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Review Article

Everything You Need to Know About Satisfying IACUC Protocol Requirements

Swapna Mohan[1] and Patricia L. Foley[2]

[1]NIH, Previously OLAW/OD, Bethesda, Maryland and [2]Georgetown University, Department of Microbiology & Immunology, Washington, DC

Address correspondence and reprint requests to Swapna Mohan, BG 38A RM B2N13Q13, 8600 Rockville Pike, Bethesda, MD 20894. Tel: 301-827-5016. E-mail: swapna.mohan@nih.gov.

Abstract

There have been recent efforts to reduce the administrative burden imposed on investigators. Although a complete and thorough review of proposed animal studies is an essential function of the Institutional Animal Care and Use Committee (IACUC), efforts to streamline and clarify this process may help investigators spend less time writing animal use protocols and responding to committee comments. The IACUC relies on well-written protocols for an efficient review process. A well-designed protocol form is also critical in guiding investigators through the process. However, it is ultimately the investigators' responsibility to ensure that the information they provide answers all the IACUC's questions with enough detail and quality for a fast and effective review. This article, aimed primarily for researchers but also IACUC administrators, provides an overview of the IACUC protocol review and approval process, the criteria that the IACUC uses for evaluations, and the type of information that should be included in the various sections of the protocol form. Some specific examples are also provided.

Key words: animal use protocol; IACUC; review of proposed procedures on animals

Introduction

The Animal Study Protocol, or the IACUC protocol, is a detailed description of the proposed use of animals prepared by the researcher for IACUC review and approval. Activities involving animals in research, testing, and teaching can begin only after they have been reviewed and approved by the IACUC. The various topics the IACUC evaluates on the protocol include the rationale for proposed animal use and numbers, search for alternatives, detailed descriptions of procedures including surgical procedures, impact on the animals' well-being, availability and use of appropriate anesthesia and analgesia, peri-procedural care, study endpoints, methods of euthanasia, and training and experience of personnel performing these procedures.[1]

General Information

Although there are no standardized protocol forms for research animal use (and no specific regulatory requirement for an animal protocol), there are many common elements that need to be addressed in proposed animal studies. The organization of forms should be such that they are easy for the investigator to complete and for the IACUC to review. There is a great degree of flexibility when designing a protocol form to serve an institution's animal program, depending on the types of species used in the institution, the types of studies carried out, and the regulatory and oversight agencies overseeing the work. A protocol also has administrative sections that help in identification of the study and to keep track of its progress during regulatory requirements such as annual review or the triennial de novo review. Information typically included are the dates of approval and expiration, institution-specific identification numbers or tags, type of study, and title. The people named on the protocol include the principal investigator (PI) and all personnel participating in the study activities. Other basic elements are described in detail below.

Federal Requirements

The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy), the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training (Principles), the US Animal Welfare Act, and Animal Welfare Act Regulations (AWRs) all have specific requirements regarding review of proposed animal activities.[2,3]

Regarding the elements of protocol review, the PHS Policy requires institutions to review the components related to animal care and use in accordance with the Policy (IV.C.1.a-g) and to use the *Guide for the Care and Use of Laboratory Animals* (hereafter referred to as the *Guide*) as a basis for evaluation,[3] which also overlap with the requirements of protocol approval listed on the AWR.[4] AAALAC accreditation is recognized by the PHS Policy, and the institutional accreditation status is indicated in the institution's Animal Welfare Assurance (documentation submitted to the Office of Laboratory Animal Welfare [OLAW], assuring institutional compliance with the PHS Policy).

Researchers may hear a lot about AAALAC accreditation at their institution. It is important to understand that AAALAC is a private nonprofit accrediting organization and has no regulatory authority. However, AAALAC accreditation is one means by which institutions assure the PHS that they are compliant with PHS policy.

