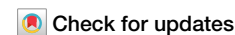


# The weights of refinement and flexibility



**IACUCs need to ensure the application of the 3Rs, while allowing researchers to conduct their work with reasonable flexibility. We invited experts from the University of Notre Dame (Eric Felde) and Northwestern University (Stefanie Bittner), along with USDA and OLAW representatives (Louis DiVincenti and Axel Wolff) to respond to a challenging situation at Great Eastern University.**

**D**r. Crick, Great Eastern University (GEU)'s IACUC Chair, called upon Dr. Jerry Silverman, GEU's most distinguished Professor, to serve as an ad-hoc consultant on a matter the IACUC was discussing. Jerry previously served as the IACUC Chair and is a subject matter expert on the nuances of the regulation governing animal care and use.

The matter at hand is with regard to Dr. Samantha Stevens' triennial renewal application. Samantha, a neuroscientist, studies neuronal pathways under a variety of conditions, such as stress, reward, sleep and peer-interactions. Samantha's IACUC protocol application, although well written, is quite lengthy and complex. As is expected of Principal Investigators (PIs), Samantha stays informed (e.g., new technologies, improved behavioral assays and equipment) so she can employ the concepts of the 3Rs in her research. For example, the triennial renewal application now includes the use of citric acid (CA) water as an alternative to traditional water restriction, which is employed to help motivate the animals to perform behavioral tests. CA water permits the animals to remain hydrated, but because of its sour taste, still motivates animals to work for access to fresh water. The challenge with the CA water is that it was only recently introduced into Samantha's field of expertise and has not yet been proven to achieve the desired goals (or at the same success rate). Samantha plans to evaluate CA water in comparison with water restriction that she has performed in the past, and consequently has requested separate groups of animals to run experiments under both paradigms.

Crick explains to Jerry that the IACUC's struggle has been whether they should require Samantha to halt all experiments using the traditional water restriction until a pilot study with the CA water has been completed to determine whether this less stressful (and less distressful) procedure can be employed for her research. The results of the pilot study would then direct the procedures that the IACUC would approve going forward (e.g., if CA water is just as effective in achieving the research outcomes needed, the IACUC may not then approve any use of the traditional water restriction). Furthermore, Crick tells Jerry the IACUC wants to ensure that PIs have the flexibility in their protocols to use the tools required for their research, but cannot resolve this with their obligation to ensure the application of the 3Rs. So, Crick asks Jerry, "What should we do?"

If you were Jerry, how would you respond to Crick?

## Response from EF

Dr. Crick described his concern that the GEU's IACUC needs to balance Dr. Stevens' ability to conduct her work with reasonable flexibility against the IACUC's need to ensure appropriate application of the principles of reduction, refinement and replacement. I believe the GEU's IACUC can accomplish both without considering the two goals to be in opposition.

GEU's IACUC is completing triennial review of Dr. Stevens' protocol, and therefore must consider whether the procedures it describes will minimize distress to the animals<sup>1</sup>. The addition of a new alternative presents a challenge: can the IACUC approve the already-approved fluid restriction when this new alternative could result in less distress to the animals?

In its review, the IACUC should reevaluate information that should have been provided in Stevens' initial protocol, including: the necessary level of fluid regulation, potential adverse consequences of fluid regulation and methods for assessing the health and wellbeing of the animals<sup>2</sup>. New information may be available for animals already included in the study, such as adverse event reports, records detailing fluid consumption and hydration status and any behavioral or clinical changes used as criteria for removal from a protocol<sup>3</sup>. If any of this information suggests that potential for distress

is not minimized, the IACUC may — at least — request modifications to Dr. Stevens' protocol.

The IACUC should carefully consider the way it approaches Dr. Stevens' proposal to add the CA water alternative because their action may influence researchers' attitudes toward refinement of experimental procedures. Without conclusive data indicating that CA water can effectively be used in place of traditional water restriction, suspending a study in progress would suggest that Dr. Stevens has not followed her IACUC protocol or regulations. By all indications, Dr. Stevens has stayed informed of advancements in practice, and she has proactively proposed refinements to the IACUC. Unless the IACUC finds in Dr. Stevens' data anything that suggests animals have experienced unexpected levels of distress to the extent that it is reportable, there does not appear to be cause to take that action.

I would instead recommend that the IACUC supports Dr. Stevens in her pursuit of refined research procedures. A pilot study looking into CA water has the potential to provide data that could benefit the IACUC in its efforts to ensure that researchers at GEU are applying the 3Rs in their work. If CA water is found to result in less distress, the IACUC might encourage other researchers who are currently using traditional water restriction to consider refining their own procedures. If there is an institutional policy on fluid restriction, it might warrant reevaluation and possible revision.

Assuming the IACUC finds no reason to suspend Dr. Stevens' protocol, allowing her the flexibility to include a pilot study of CA water as an aim of her current protocol will give her information that could support refinement of her own work, and possibly even benefit the IACUC and the entire animal program at the same time.

## Response from SB

As an experienced IACUC Chair and knowing that regulations around IACUC processes are not always black and white, Jerry understands that this can lead to great deliberation and disagreement amongst the Committee and sympathizes with the Committee's current struggle.

Jerry discusses with Crick that Dr. Stevens should be applauded for not only doing what is expected by continuously researching alternatives, but also proactively including the possible

## Box 1 | A Word from USDA and OLAW

### A Word from USDA

In 2021, APHIS amended the Animal Welfare Regulations (AWR) to replace the requirement for annual continuing reviews with a complete resubmission and review at least every 3 years. The triennial review should be conducted as if it were a new activity. This “de novo review” is meant to prevent a study from continuing “indefinitely without ever being fully revisited to ensure its underlying design or foundational assumptions are in step with current science and regulatory policy relating to animal welfare<sup>3</sup>.” Dr. Crick’s approach to the triennial review of Dr. Stevens’ protocol is appropriate.

If the animals are USDA-covered species, the AWR also require the IACUC, as part of its review, to determine that procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals and that the principal investigator (PI) has considered alternatives to procedures that may cause more than momentary or slight pain or distress<sup>7</sup>. Importantly, the AWR do not require the IACUC to determine that there are no alternatives, only that the PI has considered alternatives based on a written narrative description of the methods and sources used. In this case, the IACUC has met its obligation in determining that Dr. Stevens has considered a possible alternative to traditional water restriction, a procedure that can cause distress. However, the alternative is unproven. Allowing Dr. Stevens to compare the alternative to the traditional method is appropriate. If Dr. Stevens determines that the alternative is equivalent or superior to traditional water restriction, the IACUC is required to carefully consider Dr. Stevens’ justification for not adopting the alternative should she propose to continue using the traditional method.

### A Word from OLAW

This scenario involves IACUC considerations regarding IACUC approval of

animal activities including assessment of the new, but unproven, use of citric acid water as an alternate to water restriction for a neuroscience research protocol. In order to approve proposed animal activities or proposed significant changes, the PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy) requires the IACUC to conduct a review and confirm compliance with the Policy, the institution’s Animal Welfare Assurance, and the Animal Welfare Act and regulations, as applicable<sup>1</sup> (and see Related links). Among these requirements, and of importance to this scenario, IACUCs are required to determine that procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals<sup>1,8</sup>. “Refinement and reduction goals should be balanced on a case-by-case basis<sup>4</sup>,” and “limited pilot studies, designed to assess both the procedure’s effects on the animals and the skills of the research team and conducted under IACUC oversight, are appropriate<sup>4</sup>.” For the pilot studies (see Related links), the IACUC should engage with Dr. Stevens and evaluate the lab’s current practices involving traditional water restriction and consider the proposed procedures, monitoring, and documentation with careful attention to the *Guide’s* Food and Fluid Regulation section<sup>4</sup> (and see Related links). Monitoring frequency may need to be increased with pilot studies that evaluate new methods, especially when procedures involve water restriction. Good communication and a collaborative effort between the IACUC and the researcher are recommended as both parties share the obligation of humane animal care and use. The IACUC is responsible for the approval of animal activities that are consistent with PHS Policy requirements and institutional policies, and all animal activities must be conducted under an approved IACUC protocol.

can begin to address their shared obligations for humane animal care and use.” While PHS Policy IV C.1.a indicates<sup>1</sup>: “Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design,” Jerry asks the Committee to consider:

- Does the PI have adequate justification to have both paradigms to remain in the protocol?
- Would requiring the PI to remove a proven method of motivation to attempt an unproven method of motivation inhibit the PI’s research plans?
- Has the PI considered if there will be strain or species variances in the acceptance of CA water treatment as an alternative to water restriction if working with various models?
- Could the IACUC require a modification to secure approval that the PI conduct the unproven CA water pilot study alongside their proven methods, and that they would only continue to use the water restriction procedure in cases where the CA water paradigm demonstrates an inability to meet the same scientific goals at the same success rate as their water restriction procedure?
  - Could the Committee request a review of the pilot study results be submitted for review at the next meeting once conducted?
  - Could the Committee request a post-approval monitoring visit to review the procedure and records with a report back to the Committee?

If the pilot study cannot achieve the goals at the same or similar success rate, then although the animals may be undergoing a less stressful procedure, more animals may be required which would be trading refinement for reduction to gain statistical significance in Dr. Stevens’ research. If asked how Jerry would cast his vote on the matter, since the protocol is well written, he would be in favor that the PI be allowed to conduct the pilot study alongside their current proven paradigm.

### Compliance considerations

The Protocol Review coordinators offer the following compliance considerations:

Refinement refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress<sup>4</sup>. During protocol review, the IACUC must consider<sup>4,5</sup>:

- The “availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation”;
- That “unnecessary duplication of experiments” is not occurring; and,
- The “impact of the proposed procedures on the animals’ well-being.”

alternative in her protocol to see if it would be a viable alternative to her current studies. The PI indicated that CA water treatment is not yet proven to work in her field; so performing a set of pilot studies to ensure that the CA water treatment not only works, but also doesn’t have any adverse effects on her specific research makes

sense. Jerry reminds the IACUC that per the *Guide*<sup>4</sup>, “the IACUC is obliged to weigh the objectives of the study against potential animal welfare concerns. By considering opportunities for refinement, the use of appropriate nonanimal alternatives, and the use of fewer animals, both the institution and the principal investigator (PI)

The *Guide* further states that “At times, protocols include procedures that have not been previously encountered...If little is known about a specific procedure, limited pilot studies, designed to assess both the procedure’s effects on the animals and the skills of the research team and conducted under IACUC oversight, are appropriate<sup>4</sup>.”

Consequently, GEU’s IACUC has a regulatory and ethical obligation to consider CA water as a refinement to a stressful/distressful procedure (i.e., water restriction).

Recall that “The IACUC may invite consultants to assist with the review of complex issues<sup>4</sup>,” which may help the IACUC determine how to proceed. Ultimately, however, the IACUC must determine, for example, whether:

- CA water is a refinement that must be implemented (i.e., can Samantha continue with water restriction whilst investigating the impact of CA water on her research, or must she halt all activities until CA water has been established as a refinement); and,
- A pilot study or some other criteria are required to establish CA water as a viable refinement (i.e., it will not introduce a new variable that could skew the data outcome(s) and/or prohibit comparison to animals already studied).

### A Word from USDA and OLAW

Comments from USDA and OLAW can be found in Box 1.

*This protocol review was coordinated by Lauren Danridge and Bill Greer.*

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### References

1. Public Health Service. *PHS Policy on Humane Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services, National Institutes of Health, Bethesda, MD, 2015). Section IV.C.1. Review of PHS-Conducted or Supported Research Projects. <https://olaw.nih.gov/policies-laws/phs-policy.htm#ReviewofPHS-ConductedorSupportedResearchProjects>
2. National Research Council. *Guidelines for the care and use of mammals in neuroscience and behavioral research* (National Academies Press, Washington DC, 2003).
3. Morton, D. B. *ILAR J.* **41**, 80–86 (2000).
4. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington DC, 2011).

5. USDA. *Animal Welfare Act and Animal Welfare Regulations*. [https://www.aphis.usda.gov/animal\\_welfare/downloads/AC\\_BlueBook\\_AWA\\_508\\_comp\\_version.pdf](https://www.aphis.usda.gov/animal_welfare/downloads/AC_BlueBook_AWA_508_comp_version.pdf)
6. AWA Research Facility Registration Updates, Reviews, and Reports. 86 FR 66919 (November 24, 2021; to be codified at 9 C.F.R. pts. 2, 3, 4). <https://www.federalregister.gov/documents/2021/11/24/2021-25614/awa-research-facility-registration-updates-reviews-and-reports>
7. Animal Welfare Regulations. 9 CFR § 2.31(d)(1).
8. Office of Science and Technology Policy. *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*. Federal Register, May 20, 1985, Vol. 50, No. 97, [FR Doc. 85-12059]. Principle IV. <https://olaw.nih.gov/policies-laws/phs-policy.htm#USGovPrinciples>

### Competing interests

The authors declare no competing interests.

### Disclaimer

The views expressed in “A word from OLAW and USDA” represent the positions of OLAW/NIH and USDA, respectively, but the remainder of the contributions in this article do not represent official support or an endorsement by NIH, USDA, or any government agency.

### Related links:

- PHS Policy on Humane Care and Use of Laboratory Animals – FAQs. Protocol Review, Question No. D.6.** <https://olaw.nih.gov/faqs#/guidance/faqs?anchor=50318>.
- PHS Policy on Humane Care and Use of Laboratory Animals – FAQs. Protocol Review, Question No. D.7.** <https://olaw.nih.gov/faqs#/guidance/faqs?anchor=50319>.
- PHS Policy on Humane Care and Use of Laboratory Animals – FAQs. Protocol Review, Question No. D.11.** <https://olaw.nih.gov/faqs#/guidance/faqs?anchor=50323>.
- PHS Policy on Humane Care and Use of Laboratory Animals – FAQs. Animal Use and Management, Question No. F.19.** <https://olaw.nih.gov/faqs#/guidance/faqs?anchor=52964>