Writing clear animal activity proposals

David M. Pinson, DVM, PhD, DACVP, DACLAM

Although IACUC-related topics are frequently discussed in the literature, there is little published information about how to write animal activity proposals. In this article, the author discusses key considerations in the writing and review of animal activity proposals. The author then describes a framework for developing and writing clear animal activity proposals that highlight animal welfare concerns. Though these recommendations are aimed at individuals writing and reviewing research proposals, the framework can be modified for other types of animal activity proposals.

The laboratory animal literature is replete with information on IACUC reviews, criteria for evaluation of animal activity proposals, IACUC-related vignettes and other IACUC-related topics[1–3]. But there is little published information explaining how to write animal activity descriptions. It is my experience that many IACUC-approved animal activity documents lack clarity, so that regulators and inspectors have considerable difficulty reconciling these IACUC-approved procedures with procedures that were carried out in the course of a research project. In this article, I identify some of the problems that may arise during the review of animal activity proposals. I also recommend a platform for developing and writing clear animal activity descriptions. I hope that this article will help IACUC proposal writers to determine what they should address in their proposals. While IACUC animal activity proposals contain many types of information required for managing research projects involving animals, this article focuses on writing protocols that highlight key required information, such as a complete listing of all procedures, clear communication of animal numbers, and pain and distress management strategies that clearly define humane endpoints.

The IACUC is federally mandated by both the Animal Welfare Act and Regulations (AWARs; part 2, subpart C, section 2.31d; ref. 4) and the Public Health Service *Policy on the Humane Care and Use of Laboratory Animals* (section IV.C.1.a–g; ref. 5). Put together, these documents apply to most animals used in research in the US. My recommendations in this article are applicable to all mammalian species commonly used in biomedical research. The IACUC is responsible for reviewing animal activity proposals for specific types of information about the procedures that will be carried out. Once the IACUC is satisfied that the required information is provided and that the animal activity has been thoroughly explained and justified, it approves the proposal and the animal activity can then begin. To help IACUC members review and interpret a proposal, all required information should be written as clearly as possible.

COMMON PROBLEMS

IACUC members quickly identify problems that commonly arise in the review of animal activity proposals. Because most US institutions follow the same general standards, similar issues are likely found at institutions across the country. Here I address some of these common deficiencies.

Consistency

A common problem encountered by IACUCs is inconsistency throughout the animal activity proposal. For example, authors might describe procedures in an abstract that are not included elsewhere in the proposal or they might state using one drug dose for a procedure in one section and a different dose for the same procedure in another section of the proposal. Another common problem is listing different numbers of animal or types of pain categories in experimental descriptions and in summary tables. Such inconsistencies almost always prompt the IACUC to request clarifications from the writer.

Pain and distress review

The AWARs require a consultation with a veterinarian for any proposed procedures causing or potentially

University of Kansas Medical Center, Kansas City, KS. Correspondence should be addressed to D.M.P. (davidmpinson@gmail.com).

causing pain or distress (part 2, subpart C, section 2.31d; ref. 4). The 2010 version of the *Guide for the Care and Use of Laboratory Animals* (the *Guide*)[6], which recommends that the attending veterinarian be charged with oversight of all aspects of animal medical care and animal well-being, clearly states that veterinarians should be involved in pain management. Given these requirements, many investigators view veterinarians as regulators or 'research police', which can prompt adversarial conditions for the veterinarian. Some research scientists also claim that veterinarians have never carried out bench research or that veterinarians cannot understand the science behind a study. These arguments may have little foundation, and nonetheless, the requirement for consultation remains. The veterinarian consultation should always be well documented in writing.

Literature review

The AWARs (part 2, subpart C, section 2.31d; ref. 4) and the United States Department of Agriculture (USDA) *Animal Care Policy Manual* require animal activity proposals to include a literature review (policy #12; ref. 7). This literature review serves two primary functions: first, to ensure that the writer has considered alternatives to procedures that cause pain or distress, and second, to ensure that the research does not unnecessarily duplicate previously published work. Most IACUCs require that research scientists carry out literature searches and list keywords in their animal activity proposal. These literature searches often include keywords for scientific terms but lack keywords for painful procedures and specific searches for alternatives. Literature reviews written by inexperienced writers frequently do not address the two primary functions of the literature review.

