

Guidelines for Establishing an NIH Animal Study Proposal

Background: The [Animal Welfare Act Regulations](#) (AWARs) and [Public Health Service Policy](#) (PHS Policy) require Animal Care and Use Committee (ACUC) review and approval of all research activities involving vertebrate animals prior to initiation. At NIH, these animal activities are described in an Animal Study Proposal (ASP).

An ASP is approved for a maximum of three years. If work is to continue after that time, the ASP must be resubmitted to the ACUC for complete review and approval. Any proposed changes to previously approved animal activities must be submitted to the ACUC for review and approval prior to implementation. Approved ASPs are subject to release under the Freedom of Information Act (FOIA). NIH will withhold any information that is exempt from disclosure, in consultation with the [NIH FOIA Office](#).

This guideline provides the required sections for an NIH ASP (sections 1-12 and lettered subsections). Maintaining the order of sections is highly encouraged to assist with reviews by the [Division of Safety](#) (DS), the [Institutional Biosafety Committee](#) (IBC), and shared/central facilities who review ASPs from multiple ICs. Individual ICs may add sections, subsections, or additional requirements, but the required sections and subsections must be retained. ICs may also use customized ACUC management systems to support ASP establishment, review, and maintenance.

General Information: The following practices may help streamline the review process:

- Include only the information needed to describe the proposed work. Unnecessary detail can slow review and lead to additional questions.
- Seek guidance from investigators experienced in animal research and ACUC processes.
- Work with the IC Animal Program Director (APD) and IC ACUC Coordinator during ASP preparation.
- Consult the IC DS Safety Specialist for guidance on submitting required materials to the IBC.

A clear and well-organized ASP can support a more efficient review process and help avoid delays in starting research. Some IC ACUCs may also invite investigators to attend meetings to address questions, which can further facilitate review and approval.

The following references provide foundational information for developing an ASP:

- [External Regulations, Policies, and Standards](#)
- [NIH Policy Manual Chapters](#)
- [NIH Animal Research Advisory Committee \(ARAC\) Guidelines](#)

Section 1 - Administrative Data:

- a) **Study Title:** Provide a concise title for the ASP.
- b) **Principal Investigator (PI):** Name the scientist with overall responsibility for the design, conduct, and reporting of research on the ASP. If the PI is not a federal employee, also list a Responsible Investigator as required in [NIH Policy Manual 3040-2](#).

- c) Contact information: Provide contact information (e.g., email, phone number) for the PI and designated emergency contacts so that they can be reached with questions related to the ASP or in the event of an emergency.
- d) Animal Users: List all personnel who will perform procedures involving live animals on the ASP. All listed personnel are required to complete NIH, IC, safety, and facility training and enroll in the Animal Exposure Program (AEP) or an equivalent occupational health program. Animal users must be adequately educated, trained, and/or qualified. Individuals who will not handle or perform procedures on live animals should not be included in this section.

Section 2 - Animal Requirements:

- a) Species and Sex: Specify the species (e.g., mouse, rat, zebrafish) and the sex(es) of animals to be used in the ASP.
- b) Housing/Holding Location(s): Provide the proposed housing location(s). If location is outside an animal facility, provide building and room number. Indicate if animals will be housed outside an animal facility for more than 12 hours. If animals will be housed outside an animal facility for more than 24 hours, a satellite facility must be established and approved by the IC ACUC. Contact the IC APD or IC ACUC Coordinator for guidance on non-standard housing arrangements.
- c) Animal Procedure Location(s): Provide the proposed location(s) for all laboratory or animal activity spaces where live animal procedures will occur. This includes surgeries, in vivo procedures (e.g., physiological or behavioral tests, injections, imaging), and euthanasia. If location is outside an animal facility, provide building and room number.

Section 3 - Study Objectives: Provide a brief (approximately 300 words) non-scientific summary of the research objectives. This should be written for a general audience and should describe why the research is important and how it may advance knowledge or benefit humans and/or animals. Avoid abbreviations, technical terms, and scientific jargon. The summary should be easily understood by the nonscientist and nonaffiliated members of the ACUC. For triennial complete reviews, also include a summary of the work completed during the previous three years.

Section 4 - Experimental Design:

- a) Description of Experimental Design: Provide a clear outline of the experimental design, including group assignments, study timeline, and what happens to each animal from entry into the ASP through the study endpoint. A flow diagram or other visual representation may be included to support the description, if helpful. Descriptions of the individual procedures are to be included in Section 5.
- b) Rationale for Animal Use: Explain why an animal model is needed for the study instead of non-animal approaches (e.g., cell cultures, computer models, organoids). Describe how the principles of the 3Rs (replacement, reduction, and refinement) were considered in the study design, including any new approach methodologies (NAMs) (e.g., human-based in vitro systems, computational models, real world data) that were evaluated.