Overview of the IACUC Protocol Review Process

Before or while writing a protocol, investigators may find it helpful to consult with experts regarding statistical analysis for required animal numbers, or database searches to identify potential alternatives to painful or distressful procedures.[5] Getting assistance with these sections up front may save wasted time later by having specific language and information in the protocol at the time of submission that the IACUC will be looking for during review. There is a regulatory requirement (AWR 2.31,d,1,iv,B) that states that a veterinarian or a qualified designee must be consulted in the planning of any procedure that could cause pain to animals and that pre- and postsurgical care should be "in accordance with established veterinary medical . . . procedures." The IACUC office should be able to guide researchers to available resources at their institution. PIs submit their protocol usually to the IACUC administrative office at their institution. The format for submission may be through an online portal or may be as simple as submitting a text-based document based on the institution's protocol template. After submission, the protocol may undergo an administrative pre-review for a general quality assurance. This pre-review helps to determine completeness of sections, inclusion of documents and attachments, verification of other required approvals (eg, biosafety), and training requirements. Many IACUCs also elect to have a veterinary pre-review at this stage to ensure that descriptions of procedures, endpoint criteria, appropriateness of drugs, and plan for pain intervention are adequately addressed. Both the administrative and veterinary pre-reviews help to smooth the IACUC review process and can result in a faster approval time.

Once a protocol is accepted for IACUC review, it then moves through the review process as described in the institution's Animal Welfare Assurance, if applicable. The initial review may either be a full committee review (FCR; conducted at a convened meeting with a quorum of members present) or a designated member review (DMR; conducted by at least one member designated by the IACUC chairperson after all members have had an opportunity to call for FCR). Depending on the IACUC, other methods of review including administrative review and veterinary verification and consultation may be available methods for certain amendment requests (NOT-OD-14-126). These methods of review often allow for a more timely approval of amendments.

The outcomes of the review process (approval, requirement of modifications to secure approval, or approval withheld) are then provided to the PI in writing along with the reasons for the decisions (AWR 2.31,d,4). In case of approval, the written record will typically include information such as approval and expiry dates, species and number of animals, and any conditions of approval.[5] If approval is withheld, the PI is notified in writing with reasons and is given the opportunity to respond to the feedback. If the outcome is requirement of modifications (to secure approval), the PI is provided with a list of the required modifications, which are then addressed and sent back to the IACUC for review. This second review is often conducted by a DMR process if there is a written and signed policy to this effect in place and if the DMR was approved by unanimous vote during a convened meeting previously (PHS Policy IV C.1-8.).[3,6] If there are concerns regarding newly proposed procedures that are unfamiliar to the research team or are particularly invasive, sometimes the IACUC will approve it as a pilot study, generally for a limited time and involving fewer animals. Pilot studies allow for evaluation of the skills of the research team and/or to assess any potential animal welfare concerns and their management. Often the IACUC will ask that these pilot studies be monitored by a member of the veterinary team. Once the results of the pilot study are reviewed by the IACUC, the PI may be allowed to submit a protocol for the full study.[7]

What the Iacuc Looks for During Review

Although the layout and design of an IACUC protocol varies with the institution, the basic elements remain uniform at institutions across the United States. This ensures a certain level of standardization in the IACUC review process to ensure that the animal activities are consistent with the most humane care and use of animals.

Study Objectives and Scientific Justification

Most protocol forms have a section asking for study objectives or a brief explanation of study aims and how it is important in the advancement of science or human or animal health. Because this section is usually written in nonscientific language, it is also referred to as a "lay summary." An IACUC constituted as per the requirements of the PHS Policy comprises of a wide range of experts, including one member whose primary concerns are in a nonscientific area, such as the nonscientific member and the nonaffiliated member.[3] The purpose of explaining the rationale of the study is to provide the IACUC with a simple and straightforward overview of the proposed animal studies that can also be easily understood by all members of the committee regardless of their scientific background. This initial overview of study objectives also helps the committee understand the potential societal implications to balance the potential benefits of the study against any animal welfare concerns. The summary should not be a reiteration of the aims of the grant application.

Sample Lay Abstract: My lab studies *** because it is expressed by cancer cells and we believe that it may be important to the ability of cancer cells to grow and to spread to other sites in the body (metastasize). We have recently discovered in tissue culture experiments that treatment of ^^^ tumor cells with antibodies against *** results in the suppression of cell growth. Furthermore, concomitant treatment with drug X already used to treat some of these tumors results in a synergistic suppression of tumor growth, and works much better than either therapy alone. We now wish to extend these findings to an animal model. To do this, we will inject human tumor cells under the skin of an immune-compromised mouse called a SCID mouse, that foreign tissue can grow in. We will then treat these mice with our antibody and see if the treatments have any impact on tumor growth or spread. If successful, we will continue to study the potential of combining this antibody therapy with other chemotherapeutic drugs.