The USDA *Animal Care Policy Manual* policy #12 (ref. 7) requires scientists to list the sources (such as databases, professional consultants or scientific journals) that they used to search for alternatives. Databases such as PubMed or Medline are often named. I recommend that writers include at least one animal welfare database, links to which can be found on the Office of Laboratory Animal Welfare website (http://grants.nih.gov/grants/olaw/links.htm#DBS), in the literature review.

Experimental design

Various problems can be found in the experimental design portion of animal activity proposals. For example scientists often use wording or jargon commonly used in grant proposals or simply copy the research plan of a grant into the animal activity proposal. A grant proposal and an IACUC proposal have two very different purposes. Writing an animal activity proposal in grant style often creates confusion and ambiguity about specific procedures, timelines and pain management. Using grant proposal jargon also makes it difficult for post-approval monitors to reconcile research records with IACUC-approved documents. Animal number justifications often lack clarity, particularly when a single power analysis is used for many experiments with different read-outs, endpoints and statistical parameters.

Surgical plan

IACUC protocol writers without formal medical training may not be accustomed to writing surgical plans or records. Inexperienced writers often leave out essential information. Veterinary professionals can make the task of writing surgical plans much easier for investigators who are not medically trained by providing templates that include details such as pre-operative preparation, surgical approach, wound closure and post-operative monitoring.

Monitoring plan

Almost all IACUCs require the inclusion of a monitoring plan in animal activity proposals, as is recommended by the *Guide*[6]. Monitoring plans are often subjective and cryptic. For example, these plans might include statements such as "we will monitor the animals for any signs of pain or distress" or "we will monitor for weight loss". These statements present challenges: for example, animals frequently hide pain, and there may be no tangible criteria given for "weight loss". Monitoring plans should be carefully crafted, taking into consideration the goal of the experiment, any experimentally created defects (such as the type of surgery, the genetic modification or the drug used). Because animals often hide pain, the monitoring criteria for pain should be as objective as possible.

Criteria for removal from study

Choosing criteria to be used to determine whether an animal should be removed from a study is a delicate process. Scientists must balance the goals of the study with the welfare of the animal subjects. In my experience, the primary problem for IACUC proposals is that the criteria for removal from a study are often subjective. For example, criteria might include statements such as "animals will be euthanized if pain or distress is observed". Ideally, criteria should be objective and should be chosen based on the proposed monitoring plan. Examples of objective criteria include a 15% weight loss over a 2-week period or a tumor size of 1 cm on a nude mouse.

Euthanasia plan

The euthanasia plan is a part of the animal activity proposal in which veterinarians should be involved. Researchers and veterinarians should consider possible side effects of different methods of euthanasia, such as acid–base abnormalities associated with carbon

dioxide euthanasia, abdominal irritation associated with the use of barbiturates and pain associated with muscle injections. When determining which euthanasia method to use, researchers and veterinarians should consider the type of data being collected (as certain euthanasia methods may preclude the collection of certain types of data) and should refer to the American Veterinary Medical Association *AVMA Guidelines on Euthanasia*[8].

Record-keeping

IACUCs and investigators are required to keep medical and research procedure records (policy #3; ref. 7). Medical records should include all diagnostic procedures, diagnoses, treatments, drugs administered, anesthesia, surgeries, outcomes and terminal pathology. Records of all research procedures should be kept in a format that is easily accessible and should include drugs administered, procedures done, personnel involved, proof that the monitoring plan has been completed as required by the IACUC and experimental endpoints. The animal activity proposal should describe how experimental and medical records will be maintained to show that the protocol has been carried out as approved. Investigators should design this record-keeping paradigm before starting the study, so that they are ready for post-approval IACUC monitoring visits and USDA regulatory visits.

