concerns the provenance of the animals or animal models (e.g., surgical and/or genetic modifications). Were they produced or procured? If the latter, what were the transport and acclimation methods, the timing of animal treatment and manipulation? For studies that involve the use of surgically modified animals, what was the period of time between the surgical procedure and experimental use? How was the animal maintained during and after the surgery and throughout the experiment?

Many institutions encourage the efficient use, sharing, and/or reuse of research animals as a way to reduce the overall numbers of animals used. When animals are used in more than one study, it is essential to specify the previous use, with an explanation of how the animals were chosen for reuse and assigned to the study in question.

3.3 Genetics

The use of internationally accepted genetic nomenclature is critical, especially in light of the dramatic increase in the use of genetically modified mice during the past decade. Furthermore, biological data are increasingly shared, analyzed computationally, and archived (Sundberg and Schofield 2009, 2010). In

[5]Such information may be particularly important when "random source" animals are used (e.g., from shelters, pounds, or other nonlicensed sources). The health and background of such animals (often simply called mongrels, for example, without any information about their source) may vary widely and yield research results that are inconsistent or difficult to interpret (NRC 2009).

addition to correct, complete genetic designations, the replicability of studies with genetically modified rodents can be supported with clear references to or descriptions of gene targeting strategies and of the breeding and gene expression methods, backcross generations, substrain designation, and specific genotype of embryonic stem cells.

There are profound differences among laboratory rodent substrains and origins, and particularly among lines of mouse strains such as the 129 and C57BL/6, both of which are used commonly in the creation of genetically modified mouse models (Doetschman 2009; Linder 2001, 2006; Simpson 1997; Yoshiki and Moriwaki 2006). The 129 mouse has been genetically corrupted over the years and it is now recognized that there are at least 16 inbred 129 lines. It is therefore important to specifically identify 129 lines as well as 129-origin embryonic stem cells (Simpson 1997).

The use of shortened stock and strain designations (e.g., Sprague-Dawley rat or C57BL mouse) instead of the fully defined genetic nomenclature is not appropriate in published animal descriptions. For example, “Crl:WI(Han) rat” indicates the origin and source of this outbred rat, compared to the term ‘Wistar Han rat,” which does not indicate the source of the rat or whether it is inbred or outbred. For gene nomenclature, allele designations should be superscripted and indicate the type of mutation and the laboratory of origin, according to international nomenclature recommendations

Rules for mouse genetic nomenclature were first published in 1940 and subsequently revised by the International Committee for Standardized Genetic Nomenclature in Mice. Rules for rat genetic nomenclature were first published by the Committee on Rat Nomenclature in 1992. In 2003, the International Committee on Standardized Genetic Nomenclature for Mice and the Rat Genome and Nomenclature Committee unified the rules and guidelines for gene, allele, and mutation nomenclature in mouse and rats. Nomenclature guidelines are now reviewed and updated annually by the two international committees; current guidelines are available on the Mouse Genome Database (MGD) and Rat Genome Database (RGD) websites (www.informatics.jax.org/mgihome/nomen/index.shtml and http://rgd.mcw.edu/nomen/nomen.shtml, respectively). The current nomenclature policies take precedence over previously published versions.

Journal policies that require authors to use current, complete genetic nomenclature for all experimental cohorts and control groups will help to minimize ambiguity and promote evaluation, interpretation, and replication.

3.4 Microbial/Pathogen Status

A great advance in laboratory animal science has been the control of common infections that plagued commercial rodent colonies in the past. Challenges persist, however, with emerging pathogens and continued infections by some of the “classical” adventitious agents, many of which induce subclinical infections (Barthold 1998; Bohr et al. 2006). The microbial/pathogen status of a research animal or animal model can influence many types of biological effects and study responses (Baker 1998; Franklin 2006; NRC 1991) and thus affect the ability to replicate findings.

One challenge for investigators in describing the microbial status of their animals is definition of the term *specific pathogen-free (SPF)* (Norin and Midtvedt 2010). There is no universal agreement about which agents are considered pathogens or which should be excluded for particular types of research or species. Use of the term SPF and the determination of pathogen exclusion status are particularly problematic with genetically modified laboratory rodents. These animals are susceptible to known or unanticipated immune function dysregulation, which can result in vulnerability to opportunistic pathogens (Franklin 2006).

Professional judgment is necessary in this section of the animal description, but ambiguity can be reduced by accompanying the term SPF with a list of the pathogens excluded, reference to the pathogen exclusion list from the commercial supplier, or reference to a guidance document such as that produced by the Federation of European Laboratory Animal Science Associations (FELASA; Nicklas et al. 2002).

In addition, a description of the equipment and procedures used to maintain microbial biosecurity during the experiment can be helpful in reducing variability based on pathogen status.

3.5 Preparation and Assignment of the Research Animal to Study

Adequate descriptions include the methods used to prepare animals for studies, including the periods and procedures for quarantine, acclimation, training, or surgery. The description of habituation methods (e.g., sham dosing, acclimation to restraint equipment) is important as animals' habituation to experimental procedures and equipment can significantly affect study outcomes (Damon et al. 1986).

Data to include in the descriptions of xenobiotic administrations used during study preparation or quarantine periods are the product name, manufacturer, dose, delivery route, method, and timing of administration. Certain commonly used prophylaxis procedures during quarantine (e.g., parasiticide treatment of rodents for pinworm or mite infestation, or of fish for external parasites) can affect both the animals and certain study endpoints (Altholtz et al. 2006; Gao et al. 2008; Johnston et al. 2006; Vento et al. 2008).

Has the author explained how animals were assigned to the study and the methods and criteria used to minimize bias (e.g., through randomization, blinding, exclusion, inclusion, and/or removal) (Bebarta et al. 2003; Martin et al. 1986)? In some instances, especially with selected strains of mice, littermates are housed together and used to form experimental groups in an effort to minimize male aggression and fighting; it is appropriate to state the use of this approach.

Information about control animals is relevant. How did the control groups relate to the experiment? Were the controls concurrent, historical, littermates? Were they matched for animal, husbandry, manipulation, or study parameters?

4 The Research Animal Environment (Study Conditions)

The study conditions of the research animal environment can be difficult to succinctly describe but are critical to interpretation and evaluation (Reliene and Schiestl 2006). Numerous aspects of the animal facility environment can affect study outcomes, not all of which can be detailed in the materials and methods section (Clough 1982). Again, it is preferable to provide more rather than less specific information to enable other investigators to effectively assess and reproduce the research.

At a minimum, the description in the materials and methods section specifies the type of diet, housing, bedding, water, and general environmental parameters (e.g., temperature, humidity, lighting) with ranges.

Effective descriptions also include aspects of the animal facility environment that are known to affect the study type or endpoints. For example, in experiments with endocrine disrupters, leaching of estrogenic substances from plastic caging or water bottles, or phytoestrogen exposure in the diet, can affect study results, so it is appropriate to describe these factors in more detail than in other types of studies (Ashby et al. 2004; Everitt and Foster 2004; Hunt et al. 2003). Dosed-feed toxicity studies in rodents may be subject to experimental confounders from cross contamination by housing control cohorts and experimental groups in the same room, so a detailed description of caging, air flow, or handling procedures may be warranted. Similarly, fish used in toxicological studies may excrete metabolites into the water column that may affect cohorts in the same tank or fish in different tanks on the same filtration system.

4.1 Diet

Diet is a potential source of variation in many types of studies, so a detailed description of food and feeding methods is important to include for every study (Haseman et al. 2003; Newberne and McConnell 1980; Newberne and Sotnikov 1996; Nold et al. 2001; Rao and Crockett 2003). Diets vary in type, form, nutrients, caloric content, levels of contaminants, and methods of preparation, and each of these characteristics can affect the animals and the study results (Barnard et al. 2009; Ford and Ward 1983).

In addition to the frequency and method of feeding (e.g., ad libitum vs. portioned), effective reports include the type of diet, source, manufacturer, catalogue or batch number, dietary form, and any dietary supplements. Specialty diets, in particular, require detailed descriptions that may include handling and storage methods. Designations such as “standard laboratory chow,” “breeder chow,” “commercial dog food,” and “fish pellets” are never appropriate.

When experimental substances are added, a description of the methods of feeding (e.g., pair feeding) and dose determination is relevant; the presentation of food consumption data may be warranted in these cases. Information about food handling and preparation procedures, such as autoclaving or irradiation, is also useful as these may adversely affect the food (e.g., its nutritional quality, palatability, or shelf life; Anderson et al. 1981; Ford 1977; Twaddle et al. 2004; Zimmerman and Wostmann 1963).

For nutrition or metabolic experiments, an adequate description notes not only the specific feedstuffs (with nutrient and caloric content if customized diets are used and the reader cannot otherwise access such information) but also, when relevant, the extent and method of any dietary restriction because caloric intake affects many experimental parameters (Deerberg et al. 1990; Laroque et al. 1997; Masternak et al. 2005). Information about food contaminant levels, diet certification, or nutrient analysis is usually appropriate for nutrition or toxicology studies (Barnard et al. 2009; Newberne and Fox 1980; Newberne and Sotnikov 1996; Silverman and Adams 1983). For articles about endocrine-related research, readers will need detailed information about food handling procedures and the animals’ diet, especially in light of numerous reported differences between studies and between laboratories that study endocrine disruptor compounds (Brown and Setchell 2001; Heindel and vom Saal 2008; Muhlhauser et al. 2009; Naciff et al. 2004; Thigpen et al. 2003, 2004; Wang et al. 2005).

4.2 Water

Specific information about drinking water source, delivery methods, and treatments (e.g., acidification, chlorination, sterilization) is important to provide; some treatments, in particular, are known to affect certain experimental parameters (Bjornsson et al. 2003; Hall et al. 1980; Hermann et al. 1982; Merne et al. 2001). In certain types of studies water delivery methods have been known to be an important component of husbandry as well (Gordon and Wyatt 2011). (Water environment for fish and other aquatic species is discussed separately in the section on Aquatic Systems.)

4.3 Housing

Adequate descriptions of housing convey the physical, microbial, and social features of the animals’ proximate environment, including the following information:

- the nature of the housing (controlled environment vs. outdoor), including temperature, humidity, lighting, with ranges;
- type of caging (e.g., static vs. ventilated, filtered vs. unfiltered, style, composition, dimensions);

- bedding and nesting materials (composition, amount, analysis);
- cage complexity (enrichment);
- housing paradigm (group/multiple vs. single);
- method of cage handling (frequency and methods, aseptic transfer, methods of sterilization); and
- nondomiciliary specialized housing such as metabolism caging, isolators, or inhalation exposure housing.

Taken together, these details will convey the animals' microenvironment (including local microbial burden and air quality), which is influenced by numerous housing variables (Keller et al. 1989; Lipman 1999; Stark 2001). For example, the air quality in a rodent cage is affected by the type of cage (solid, filter-capped, ventilated), whether it contains direct contact bedding, the animals' diet, and the number of animals (Keller et al. 1989; Krohn and Hansen 2002; Lipman et al. 1992; Macy et al. 2002; Memarzadeh et al. 2004; Rosenbaum et al. 2009).

Caging type, size, and composition can affect behaviors and physiologic responses (Abramov et al. 2008; Freed et al. 2008; Gordon and Fogelson 1994; Kallnik et al. 2007; Mineur and Crusio 2009; Stark 2001; Steplewski et al. 1987; Tsai et al. 2003).

Similarly, bedding type, manufacturer, source, treatment and storage before use, and quantity can be important because bedding is known to influence study outcomes through effects on the animals and/or their microenvironment, including through the presence of contaminants (Becker et al. 2010; Bohonowych et al. 2008; Buddaraju and Van Dyke 2003; Gordon 2004; Perkins and Lipman 1995; Potgieter and Wilke 1997; Potgieter et al. 1996; Rosenbaum et al. 2009; Sanford et al. 2002; Silverman and Adams 1983; Smith et al. 2004).

Growing international interest in the welfare of research animals has led to support for the provision of environmental complexity and enrichment and, when possible, the housing of research animals in socially compatible groups. These elements of the housing environment have many effects both known and unknown (Bayne 2005; Gortz et al. 2008; Haemisch and Gartner 1994; Jankowsky et al. 2003; Lawson et al. 2000; Tsai et al. 2002; Whitaker et al. 2009). To account for possible effects that might introduce variability in the results, it is important to provide detailed descriptions (including source information) about all cage additions, including nesting and other materials used for enrichment, in the materials section.

A description of the grouping of animals and details of their housing are relevant as a number of studies have reported dramatic differences in scientific outcomes based on single versus group housing (Andrews et al. 2000; Haseman et al. 1994, 2003; Nevalainen et al. 2007; Nyska et al. 1998).

The handling of cages—for example, the frequency and method of cage changing—can affect study outcomes (Burn et al. 2006; Vesell et al. 1976). If microbial status is important in an experiment, description of cage sterilization methods and aseptic cage changing methods may be warranted.

Because cage placement, both in rooms and on racks, has been associated with effects in long-term studies (e.g., for toxicity/oncogenicity or inhalation research), a description of methods to rotate cages on racks to minimize any environmental bias is useful (Herzberg and Lagakos 1992).

4.4 Macroenvironment

The macroenvironment of the animal room—temperature, humidity, lighting, ventilation—influences the microenvironment and therefore is relevant information (Dauchy et al. 2011; Rosenbaum et al. 2010). For most studies the materials section includes specifics such as temperature range, relative humidity range, and aspects of lighting such as the timing of light:dark cycles and dimming to mimic circadian cycles. Ambient temperature affects many research endpoints (Jhaveri et al. 2007; Swoap et al.

2004; Zhao et al. 2010), and relative humidity can directly affect animals and interact with other environmental parameters such as temperature to influence study outcomes (Ashida and Denda 2003; Diercks et al. 2010; Drickamer 1990; McJilton et al. 1976). Other aspects of the physical environment, such as sound, ventilation, and vibration, can affect the outcome of certain types of studies (NRC 2010, 45-47).

Discussion of the degree to which the animal environment was controlled and to what extent there was variance from the reported values will assist readers in interpreting and reproducing the results.

5 Basic Animal Methodology

5.1 Description of the Research Animal during the Study

A description (in the results and/or discussion) of any significant effects of the study on the animal subjects, including clinical effects or the removal or loss of animals, will be of interest to readers. Criteria for removal from the study are relevant, accompanied by a description of any clinical assessment or scoring systems (Ray et al. 2010; Toth 2000). This portion of the report is also the place for authors to note animals that died or were euthanized during the study and to discuss the cause of death and its implications for the study. (Euthanasia is further discussed in the section on Basic Animal Methodology.)

5.2 Administration of Anesthetics, Analgesics, and Other Substances

It is important to identify all substances administered to research animals, including those not part of the experiment (e.g., treatments for clinical conditions that arise during the study), with generic description, trade name, catalogue or batch number if relevant, and vendor name and address, together with a description of the preparation and handling of the substances, including any modifications to concentration. Similar details are appropriate for vehicles and excipients. Adequate reporting also includes information about the relationship of dose administration to feeding or fasting (Adams et al. 2009) and about methods to minimize bias such as timing (and order) of dose administration, food or water removal, or blinding.

All preanesthetic agents, anesthetics, and analgesic drugs have the potential to induce numerous and varied effects on studies and consideration and discussion of these effects is warranted (Adams et al. 2008; Avsaroglu et al. 2007; Flecknell 1993; Hampshire et al. 2001; Heavner 2003; Katz et al. 2002; Murphy et al. 2001; Nakai et al. 2005; Suliburk et al. 2005). If drugs are dosed to effect, the discussion will include strategies for dose determination as well as monitoring methods.

It is appropriate to describe the frequency, route, buffering (e.g., for fish anesthetic), and method of substance administration. The choice of enteral administration method, for example, can affect animals and study results (Atcha et al. 2010; Brown et al. 2000; Craig and Elliott 1999; Nickerson et al. 1994), so specific information about the method used (e.g., oral via syringe, dosed water, or dosed feed; gavage tube into the stomach; or intragastric injection) is appropriate in reports of studies involving enteral administration. The same is true for the administration of substances via vascular and other parenteral routes.

Clear descriptions of the treatment of control animals will indicate whether they were treated identically to dosed animals (e.g., subject to sham handling and vehicle treatment, identical diet in a dosed-feed study, inhalation exposure apparatus).

5.3 Use of Infectious Agents

Aside from obvious considerations regarding biohazard containment, studies involving infectious agents require specific experimental detail to allow reproducibility of results. The outcome of infection of experimental animals with microbial agents is highly dependent on dose, pathogen strain (virulence), route of inoculation, particle size (in the case of inhalants as it determines delivery level in the respiratory tree), vehicle and volume of inoculum, and the animal's age, genetic background, and environment. Furthermore, the site of inoculation can profoundly influence the outcome of infection; for example, lesion distribution and severity, organ distribution, and host immune response of rodents to *Borrelia burgdorferi* inoculation is highly dependent on the specific site of inoculation (deSouza et al. 1993).

5.4 Tissue and Fluid Sample Acquisition

Adequate descriptions of tissue and fluid sample acquisition procedures provide specific information about the frequency, technique, equipment, site, and quantity of sampling when tissues or body fluids are obtained from research animals (Kurien et al. 2004). The site of blood removal can affect some types of research and endpoints (Fernandez et al. 2010; Mahl et al. 2000; Neptun et al. 1985; Rogers et al. 1999; Smith et al. 1986). Furthermore, because circadian cycle is frequently important to research endpoints, the timing of sample collection may be pertinent (Bertani et al. 2010; Bertolucci et al. 2005; Gachon and Firsov 2011; Pinotti et al. 2005), to ensure correlation with either light cycles or feeding patterns (Laakso et al. 1990).

5.5 Euthanasia

It is always appropriate to include a detailed description of the method of euthanasia, which can have numerous and varied effects on study endpoints depending on the methods and agents used (Al-Mousawi et al. 2010; Artwohl et al. 2006; Berger-Sweeney et al. 1994; Butler et al. 1990; Hauser et al. 2001; MacLusky 2009; Reed et al. 2009; Traslavina et al. 2010). It can also be important to describe the relationship of terminal procedures (e.g., anesthetic administration or tissue perfusion) to the final euthanasia procedures. If animals are fasted before euthanasia or terminal acquisition of samples, this and any other ancillary procedures will be described with details such as timing, rationale, and duration. The report will also describe any methods to reduce bias (e.g., randomization of animals or groups) in the implementation of euthanasia.

6 Aquatic Systems

6.1 Water Quality

In addition to some of the micro- and macroenvironmental parameters discussed above, animals that live in an aquatic environment have requirements particular to their liquid medium. Fish have species-specific and sometime even life stage–specific optimal ranges for each water quality parameter; when parameters fall outside the acceptable range, fish become stressed and more susceptible to disease.

Standard (i.e., control) and experimental water quality parameters (e.g., temperature, ammonia, nitrite, nitrate, pH, dissolved oxygen, carbon dioxide, hardness, alkalinity, supersaturation, salinity, chlorine, chloramine, suspended solids, and heavy metals such as copper, zinc, and cadmium) are to be documented as thoroughly as possible so that the study can be properly assessed or replicated. Most of these parameters can directly or indirectly affect the behavior, physiology, metabolism, reproduction, and immunology of fish (Haywood 1983; Kroupova et al. 2008; Lewis and Morris 1986; Randall and Tsui 2002; Tomasso 1994).

Many water quality parameters are affected by others. For instance, the temperature of the water directly affects the amount of dissolved oxygen in the water—as the water temperature increases, oxygen levels decline. The pH of the water affects the amount of the relatively more toxic un-ionized ammonia in the water versus the amount of ionized ammonia. It is therefore important to document as many water quality parameters as possible to reduce variability in experimental outcomes.

Without adequate filtration, nitrogenous wastes and other excretory products accumulate in an aquatic system (Burrows 1964). The exchange rate and water velocity may also affect the behavior and growth of fish in both flow-through and closed recirculating systems (d'Orbcastel et al. 2009). Thus it is usually relevant to describe the type of mechanical and biological filtration used, including any supplementary equipment (e.g., mechanisms that use UV, ozone, or oxygen).

6.2 Diet

As with terrestrial animals, the source, type, form, quantity, and nutrient and caloric content of the diet can affect aquatic animals and study results. If the food is presented in pellet form, the pellet size is relevant information to provide as certain fish ingest only certain size ranges of food. The number of feedings per day can influence the growth of many fish species (Lambert and Dutil 2001) and uningested food can compromise water quality.

Reports of unintended exposure of aquatic animals used in research to contaminants (e.g., endocrine disruptors, dioxin, melamine) in commercial diets have highlighted the importance of documenting the source and components of diets (Andersen et al. 2008; Fiedler et al. 1998; Rappe et al. 1998; Yan et al. 2009). These compounds can cause changes to genetics and metabolism, with resulting pathologies in the reproductive, immune, and neurological systems (Andersen et al. 2003; Fenske et al. 2005; Länge et al. 2001; Örn et al. 2003), thus potentially confounding research results.

6.3 Housing

An adequate description of housing for aquatic animals used in research will include the type of system (e.g., raceway, tank, aquarium, cage), including the material of which the system is constructed (e.g., concrete, fiberglass, polyethylene, glass) (Arndt et al. 2001) and lighting (e.g., intensity, hours, and circadian cycle) (Bayarri et al. 2002; Downing and Litvak 2001; Head and Malison 2000; Hossain et al. 1998; Karakatsouli et al. 2008). The color of the inside of a tank can also be relevant as it may compromise research results by affecting the behavior, physiology, and stress level of fish (Barcellos et al. 2009; Papoutsoglou et al. 2000; Rotllant et al. 2003; Strand et al. 2007).

Although the presence of structures in a tank or aquarium reduces the ability to observe and monitor the animals, most aquatic animals prefer refugia to avoid tank mates or perceived predators. Aquatic animals maintained in glass or plastic tanks can also become stressed by cohorts in adjacent tanks or by activities in the room. For these reasons bare tanks are neither scientifically nor behaviorally, socially, or environmentally advisable.

As international welfare principles increasingly include fish and other aquatic animals, it is appropriate for study reports to fully characterize approaches to environmental enrichment, such as adjustments in tank size, the provision of substrate or structures, water movement, artificial vs. natural light, conspecifics and sex ratio, artificial or real plants, and varied diet.

6.4 Animal Numbers

Information about stocking density and male:female ratio is a basic requirement in all study publications. Although many species of fish prefer to exist in schools, others are more solitary. As with mammals, maintaining fish species and other aquatic animals according to their behavioral preference will minimize stress in individuals.

Stocking density and sex ratio are known to have a profound influence on feed intake, growth, performance, behavior, and survival of aquatic animals (Correa and Cerqueira 2007; Di Marco et al. 2008; Hecht and Uys 1997; van de Nieuwegiessen et al. 2009). In general, greater stocking density leads to decreased performance and increased aggression in most aquatic animals. However, in the larvae and fingerlings of some fish species increased stocking density has been associated with greater feed intake, more swimming activity, and less aggression (van de Nieuwegiessen et al. 2009).

Subtle effects of confinement, such as changes in social behavior, breeding dynamics, and genetic integrity, are becoming increasingly recognized in aquatic animals (Saxby et al. 2010). Dominance hierarchies have also been documented in aquatic animals maintained in captivity (Paull et al. 2010; Pullium et al. 1999).

7 Summary and Conclusions

The interpretation, evaluation, and reproducibility of research are a cornerstone of scientific progress that depends on the publication of adequate and specific information about all relevant aspects of the reported study. Considerable variation in the amount of information required by scientific publications and reported by authors undermines this basic scientific principle and results in the unnecessary use of animals and other resources in failed efforts to reproduce study results.

The editors of scientific publications have a role to play in promoting high-quality research reporting by adopting tailored guidelines for their authors and reviewers to ensure adequate descriptions that enable assessment and replication of the reported study. That said, the committee members recognize that editors, reviewers, and authors have numerous claims on their time and attention; that a one-size-fits-all approach for articles and journals is unrealistic and unreasonable; and that space may be limited in print journals. To address these considerations, journal editors may consider the following options:

- Journals provide links on their websites (e.g., in their instructions to authors and reviewers) to this report and/or other resources and checklists.
- Procedural details and data are published, after review, in an online article appendix or journal-specific repository for such information.
- Authors cite previous peer-reviewed publications that convey the appropriate methods and details and include specific descriptions only of changes relevant to the newly reported experiment. Supplemental information, if published, would include all the relevant details.