Although IACUCs are not required to evaluate the scientific content of a protocol for quality, the benefits of the study against potential animal welfare concerns have elements of scientific merit review in them. US Government Principle II states that "procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society."[3] Some IACUCs take into consideration whether scientific peer-review has already occurred (based on funding source) and may require additional internal review (eg, departmental chair approval) for those studies that have not received peer review.

Example of Rationale for Animal Use: Acute kidney injury remains a serious medical condition that is often fatal despite years of research in disease pathogenesis and treatment. Understanding the various causes of acute kidney injury as well as its potential progression to chronic kidney disease is the main focus of my laboratory's research. The kidney is a vastly complex organ and integral to maintaining homeostasis, a system that cannot be fully recapitulated in vitro. Animal models are essential to this research, helping us to better clarify the underlying mechanisms and to identify potential novel therapeutic approaches. Research in in vitro models has yielded promising results associating various biomarkers to different stages of acute kidney injury. The function and interactions of these biomarkers in specific signaling cascades within the in vivo system will provide insight into the development and pathogenesis as well as shed light on possible interventions that may control the progression of the disease.

Rationale for Animal Use and Search for Alternatives (Consideration of 3Rs)

The investigator is asked to provide a rationale and purpose for use of animals to achieve study aims and to consider the 3Rs (replacement, reduction and refinement) first developed by Russel and Burch in 1959 as an ethical framework for determining the scope of animal use, species chosen, and how they are used.[8,9] The rationale for animal use should be scientifically based and relate directly back to the study objectives.

The overall precept of the 3Rs is that animal use in scientific research should be carried out only after exhaustively searching options for nonanimal alternatives. And when animals are used, the research should be performed in the most humane manner with the minimum number of animals required for obtaining valid results. Specifically, the principle of replacement will address why nonanimal models (in vitro, computational) or phylogenetically lower animal models cannot be used. The principle of reduction will be addressed by providing justification for the number of animals proposed. This should include an explanation of the different arms of the study, group sizes needed, time points, etc. A flow chart or table is often helpful when explaining animal numbers, especially if there are several different studies combined in a single study and/or complicated breeding schemes. The IACUC will want to know how group sizes were determined, the statistical parameters used, and the power calculations used for achieving statistically valid results.[7] Refinement can be addressed by design of procedures that minimize pain, distress, and discomfort.[10] It is helpful to give specific examples of how the procedures have been refined to minimize pain or distress, such as use of postoperative analgesics, noninvasive imaging procedures, study designs that minimize the need for frequent handling or restraint of animals, procedural training that occurs before using live animals, etc.

Example of Consideration of Refinement: The surgery to implant an osmotic implant requires only a skin incision and is minimally invasive. Mice receive anesthesia during surgery and analgesics postoperatively. We considered daily IP injections as a nonsurgical alternative but believe a single relatively minor surgery will be less distressful than daily IP injections for 3–4 weeks and will provide more consistent drug blood levels.

Additionally, the AWRs (9CFR, §2.31 (d)(1)(ii)) and the *Guide* require an assurance that animal activities do not unnecessarily duplicate previous studies and also a description of the measures used for search of alternatives to painful and distressful procedures. The former can be achieved by a simple check box or statement on the protocol form. The latter description should include information such as the databases used for the search, date of search, the time period covered by the search, and the search strategy used. Provision of the keyword combinations and Boolean operators used allows the committee to determine whether the database searches are likely to be relevant and effective in searching for alternatives.[11]

Species and Strain Justification

You will be prompted to provide a justification for the choice of species and specific animal model (genetic model, spontaneous model, etc.). This provides the reviewers with assurance that the animal model has validity to address the specific hypotheses and research questions being studied. It is also an opportunity to explain why a species lower on the phylogenetic scale would not be equally (or more) appropriate.

