

June 28, 2010

Office of the Chief Health and Medical Officer

TO: Associate Administrator for Exploration Systems Mission Directorate
Associate Administrator for Space Operations Mission Directorate
Associate Administrator for Science Mission Directorate
Associate Administrator for Aeronautics Research Mission Directorate

FROM: Office of the Chief Health and Medical Officer

SUBJECT: NASA Interim Directive (NID): Vertebrate Animal Scientific Review

The purpose of this NID is to issue an interim policy directing that a Vertebrate Animal Scientific Review take place for all NASA solicited research that involves the use of vertebrate animals as subjects. This NID describes the requirements and responsibilities for conducting this review.

NASA has been at the forefront of biomedical research ethics for the protection of animal subjects in NASA supported research. Recently, the National Institutes of Health (NIH) adopted a series of specific questions that investigators, who are applying for NIH grants, must answer regarding why vertebrate animals are required as research subjects and to demonstrate that there are no alternatives for their use. Additionally, the researcher must show how the animals will be treated throughout the study, how they will be handled and protected from undue pain or suffering, what veterinary care will be available, and what will happen to the animal after the research has been completed. The answers to these questions must be evaluated during the scientific merit review. Moreover, for studies involving non-human primates, the NIH also requires that the review be performed by experts on the specific species being requested for research consideration.

With this NID, the Office of the Chief Health and Medical Office, under its responsibility for oversight of NASA's research subject protection activities, and in order to enhance the Agency's animal care and use policies, is adopting the NIH policy and procedures for all research proposals that require vertebrate animals. NASA now requires that any and all research proposals that request funding for vertebrate animal research shall be reviewed as described in this NID.

This interim policy complements the existing NPD 8910.1, Care and Use of Animals, and NPR 8910.1, Care and Use of Animals. All policies contained in those documents remain in effect. This NID applies to NASA Headquarters and NASA Centers, including Component Facilities and shall be enforced for 1 year from the effective date of this memorandum, or until superseded by appropriate revision of NPR 8910.1, Care and Use of Animals.

Vertebrate Animal Scientific Review

1.0 Policy

1.1 Any research proposal submitted to NASA that requests funding for vertebrate animal research shall undergo a Vertebrate Animal Scientific Review (VASR) conducted by the scientific merit review panel.

1.2 All NASA research solicitations requesting studies that use vertebrate animals, or that could result in studies using vertebrate animals being submitted, shall contain at a minimum the text provided in Appendix A of this NASA Interim Directive (NID), regarding the VASR.

1.3 A proposal using vertebrate animals shall be coded as “no concerns/acceptable” by the VASR before any funds are awarded.

1.4 Any issues related to a proposal coded as “concerns/unacceptable” by the VASR shall be resolved by the NASA Chief Veterinarian, through the Office of the Chief Health and Medical Officer, and the proposal re-coded as “no concerns/acceptable” before any funds are awarded.

2.0 Responsibilities

2.1 NASA program staff shall be responsible for:

2.1.1 Ensuring that the VASR is conducted according to the requirements and stipulations of this NID.

2.1.2 Including the text contained in Appendix A in all appropriate research solicitations. The material in Appendix C is additional material that may be helpful to applicants. Its inclusion in research solicitations is at the discretion of NASA staff, although the “Detailed Instructions for Preparation of the VASR Worksheet” is strongly recommended;

2.1.3 Performing an administrative review of each VASR worksheet, checking that all five points are addressed;

2.1.4 Providing scientific merit reviewers with instructions for conducting the VASR, noting that all five points must be evaluated as appropriate for the proposal to be coded as “acceptable.” Appendix B provides some additional information that can be used for instructing reviewers;

2.1.5 Ensuring the scientific merit review panels evaluate the information provided in the VASR worksheet to determine if it is complete and if plans for the use of vertebrate animals are appropriate relative to the scientific work proposed, and code the proposal;

2.1.6 Ensuring that if there is a proposal(s) to be reviewed using non-human primates, that the scientific merit review panel includes at least one member with expertise and knowledge of the species to be used;

2.1.7 Including reviewers' comments, and any concerns, in the proposal review summary statement, subsequent to the scientific review panel VASR;

2.1.8 Obtaining additional information or clarification from the applicant to resolve concerns for any proposal found to be "unacceptable," if it is to be recommended for funding;

2.1.9 Providing the NASA Chief Veterinarian with all relevant information to allow a determination of the final disposition of the VASR coding of the proposal; and

2.1.10 Verifying that the institutional Animal Welfare Assurance number is provided and obtains verification of IACUC approval before funding is awarded.

2.2 The NASA Chief Veterinarian shall be responsible for:

2.2.1 Reviewing all material associated with a proposal that would otherwise be funded, but originally coded as "unacceptable" during the VASR, and making a determination if it can be deemed "acceptable."

