

ARVO Statement for the Use of Animals in Ophthalmic and Vision Research

1. Introduction

Research in vision and ophthalmology expands our understanding of the nervous system and paves the way for the discovery of new treatments to prevent blindness and improve human and animal vision. Currently there are no *in-silico* or *in-vitro* models that adequately recapitulate the structure and function of the complex and intricately connected biological systems in the eye and the brain. Hence, studies with living animals is vital to continued progress in many areas of clinical and basic research on vision. The proper use of animals in research is, therefore, an honorable and essential contribution to the improvement of both human and animal lives.

Our concern for the humane treatment of animals obliges us to always establish that the potential benefits to human and animal health outweigh the cost in animal lives. It is therefore essential for scientific societies such as The Association for Research in Vision and Ophthalmology to formulate guidelines for the humane use of laboratory animals in research.

The remainder of this document provides guidelines for the humane treatment and ethical use of animals for vision research. These are based on guidelines that are generally considered acceptable and reasonable by the biomedical research community and are intended for the investigator who is responsible for the humane, ethical care and use of animals in vision research. The discussion deals mainly with endothermic (warm-blooded) vertebrates, but the principles can be applied generally. Ethical issues involving the use of any species should be considered in relation to the complexity of its central nervous system and its apparent awareness of its environment.

2. Guidelines for the design of experiments

ARVO strongly advocates for the responsible use of animals in biological and biomedical research and follows two fundamental principles: (1) Although animal models are vital and irreplaceable for scientific progress, combating devastating ocular diseases, and improving human and animal health, the investigator's first concern must be to avoid the use of animals when possible, and (2) The principles of the 3R's: replacement, reduction and refinement, should be used as a framework for conducting high quality science.

When designing experiments, first and foremost, the investigator and other qualified personnel should determine that the use of animals is a necessity for scientific progress, with consideration given to the use of mathematical models, computer simulation and/or *in vitro* biological systems. All experiments and procedures involving animals must be designed and performed with specific consideration of their relevance to human or animal health, the advancement of knowledge and the benefit to society.

When embarking on studies that cannot be completed without animals, the investigator must justify the

use of animals, identify the appropriate species, and use the minimum number needed to provide reliable and valid results. Thoughtful experimental design may include performing pilot studies to estimate the minimum number of animals required to obtain answers without compromising scientific quality. Knowledge from pilot studies to evaluate safety and/or efficacy of experimental procedures, interventions and/or treatments can help identify unanticipated problems in an experiment before larger numbers of animals are used. Pilot studies may provide critical insight into study outcomes or effect sizes to allow for appropriate power calculations for future studies, as well as enabling the refinement of experimental protocols to minimize subsequent animal use and/or discomfort.

With recent advances in ophthalmic imaging, researchers should strive to use appropriate non-invasive imaging methods for longitudinal animal follow-up, to reduce the number of animals used and to enhance the research reliability and validity.

Additionally, in accordance with the National Institutes of Health's commitment to improving the health outcomes of men and women, all research experiments must consider sex as a biological variable. Strong justification from the scientific literature, preliminary data, scarce research resources such as nonhuman primates, or other relevant considerations must be provided for experiments that study only one sex. Finally, experiments should be designed to avoid depletion of endangered species.

Critical to experimental design and implementation is disciplined adherence to proper use and care of animals including the avoidance or minimization of distress, discomfort and pain. This includes following strict attention to anthropomorphic judgements made by qualified, experienced and prudent human observers. Unless otherwise demonstrated, investigators should consider that procedures which may cause pain or distress to human beings may also cause pain in animals. Although most research on animals causes little or no distress or discomfort, certain important scientific questions may demand experimental studies that inevitably give rise to some discomfort or short-term distress. Decisions related to the design and implementations of these experiments should be made in consultation with appropriate review groups such as the institutional animal care and use committee (IACUC) as well as the institution's appointed veterinarian/s.

In all experiments, discomfort or distress must be minimized by careful protocol design outlining pre- and post-procedural use of analgesics, anesthesia, sedation and when necessary, euthanasia. There is no difference between distress and discomfort that result from either the design of a study or its unintended side effects. The investigator must therefore identify and eliminate all avoidable sources of discomfort or distress, taking advantage of veterinary expertise. The subsequent recovery of animals from procedures must also be monitored to ensure their welfare.

The investigator is responsible for making sure that all personnel working with animals receive adequate training before beginning animal work. The research team should be familiar with the national and local laws on animal research, ethics of animal use and the concepts of the Three Rs, methods for reporting concerns about animal use, and the relevant occupational health and safety issues related to animal use.