Species Justification Example: A nonmammalian species would not adequately address our scientific needs. Decades of studies on mouse cancer models have provided substantial background, standard techniques, and reagents to extensively evaluate the role of oncogenes and genetic mutations during mammary tumorigenesis. Further, the murine xenograft and allograft models described in this protocol faithfully reproduce the histological lesions, the pattern of gene and protein expression, as well as the stromal responses observed during human breast cancer progression. Additionally, animals higher on the phylogenetic scale are not being used for this study because XXX knockout constructs are at present available only in mice. Many immunologic agents required for this research are also only available for mouse tissues.

Justification of Animal Numbers

Justifying animal numbers is a critical section of the protocol and will receive particular scrutiny because of the ethical requirement to use as few animals as absolutely necessary. A sample size that is too large may unnecessarily use more animals than is needed. However, using too small a sample size may result in missing a true effect and thus also waste animals. Statistical methods such as a power analysis should be used when possible to determine appropriate group sizes (N) for the experiments and the total number of experimental groups stated. For example, a study might have 4 different experimental groups, with 3 different time points and require an N of 12 based on a power analysis, equaling a total of 144 animals. Any expected attrition should also be factored in, such as surgical mortalities, less than 100% tumor take rate, etc. There are several sample size calculators available online. However, a power analysis is not appropriate for all studies, and the AWRs allow other methods such as published literature that uses the same or similar animal model or results from pilot studies. For in vitro studies where animal tissue is needed, the justification may be based on amount of tissue or cells needed and that can be retrieved from the particular tissue or organ. Justifying animal numbers based on how many experiments can be completed in a certain amount of time, rather than specific scientific goals, is generally not adequate. Breeding colonies should be sized to provide the number of experimental animals needed without producing an abundance of extra animals that then must be needlessly euthanized. Unwanted genotypes should be explained and any potential use included (eg, as practice animals for training new staff, use in pilot studies). There should be appropriate control groups, randomization, and a discussion of sex choice given the increased emphasis on performing animal studies using both males and females.[12]

Description of Experimental Procedures

Complete, concise descriptions of all procedures to be employed in the study are required in this section. As mentioned previously, the layout of this section varies with the IACUC, and IACUCs may require a brief explanation of the experimental design (eg, flowchart of procedures) or a checklist of major procedures prior to the detailed description. It is important for reviewers to be able to discern the total "picture" of what will happen to animals from beginning to end to assess overall impact of the experiments on animal well-being. One aspect investigators often struggle with is how much detail to include. It might help to consider the "Goldilocks Effect" in this context: too little detail limits the ability to conduct a proper review; too much detail restrains the researcher's ability to make minor procedural changes during the course of the experiments and increases probability of an off-protocol (noncompliant) event.

Surgical or Invasive Procedures

Indication of the type(s) of surgical procedure(s) (major vs minor, survival vs nonsurvival) is useful here, along with information on who (personnel) and where (location, procedure room). Major survival surgery is generally defined as one that "penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection."[7] Nonsurvival surgery is one in which the animal is euthanized and does not recover from anesthesia. Describe any preoperative procedures such as food and fluid restriction (presurgical fasting), baseline data acquisition (eg, body weight, blood work), presurgical sedation if needed, and acclimation to devices or jackets that might be in use after surgery. For description of the surgical procedure, include information such as aseptic techniques that will be utilized, plans for anesthesia and analgesia, incision site and its preparation, approximate duration of procedure, supportive measures during procedure (fluids, ventilation), and monitoring parameters (eg, depth of anesthesia, vital signs, oxygen saturation levels) both during and in recovery from procedures.

For survival procedures, a detailed description of the postprocedural care is required including housing and palliative care, suture removal, provision of analgesia and other postoperative medications to be administered, parameters for monitoring pain and distress during recovery, other assessments for postoperative recovery, management of potential complications, and any humane endpoints in case of unrelieved pain or distress. Protocols should also indicate the personnel in charge of postprocedural care and assessment and regular update of records, including on weekends, after hours, and holidays.