Appendix A

Required Vertebrate Animal Scientific Review (VASR) Text for NASA Research Solicitations

Vertebrate Animal Scientific Review (VASR)

If vertebrate animals are to be used, the following five points must be addressed completely by applicants in the VASR worksheet of their proposal:

1. Detailed description of the proposed use of the animals, including species, strains, ages, sex and number to be used;
2. Justification of the use of animals, choice of species and numbers to be used, and proposer's assessment of potential benefits and knowledge to be gained;
3. Information on the veterinary care of the animals;
4. Description of procedures for ensuring discomfort, distress, pain and injury is minimized; and
5. Method of euthanasia and the reasons for its selection.

Each of the five points must be addressed, for all performance sites, in the VASR worksheet. The VASR worksheet will be reviewed by the scientific merit review panel and the proposal coded as either "No Vertebrate Animals," "No Concerns/Acceptable," or "Concerns/Unacceptable." If coded as "Unacceptable," NASA staff will work with the applicant to resolve concerns prior to award. Coding of the proposal as Acceptable or No Vertebrate Animals is required prior to award.

In order to be coded as "No Vertebrate Animals," the vertebrate tissue used in the study will be obtained from other sources (e.g., tissue repository, animals euthanized for an unrelated purpose). The source of the tissue should be included in the VASR to validate the coding as no vertebrate animals used. If vertebrate tissues are obtained through euthanasia for tissue harvest, the proposed research is coded as use of live vertebrate animals. The generation of custom antibodies is coded as use of live vertebrate animals.

A "performance site(s)" 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 and must address the five points.

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

Appendix B

Instructions for Scientific Reviewers

These instructions are to assist Scientific Merit Review Panel (SMRP) members in the VASR review of the proposal.

Subsequent to evaluation of the VASR worksheet by a SMRP, all proposals are coded as either “No Vertebrate Animals,” “No Concerns/Acceptable,” or “Concerns/Unacceptable.”

Coding as “NO VERTEBRATE ANIMALS” - If vertebrate tissue used in the study is obtained from other sources (e.g., tissue repository, animals euthanized for an unrelated purpose), the proposal is coded as no vertebrate animals used. The source of the tissue should be included in the VASR to validate the coding as no vertebrate animals used. If vertebrate tissues are obtained through euthanasia 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” or “CONCERNS/UNACCEPTABLE” - Coding is based on the 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 and must address the five points.

Appendix C

Supporting Material

Detailed Instructions for Preparation of the VASR Worksheet

These instructions are to assist applicants in preparing their VASR information.

Preparation of the VASR worksheet:

Typically, all of the required elements for the VASR can be addressed within 1-2 pages.

Point 1 - Description of animals and how they will be used

A concise, complete description of the proposed procedures must be included in the VASR. 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 VASR. The description must include sufficient detail to allow evaluation of the procedures. Examples of the types of procedures that should 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 and/or strain to be used:

- 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. The justification must indicate why alternatives to animals (e.g., computer models, cell culture) cannot be used and should indicate 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. The rationale should indicate the advantages of the species chosen and why alternative species are not appropriate. If less highly evolved or simpler animal models are available, justification must 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, provide an additional rationale for their selection and the number of animals used.

Estimates for the number of animals to be used should be as accurate as possible. Justification for the number of animals to be used should 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 VASR 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 should be stated. If survival surgeries are proposed, veterinary involvement or post-surgical monitoring should be described. For example, if animal use involves invasive approaches that might result in discomfort, distress or pain, the investigator should indicate if or when veterinary care is necessary. The indicators for veterinary intervention to alleviate discomfort, distress or pain should be described. The ways in which veterinary staff may intervene should be described.

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) should be specified by name or class. Any additional (e.g., non-pharmaceutical) means to avoid discomfort, distress, pain or injury should be described briefly. The manner, circumstances, and duration of all post-surgical provisions and care should be described. If special housing is necessary following surgery or manipulations, the VASR should describe these provisions, the duration, and type of monitoring provided. 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) should be described. All of these issues are particularly important for survival surgeries. If multiple surgeries are proposed, these must be well justified and provisions to avoid any potential complications must 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) should 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 NASA and Public Health Service (PHS) Policy, and federal requirements. The NASA and PHS Policy incorporate the standards in the *Guide for the Care and Use of Laboratory Animals* and require 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>).

NASA Policy and Requirements (<http://nodis3.gsfc.nasa.gov/displayDir.cfm?t=NPR&c=8910&s=1B>)

PHS Policy (<http://grants.nih.gov/grants/olaw/references/phspol.htm>)

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)

Checklist to Assist in Addressing the Required Five Points of the VASR

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 for each site provided?

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, but 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)

Point 3 - Provide a general description of veterinary care, including veterinary support that is specifically relevant to the proposed procedures. Indicate the following:

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. Indicate the following:

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

Example of a complete VASR Worksheet

(This VASR worksheet has been modified from the original. It addresses all five points concisely)

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 titring. 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