

Once Used, Twice Counted

Dr. Jerry Silverman, a relatively new faculty member at Great Eastern University (GEU) completed his first triennial renewal application since he joined GEU. A week later, Jerry received notification that the renewal application was approved. Relieved, Jerry and his lab continued their animal research.

Three months later, when Dr. Matt, Jerry's Post-doctoral fellow, placed

an animal order, he was informed that he could not order additional animals until he filed an amendment increase in his animal numbers with an appropriate justification. Considering that the protocol was just renewed, Matt was certain an error had occurred. Matt and Jerry called Gwen Skladnost, the IACUC Administrator, to discuss the confusion. Jerry explained that some

animals used in his research are ordered from a vendor while others are obtained from his in-house breeding colony. His understanding was that animals are deducted at the time of an order or at weaning. Consequently, he didn't understand why the animal deductions were carried over at the time of his triennial renewal.

Skladnost informed Jerry that there was no error. She explained that, at GEU, when protocols are renewed, the number of animals that are in the vivarium at the time of the protocol renewal are "subtracted" from those animals on the renewal protocol at the time of the renewal's approval, thereby reducing the number available on the newly approved protocol. Jerry was surprised and a bit perplexed by the explanation since he already accounted for the animals he used on the prior protocol; why were the animals counted twice?

Nonetheless, Jerry accepted the process as Skladnost explained, without further inquiry to avoid an ongoing discussion with the IACUC. Matt prepared an amendment to increase the animal numbers, but the reviewers required significant explanation for why so many animals were needed so soon after the renewal. Eventually the amendment was approved, but only after Skladnost helped both parties understand what was required and why.

Unfortunately, Jerry and Matt continued to see the available animal balance decrease when the animals transferred from the prior protocol were euthanized. Jerry called the IACUC Chair to discuss the matter.

What do you think:

- Are the IACUC's processes regarding animal numbers and triennial renewals acceptable?
- Are they aligned with federal mandates?
- At what point are animals considered "used"?

Lauren Danridge [✉] and Bill Greer [✉]
Animal Care & Use Office, University of Michigan, Ann Arbor, Michigan, USA.
[✉]e-mail: danridlm@umich.edu; wgreer@umich.edu

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A WORD FROM OLAW AND USDA

Response from OLAW

As mentioned by other Reviewers, the PHS Policy requires that proposals to use animals include a rationale for the approximate number of animals to be used and limit the number to what is necessary to obtain valid results¹. It is the institution's choice to document and monitor the number of animals acquired and used as works best for their program². The documentation should include not only animals acquired or bred and then used in approved activities, but also animals produced and not used for the original purpose³. While increases in animal numbers are a significant change to an approved animal activity, with an existing policy in place an IACUC may administratively handle such changes and reduce burden³. In this case, the lack of clear communication with the researchers about the IACUC's process has created an unnecessary complication and increased burden on those involved.

Response from USDA

At issue in this scenario is confusion or uncertainty on the part of the Principal Investigator regarding this institution's standard operating procedure (SOP) for reporting animal numbers, with focus on determining when an animal is deemed "used". As noted by one Reviewer and in the *Compliance Considerations*, the AWA and regulations do not provide a definition of "used" in this context. To reiterate previously raised points regarding reporting of animal numbers used in research facilities, the AWA regulations only address this in two areas. First, proposals to conduct activities with

animals (or make significant changes in an approved protocol) must provide an estimate of the number of animals and a rationale for the number and species used⁴. Second, the annual report must state the common names and numbers of animals used in each of the pain categories (i.e., columns C, D and E of the report)⁵. While it is entirely at the discretion of the institution and its IACUC how these numbers are tracked internally, having a system that facilitates accurate and consistent reporting under the AWA would seem advantageous. □

Patricia Brown¹ [✉] and Betty Goldentyer² [✉]

¹Director, Office of Laboratory Animal Welfare, OER, OD, NIH, HHS, Bethesda, MD, USA.

²Deputy Administrator, Animal Care, APHIS, USDA, Riverdale, MD, USA.

[✉]e-mail: brownp@od.nih.gov;

betty.j.goldentyer@usda.gov

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3. National Institutes of Health. *Guidance on Significant Changes to Animal Activities*. Notice NOT-OD-14-126 [online]. (National Institutes of Health, Bethesda, MD, August 2014). <https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-126.html>
4. 9 CFR, Chapter 2, Subpart C – Research Facilities, § 2.31(e)(2).
5. 9 CFR, Chapter 2, Subpart C – Research Facilities, § 2.36(b)(5-7).

Once Used, Once Counted

Are the IACUC's processes regarding animal numbers and triennial renewals acceptable?