Anesthetics, Analgesics, Sedatives, and Tranquilizers

For anesthetics, analgesics, sedatives, or tranquilizers that may be used, the name or class of agent, dose, routes, and schedule of administration should be indicated. It may be acceptable to provide a range for the dosage to allow flexibility in the procedure.[13] If halogenated inhalant agents are used such as isoflurane, methods for scavenging waste anesthetic gas should be described. Many institutions have a formulary of recommended anesthetics and analgesics developed by the attending veterinarian; agents from this formulary may be used preferentially. If your study requires use of an anesthetic, analgesic, or sedative that is not recommended by the attending veterinarian, you should consult with your veterinarian to determine an option that best meets both scientific needs and clinical recommendations. Nonpharmaceutical grade anesthetics such as tribromoethanol will require additional information and justification.[7]

If multiple survival surgical procedures are planned (ie, multiple anesthetic events), a scientific justification for the multiple survival surgeries will be required in addition to information on whether the surgeries are major or minor (as defined by your IACUC) and the time period (minimum) between 2 surgeries. If major surgery is conducted on animals prior to purchase and/or transfer to your animal facility, a brief description of the procedure and any measures taken during care and acclimation should be described. Performing multiple survival surgeries on animals as a way of reducing animal numbers is not generally accepted.[7]

Nonsurgical Procedures

Detailed, sequential description of all nonsurgical procedures is required. We recommend to the extent acceptable by your IACUC providing ranges for specific items, such as volume of blood being collected or drug dosages being administered. Acceptable ranges allow flexibility in the procedure while minimizing potential noncompliance as a result of going "off protocol."[13] Information that should be included:

- Administration of substances (route, frequency, volume). If there is known toxicity with any of the substances, provide details on monitoring parameters and planned intervention

if adverse effects are observed. Substances that are also hazardous to humans, either chemically or biologically, usually require additional information elsewhere in the protocol form.

- Blood withdrawal (site, frequency, volume, methodology). IACUCs may have established guidelines on blood sampling procedures such as a maximum limit of total blood collected per week, limit on the number of times a collection site is used, and minimum recovery period between collections. Any deviations from the guidelines should be scientifically justified.
- Imaging procedures (including methodology such as anesthesia, monitoring of animals and/or depth of anesthesia during imaging, maintenance of body temperature, etc.). The IACUC may also ask for other information such as location of equipment, transport to and from the location, any effect on animal well-being, and how equipment is sanitized between cohorts.
- Behavioral tests (methodology, equipment used, monitoring, and acclimation process). Any training given to animals prior to experiments, such as performing tasks for rewards, must be described along with any food or fluid regulation that is part of the motivation-reward process. Positive reinforcement methods are preferred over negative reinforcement as motivators to perform and the latter requires clear justification.
- In case of tumor cell lines injected for producing tumors in the animal, include additional details such as how tumor growth is measured, frequency of measuring, anticipated impact on health and well-being, endpoints for the study such as maximum size of tumor, ulceration, decreased body condition score or body weight, or other clinical impairments depending on tumor location.
- If prolonged physical restraint is performed for any purpose, the IACUC will ask for scientific justification and ensure that the duration of restraint is the minimum necessary for the research objectives.[7] Information in this section should include a description of the physical restraint device and a description of the acclimation procedure to the device and the prolonged restraint, including measures to minimize animal distress during the prolonged restraint procedure.

Postprocedural Care

Any veterinary or clinical care provided after a procedure—for example, tranquilizers, analgesics, antibiotics, heated environment, extra bedding—must be described. The anticipated clinical signs (lesions, behavioral changes, changes in vital signs), the plan of action, or signs for early euthanasia must be described.

Pain and Distress

Tolerance to pain and clinical signs exhibited in response to various painful stimuli vary between species, between strains and breeds, and even between different genders or individuals of the same species. Nonetheless, general assumptions are made regarding types of experiments as to whether pain or distress induced by experimental manipulations will be absent, momentary, or more sustained, and for the latter whether it will be relieved with anesthetic, analgesic, and/or tranquilizer drugs. Any nonpharmacological methods used to minimize pain or distress should also be described. Many IACUCs use the USDA's pain and distress classification, which is used for annual reporting on USDA regulated species (9CFR §2.36 a. 5-8). Briefly, the USDA pain categories are:

- Classification B: Animals being bred or held for use in research but not yet used for such purposes.
- Classification C: Animals upon which experiments will be conducted involving no or only momentary pain, distress, or use of pain-relieving drugs.
- Classification D: Animals upon which experiments will be conducted involving pain or distress, for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used.
- Classification E: Animals upon which experiments will be conducted involving pain or distress but use of anesthetic, analgesic, or tranquilizing drugs will adversely affect the experiments.