Training plan

US federal animal research regulations and policies require adequate training of animal care and use personnel (AWARs; part 2, subpart C, sections 2.31d (viii) and 2.32; refs. 4–6). Training programs often include laws, policies, use of drugs and anesthetics and recognition of pain in animals, among other topics. However, the intent of the AWARs is for personnel to be trained to carry out the specific procedures required of them. The AWARs state that "[p]ersonnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures" (subpart C, section 2.31d (viii); ref. 4). This implies that training should be tailored to the research project. Thus, when writing an animal activity proposal, investigators should consider what each person will be doing in the project and how each person is trained (or must be trained) in order to carry out the work required of him or her. Records of that research-specific training should be available to document the training and competence of each individual involved in the research project. The AWARs also require that training be reviewed by the IACUC. Specifically, the AWARs state that "[t]raining and instruction shall be made available, and the qualifications of personnel reviewed, with sufficient frequency to fulfill the research facility's responsibilities under this section and [section] 2.31" (subpart C, section 2.32b; ref. 4). This means that personnel qualifications should be reviewed to be certain that training is up-to-date. It is prudent for the investigator to consider specific training programs for his or her employees, to include documentation of these training programs in the animal activity proposal and to have these documents available for regulators and monitors.

A FRAMEWORK FOR WRITING PROPOSALS

To help address some of the common problems with animal activity proposals, I developed a framework for IACUCs and for investigators writing animal activity proposals. These suggestions are focused primarily on research proposals but can be modified for other types of animal activity proposals. My recommendations for animal activity proposals are that they should be clearly written and should focus primarily on animal welfare issues, without ignoring the science of the study. The primary premise of these recommendations is that if it is easier for IACUCs and regulators to find and evaluate the animal welfare information that is required for review, then the review process or the regulatory site visit will be easier as well.

Pain and distress consultation form

The AWARs (part 2, subpart C, section 2.31d(iv)B; ref. 4) require that investigators consult with veterinarians to determine how to manage potential animal pain and distress. I recommend that veterinary consultations for the development of a pain and distress proposal be documented using a pain and distress consultation form. For example, the pain and distress consultation form used by the University of Kansas Medical Center includes the following information: study title and investigator name and contact information; a brief project overview; a table listing all experimental procedures (injections, surgeries, drugs and activities) and the frequency with which they will be carried out; the investigator's plan for managing pain or distress; the veterinarian's comments or suggestions for improving the plan; any proposed euthanasia methods; and date and signature blocks for the investigator and the veterinarian.

When filling out the form, the veterinarian scores the potential for pain and distress and the duration of that distress for each procedure listed (**Table 1**). This scoring paradigm is subjective, and each veterinarian's perspectives on the level of pain associated with a particular procedure are influenced by his or her training, experience and personal empathy for what the animals will experience. Though it is subjective, the scoring paradigm allows veterinarians to categorize the level and duration of pain or distress they think will result from specific procedures. The AWARs[4] do not require that the level of and duration of pain be scored but do require

TABLE 1 | Pain and distress consultation form for animal activity proposals

Species	Procedure	How many times per animal?	Pain/distress intensity score	Pain/distress duration score
Mouse	Ovariectomy	Once	3	2
Mouse	Pellet implant	Twice	2	1

The pain/distress intensity score uses a scale of 1–5 to describe the overall intensity of pain or distress potentially caused by the procedure based on the veterinarian's understanding (1 is the least painful; 5 is the most painful. The pain/distress duration score uses a scale of 1–5 to indicate how long pain or distress potentially caused by the procedure will last (1, 1 d; 2, 2–4 d; 3, 1 week; 4, 2 weeks; 5, constant, unremitting pain).

that the consultation be carried out and that consideration be given to pain and distress management. It is the IACUC's responsibility to decide whether the investigator has included the veterinarian's suggestions in the proposal development. The veterinarian has no authority to mandate the recommendations, but the IACUC does have this authority (subpart C, section 2.31d; ref. 4).