COMPLIANCE CONSIDERATION

The Protocol Review coordinators offer the following compliance considerations:

1. At what point are animals considered "used"?

Federal mandates establish guidelines for the proper care, treatment, housing *etc* of animals "to be used in" research, teaching..., but do not define when an animal is considered "used". The only cause for discussion about the word "used" is the need to track and report animal numbers to the federal agencies. The reporting requirements differ for USDA and OLAW:

The USDA Annual Report¹ :

- Requires a report of the total yearly number of animals used, by column (i.e., pain and distress classification) and by species; and
- Columns C, D, and E are in past tense (how many *were* used), whereas Column B is in present tense (how many are *being* held, ...).

OLAW, on the other hand, "only requires reporting of average daily inventories of animals, by species, in new and renewal Animal Welfare Assurances²."

IACUC protocols:

- Are approved for a 3-year term;
- Require an estimated number of animals for the proposed activities;
- Can be amended at any time to add experiments (and animals);
- Do not require activities to be completed prior to triennial renewal.

Consequently, the activities and number of animals proposed in a 3-year protocol can (and often) spill over into the triennial renewal application. The animals are still being "used" for research, will continue to be reported in inventories of animals, and could be reported across more than one USDA annual report. These matters, however, are independent of Jerry's problem and the issue of animal deductions.

2. Are the IACUC's processes regarding animal numbers and triennial renewals acceptable?

OLAW expects that IACUCs ensure that "proposed research projects are in accordance with this Policy" and that

The IACUC at Great Eastern University may have lost perspective in the reason for tracking animal numbers. Their current

they "conduct continuing review of each previously approved, ongoing activity covered by this Policy at appropriate intervals as determined by the IACUC, including a complete review in accordance with [PHS Policy] IV.C.1.-4. at least once every three years³." Consequently, IACUCs should ensure that triennial renewal applications:

- Indicate the approximate number of animals to be used;
- Include a rationale for the requested number of animals; and
- Request only the minimum number of animals to obtain valid results.

In summary, there are no federal mandates requiring a "transfer" of animals between the expiring and the renewal applications. This is, in our opinion, an administrative task that is required for certain business process to continue (e.g., updating cage cards with new protocol number(s) and/or dates of approval). Furthermore, there is no need to duplicate the number of animals across protocols (i.e., deducting animals from the expiring protocol when purchased or bred and then also deducting from the renewal protocol is self-imposed burden).

3. Are they aligned with federal mandates?

OLAW² indicates that institutions must "monitor and document numbers of animals acquired (through breeding or other means) and used in approved activities", presumably to ensure that the number of animals used is "limited to the appropriate number necessary to obtain valid results" but does not prescribe the method(s) for monitoring. □

Lauren Danridge ✉ and Bill Greer ✉

Animal Care & Use Office, University of Michigan, Ann Arbor, Michigan, USA.

✉e-mail: danridlm@umich.edu; wgreer@umich.edu

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3. 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).

process of recounting animal numbers during the triennial review, and potentially again at euthanasia, could produce an inflation to the actual number of animals within a project. This could become a sticky situation, especially when disclosing animal numbers for category E studies, or for USDA-covered species. Regulatory and accreditation agencies may question the high animal numbers listed, bringing unwarranted attention to these protocols. We would caution against this idea of "pumping" animal numbers onto a protocol, as this seems counterintuitive to the goal of reducing animal numbers. Furthermore, by having Dr. Silverman submit multiple amendments to continually add animals to meet a moving target, produces additional administrative burden on the Principal Investigator (PI) and the IACUC Office. Instead, the IACUC could use the triennial review form as an opportunity to provide a retrospective look at the protocol, by addressing the number of animals used over the previous three years, and by justifying an adequate number of animals for the next three years. Consequently, the animals remaining housed in the vivarium may require a new cage card to display the appropriate protocol number, but should only be counted once. Therefore, we believe the IACUC should re-evaluate their process regarding animal numbers and triennial renewals.

Are these processes aligned with federal mandates?

There are no federal regulations outlining how to approach the counting of animals especially at the time of protocol renewal^{1,2}. However, institutional and IACUC guidelines should be set to address how to handle animals that are still on a protocol at the time of renewal. Asking questions such as: What is the previous protocol number (if applicable)? How many animals have been used? Are there any animals currently housed in the vivarium? To reduce confusion, both the PI and IACUC should put special attention to the animal numbers used, being requested, and the number that is being carried over from the previous protocol.

At what point are animals considered "used"?

If we go by the strict definition, animals would be considered "used" when they have been euthanized. However, from an administrative and oversight

point of view, it would require the animal facility and/or IACUC to know and follow each animal throughout their time in the facility. To reduce administrative burden, we suggest that “used” should equal “purchased and arrived safely into the vivarium” (or weaned when bred in house). This means that animal numbers would not be carried over to the renewal protocol and would be counted only once, as they should be.

It is imperative to outline a procedure during protocol renewal to avoid confusion amongst PI, animal facility and IACUC and to ensure a proper animal count. Communication is key! □

Christina Aguilar ✉, **Karina Garcia** ✉ and **Nathalie Sumien** ✉
Office of Research Compliance, University of North Texas Health Science Center, