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Scientific justification for exemptions

Several studies have indicated that different stressors can affect research outcomes when using laboratory animals. David Fuller was of the opinion that euthanasia by carbon dioxide asphyxiation would be stressful to his mice because of the sound of the gas entering the euthanasia chamber, the potential for pain as the gas traversed the mucous membranes of the animal's nose and the possibly distressful sensation of hypoxia as the gas gradually displaced the air in the chamber. Carbon dioxide inhalation was the most common means of euthanasia used in the Great Eastern University animal facility; therefore, Fuller requested permission from the IACUC in his protocol to bring mice to his lab where they would be immediately euthanized by cervical dislocation, a technique with which he had many years of experience. Because the

IACUC also required scientific justification for any request to remove animals from the vivarium, even for a 'one way' trip, Fuller explained that his lab was in a far wing of the building, that it would take 10 minutes to bring tissues from the vivarium to the lab and that this delay between euthanasia and the enzyme extraction process that he planned to use was simply too long.

"Why don't you euthanize the mice in the animal facility by cervical dislocation, remove the tissues, put them in a Petri dish over ice, and then bring them to your lab?" asked Remy Snyder, the school's attending veterinarian, during her pre-review of Fuller's protocol.

Fuller responded, "These are very delicate neural tissues, and they have to be used when they are as fresh as possible. The 10-minute delay, even with ice, is not acceptable."

"But," queried Snyder in return, "even if you take the mouse to your lab, isn't the 10-minute transport just as much of a stressor on the animal as the carbon dioxide? And won't cervical dislocation affect the brain tissue?"

"Not in my experience," said Fuller. Snyder shook her head and returned the protocol to the IACUC office where, at her request, it was scheduled for full committee review.

Fuller did what the IACUC asked of him: he provided scientific justifications for bringing animals to his lab and for euthanizing them there by cervical dislocation. But are the justifications he provided sufficient for the IACUC to approve the request? Must the IACUC accept plausible scientific justifications even if the committee does not think they are fully adequate?

RESPONSE

Experience is not evidence

Darrell E. Hoskins, DVM, DACLAM

A number of questions must be addressed in order for the Great Eastern University IACUC to determine an appropriate course of action.

Is the opinion of Fuller that euthanasia by carbon dioxide asphyxiation would be stressful to his mice grounded in sound scientific evidence? This question, when disarticulated from the remainder of the issues, is not highly contentious. There are published scientific data indicating that asphyxiation by carbon dioxide can be stressful. It would not be hard for Fuller to find scientific data to support that opinion.

Is Fuller's belief that 10 minutes of transport time is simply too long sufficiently justified? Although Fuller's statement does reflect a common belief and practice in neurobiology research, the onus should

rest on Fuller to provide some supporting scientific data to that end.

Is Snyder's assertion that 10 minutes of transport time is just as much a stressor on the animal as carbon dioxide grounded in sound scientific evidence, and is it within her purview as the attending veterinarian and as an IACUC member to ask the question? Was Fuller's response adequate? Attending veterinarians are typically among the best-qualified individuals to explore and propose refinements. Snyder should remain steadfast in her efforts in that regard. As an IACUC member, Snyder is certainly allowed to pose any question necessary to ensure that she completely understand the ramifications of the research proposal. Therefore, she was acting appropriately when she asked her questions. However, she, too, has made an assertion that she has not substantiated with data. It would be advisable for her to do a literature search of her own regarding her assertion. Having said that, Fuller's response, "not in my experience," is not a sufficient scientific justification and also should be

backed up by documented scientific data. Both individuals have some homework to do.

Are the justifications Fuller provided sufficient for the IACUC to approve the request? Fuller's requests seem to be reasonable and to fall within the boundaries of common practice in neurobiology research. In its full-committee deliberations, the IACUC should consider whether Fuller's requested protocol would be more distressful to his mice than the standard practices it typically approves. If Fuller's lab personnel have the experience and documented qualifications to conduct cervical dislocation, there may be no substantial reason to deny his request. If the request is approved, the IACUC would need to be sure that Fuller's lab is added to the facilities inspected semi-annually. However, Fuller has not provided any scientific evidence to support his opinions and experiences.

Must the IACUC accept plausible scientific justifications even if the committee does not think they are adequate? No; it is incumbent upon the IACUC not to accept

justifications at face value if it does not think they are adequate. If the IACUC needs additional information to fully understand the ramifications of the requests, it should press the investigator to provide supporting published or personal lab data. If Fuller is unable to produce the supporting data, a pilot study to answer key questions could be pursued.

Ultimately, Fuller should be prepared to defend his opinions and experiences with sound scientific data. The attending veterinarian and the IACUC should feel empowered to require that Fuller provide as much scientific evidence as they deem necessary to adequately address their concerns.

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RESPONSE

Scientific justification needed

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Snyder is justified in requesting full committee review of this protocol. Fuller's opinion that carbon dioxide euthanasia is stressful to the mice may be correct, but he has not provided scientific justification for his requests to transport mice to his lab and to use cervical dislocation for euthanasia. These are the two primary IACUC concerns in this case.

Regarding transport, Fuller states that neural tissues are delicate and must be used as quickly as possible but has not provided data to support his claim that a 10-minute delay is unacceptable. The institution has a policy governing removal of animals from the vivarium, and it is the IACUC's responsibility to enforce this policy and to require scientific justification for exemptions from it. The IACUC has the authority to request scientific data from Fuller to back up his claim that a 10-minute delay will affect his results¹. If Fuller cannot furnish the data, a pilot study can be recommended to

confirm Fuller's justification for transporting mice to his laboratory.

Regarding euthanasia, if Fuller feels that the stress of carbon dioxide euthanasia will affect the results of his study, then he should choose a method that is consistent with the goals of the project¹. Although carbon dioxide asphyxiation and cervical dislocation are methods of euthanasia that are considered acceptable with conditions for small rodents, cervical dislocation requires a higher level of training and skill to perform, and thus additional oversight is needed to ensure that it is being done correctly². If the IACUC approves his request for the use of cervical dislocation, it should observe Fuller carrying out the luxation technique to confirm his proficiency¹.

As the attending veterinarian, Snyder is responsible for the health and well-being of all laboratory animals used at the institution³. If, in her opinion, transport of the animals to Fuller's lab will cause as much stress as carbon dioxide euthanasia (and thus negatively affect animal well-being), it is her duty to bring this to the attention of the committee¹. The IACUC is also charged with assessing the effects of the proposed procedures on the animals' well-being, and so it is important that Snyder's concerns be considered by the committee³.

Although Fuller's requests are not unreasonable, he has not provided adequate scientific justification or demonstrated technical proficiency to support his requests. It is the IACUC's responsibility to ensure there is sound scientific justification for exceptions to the institution's policies and the *AVMA Guidelines for the Euthanasia of Animals*. Plausible ideas are not sufficient justification; requests should be backed up by scientific data.

1. ARENA/OLAW. *Institutional Animal Care and Use Committee Guidebook* 2nd edn. (Office of Laboratory Animal Welfare, Bethesda, MD, 2002).
2. American Veterinary Medical Association. *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* (AVMA, Schaumburg, IL, 2013).
3. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).

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RESPONSE

Review institutional policies

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If weak scientific justification were the only problem in this scenario, resolution might be as simple as requesting recent references from the literature to support Fuller's claims that carbon dioxide euthanasia would interfere with his research but cervical dislocation would not and that tissue samples would be adversely affected by a 10-minute delay. Requiring a principal investigator to provide objective data supporting a non-standard approach is a valid way to address the potential inadequacy of the "in my experience" approach. There are, however, other points to consider when assessing procedures for which scientific justification is requested.

According to the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*¹, euthanasia by cervical dislocation is 'acceptable with conditions'. Specifically, it is humane "when performed by individuals with a demonstrated high degree of technical proficiency." On the basis of that condition, concerns about the competence of Fuller or his staff members could be resolved to the satisfaction of the IACUC through a demonstration of proficiency. It is important to note that the AVMA guidelines no longer stipulate scientific justification for use of cervical dislocation as a primary euthanasia method. The requirement for scientific justification to remove live animals from the vivarium is strictly institutional policy rather than an externally imposed current or even legacy regulation. The IACUC has the right to develop internal policies beyond the scope of any applicable regulatory documents^{2,3}, but such rules should be flexible enough to permit reasonable exceptions. Institutional objections to the use of live animals outside the central facility might include risk management concerns (exposure of non-lab personnel to allergens), administrative issues (limited resources for lab inspections) or compliance history (previous non-compliance in less supervised settings).

To provide a fair opportunity for Fuller to justify his requests, the IACUC must be

A word from OLAW

In response to the questions posed in this scenario, the Office of Laboratory Animal Welfare (OLAW) offers the following clarification and guidance, with the assumptions that Great Eastern University has an Animal Welfare Assurance with OLAW and that the study is funded by the Public Health Service.

This column asks whether the IACUC must accept scientific justifications that the committee does not consider adequate. Broadly, the IACUC must determine that investigator-provided scientific justifications are adequate in order to approve the proposed activities. It is the IACUC’s responsibility to review the investigator’s request in the context of federal requirements and local policies or guidelines.

In the scenario, the attending veterinarian questions the plausibility of the investigator’s scientific justification for euthanasia by cervical dislocation. The Public Health Service *Policy on Humane Care and Use of Laboratory Animals (PHS Policy)*¹ directs Assured institutions to follow the recommendations of the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition (the AVMA Guidelines)*². The 2013 *AVMA Guidelines* have refined the acceptability of cervical dislocation, stating that “when performed by well-trained individuals on appropriate animals, [it] appears to be humane”². If the IACUC decides to approve the investigator’s request, it should ascertain and document demonstrated technical competency by all staff conducting the procedure².

The scenario also questions the legitimacy of the investigator’s request to euthanize rodents in his laboratory. This institution requires investigators to provide scientific justification to remove animals from the vivarium. The institution is within its rights to develop and enforce institutional policies, such as a policy requiring investigators to provide scientific justification for conducting animal procedures outside a central animal facility. The following concerns may prompt such a policy: (i) occupational risks to personnel through exposure to animals in the investigator’s laboratory; (ii) transportation of live animals through the campus and building corridors to the laboratory; (iii) disposal of animal carcasses after tissue collection; and (iv) aesthetics of the euthanasia method to uninformed observers present in the laboratory^{2,3}.

Consideration of the adequacy of scientific justifications is part of the IACUC’s overall protocol review responsibility. During protocol review, IACUCs are required to evaluate proposed activities to ensure that they are consistent with the *Guide for the Care and Use of Laboratory Animals*³, unless a scientific justification for a departure is presented and is acceptable to the IACUC; that they conform with the institution’s Animal Welfare Assurance; that they will be conducted in accordance with the USDA Animal Welfare Regulations⁴, if applicable; and that they meet the requirements of the *PHS Policy* (section IV.C.1; ref. 5). Should a proposal fail to address any of these items to the IACUC’s satisfaction, the committee may require modifications to secure its approval.

1. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).
2. American Veterinary Medical Association. *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* (AVMA, Schaumburg, IL, 2013).
3. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th ed. 134–135 (National Academies Press, Washington, DC, 2011).
4. Code of Federal Regulations. Title 9, Chapter 1, Subchapter A - Animal Welfare: Part 2 Regulations. §2.31(d).
5. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals—Frequently Asked Questions*. Protocol Review, Question No. D.6. (US Department of Health and Human Services, Washington, DC, 2006, revised 2013).

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very clear about its reasons for originally creating and enforcing these policies. If the policies have been in place without further review for some time, general discussion among the entire IACUC may be beneficial. Information addressing the relevant issues identified by the IACUC could then comprise the comprehensive justification required for approval.

As part of ongoing program review, policies should be regularly reassessed to ensure that they are still relevant and necessary to promote optimal animal care and use. Whereas the *Guide for the Care and Use of Laboratory Animals*⁴ (the *Guide*) and *IACUC Guidebook*³ focus on review of policies as they relate to provisions of the *Guide*, *The IACUC Handbook*⁵

lists institutional policies separately, emphasizing the effects of local decisions on the conduct of research activities.

It is important to keep in mind that the IACUC has multiple roles. Focus tends to be on the regulatory aspects of the IACUC’s responsibilities, which is understandable given the complex regulatory framework. At the same time, there is an obligation to “not only oversee but also support animal users”⁴. Internal policies that place an extra burden on researchers must be carefully evaluated to confirm that they are both reasonable and necessary to meet the overarching goal of ensuring humane care and use of animals while supporting the advancement of scientific knowledge.

1. American Veterinary Medical Association. *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* 38 (AVMA, Schaumburg, IL, 2013). <<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>>
2. American College of Laboratory Animal Medicine. Position on Adequate Veterinary Care. <http://www.aclam.org/Content/files/files/Public/Active/position_adeqvetcare.pdf>
3. ARENA/OLAW. *Institutional Animal Care and Use Committee Guidebook* 2nd edn. (Office of Laboratory Animal Welfare, Bethesda, MD, 2002).
4. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. 13 (National Academies Press, Washington, DC, 2011).
5. Silverman, J., Suckow, M.A. & Murthy, S. *The IACUC Handbook* 2nd edn. 430 (CRC Press, Boca Raton, FL, 2007).

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