The investigator and the research personnel should be well-trained for all animal procedures relevant to the study. It is the investigator's responsibility that the research team receives continuing education in a

timely manner, to reinforce training and provide updates on legislation changes, new research technologies, and other relevant areas.

3. Guidelines for the conduct of experiments

The quality of the information obtained through research depends in no small measure on the health and general condition of the animals used in experimentation. Proper animal husbandry is fundamental to the success of any research effort that uses animals.

Research animals must be obtained and cared for in accordance with the recommendations of the [Guide for the Care and Use of Laboratory Animals](#), the [National Academies Institute of Laboratory Animal Resources](#), the [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#) and the [Guide to the Care and Use of Experimental Animals by the Canadian Council of Animal Care](#) (if conducting research in Canada). In the United States, the [Office of Laboratory Animal Welfare \(OLAW\)](#) provides guidance and interpretation of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals and monitors compliance with the Policy.

Investigators in the United States must comply with relevant local, state and federal laws, including the [U.S. Animal Welfare Act](#), as amended and its accompanying regulations. An [Institutional Animal Care and Use Committee](#) must review and approve the use of animals in research in the United States and Canada.

Surgery should be carried out or directly supervised by persons with appropriate levels of experience and training. Surgery performed on animals that will survive (for example, on animals intended for long-term studies) should be undertaken with careful attention to aseptic technique and prevention of infection. Major surgical procedures should be completed under anesthesia that will render the animal insensitive to pain. Muscle relaxants and paralytics have no anesthetic action and must not be used as a substitute for anesthesia. Postoperative care must include efforts to minimize pain, distress, discomfort and the risk of infection.

Some studies require surgical preparation of animals that are not intended to survive. In such cases the animals ordinarily should be maintained unconscious throughout the experiment. At the end of the experiment animals must be euthanized without recovering consciousness. In this and all other cases of euthanasia, the investigator should follow the [AVMA Guidelines for the Euthanasia of Animals](#).

Where experiments require physical restraint and/or the withholding of food or water, the effects of which are not themselves the objects of study, care must be taken to minimize discomfort or distress and to ensure that good general health is maintained. Only when there is no alternative procedure should animals be subjected to immobilization or restraint to which they cannot be adapted readily. Whenever it is possible, the experimental schedule should be designed to include reasonable periods of rest and readjustment. In the rare cases where distress and discomfort are unavoidable attributes of a well-designed study, the investigator must, within the limits of the design, take all possible steps to minimize these effects and to minimize the duration of the procedure and the number of animals used.

ARVO will amend The Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research pending changes to US and EU guidelines ([FELASA](#) and [EU legislation](#)).

4. Factors that relate specifically to the conduct of vision and ophthalmology experiments

Besides the considerations generally applicable to all animal experiments, production of visual disability is a special animal welfare consideration that may apply to vision research protocols. Visual disability of experimental animals may be either an intrinsic or an unplanned consequence of experimental design. In its definition of major survival surgery, the Guide to the Care and Use of Laboratory Animals includes any surgical intervention that “produces substantial impairment of physical or physiologic functions” in an animal that is expected to recover. Hence, any experimental procedure that results in, or has the potential to result in, a level of visual disability sufficient to disrupt an animal's normal daily activity should be considered a major survival procedure. Such procedures require appropriate justifications and suitable animal care accommodations.

Protocols involving bilateral survival ocular procedures require special consideration and justification, with particular attention to the likelihood of adverse events and rate of onset, nature and duration of any consequences to visual function. Such procedures include, but are not necessarily limited to, bilateral ocular surgeries, injections or implantations, whether performed simultaneously or sequentially, and any other experiments with the potential to affect vision bilaterally. In the interests of transparency, rates of adverse events following ocular procedures should be clearly documented and included by investigators in reports arising from their studies. Investigators should consider species differences in ocular anatomy and physiology, importance of visually-guided behaviors, likelihood of adverse events following specific ocular procedures, and response to experimental manipulations and drugs when designing experiments. The Guide to the Care and Use of Laboratory Animals strongly recommends that animals not be subjected to multiple major survival surgical procedures unless they are related components of a particular research project. Accordingly, a visually disabling procedure should not be performed bilaterally unless the two procedures are related and unavoidable components of a specific project. As noted in the Guide to the Care and Use of Laboratory Animals, cost savings alone is not an adequate justification for performing multiple survival surgical procedures. Investigators should carefully consider whether or not inclusion of the contralateral eye represents an appropriate control under their specific experimental design.