It should be noted that these are not necessarily step-wise levels of invasiveness. The USDA Pain Categories specifically focus on the use of drugs to alleviate pain or distress. For studies that induce a chronic disease condition such as tumor studies or infectious disease studies, the appropriate pain category may not always be clear. The investigator should seek guidance on how his/her IACUC would categorize a study of this type.

An Example of a Study Requiring Pain Category E: An efficacy study of a new analgesic drug being evaluated in a pain model would likely have a control group of animals that do not receive the test agent. These animals may have unalleviated pain by necessity of study design and thus would fall into Pain Category E. The use of a control group is scientifically justified but should be explained.

As stated in the *Guide* "for certain animal use protocols . . . the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns." This has been interpreted by some, such as AAALAC, as performing a harm-benefit analysis and may come into play for particular procedures that have potential to elicit pain or distress.

Category E studies raise the bar for a harm-benefit analysis, and a detailed explanation of why appropriate analgesics or other drugs cannot be used in these procedures is required.[14] For USDA regulated species, the number of animals on Category E studies will be reported to the USDA on an annual basis with the explanation for why pain-alleviating drugs were not used. IACUCs may recommend additional requirements such as use of nonpharmacological measures to mitigate pain or distress, increased frequency of monitoring, and establishment of humane endpoints to facilitate removal of animals from study as soon as research objectives are met. A clinical (or pain) scoring sheet is often helpful for studies where unmitigated pain or an advanced disease state is anticipated.[15] Such scoring systems may be used to predict death and permit early intervention with euthanasia to minimize the length of time an animal may experience pain or distress. Scoring systems also establish minimum requirements for frequency of animal observations. This level of detail of clinical monitoring, the stated frequency of monitoring, and specified endpoints (eg, attaining a numeric score greater than the stated threshold for euthanasia) help provide assurance to the IACUC that animals will be closely monitored and that criteria for euthanasia will be adhered to in an objective manner. The attending veterinarian should be consulted and may have examples of clinical scoring sheets as a starting point.

Endpoints and Euthanasia

Humane endpoint criteria are established to determine when euthanasia should be performed. Humane endpoints are not necessarily the same as experimental endpoints, as experimental endpoints are planned based on experimental and scientific needs, whereas humane endpoints recognize that some animals may need to be euthanized for ethical reasons prior to reaching their experimental endpoint. Some examples for indicators for euthanasia are tumor size, percentage body weight loss, inability to eat or drink, behavioral abnormalities, signs of toxicity, or clinical signs of severe infection or respiratory distress. These must be described along with an appropriate plan of action. Anticipated clinical signs and endpoints must be described for any studies that are known to cause significant signs or that may be potentially lethal, such as administration of tumor cells, biologics, infectious agents, radiation, or toxic chemicals. Euthanasia is usually expected at the earliest stage when scientific objectives have been achieved. If death of the animal is used as the experimental endpoint, the study will be rigorously evaluated by the IACUC. Therefore, the requirement for using death as an experimental endpoint and unrelieved pain and distress must be scientifically justified.

Sample Clinical Monitoring and Endpoints Language: Lack of grooming, dehiscence of surgical site, hunched posture, weight loss, and abnormal behavior that might indicate pain or distress will be monitored for at least once daily until incision is healed. We will ensure that the animals are eating, drinking, eliminating, and moving normally. If weight loss is >15% from presurgical weight or if we observe bleeding, wound dehiscence, evidence of infection (redness, swelling, and/or discharge), abnormal behavior, or signs of pain, the veterinarian will be consulted or the animal will be promptly euthanized.

The proposed method of euthanasia must be indicated and described. For example, if a chemical agent is used, specify the dosage range and route of administration. The method of euthanasia must be consistent with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals.[16] If the method of euthanasia is not consistent with these guidelines, a scientific justification must be provided and approved by the IACUC. Most protocols also ask the researcher to state how death will be confirmed, for example, use of cervical dislocation following CO_2 euthanasia of mice or confirmed lack of respiration for a specified period of time.

Other Relevant Information

Nonstandard Housing or Husbandry

Deviations from standard husbandry practices should be described in detail along with justifications for the same and any anticipated effects. This could include metabolic or other specialized caging, changes in light cycle, single housing, special diets, medicated water, food or fluid regulation, or a variety of other special study needs. Social housing of species that normally live in social groups is considered the default by OLAW and AAALAC; requests for single housing will be closely scrutinized by the IACUC and must have specific justification. If a departure from the recommendations of the *Guide* is required for the study, a scientific justification must be provided. Such departures must be approved by the IACUC and will be reported in the semiannual report to the Institutional Official by the IACUC.[17]

In the case of genetically engineered animals, it is useful to mention any anticipated phenotypic consequences (small size, aggressiveness, atypical behaviors) and any special care required (feed placed on the cage floor, separating individuals, need for extra enrichment).

Movement of Animals in and out of Housing Facility

Most institutions have transportation guidelines/policies and are subject to federal regulations when transporting animals on public roads and out of state. However, if animals are to be transported between facilities within the institution, such as for imaging or experimental procedures, it should be described on the protocol. Descriptions of the method, schedule, and route of transportation will help assess whether there will be any exposure risk to nonresearch personnel (eg, if a public elevator is used), if special containers/cages are required for transport, and whether special training is required for personnel transporting the animals. If vehicles are needed for animal transport, additional information should be provided regarding transportation procedures.

Species-Specific Enrichment

Animal programs are expected to provide appropriate enrichment to animals to promote physical and psychological well-being and expression of species-specific behaviors.[18] If seeking an exemption from enrichment, scientific justification must be provided to the IACUC along with the time frame during which enrichment will be withheld. In particular, any restrictions on the institutional plan for environmental enrichment for nonhuman primates or restrictions or the exercise and socialization plan for dogs must be described and scientifically justified.

Use of Nonpharmaceutical Grade Substances

When using nonpharmaceutical grade substances on live animals, the IACUC will require scientific justification for their use if a pharmaceutical grade alternative is available.[19] Investigators will also need to address the purity, sterility, storage, date of expiration, known side effects, and adverse reactions of the nonpharmaceutical grade substance.[7]

Hazardous Agents

When hazardous materials are used in animal research, many institutions require additional authorizations or approvals from other committees such as Environmental Health and Safety (EHS), Radiation Safety, or the Institutional Biosafety Committee. This is to ensure that appropriate approvals are in place prior to the conduct of the work with a hazardous material in the vivarium. These are usually categorized as biological, chemical, or physical hazards. The researcher should also be aware that additional training requirements will likely be triggered.

The IACUC may require the protocol to contain a description of the hazardous material (name, class, half-life), its use (administration, dosage, disposal), and how it may pose a risk to humans or other animals in the facility (metabolites produced, decontamination, toxicity levels). Additionally, the investigator may be asked to describe what necessary precautions (such as signage, notification of facility personnel, special personal protective equipment, husbandry measures) are being taken.

Biological Materials

Research involving the use of biological materials in animal research subjects is usually subjected to additional review and approval procedures. Materials such as cells of human or animal origin, recombinant or synthetic DNA, bacteria or viruses, viral vectors, tissues, and body fluids harvested outside the animal facility are required to go through screening procedures to ensure their safe use. IACUCs may require investigators to submit Environmental Health and Safety (EHS) and Institutional Biosafety Committee approval, results of pathogen profile, documentation of origin, and storage of the materials.

Investigators may also be asked to complete relevant sections of the protocol form to ensure that animal test subjects are housed appropriately, such as in Animal Biosafety Level 2 or 3 housing, with appropriate engineering standards, including biosafety cabinets and safety mechanisms such as suitable personal protective equipment for the animal care staff.

Personnel on the Protocol

The PI is the primary person responsible for the research proposal, the use of animals on the protocol, oversight of personnel in the laboratory, and implementation of research in compliance with institutional rules and federal laws and regulations. Additionally, the names, contact information including phone numbers for after hours and emergency situations, brief descriptions of qualifications and training, and any health and safety clearance information of personnel named on the protocol is required in this section. All individuals who conduct the procedures described in the protocol, including students, research assistants, other laboratory staff, visiting scholars, and/or volunteers should be listed on the protocol.

