Worksheet for Review of the Vertebrate Animal Section (VAS)

This worksheet is provided to assist applicants in preparing the VAS for submission to the NIH, and as guidance to reviewers in evaluating the VAS of grant applications and cooperative agreements. The responsibilities of extramural scientists and NIH staff are clarified on page 1. A worksheet to assist in preparing or evaluating the VAS is provided on page 2, with more detailed instructions provided on pages 3-4. An example of a complete VAS, coded as ACCEPTABLE, is presented on page 5.

I. Instructions for Applicants, Reviewers and NIH Staff

Overview of requirements

If live vertebrate animals are to be used, federal policy requires that the following five points are addressed in all applications.

1. Provide a detailed description of the proposed use of the animals in the work outlined in the Research Strategy section. Identify the species, strains, ages, sex and number of animals to be used in the proposed work.
2. Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.
3. Provide information on the veterinary care of the animals involved.
4. Describe the procedures for ensuring that discomfort, distress, pain and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.
5. Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the *AVMA Guidelines on Euthanasia*. If not, include a scientific justification for not following the recommendations.

Applicants should be aware that NIH may release information contained in funded applications pursuant to a Freedom of Information Act request.

Applicant responsibilities

Each of the five points must be addressed in the VAS of NIH grant applications. Failure to address the five points may result in the application being designated as incomplete and will be grounds for the PHS to defer the application from the peer review round. Alternatively, the application's impact/priority score may be negatively affected.

Scientific review group (SRG) responsibilities

The SRGs evaluate the involvement of live vertebrate animals as part of the scientific assessment of the applications submitted to NIH according to the five points.

NIH Staff responsibilities

- *Review staff* a) performs an administrative review of each application, checking that it includes a VAS if the use of vertebrate animals is indicated; b) provides reviewers with instructions for reviewing the VAS (VAS Worksheet, PDF) instructing them that the responses to all five points must be appropriate for the VAS to be acceptable; c) codes the application according to the SRG's recommendation and includes reviewers' comments in the Resume of the summary statement.
- *Program staff* a) obtains additional information or clarification to resolve concerns related to any application for which the VAS is found to be unacceptable, if the application is to be recommended for funding; b) works with the applicant to provide revisions to OLAW, facilitating approval of the VAS.
- *Grants Management staff* a) verifies that the organization's Assurance number is provided; b) obtains verification of IACUC approval.

II. Worksheet to Assist in Addressing the Required Five Points of the VAS

Performance site(s): The five points must be addressed for all performance sites.

__ If the applicant's institution is not where animal work will be performed, are all collaborative performance site(s) identified?

__ If more than one performance site is planned, are descriptions of animal care and use addressing the five points provided for each site?

Point 1 Describe the animals and their proposed use; address the following for all species to be used:

__ Species
__ Strains
__ Ages
__ Sex
__ Number of animals to be used
__ A concise, complete description of proposed procedures (i.e., sufficient information for evaluation)

Point 2 Provide justifications for:

__ The use of animals
__ Choice of species
__ Number of animals to be used (cite power calculations, if appropriate) with specific justification for large numbers of animals
__ Use of animals that are in short supply or are costly

Point 3 Provide a general description of veterinary care, including veterinary support that is relevant to the proposed procedures. Examples of the kinds of items that may be appropriate to include are:

__ A brief account of veterinary staff and their availability
__ The regular schedule of monitoring of animals by veterinary staff
__ Any additional monitoring and veterinary support that may be required to ensure humane care, if relevant to the procedures proposed (e.g., post-surgical)
__ Indicators for veterinary intervention to alleviate discomfort, distress or pain, if relevant

Point 4 Describe procedures to minimize discomfort, distress, pain and injury to that which is scientifically unavoidable in the conduct of research. Examples of the kinds of items that may be appropriate to include are:

__ Circumstances relevant to the proposed work, when animals may experience discomfort, distress, pain or injury
__ Procedures to alleviate discomfort, distress, pain or injury
__ Identify (by name or class) any tranquilizers, analgesics, anesthetics and other treatments (e.g., antibiotics) and describe their use
__ Provisions for special care or housing that may be necessary after experimental procedures
__ Plans for post-surgical care, if survival surgeries are proposed
__ Indicators for humane experimental endpoints, if relevant
__ Describe the use of restraint devices, if relevant

Point 5 Describe methods of euthanasia:

__ Describe the method(s) of euthanasia and rationale for selection of method(s)
__ Indicate if the method is consistent with *AVMA Guidelines on Euthanasia*
__ Provide a scientific justification for the choice of method if not AVMA recommended

III. Detailed Instructions for Preparation, Review and Coding of the VAS

Subsequent to evaluation of the VAS by a SRG, all applications or proposals are coded as NO VERTEBRATE ANIMALS (10), NO CONCERNS/ACCEPTABLE (30) or CONCERNS/UNACCEPTABLE (44).

Coding as NO VERTEBRATE ANIMALS (10)

If animal tissue used in the study is obtained from other sources (e.g., tissue repository, animals euthanized for an unrelated purpose), the application is coded as no vertebrate animals used. The source of the tissue should be described in the application to validate the coding as no vertebrate animals used.

Vertebrate animals: If animals are obtained or euthanized for tissue harvest, the proposed research is coded as use of live vertebrate animals. The generation of custom antibodies must be coded as use of live vertebrate animals.

Coding as NO CONCERNS/ACCEPTABLE (30) or CONCERNS/UNACCEPTABLE (44)

Coding is based on peer review of the five required points for each of the performance sites.

Performance site(s): This is defined as the institutions where procedures with animals will be performed. If the applicant institution is not the site where animal work will be performed, the performance site must be identified. If there is more than one performance site, the description of animal care and use at each site must be included in addressing the five points.

