

Worksheet for Applications Involving Animals

This worksheet describes the information that must be included in applications submitted to NIH for activities involving the care and use of animals. It provides an overview of the requirements, a checklist for applicants and reviewers, detailed instructions, responsibilities of applicants, reviewers and NIH staff, and an example of an acceptable application.

Applicability

If the proposed work involves live vertebrate animals, federal policy requires applicants to address the criteria noted below. This includes work involving animals obtained or euthanized for tissue harvest and generation of custom antibodies.

If the applicant institution is not the site where animal work will be performed or if the work will be performed at several sites, these performance sites (institutions) must be identified. The proposed work involving live vertebrate animals should be described for every performance site.

Requirements

Applications involving live vertebrate animals must address the following criteria:

- 1. Description of Procedures (Vertebrate Animals Section).** Provide a concise description of the proposed procedures to be used that involve vertebrate animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex, and total number of animals by species to be used in the proposed work. If dogs or cats are proposed, provide the source of the animals.
- 2. Justifications (Vertebrate Animals Section).** Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g., computational, human, invertebrate, *in vitro*).
- 3. Minimization of Pain and Distress (Vertebrate Animals Section).** Describe the interventions including analgesia, anesthesia, sedation, palliative care, and humane endpoints to minimize discomfort, distress, pain, and injury.
- 4. Method of Euthanasia (Cover Page Supplement).** Provide a justification for methods of euthanasia that are not consistent with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.
 - If answer is “No” to the question “Is method consistent with AVMA guidelines?”, describe the method and provide scientific justification in the text field provided.
 - Note, for applications with due dates of January 25, 2016 to May 24, 2016, the method of euthanasia is addressed as the fourth criterion in the Research Plan, Vertebrate Animals Section: State whether the method of euthanasia is consistent with the recommendations of the AVMA Guidelines for the Euthanasia of Animals. If not, describe the method and provide a scientific justification.

Note, for applications with due dates on or after May 25, 2016: 1) a description of veterinary care is no longer required; and 2) the rationale for the number of animals and consideration of sex as a biological variable should be addressed as part of experimental design in the Research Strategy.

Checklist for Applicants and Reviewers: Vertebrate Animals

Performance Site

- | | |
|--------------------------|---|
| <input type="checkbox"/> | If the applicant's institution is not where animal work will be performed, are all collaborative performance sites identified? |
| <input type="checkbox"/> | If more than one performance site is planned, are descriptions of animal use addressing the required criteria provided for each site? |

1. Description of Procedures

Are the following addressed for all species?

- | | |
|--------------------------|--|
| <input type="checkbox"/> | Species |
| <input type="checkbox"/> | Strains |
| <input type="checkbox"/> | Ages |
| <input type="checkbox"/> | Sex |
| <input type="checkbox"/> | Total number of animals by species |
| <input type="checkbox"/> | Concise, complete description of proposed procedures (i.e., sufficient information for evaluation) |
| <input type="checkbox"/> | Source, only if dogs or cats are proposed |

2. Justifications

Are justifications provided?

- | | |
|--------------------------|---|
| <input type="checkbox"/> | Choice of species is appropriate for proposed research |
| <input type="checkbox"/> | Why research goals cannot be accomplished using an alternative model (e.g., computational, human, invertebrate, <i>in vitro</i>) |

3. Minimization of Pain and Distress

Are interventions to minimize discomfort, distress, pain, and injury described? (Examples below)

- | | |
|--------------------------|---|
| <input type="checkbox"/> | Circumstances relevant to the proposed work, when animals may experience discomfort, distress, pain, or injury |
| <input type="checkbox"/> | Procedures to alleviate discomfort, distress, pain, or injury |
| <input type="checkbox"/> | Identify (by name or class) any tranquilizers, analgesics, anesthetics, and other treatments (e.g., antibiotics) and describe their use |
| <input type="checkbox"/> | Provisions for palliative care or housing that may be necessary after experimental procedures |
| <input type="checkbox"/> | Plans for post-surgical care, if survival surgeries are proposed |
| <input type="checkbox"/> | Indicators for humane experimental endpoints, if relevant |