Training and Qualification

The IACUC will ask for the qualifications of the personnel listed on the protocol as well as information on the training these individuals have received. The training and qualifications review is often performed during the administrative pre-review of the protocol. This is to ensure that they are qualified to perform specific procedures listed on the protocol and are aware of the risks associated with the study.[20] Investigators may be asked to provide proof of additional training on topics such as laboratory animal allergies, good laboratory practices, or training on the use of hazardous agents.[21]

External Performance Sites and Collaborations

Field Studies

Scientific research on free-living wildlife in their natural habitats, or field studies, require unique IACUC consideration if the activities impact the animals or their environment in any way.[22] Activities that affect animals may be handling procedures with potential to cause harm, such as capturing, banding or tattooing, and biological sampling, or may be procedures with no direct contact that still affect animals such as playback of sounds, habitat manipulation, or approach by humans. To evaluate the potential impact of the studies, the IACUC may consult with experts (wildlife biologists, ecologists). Some of the aspects that the IACUC considers when reviewing field studies are applicable federal, state, and local regulations, permitting requirements, trapping methods, minimization of pain and distress, emergency euthanasia methods available, training of personnel performing these procedures, and whether the occupational health and safety program adequately addresses the risks associated with activities in the field.[23,24]

Contract Research Organizations (CRO)

For research conducted by external groups such as a CRO, the institution's IACUC is responsible for ensuring the humane care and use of animals in that project. The IACUC may request additional information such as study protocols, certain veterinary or husbandry information, or documentation on the technical staff for evaluation of the CRO. The extent and depth of evaluation by the IACUC may also depend on any certifications the CRO has, such as AAALAC accreditation, an Animal Welfare Assurance with OLAW, and/or active registration with the USDA.[25]

Updates and Revisions

During ongoing research work, investigators wishing to request changes to already approved IACUC protocols do so by submitting an amendment for IACUC review. This is often an unintentional but easy way in which a PI finds him/herself out of compliance by gradually deviating from the approved protocol as the research progresses and new experiments or refinements to existing experiments evolve. Minor changes (changes in personnel other than the PI, changes in funding, etc.) are usually reviewed and approved administratively. However, significant changes (change in species, surgical procedures, method of euthanasia, change in duration, number or frequency of a procedure) have the potential to negatively impact animal welfare and therefore must be approved through either FCR or DMR, as all amendments must be reviewed and approved by the IACUC prior to implementation. Some institutions have IACUC-reviewed and -approved policies by which certain significant changes can be handled administratively in consultation with a veterinarian authorized by the IACUC (NOT-OD-14-126).[26] As mentioned earlier, this process (also known as the veterinary verification and consultation) could have a faster turn-around time than FCR or even DMR and can help to reduce regulatory burden.

PI Assurances

Signed assurances from PIs on protocols vary with the IACUC and institutions; however, the common elements in most PI assurances concern appropriate use of animals and the safety and well-being of humans working with the animals. These assurances serve as statements from the PIs promising compliance with federal and institutional regulations, humane care and use of animals on their protocols, adequate training for research personnel, and participation on health and safety programs. A statement indicating that the research is not unnecessarily duplicative of previously published research is also often included.

Conclusions

Through the protocol review process, the IACUC evaluates proposed procedures and facilitates the conduct of ethical use of animals in research and education. The protocol review process is complex, and the quality of the protocol being reviewed can make all the difference in the efficiency with which an IACUC can conduct a thorough and complete review. Investigators should aim to provide all required information (using nontechnical language when possible) so that any IACUC member

reading the protocol can get a comprehensive understanding of the research that is proposed. Institutions should also work towards making this process as user-friendly as possible to minimize the associated administrative burden.[27] Well-designed protocol templates, use of standardized SOPs, availability of veterinary consultation and protocol pre-review, written examples of procedures, assistance with database reviews, tutorial assistance (either online or in person), coordination with other approval activities (EHS), and consistency in the review process are all possibilities that contribute to more efficient and straightforward reviews.[27]

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