Preparation of the VAS: Typically, all of the required elements for the VAS can be addressed within 1-2 pages. Following the detailed guidelines below, an example of a concise, but complete VAS section is included on the last page of this document.

Point 1 Description of animals and how they will be used

A concise, complete description of the proposed procedures must be included in the VAS. 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 within 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 and post-irradiation procedures. In describing the animals, investigators must provide the following information for each species or strain:

- Species
- Strain
- Ages
- Sex
- Number of animals to be used

Point 2 Justifications for use of animals

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 alternatives to animals (e.g., computer models, cell culture) cannot be used and the potential benefits and knowledge to be gained. In addressing this point, researchers are encouraged to consider means to replace, reduce and refine the use of animals. Rationale for the choice of species must be provided (e.g., advantages of the species chosen and why alternative species are not appropriate). If less highly evolved or simpler animal models are available, justification should be provided for using more advanced species. For example, the use of non-human primates (NHP), dogs or cats should be thoroughly justified. If NHP species are to be used, a comparison to other NHP species may be appropriate. If animals are in short supply, costly, or to be used in large numbers, an additional rationale for their selection and the number of animals to be used is required.

Estimates for the number of animals to be used should be as accurate as possible. Justification for the number of animals to be used may include considerations of animal availability, experimental success rate, inclusion of control groups and requirements for statistical significance; cite power calculations where appropriate.

Point 3 Veterinary care

Descriptions of veterinary care should indicate the availability of veterinarians or veterinary technicians. For example, the VAS might indicate the number of veterinarians and veterinary technicians associated with the applicant institution, and their proximity to the performance site(s). The frequency with which veterinary staff observe or monitor animals may also be stated.

If survival surgeries are proposed, descriptions of veterinary involvement or post-surgical monitoring may be described. For example, if animal use involves invasive approaches that might result in discomfort, distress or pain, the investigator may describe the indicators for veterinary intervention and the ways in which veterinary staff may intervene.

Point 4 Provisions to minimize discomfort, distress, pain and injury

Procedures or circumstances that may result in more than momentary discomfort, distress, pain or injury should be identified. Methods to alleviate discomfort, distress or pain should be described. If pharmacological agents are used, the agent(s) may be specified by name or class. Any additional (e.g., non-pharmaceutical) means to avoid discomfort, distress, pain or injury may be briefly described. The manner, circumstances and duration of all post-surgical provisions and care may be described. If special housing is necessary following surgery or manipulations, the VAS may describe these. If procedures (e.g., pharmacological or surgical) 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) may be described. 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. Describe how restraining devices will be used, if applicable.

Point 5 Euthanasia

The method(s) of euthanasia must be described and must comply with the *AVMA Guidelines on Euthanasia*. If the method(s) do not comply with AVMA recommendations, the rationale and scientific justification for use of the method(s) must be provided. The indicators for euthanasia (i.e., termination of experiment or humane endpoints) may be stated. It is not sufficient to state simply that humane methods will be used, that are consistent with the recommendations of the *AVMA Guidelines on Euthanasia* or the Institutional Animal Care and Use Committee (IACUC).

References

Guidance in this document is based on PHS 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 on Euthanasia*. Additional background information and references are available on the Office of Laboratory Animal Welfare 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/openbook.php?record_id=5140
- *AVMA Guidelines on Euthanasia* - http://www.avma.org/issues/animal_welfare/euthanasia.pdf
- NIH Guide for Grants and Contracts Notice - NOT-OD-10-027

IV. Example (This VAS has been modified from the original. It addresses all five points concisely.)

F. Vertebrate Animals

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

1. 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. Based on prior experience, 70 groups, each including 10 mice will be required over five years to achieve adequate statistical power. 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. 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. 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: 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; c) The entire mouse genome is known and easily manipulated genetically, allowing extension of the work in future genetic studies. Female mice will be used due to compatibility issues. Balb/c mice will be used because they have intermediate resistance to infection. ABC-4 knockout and ABC-4 test-strains will be used. For the enzyme study, we will use 4 treatment groups: enzyme-1, enzyme-2, enzyme-3, and mock treated virus. We will also use different amounts of inoculum for each condition allowing a more accurate calculation as to the effect of the digestions on infectivity. For the test-article peptide study, we will use two formulations (one aqueous and one hydrophobic), test 4 different concentrations and also vary the treatment protocol. Two groups will receive a single dose of drug in each of the two formulations prior to the addition of virus to assess prophylactic activity. These groups will not receive any additional enzyme treatments. Two groups will be infected with virus and beginning 4 h post-infection, we will treat with each formulation and concentration 4 times daily for 7 days.

3. All mice are housed in the Animal Resources Center of the University. Animal housing rooms are under temperature and humidity control. The mice will not be subjected to water or food restrictions, and bedding material is placed in each cage. The facility is staffed by four full time veterinarians and six veterinary technicians; the veterinary staff is on site and a clinical veterinarian is available at all times. Animal care staff conducts routine husbandry procedures (e.g., cage cleaning, feeding and watering) and checks animals daily to assess their condition. Laboratory staff monitors mice when treatments are given, disease is scored or samples are collected for titering. The veterinary staff monitors mice in their home cages, weekly. If animals exhibit any indication of infection or distress, the veterinary staff confers with laboratory personnel to recommend appropriate antibiotics, analgesics or other pharmaceuticals. The veterinary staff may intervene or recommend euthanasia based on animal welfare concerns.

4. 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.

5. All mice will be euthanized by cervical dislocation under isoflurane anesthesia. Isoflurane ensures that the mice are unconscious, while dislocation ensures quick death. This minimizes animal distress, is effective and efficient; it is consistent with the recommendations of the *AVMA Guidelines on Euthanasia*.