Literature review

The literature review section of the animal activity proposal should include the search terms used and the results of the literature search. Search terms should include both the keywords for the scientific topics being studied and the keywords for the painful procedures. The name of a painful procedure should be searched for, together with words such as 'pain', 'distress' and 'alternatives'. At least one animal welfare database should be used (such as Agricola or Altweb). In the results summary, the investigator should write a paragraph describing how he or she has looked for alternatives to procedures that produce pain or distress (and whether these alternatives may or may not be appropriate for use in this study) and a paragraph confirming that the research will not be unnecessarily duplicating previous studies. As these two points are required by federal regulations, investigators should label the paragraphs with headings, such as 'Alternatives to painful procedures' and 'Nonduplication of previous research,' to ensure that they stand out.

Experimental design

In the experimental design section of the IACUC proposal, scientific jargon should be avoided. This section should cover each experiment that will be carried out during the study. To avoid confusion, I recommend that writers divide up the study into simple, small experiments that are easy to understand. Before writing this section, it is helpful to make an outline that highlights animal welfare issues that will be discussed in this section. For each experiment being described in the experimental design section, I recommend writing the following (clearly labeled) sections.

Experimental overview. The overview is a very brief paragraph describing the science behind a particular experiment. It should state what specific question is being asked and answered by the experiment and should provide a scientific context for the experiment. This paragraph should be free of jargon and should be written for a lay audience.

Bulleted list of all animal procedures being carried out in this experiment. A comprehensive list of all surgical procedures, nonsurgical procedures, activities, drugs administered to the animals (including dose and administration route) and euthanasia method(s) should be included. Identifying all of these procedures in one location makes it easy to find the descriptions and to verify which procedures were approved of in the protocol. USDA inspectors and post-approval IACUC monitors can also quickly and easily find approved procedures.

Experimental plan. This is a description of the experimental approach being used to answer the experimental question. This section should include tables identifying animal groups, treatments, numbers of animals in each group and the expected pain classification for each group. Use of the pain categories (B, C, D and E) required for USDA annual reports (AWARs, subpart C, section 2.36; ref. 4) is recommended. The experimental plan should state the only names of the surgical or non-surgical procedures; details of these procedures should be provided in two separate sections to keep the experimental plan section clear and concise.

Justification of the number of animals required. Researchers can use either a power analysis or any reasonable and logical argument to justify the number of animals required for their experiments. The justification might include a literature review of the number of animals typically required for this type of experiment. Justification should be provided for the number of animals used in each experiment, because experiments with different objectives and endpoints will have different statistical parameters.

Timeline. A timeline should be provided for each experiment. When reading animal activity documents, IACUC members and USDA officers often have difficulty understanding when a procedure will be done in an experiment and how many times this procedure will be carried out. Readers are often confused about when the

experiment will end. If they are well presented, experimental timelines should help clarify these issues.

Pain and distress management plan. This section should describe the pain and distress management plan that will be used in a particular experiment. This plan should identify any drugs that will be given and the dosage, administration route and frequency of drug administrations. Any other environmental modifications being used to increase the comfort of the animals should also be included. This plan should agree with the pain and distress consultation, and justification should be provided for any deviations.

Humane experimental endpoints. Investigators should clearly define what constitutes the end of the experiment, as well as any criteria used to determine whether animals will be euthanized or removed from the study in order to minimize pain and distress to animals. These endpoints should be objectively, rather than subjectively, defined. The IACUC ultimately decides whether or not these endpoints are clearly stated and reasonable and can require modifications of such endpoints (subpart C, section 2.31d; ref. 4). Regulators can and will evaluate experimental data records to ensure that the endpoints used in the study match with those described in the proposal[9]. Post-approval IACUC monitors should do likewise.

Breeder management. It is often unclear how many unusable animals will be produced in various breeding paradigms, particularly when genetically modified animals are being bred. Unusable animals, those animals of inappropriate genotypes for a specific experiment, are still a part of the experimental paradigm and must be accounted for in the experimental design, so that adequate animal numbers are requested to produce sufficient animals for the experiment.