4. Method of Euthanasia

- | | |
|--------------------------|--|
| <input type="checkbox"/> | If answer is "No" to the question "Is method consistent with AVMA guidelines?", is the method described and a scientific justification provided? |
|--------------------------|--|

Instructions

Vertebrate Animals Section (VAS)

Typically, all of the required elements for the VAS can be addressed within 1-2 pages. The VAS must not be used to circumvent page limits. Applicants should be aware that NIH may release information contained in funded applications pursuant to a Freedom of Information Act request. Note, for applications with due dates on or after May 25, 2016: 1) a description of veterinary care is no longer required; and 2) the rationale for the number of animals and consideration of sex as a biological variable should be addressed as part of experimental design in the Research Strategy.

1. Description of Procedures

Investigators must include a concise, complete description of the proposed procedures. While additional details may be included in the Research Strategy, a coherent, albeit brief, description of the proposed use of the animals must be provided in the VAS. The description must include sufficient detail to allow evaluation of the procedures.

Examples of the types of procedures that may be described include:

- Blood collection
- Surgical procedures
- Administration of substances
- Tumor induction
- Post-irradiation procedures

In describing the animals, investigators must provide the following information:

- Species
- Strains
- Ages
- Sex
- Total number of animals to be used by species
- Source of the animals, if dogs or cats are proposed

2. Justifications

Investigators must justify the use of animals in the proposed research. [U.S. Government Principles](#) require grantees to consider mathematical models, computer simulation, and *in vitro* biological systems. The justification should:

- Indicate why the research goals cannot be accomplished using an alternative model (e.g., computational, human, invertebrate, *in vitro*).
- Provide rationale for the choice of species (e.g., advantages of the species chosen and why alternative species are not appropriate).
- Discuss why less highly evolved or simpler animal models are not appropriate. For example, the use of non-human primates, dogs, or cats should be thoroughly justified.

3. Minimization of Pain and Distress

Investigators should identify procedures or circumstances that may result in more than momentary discomfort, distress, pain, or injury and describe:

- Interventions to alleviate discomfort, distress, or pain.
- Name or class of pharmacological agents, if used.
- Any additional (e.g., non-pharmaceutical) means to avoid discomfort, distress, pain, or injury, including palliative care.

- Manner, circumstances, and duration of all post-surgical provisions and care.
- Special housing following surgery or manipulations, if necessary.
- Procedures (e.g., pharmacological, surgical) that might lead to severe discomfort, distress, pain, or injury.
- Indicators for humane endpoints and euthanasia (e.g., severe infection, respiratory distress, failure to eat, tumor size).

All of these issues are particularly important for survival surgeries. If multiple surgeries are proposed, these should be well justified and provisions to avoid any potential complications may be described.

4. Method of Euthanasia (Cover Page Supplement)

If investigators are proposing to use a method of euthanasia that is not consistent with AVMA guidelines, they must describe the method and provide a scientific justification in the text field provided.

Note, for applications with due dates of January 25, 2016 to May 24, 2016, the method of euthanasia is addressed as the fourth criterion in Research Plan, Vertebrate Animals Section: State whether the method of euthanasia is consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. If not, describe the method and provide a scientific justification.

Applicant Responsibilities

Each of the criteria must be addressed in NIH applications. Failure to address the criteria will result in the application being designated as incomplete and it will not be reviewed. The Vertebrate Animals section must not be used to circumvent page limits.

Reviewer Responsibilities

Members of scientific review groups (SRGs) must evaluate the criteria to determine if plans for the use of vertebrate animals are appropriate relative to the scientific work proposed. All of the items must be evaluated by reviewers as appropriate for an application to be rated as ACCEPTABLE. An application will be rated UNACCEPTABLE if all required items are not addressed adequately or found inappropriate. Reviewers will assess the criteria for applications proposing the use of chimpanzees as they would any other application.

NIH Staff Responsibilities

- **Review staff** a) performs an administrative review checking that all criteria are addressed; b) provides reviewers with instructions (e.g., worksheet), noting that all criteria must be evaluated as appropriate for the application to be ACCEPTABLE; c) subsequent to SRG review, codes the application and includes reviewers' comments in the summary statement.
- **Program staff** a) obtains additional information or clarification to resolve concerns for any application found to be UNACCEPTABLE if it is to be recommended for funding; b) works with the applicant to provide information to the Office of Laboratory Animal Welfare (OLAW) allowing resolution of the animal welfare concerns.
- **Grants Management staff** a) verifies that the institutional Animal Welfare Assurance number is provided; b) obtains verification of IACUC approval.

