## Tough decisions about protocol review

**To the editor:**

How Institutional Animal Care and Use Committees (IACUCs) balance protocol review ideology with reality was the topic of a panel discussion held at the 66th National Meeting of the American Association for Laboratory Animal Science. The discussion began by characterizing the essence of the IACUC. The term "essence" refers to the intrinsic nature or indispensable quality of something, especially something abstract that determines its character. The Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy) and the Animal Welfare Act and Regulations (AWAR) require an IACUC to be composed of representatives from various constituencies that collectively set a tone for the committee's character[1,2]. These representatives include members who understand the science, those who contribute the voice of nonscientists, and individuals unaffiliated with the IACUC's institution. At a full committee meeting, decisions regarding animal use requests can only be conducted when a quorum of voting members is present. This requirement meets the intent of having the "essence" of the committee participating in the deliberations.

Key to discussing tough protocol issues and making decisions about animal use requests is to define what constitutes a "protocol review." Both the PHS Policy[3] and AWAR[4] require the IACUC to review those components of the activity (the protocol) that describe the proposed animal use, and specify some topics that must be addressed by the Principal Investigator (PI) in the animal use proposal. Many of the required topics can be categorized according to **Table 1.**

**TABLE 1** | Topics that must be addressed by the Principal Investigator (PI)

| |
|---|
| **Rationale and purpose of the proposed animal use, including:** |
| Relevance of the study to the advancement of knowledge for the benefit of humans and other animals |
| Aims of the study |
| Assurance that the proposed work is not unnecessarily duplicative |
| Rationale for the species to be used |
| Rationale for the number of animals to be used |
| Consideration of alternatives to the use of animals |
| |
| **Assurance that discomfort and pain will be limited to that which is unavoidable for the conduct of scientifically valid research, including:** |
| Alternatives to painful or distressful procedures and measures to minimize pain and distress |
| Pre- and post-procedural care |
| Assessment parameters for post-procedural monitoring and the frequency of monitoring |
| Determination of how clinical signs will be managed |
| Selection of study endpoints and the rationale for their selection |
| Criteria for removing animals from the study before the selected endpoints |
| Skill and experience of the research team |

Despite regulatory language about topics that must be deliberated on during protocol review, research suggests that IACUCs might not be fulfilling this federally mandated responsibility according to the intent of the regulations and the PHS Policy. **Table 2**, from a recent study, lists the top seven topics most frequently discussed and four of the five topics least frequently discussed during protocol review[5].

The total number of times that each topic was mentioned was subdivided to indicate the role of the IACUC member that initiated the discussion on that topic[6]. For the five most frequently discussed topics, different IACUC members mentioned them in nearly the same rank order, suggesting that the "essence" of the committee was thinking alike, despite the varying constituencies they represent, i.e., scientist, veterinarian, non-affiliated, etc. Similarly, for the four topics discussed the fewest number of times, the rank order was similar between IACUC members representing different constituencies.

The topics that were most often addressed during the protocol review were related to statistics, study design, selection of the appropriate anesthetic, and appropriate monitoring interval. Such issues are easier to objectively evaluate and quantify, and, thus, more likely to require revision prior to approval.

The tougher issues for the IACUC to address are those that are more subjective and less easily quantified. Addressing these relies on multiple perspectives and on engagement of the collective essence of the committee. Such issues include the societal value/benefit of the studies, knowledge advancement, species choice rationale, and alternatives to using live animals as research subjects. These topics were discussed least often. As a result, there is a lower likelihood that the investigator will be required by the IACUC to clarify or expand on these topics, and the IACUCs may run the risk of missing critical information that could impact their decisions regarding the proposed activity.

**TABLE 2** | Least and most frequently discussed topics during protocol review

| Most discussed topics | Number of times mentioned (times/protocol) |
|---|---|
| Pain/distress | 816 (9.4) |
| Procedures performed | 770 (8.9) |
| Study design | 659 (7.8) |
| Form complete | 561 (6.4) |
| Animal death (study endpoints, euthanasia) | 543 (6.2) |
| Skill/experience | 358 |
| Numbers justification | 297 |
| | |
| **Least discussed topics** | |
| Species justification | 171 (2.0) |
| Purpose/aims justify animal use | 83 (1.0) |
| Scientific/clinical benefit justify animal use | 43 (0.5) |
| Alternatives | 29 (0.3) |

The top seven topics most frequently discussed (topics 1–7) and four out of the five topics least frequently discussed (topics 13–16) during protocol review are listed.

At the conclusion of the review, subsequent IACUC approval is interpreted to mean that the IACUC is satisfied that it has fulfilled its responsibilities in the review of the protocol and that the proposed animal use meets federal requirements. Approving animal use requests with minimal or no discussion by the essence of the committee on these "tougher issues" therefore raises the question of whether or not IACUCs are "doing their job."

During the panel discussion, five scenarios were presented to the audience in which the animal study proposal involved a "tough decision." Following the presentation of each scenario and the relevant federal regulations, the audience was asked for its thoughts on the issue, which was then followed by comments from the OLAW and USDA representatives on the panel.

**Scenario 1**

*The Great Southwest University IACUC unanimously approves a policy allowing all-new and three-year renewal protocol applications to be reviewed by means of a Designated Member Review (DMR), unless a Full Committee Review (FCR) is requested by a committee member.*

*Additional information: The IACUC meets monthly and has a business process for processing and reviewing protocols on a monthly cycle for both FCRs and DMRs.*

The relevant federal language related to this scenario comes from the AWAR section §2.31, d, 2 (ref. 7) and the PHS Policy, section IV, C, 2 (ref. 8): "If full committee review is not requested, at least one member of the IACUC qualified to conduct the review . . . shall review those research projects and have the authority to approve, require modifications to secure approval, or request full committee review of the project."

The scenario raises the following question: given that the Great Southwest University IACUC meets monthly, does the DMR process, which entails review by at least one IACUC member designated by the Chair but not a discussion by at least a quorum of the full committee, have the same thoroughness as when the full committee deliberates the animal use request? In other words, does the essence of a FCR differ substantially from that of a DMR, when only the assigned IACUC member and perhaps one other individual read the entire protocol?

Relevant to this scenario are recent unpublished data from a study conducted by J. Silverman *et al.* that calculated the odds ratio of another IACUC member raising a topic during the review of a protocol if the topic was mentioned by an assigned primary reviewer or veterinary reviewer during the introductory summary of the protocol application to the committee. The data showed that the topics with the highest odds ratio (i.e., the topics most frequently brought up by other IACUC members once they were mentioned by the primary reviewer) are in the group of "tough topics" described above. This finding may indicate that IACUC members have a subconscious reluctance to be the first to raise tough issues, but have less hesitation once a touchy topic is mentioned. Summary responses from the audience and from OLAW and USDA can be found below:

**Audience**

- If one or two IACUC members assigned to conduct DMR are the only individuals thoroughly reviewing the protocol and making the outcome decision, then the essence of the committee and the intent of a FCR may not be met.
- DMR is unlikely to be equivalent to FCR in terms of the diversity of represented views and detail of deliberations, but a well-run DMR process may accomplish a high quality protocol review, satisfy the essence of the committee, and satisfy the regulatory expectation of a committee review.
- The regulations require that all IACUC members have access to the protocol under review, including descriptions of all proposed animal use, but provide no specific criteria for IACUC members to consider when deciding whether to request FCR.
- If DMR is being used the way it was designed, it is believed that the intention is for each member to have read the protocol in order to decide whether to call for FCR, and if the member feels that there is no need to call for FCR, then the member has effectively weighed in on the need for full committee deliberations. Failure of members to do the above is misapplication of the DMR mechanism.

**OLAW/USDA**

- The DMR process is an acceptable process for reviewing proposed animal use and approving that use.
- There is truth to the idea that having more individuals contributing to the deliberations is of value, and this, in fact, is the idea behind requiring more than one IACUC member to participate in other committee responsibilities, such as semiannual evaluations of animal programs and facilities.
- So, the devil is in the details of the DMR process for ensuring that the IACUC is functioning as intended: for example,
  - Does the IACUC Chair select the DMR members based on the specialized knowledge critical for evaluating the proposed animal use?
  - Are the designated reviewers the same individuals regardless of the proposed use?
  - Are *ad hoc* consultants brought in to round out the expertise of the designated reviewers?
  - How is the input from non-scientists and the unaffiliated members received, i.e., how are the public's concerns brought into the discussion?
- Do IACUC policies and institutional culture encourage IACUC members to ask for more information and to provide a critical evaluation, even if they are not designated reviewers?

**Scenario 2**

*For FCR at the Great Southwest University, both the protocol and the reviewers' questions and comments are made available electronically to committee members before and during the meeting.*

The relevant federal language related to this scenario comes from the AWAR section §2.31, d, 2 (ref. 7) and the PHS Policy section IV, C, 2 (ref. 8): "If full committee review is requested, approval of those projects may be granted only *after review* at a convened meeting of a quorum of the IACUC and with the approval vote of a majority of the quorum present."

The scenario raises the question: Does having written comments available constitute a full committee review equivalent to IACUC deliberation, or is a verbal discussion needed? Summary responses from the audience and from OLAW and USDA can be found below:

**Audience**

- Having reviewer comments available at the committee meeting promotes more discussion than would occur if the reviewers' comments were processed without presentation to the committee. Comments should be viewed more as a stimulus for further discussion, not as "the discussion."

- It is important that the committee chair's, the veterinarian's, and the primary reviewer's concerns are available to call Committee members at the meeting.

**OLAW/USDA**

- The OLAW's and USDA's position is that real-time communication should occur during convened meetings[9].

- However, how much discussion is appropriate depends on the topic and the pre-review process.

- Available reviews at the time of the committee meeting make a good launching point for further deliberations and can focus discussions.

**Scenario 3**

*At the Great Northwest University, the IACUC is reviewing a protocol in which it is being proposed that a group of 10 different experiments be conducted on each of 24 different genetically modified mouse lines.*

The relevant federal language related to this scenario comes from the AWAR section §2.31, d, 1, iii[7] and the 8th edition of the *Guide*[10], page 26: "The principal investigator has provided written assurance that the activities do not unnecessarily duplicate previous experiments."

The scenario raises the question: When is repeating the same experiment in different genetically modified mouse lines considered duplication of research? Summary responses from the audience and from OLAW and USDA can be found below:

**Audience**

- The concept of replication is separate from that of unnecessary duplication:

  - "Replication" is just that, replication, i.e., the exact same experiment was repeated.

  - Replication is necessary to establish reliability of the data.

  - "Duplication" means copying something, in this case the experiment.

- Repeating the same experimental paradigm in different mouse lines is not replication of an identical experiment even if the methodology is identical, because outcomes and scientific data may differ depending on the genetic background.

- To demonstrate that the experiments in the different mouse lines are not unnecessarily duplicative, the PI needs to explain the basis for using each mouse line: i.e., what objectives are being sought with the various mouse lines? How is each mouse line contributing to testing the study hypothesis?

**OLAW/USDA**

- The federal regulations are clear: unnecessary duplication is not allowed.

- The IACUC is responsible to ensuring that proposed animal use is not unnecessarily duplicative.

  - The IACUC should require that the PI provide an explanation that demonstrates the unique value of each mouse line to the study hypothesis.

- The IACUC must be provided with a rationale for the number of animals that will be used to achieve the scientific goals.

**Scenario 4**

*At the Great Eastern University, the IACUC is reviewing a protocol with a hypothesis in which it is difficult for the committee to envision the experimental outcomes being translatable to the clinical, scientific, or environmental setting.*

*Proposal: A senior investigator proposes to use a mouse model of Disease A, a disease commonly found in humans, to evaluate the efficacy of a drug in suppressing Gene 1, a gene thought to be involved in the disease's progression. The hypothesis is that in the mouse model the drug will suppress Gene 1 expression, minimize disease progression, and allow determination of the role of Gene 1 in disease progression.*

*An IACUC reviewer knowledgeable about the human disease and the mouse model brings forth a recent publication that suggests that in humans, Gene 1 expression is naturally suppressed as the disease progresses, while in the mouse model Gene 1 expression increases with disease progression.*

*The IACUC member asks if the mouse study will have any relevance to understanding the role of Gene 1 in the human disease.*

The relevant federal language related to this scenario comes from the AWAR section §2.31, e, 2 and the US Government Principle 2 (ref. 6): "Procedures involving animals should include a rationale for using animals [AWAR] and be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge or the good of society [US Government Principles]."

The scenario raises the question: How is knowledge advancement evaluated for basic science studies when their translatability

to clinical or another important use is unclear? How is knowledge advancement determined? Summary responses from the audience and from OLAW and USDA can be found below:

**Audience**

- A single publication that "suggests" that the model is not appropriate is not sufficient reason for concluding that the mouse model will have no value.
    - The mouse model may elucidate differences between the mouse and human condition that may be informative for developing new therapeutic approaches.
    - If the PI did not mention the single publication in the protocol, he/she should be asked to explain why the mouse model is of value.

**USDA/OLAW**

- The onus is on the IACUC to get clarification from the PI about the potential impact of the publication on the rationale for performing the study.
- The IACUC should have a clear understanding of the PI's premise for the study and the relevance of potential findings to the stated aims.

**Scenario 5**

*At the Great Southern University, the IACUC is reviewing a protocol for an internally funded project designed to meet a degree requirement for a "research experience."*

*Proposal: Using 10 rats for each closure method, a surgery resident proposes a project to determine if it is more cost-effective to close a clean surgical skin wound with an absorbable multifilament skin suture or a monofilament skin suture. Both have been shown to be clinically effective. Cost-effectiveness will be evaluated as the time required to suture the wound and time required for post-surgical care.*

Regarding relevant federal language: there is nothing specific in US regulations concerning a requirement to perform a harm:benefit analysis. The European Union Directive (Art. 38,2,d)[11] requires "a harm-benefit analysis of the project to assess whether the harm to the animals in terms of suffering, pain and distress is justified by the expected outcome."

However, US Government Principle 2 (ref. 12) and PHS Policy[3] review criteria include consideration of the research design, justification for using animals, the advancement of knowledge, and the good of society. In addition, the *Guide* (page 4) states: "Using animals in research is a privilege granted by society to the research community with the expectation that such use will provide either significant new knowledge or lead to improvement in human and/or animal well-being."

This scenario raises the following questions: (a) What is meant by an adequate harm:benefit analysis? (b) How does the option of conducting the study in humans weigh into the harm:benefit analysis of using live animals? (c) Should the analysis be similar for projects that fulfill an academic degree or residency requirement for a "research experience," as may occur in research training programs? Summary responses from the audience and from OLAW and USDA can be found below:

**Audience**

- With a component of animal welfare, i.e., post-op care, included as part of the outcome measures, this may be considered an acceptable study by the IACUC.
- The protocol must address alternatives, and the IACUC is responsible for ensuring that this information is in the protocol.
- The harm:benefit analysis should be the same as for any study using live animals.
    - The difficulty is establishing the appropriate factors on which to evaluate harm and benefit.
- Assuming this was internally funded and a scientific review of the animal use was not conducted, the IACUC should engage an outside consultant to review the protocol, unless the IACUC has the appropriate scientific expertise.

**OLAW/USDA**

- Required animal-based research opportunities for students may fall under the research facility's program for humane care and use of animals if it involves animals in the research. Therefore, the IACUC may wish to review this portion of the program in detail. For example, IACUCs may wish to understand the process for choosing and developing projects and to address questions regarding the scientific rationales for using animals in such projects. For instance, IACUCs may wish to ask if there are measures in place to ensure availability of student research opportunities that are deemed appropriate by the IACUC, such as provisions to join an ongoing project rather than undertaking a project that might have an unclear value.
- USDA: IACUCs are placed in tough position in this scenario because AWAR section §2.31[7] says, "Except as specifically authorized by law or these regulations, nothing in this part shall be deemed to permit the Committee or IACUC to prescribe methods or set standards for the design, performance, or conduct of actual research or experimentation by a research facility"; and yet the law itself, section 2142(§2143(b)(1)), states, "Such members shall possess sufficient ability to assess animal care, treatment, and practices in experimental research as determined by the needs of the research facility and shall represent society's concerns regarding the welfare of animal subjects used at such facility." (2.31(a) and §2143(b)(1). So, one way to address this is to obtain additional information from the PI about the exact benefit envisioned. If it's a teaching activity: What are the teaching benefits? What are the scientific benefits?
- This scenario reinforces the fact that the onus is on the IACUC to ensure that the protocol provides adequate information for the IACUC to understand the benefits of the project before granting approval to use live animals for the study. It is up to the IACUC

to ensure that they have satisfactory explanations in response to any concerns raised by committee members.

## Conclusion

There were two common themes throughout the discussions. (1) The federal regulatory requirement that the IACUC represent multiple constituencies, including scientists, veterinarians, non-scientists, and individuals unaffiliated with the IACUC's institution, is meant to ensure that the essence of each constituency is part of the deliberations about each proposed animal use. IACUCs can use either the FCR or DMR process, but the DMR process must ensure that the protocol is reviewed by content experts and that there are a culture and process that encourage input from all constituencies. (2) The content addressed least frequently in IACUC deliberations relates to the "tough issues" described previously: the societal benefit of the studies, knowledge advancement, justification for the species selected and for the number of animals that will be used, and alternatives to using live animals as the research subjects. While not all of the "tough issues" may be highly relevant in every proposed animal use that an IACUC considers, the onus is on the IACUC to ensure that this information is included in the protocol in a manner understandable to all committee members prior to the protocol being approved.

These themes reinforce the responsibility of the IACUC to ensure that animal care and use follows all applicable federal regulations, the standards of the *Guide*, and addresses the public's concern for animal welfare.

**COMPETING FINANCIAL INTERESTS**
The authors declare no competing financial interests.

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