Surgical design

Investigators should provide several types of information, as described below, for each surgical procedure being carried out in the study.

Pre-operative preparation. First, investigators should describe any pre-operative preparation, such as overnight fasting and pre-administration of analgesics or sedatives to reduce distress.

Anesthesia plan. Second, an anesthesia plan, describing the induction and maintenance of anesthesia, should be included. This plan should also describe how personnel will monitor the depth of anesthesia throughout the procedure.

Surgical plan. In the surgical plan, investigators should define all of the parameters of the surgery, including specific site location, site preparation (such as hair removal and disinfection), surgical approach, manipulation and specific closure methods (such as layered closure or wound clips).

Post-operative plan. In this section, investigators should describe where recovery will occur, which types of monitoring will be carried out and the frequency and duration of monitoring. Additionally, investigators should explain how they will determine that animals have recovered from anesthesia. The IACUC can require modifications here, if necessary, to ensure the well-being of animals (subpart C, section 2.31d; ref. 4).

Non-surgical procedures

The proposal should include a clear description of all non-surgical procedures and activities, such as behavioral tests, injections and administered drugs (dose, route and frequency), compounds (dose, route, frequency and toxicity) immunogens or infectious agents. For each procedure, investigators should state the clinical expectations and outcomes.

Experimental monitoring plan

Many research manipulations on animals result in distress or pain. IACUCs must review the monitoring plan to make sure that it addresses any physiologic problems that could be caused by the research (AWARs, subpart C, section 2.31d; ref. 4). This plan should be evaluated in the context of the pain and distress consultation and management plan. The monitoring plan should be based on the experimental manipulations and should be relevant to those manipulations. The more objective the post-procedural monitoring criteria, the clearer the monitoring plan will be. Investigators should maintain records so that they can prove to post-approval IACUC monitors and regulators that a monitoring plan has been used during the study as approved by the IACUC.

Criteria for removal from study

Investigators should clearly link the criteria for removing an animal from the study to the monitoring plan. These criteria should be as objective as possible. If applicable, investigators should maintain records that indicate when the criteria were met for specific animals that were removed from a study.

Euthanasia plan

Investigators should choose a euthanasia method that is appropriate given the type of data being collected. The *AVMA Guidelines on Euthanasia* provides valuable information on the various methods of euthanasia[8].

Record-keeping plan (preparing for post-approval monitoring visits)

The 2010 version of the *Guide* suggests implementation of post-approval monitoring programs[6]. USDA

inspectors reviewing research facilities can and do request research and medical records[9]. Investigators should maintain research and medical records sufficient to prove that the animal activity protocol was carried out as approved by the IACUC. Institutions should create policies that require investigators to keep such records. These records should clearly identify all research procedures, personnel involved in those procedures, drugs and materials used during these procedures and all monitoring points indicated in the approved IACUC proposal. Medical records, as required by the USDA *Animal Care Policy Manual*[7], must include all treatments, procedures, drugs (include route and dose), clinical findings (monitoring points), laboratory diagnostics and pathology results. Personnel should develop record templates unique to each protocol; these templates should be fully completed and maintained in a place where they are readily accessible for review. Veterinarians can assist personnel who are not medically trained to develop an acceptable medical record. It is just as important to have clear medical and research records as it is to have a clear IACUC proposal.

Personnel and training plan

Institutions are required to establish training programs and to document that laboratory animal care and use personnel receive adequate training[4–6]. Moreover, the IACUC must review personnel training with sufficient frequency to ensure that training is current and relevant for the role of personnel in the particular research project. To document that personnel have received effective and appropriate training, detailed training records must be maintained. An individual should receive training that is relevant to the tasks he or she is asked to carry out in any animal activity protocols or the animal care and use program. As a result, training programs should be unique to the individual, should be updated periodically and should be reviewed by the IACUC or the post-approval monitor for the IACUC.

CONCLUSIONS

Though not comprehensive, the above recommendations address many of the problems that IACUCs face on a regular basis. I hope that this document will provide some guidance for writers about how to develop and write clear animal care and use proposals.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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