- c) Justification of Species: Explain why the selected species is appropriate for the proposed study and why alternative species are not appropriate. If more than one species is used, provide a justification for each.
- d) Sex as a Biological Variable: For conditions that affect both sexes, include both males and females to allow analysis of sex as a biological variable. If the study uses only animals of the same sex in all experimental groups, provide a scientific justification. For example, studies of prostate cancer typically involve only male animals, while studies of pregnancy may use only female animals.
- e) Estimated Number of Animals: Provide an estimate of the minimum number of animals needed to achieve the research goals. Present this information clearly for each species using a table, outline, or flow chart, if helpful. The following approaches may be used to justify the animal numbers:
 - Statistical justification: Whenever possible, the number of animals and experimental group sizes should be statistically justified.
 - i. New experimental paradigms: describe the scientific rationale used to determine group sizes.
 - ii. Continuation of ongoing work: summarize the statistical approach based on known effect sizes and variability.
 - iii. When statistical justification is not feasible (e.g., pilot study), provide a scientific rationale for the proposed group sizes.
 - Organ, fluid, or tissue harvest: When animals are used to obtain tissues or fluids, estimate the amount needed and describe expected use. If prior data are not available, provide an estimate of yield per animal and describe the collection process.
 - Breeding: Account for all animals produced through breeding, regardless of their final use. Offspring are counted at first cage change or experimental manipulation, whichever comes first. Estimate the number of offspring expected:
 - For example, 4 breeding females producing 12 litters per year with 8 pups per litter over 3 years would generate: $4 \times 12 \times 8 \times 3 = 1,152$ pups.

Section 5 - Animal Procedures: Provide a clear description of all animal procedures. If permitted by the IC, ACUC-approved standard operating procedures (SOPs) may be referenced.

- a) Administered Substances: Describe all substances administered to animals, including injections, inoculation, biological materials, anesthetics, analgesics, and other agents used in the study. For each substance, include the name, grade (e.g., USP or non-pharmaceutical), dose, route, frequency, and duration of administration. This information may be presented in a list, table, or other format. If non-pharmaceutical grade compounds are used, provide justification in accordance with the ARAC [Guidelines for the Use of Non-Pharmaceutical Grade Compounds in Laboratory Animals](#). Note any substances that may be hazardous or require special handling/storage practices (e.g., controlled substances). If rodent derived biological materials are administered, include MAP/RAP/HAP/PCR testing results.
- b) Blood Collection: Describe the collection site or technique, frequency, and volume collected at each time point. Ensure collection volumes align with the ARAC [Guidelines for Blood Collection in Mice and Rats](#) and any IC-specific requirements.

- c) Surgical Procedures: Describe all surgical procedures, and indicate whether they are survival or non-survival, major or minor. Include details on preoperative preparation, aseptic technique, anesthesia, perioperative and postoperative monitoring, surgical methods, and postoperative analgesia. If multiple survival surgeries are planned for a single animal, provide scientific justification. Refer to the ARAC [Guidelines for Survival Rodent Surgery](#) for additional guidance.
- d) Ionizing/Non-Ionizing Radiation: Describe any use of ionizing radiation, including radionuclides with dose and schedule, and radiation producing equipment (e.g., x-ray, irradiator, micro-CT, etc.) or non-ionizing radiation producing equipment (e.g., UV, lasers – Class 3b/4).
- e) Methods of Restraint: Describe any restraint that exceeds routine handling (e.g., longer than 30 minutes or as defined by the IC). For this prolonged restraint, include:
- Purpose and duration
 - Adaptation or training procedures, if applicable
 - Monitoring frequency during restraint
 - Criteria for removal from the device or study
 - Consideration of alternatives (e.g., chemical restraint)
- f) Animal Identification Methods: Describe any methods used to identify animals (e.g., ear tags, tattoos). Indicate whether anesthesia will be used, if applicable.
- g) Behavioral Assessments: Describe each behavioral test, including how it is conducted and how it is necessary to meet the aims of the study. Include the number of tests, frequency, intervals between tests, and whether tests are combined with other procedures. If a test may cause pain or distress, also describe:
- The nature and intensity of the stimulus
 - Expected level of pain or distress
 - Measures to minimize or end pain or distress
 - Duration of exposure to the stimulus
 - Criteria for removal from the assessment or study
- h) Breeding: Describe the overall breeding plan, including the strategy, colony management, disposition of surplus animals, and genotyping.
- i) Other Procedures: Describe any procedures not covered above, including all non-surgical procedures (e.g., swabs, injections, gavage, imaging). Describe the procedure, use of analgesia or sedation (if applicable), frequency, number of procedures, intervals between procedures, and any expected complications.