In all instances, appropriate pre-emptive analgesia strategies of recognized efficacy in the management of ocular pain in animals should be incorporated in study designs to minimize discomfort. When necessary, species-appropriate environmental adaptations should be made in order to minimize the impact of visual compromise.

Vision investigators are encouraged to distribute unrelated tissues to investigators in other research areas and, where practical, to obtain suitable ocular tissues from investigators working on other organs. This recommendation applies to all species.

Inherited disorders of the visual system are significant health problems for both humans and animals. The impact of visual compromise on normal physiological functions differs significantly among diverse species, strains and disorders. The deliberate breeding of and generation of animals with genetic disorders leading to blindness requires scientific justification, and consideration of appropriate modification of standard husbandry procedures as needed to minimize the impact of visual compromise on normal behaviors and physiological functions. Investigators who breed genetically impaired animals are encouraged to share such animals and tissues with qualified investigators having complementary expertise, including those outside their own institution, and to develop a plan for resource sharing.

5. Investigators outside the United States

Although the laws that regulate the care and use of animals in the United States are not directly applicable to citizens of foreign countries, ARVO endorses the policies in the [Guide for the Care and Use of Laboratory Animals](#), the [Public Health Service Policy on the Humane Care and Use of Laboratory Animals](#) (revised 2015), and the [U.S. Animal Welfare Act](#), as amended. If ARVO is to support a vision scientist under scrutiny by animal activists, the vision science experiment involving animals must conform to the guidelines established in these documents, even though they are not necessarily enforceable by law in the country in which the experiment is performed.

6. Additional resources

In addition to these guidelines, references in the following resources are recommended. More details can also be found in the [ARVO Toolkit for Conducting Secure Biomedical Research in Animals](#).

6.1. Animal use guidelines

- [The Guide for the Care and Use of Laboratory Animals](#) (NRC 2011)
- [Public Health Service \(PHS\) Policy on Humane Care and Use of Laboratory Animals](#) (from the US Department of Health and Human Services)
- [OLAW PHS Humane Care and Use of Laboratory Animals Tutorial](#)
- [U.S. Animal Welfare Act](#) (from the US Department of Agriculture)
- [Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching](#) (from the American Dairy Science Association, the American Society of Animal Science, and the Poultry Science Association)
- [NIH Grants Policy Statement \(2021\)](#)
- [NIH Medical Research with Animals website](#)
- [Guide to the Care and Use of Experimental Animals](#) (from the Canadian Council on Animal Care)
- [Institutional Animal Care and Use Committee \(IACUC\) Guidebook](#)
- [IACUC Bookmarks](#)
- [Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research, National Academies Press](#)
- [Medical Research Council guide for the use of animals. Responsibility in the use of animals in bioscience research.](#)

6.2. Alternatives to animal research

- Bibliography on alternatives to animal testing: [ALTBIB](#)
- Global clearing house for information on alternatives to animal testing: [AltWeb](#)
- Center for Alternatives to Animal Testing (Johns Hopkins University): [CAAT](#)
- Center for Animal Alternatives (UC Davis) [CAA](#)
- Animal use alternatives terminology (National Agricultural Library): [USDA Thesaurus](#)
- Interagency Coordinating Committee on the Validation of Alternative Methods [ICCVAM](#)
- Alternative Methods Accepted by U.S. Agencies [Alternative Methods](#)

6.3. Animal use resources

The purpose of these resources is to provide information about model species (traditional and non-traditional) that are used for biomedical research:

- [NIH Model Organisms for Biomedical Research](#)
- [NIH Using Model Organisms to Study Health and Disease](#)
- Information on non-traditional model species
- [Model Organisms: Beyond the Inner Circle](#)

6.4. Organizations that promote proper and ethical use of laboratory animals

- [Office of Laboratory Animal Welfare \(OLAW\)](#)
- [American Association for Laboratory Animal Science \(AALAS\)](#)
- [American College of Laboratory Animal Medicine \(ACLAM\)](#)
- [Association for Assessment and Accreditation of Laboratory Animal Care \(AAALAC International\)](#) This site contains a listing of international regulations and resources by country.
- [Asian Federation of Laboratory Animal Science Associations \(AFLAS\)](#)
- [Canadian Association for Laboratory Animal Science \(CALAS/ASCAL\)](#)
- [Federation of European Laboratory Animal Associations \(FELASA\)](#)
- [International Council for Laboratory Animal Science \(ICLAS\)](#)
- [National Centre for the Replacement, Refinement and Reduction of Animals in Research \(NC3Rs\)](#)
- [National Association for Biomedical Research \(NABR\)](#)
- [Foundation for Biomedical Research \(FBR\)](#)