In addition, the ILAR website (http://dels.nas.edu/ilar) will indicate journals and, as applicable, sponsoring agencies that endorse/have adopted this and other guidelines for animal reporting.[6]

The purpose of this report is to serve as a resource for editors to consider in crafting policies to ensure the inclusion of adequate animal descriptions in published research articles. The report is not meant to be prescriptive but rather to complement existing checklists and other resources by providing

[6] For example, the NC3Rs website includes such information for the ARRIVE guidelines.

guidance and scientific evidence for the specific types of information to be included in research publications in order to promote the advancement of science involving animal studies.

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Appendix

Animal Research: Reporting *In Vivo* Experiments: The ARRIVE Guidelines[7]

ITEM		RECOMMENDATION
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

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		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.
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†. 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.

The guidelines are intended to:

— Improve reporting of research using animals.

— Guide authors as to the essential information to include in a manuscript, and not be absolutely prescriptive.

— Be flexible to accommodate reporting a wide range of research areas and experimental protocols.

— Promote reproducible, transparent, accurate, comprehensive, concise, logically ordered, well written manuscripts.

— Improve the communication of the research findings to the broader scientific community.

The guidelines are NOT intended to:

— Promote uniformity, stifle creativity, or encourage authors to adhere rigidly to all items in the checklist. Some of the items may not apply to all studies, and some items can be presented as tables/figure legends or flow diagrams (e.g. the numbers of animals treated, assessed and analysed).

— Be a guide for study design and conduct. However, some items on the checklist, such as randomisation, blinding and using comparator groups, may be useful when planning experiments as their use will reduce the risk of bias and increase the robustness of the research.

What kind of research areas do the guidelines apply to?

— The guidelines will be most appropriate for comparative studies, where two or more groups of experimental animals are being compared; often one or more of the groups may be considered as a control. They apply also to studies comparing different drug doses, or, for example, where a single animal is used as its own control (within–subject experiment).

— Most of the recommendations also apply to studies that do not have a control group.

— The guidelines are suitable for any area of bioscience research where laboratory animals are used.

Who are the guidelines aimed at?

— Novice and experienced authors

— Journal editors

— Peer reviewers

— Funding bodies

How might these guidelines be used?

The guidelines provide a checklist for those preparing or reviewing a manuscript intended for publication.

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
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ 340:c332.

Acknowledgements

The NC3Rs gratefully acknowledges the expertise and advice that all the contributors have given to developing the guidelines. We would particularly like to acknowledge the contribution of the NC3Rs Reporting Guidelines Working Group† – Professor Doug Altman, Centre for Statistics in Medicine, University of Oxford UK, Professor David Balding, Department of Epidemiology & Public Health, Imperial College, London UK, Professor William Browne, Department of Clinical Veterinary Science, University of Bristol UK, Professor Innes Cuthill, School of Biological Sciences, University of Bristol UK, Dr Colin Dunn, Editor Laboratory Animals (Royal Society of Medicine press), Dr Michael Emerson, National Heart and

Lung Institute, Imperial College, London UK, Dr Stella Hurtley, Senior Editor Science, Professor Ian McGrath, Editor-in-Chief British Journal of Pharmacology (Wiley Blackwell Publishers) and Dr Clare Stanford, Department of Psychopharmacology, University College, London UK. We would also like to thank NC3Rs grant holders, the Medical Research Council, Biotechnology and Biological Sciences Research Council (BBSRC), Wellcome Trust, Parkinson's Disease Society, British Heart Foundation and their grant holders and funding committee members who provided feedback on the guidelines; and Kathryn Chapman and Vicky Robinson (both NC3Rs) for their help with the manuscript.

†Please note that the working group members who contributed to these guidelines were advising in their personal capacity and their input does not necessarily represent the policy of the organisations with which they are associated.

Funding

The reporting guidelines project was funded by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).