Resources

- [Frequently Asked Questions](#)
- [Grant Application VAS Worksheet](#) (PDF) – a tool for grant application review
- [Contract Proposal VAS Worksheet](#) (PDF) – a tool for contract proposal review
- [VAS Factsheet](#) (PDF)
- [What Investigators Need to Know About the Use of Animals](#) (PDF)
- [NOT-OD-16-006](#): Simplification of the Vertebrate Animals Section of NIH Grant Applications and Contract Proposals

References

The guidance in this worksheet is based on Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy) and federal requirements. The PHS Policy incorporates the standards in the *Guide for the Care and Use of Laboratory Animals* and the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training*, and requires that euthanasia be conducted according to the *AVMA Guidelines for the Euthanasia of Animals*. Additional background information and references are available on the OLAW website (<http://olaw.nih.gov>).

- PHS Policy
<http://grants.nih.gov/grants/olaw/references/phspol.htm>
- U.S. Government Principles
<http://grants.nih.gov/grants/olaw/references/phspol.htm#USGovPrinciples>
- Guide for the Care and Use of Laboratory Animals
http://www.nap.edu/catalog.php?record_id=12910
- AVMA Guidelines for the Euthanasia of Animals
<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>

Example

(This application has been modified from the original. It addresses all required criteria for applications with due dates on or after May 25, 2016.)

Vertebrate Animals Section

Aims 1-3 will be addressed *in vitro*; Aim 4 will be addressed using a mouse model of ocular infection.

1. Description of Procedures: Male and female Balb/c mice will be used to determine if virions treated with enzyme can cause viral keratitis, and to test the *in vivo* efficacy of the test articles. The studies will require 700 mice, 4 to 6 weeks old. Ocular infection is accomplished by scratching the cornea of anesthetized mice with a sterile needle and exposing the scarred portion of the cornea to inoculum. Test articles are applied directly to the scarified cornea as liquid or cream. Following inoculation and recovery, mice are monitored for 30 days. With the mice under anesthesia, the eyes will be examined at intervals, microscopically, and are flushed with medium with 2% serum to determine viral titers. Thirty days post-infection, with the mice under deep anesthesia, the trigeminal ganglia are removed aseptically for viral assay, followed immediately by euthanasia.

2. Justifications: The proposal is to study mechanisms for the prevention of ocular disease caused by viral infections, a leading cause of blindness in the US. Mice are needed for these experiments because no alternative *in vitro* model incorporates all elements of the mammalian ocular immune system; too little is known about this system for the development of computer simulations or for clinical studies to be considered. Mice are a well-accepted model for studying viral keratitis, assessing the virulence of viral strains, and testing the efficacy of antivirals. Mice provide several advantages over other models for these studies: a) The murine ocular immune system is similar enough to that of humans to allow extrapolation of the results; b) Their small size allows the use of smaller amounts of drugs for testing; and c) The entire mouse genome is known and easily manipulated genetically, allowing extension of the work in future genetic studies. Balb/c mice will be used because they have intermediate resistance to infection.

3. Minimization of Pain and Distress: Mice will be anesthetized with isoflurane (3-5%) during the infection process, when treatments are administered, and titer samples are collected. This eliminates the need for restraint devices and topical anesthetics that would interfere with the infection and disease process. For post-procedural pain relief, we will administer buprenorphine twice daily for the duration of the experiments (i.e., approximately two weeks post-inoculation). Death is not an endpoint for the studies; the Balb/c strain was chosen because of its resiliency and resistance to this particular virus. Our goal is to avoid severe infections leading to death. Though unlikely, if an animal reacts severely, it will be euthanized, based on humane indicators (e.g., failure to groom or feed). These experiments involve no post-surgical survival animals.

Cover Page Supplement: Method of Euthanasia

Are vertebrate animal euthanized?

Yes

Is method consistent with American Veterinary Medical Association (AVMA) guidelines?

Yes