Section 6 - Monitoring, Endpoints, and Euthanasia:

- a) Monitoring: Describe the parameters that will be monitored during the study (e.g., body weight, behavior, tumor size), how often monitoring will occur, who is responsible for monitoring, and what actions will be taken if concerns arise. If monitoring differs by procedure or study group, describe those differences. This information may be presented in text or in a table.
- b) Endpoint Criteria: Describe the experimental endpoint, such as a time point or clinical condition

(e.g., tumor size), that defines when the study will end. For studies in which adverse outcomes are expected, also describe humane endpoints. A humane endpoint is the earliest scientifically justified point at which pain or distress can be prevented, reduced, or relieved while still meeting the study objectives. Humane endpoints are preferred over death or moribundity because they minimize pain and distress. Effective use of endpoints requires trained personnel to perform routine study-specific observations at appropriate intervals. Clearly define assessment criteria and the actions to be taken in response to findings. Consider using study-specific animal assessment records to support monitoring. Death as an endpoint should be minimized and must be scientifically justified when necessary. For additional guidance, refer to the ARAC [Guideline for Endpoints in Animal Study Proposals](#).

- c) Euthanasia/Disposition: Describe what will occur when animals reach the described endpoints, including how euthanasia will be performed (e.g., method, dose, route). If the method is not consistent with the AVMA Guidelines for the Euthanasia of Animals, provide a scientific justification. If animals will not be euthanized, describe the planned disposition, such as transfer to another approved ASP or adoption. For additional information, refer to the following guidance documents:
- ARAC [Guidelines for Euthanasia of Rodents Using Carbon Dioxide](#)
 - ARAC [Guidelines for Use of Zebrafish in the NIH Intramural Research Program](#)
 - ARAC [Guidelines for Post-Study Research Animal Disposition](#)
 - [AVMA Guidelines for the Euthanasia of Animals: 2020 Edition](#)

Section 7 - Pain and Distress:

- a) Potentially Painful and/or Distressful Procedures: Identify any procedures, phenotypes, or conditions that may cause pain or distress. If pain or distress is expected and cannot be relieved with appropriate anesthetics, analgesics, or tranquilizers, provide a clear scientific justification. This justification should explain both the need for the procedure and why the pain or distress cannot be alleviated. For additional guidance, refer to the ARAC [Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition, and Intervention](#).
- b) USDA Pain and Distress Classification: NIH requires all animals used in research be assigned to one of three columns of pain and/or distress. In this section, identify how many are assigned to each pain column. The total here must match the total number of animals requested in Section 4:
- Column C: Total number of animals that did not experience more than momentary pain or distress. Examples include: research-related breeding, blood collection, fluid administration, gastric gavage, and use of sedation for restraint purposes (e.g., imaging).
 - Column D: Total number of animals that experienced more than momentary pain or distress that was appropriately alleviated with anesthetics, analgesics, or tranquilizers. Examples include: surgery (survival and non-survival) and implantation of devices.
 - Column E: Total number of animals that experienced unalleviated pain or distress. Examples include: sepsis, heart failure, and chronic inflammation. In addition to such procedures, studies that use moribundity or mortality (death) as an endpoint are also classified in column E. For USDA-regulated species, completion of a [Column E Explanation](#) form is also required ([USDA APHIS Form 7023B](#)).
- c) Alternatives Search Documentation: For animals in USDA pain columns D or E, conduct a literature search to identify alternative methods or procedures that may reduce or avoid pain or distress.

The search must include at least two databases (e.g., MEDLINE, AGRICOLA, ALTWEB). Provide a brief narrative describing the search, including the databases used, the date of the search, the years covered, and the keywords or search strategy. Summarize the results of the search. NIH Library staff can provide guidance on selecting appropriate databases and search terms (<https://www.nihlibrary.nih.gov/>).

For examples and more information, refer to the following ARAC guidance documents:

- [Guidelines for Preparing USDA Annual Reports and Assigning USDA Reporting Columns](#)
- [Guidelines for Pain and Distress in Laboratory Animals: Responsibilities, Recognition, and Intervention](#)

Section 8 - Hazardous Agents: Consultation with the DS Safety Specialist is recommended, as the use of hazardous agents requires review and approval by DS and/or the IBC prior to ASP approval. The information provided in this section helps determine the appropriate animal biosafety level (ABSL) for the ASP. Include the following:

- a) Biological Agents with Pathogenic Potential and Recombinant DNA: List all relevant agents, including human pathogens; other pathogens of concern; human blood, tissues or body fluids; biological toxins; materials from Old World nonhuman primates that will be administered to animals; and any non-exempt recombinant and/or synthetic materials administered to animals (e.g., constructs used for development of transgenic or knock-out animal models). IBC approval is required and PIs should submit an IBC registration or amendment as soon as possible to ensure timely review. Include the IBC approval number and the approved ABSL. Refer to the NIH Guidelines for [Research Involving Recombinant or Synthetic Nucleic Acid Molecules](#) or consult the [NIH IBC](#) for additional guidance.

For PI taking samples or specimens from NHPs, the work with blood and body fluids from animals must also be registered. Refer to the Pathogen Registration Document and [NIH Policy Manual 3044-2](#) for additional guidance.

- b) Hazardous Chemicals or Drugs: List all hazardous chemicals or drugs used in the animal facility (e.g., carcinogens, mutagens, formaldehyde, inhalant anesthetics). The IC Safety Specialist can assist in identifying hazards and providing safety language to communicate safe handling, administration, and disposal.
- c) Ionizing/Non-ionizing Radiation: Identify radioactive isotopes and other ionizing radiation sources used *in vivo*. Contact the DS [Radiation Safety Operations Branch](#) (RSOB) for additional guidance. For the use of non-ionizing radiation (Class 3b/4 lasers) contact the DS [Laser Safety Program](#) for guidance.
- d) Other Hazards: Describe any additional hazards such as noise exposure or physical risks (e.g., falling hazards), that may affect personnel working with animals.

Section 9 - Special Concerns or Requirements: Describe any items or procedures that may require special care or attention by the PI or the animal facility during the study. Examples include:

- Deviations from the *Guide*
- Single housing of social species
- Exemptions from standard enrichment

- Specialized caging
- Special husbandry (e.g., diet, water, bedding)
- Environmental modifications (e.g., light, humidity, temperature)

For any item or procedure that may adversely impact animal health or well-being, scientific justification must be provided. The ASP should also describe how animals will be cared for and how pain or distress will be minimized.

Section 10 - Principal Investigator Certifications: NIH requires the signature of the PI, which certifies the following:

- a) The PI has completed the NIH OACU course [Using Animals in Intramural Research: Guidelines for Principal Investigators](#) and will complete the NIH OACU course [Using Animals in Intramural Research: REFRESHER](#) triennially.
- b) The proposed research is not unnecessarily duplicative of previous research.
- c) All individuals working on the proposal who have animal contact are participating in the NIH Animal Exposure Program (or equivalent program, for contract personnel) and have completed required laboratory safety training.
- d) All individuals authorized to conduct procedures involving animals on the proposal, have completed the NIH OACU course [Using Animals in Intramural Research: Guidelines for Animal Users](#), will complete the NIH OACU course [Using Animals in Intramural Research: REFRESHER](#) triennially, and have received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns. The PI is responsible for the professional conduct of all personnel working on the proposal.
- e) For all Column D and E procedures, the PI has reviewed the pertinent scientific literature and the sources and/or databases (two or more) as noted in Section 7 and have found no valid alternative to any procedures which may cause more than momentary pain or distress, whether it is relieved or not.
- f) The PI will obtain approval from the IC ACUC before initiating any changes to the study.
- g) Animal transportation will comply with applicable NIH and IC policies.
- h) If generative AI is used in the preparation of this ASP or subsequent amendments, all output will be verified in accordance with applicable NIH and IC policies (<https://osp.od.nih.gov/policies/artificial-intelligence/>).

Section 11 - Concurrences: NIH requires the signature of the laboratory or branch chief. This signature reflects approval of the scientific merit, consideration of sex as a biological variable, and other relevant factors. NIH also requires a safety specialist signature to confirm that the ASP meets DS requirements, including IBC approval, when applicable. Individual ICs may require additional signatures, such as from the Attending Veterinarian, Facility Manager, or others.

When facility staff signatures are required, they indicate that appropriate infrastructure and veterinary care resources are available to support the ASP. These signatures are not intended to delay or withhold approval for other reasons. Questions outside of infrastructure or veterinary care should be submitted in writing to the IC ACUC Chair.

Section 12 - Final Approval: NIH requires the signature of the IC ACUC Chairperson to indicate final approval of the ASP. Written notification is sent to the PI denoting the date of approval and authorizing the conduct of approved animal activities.

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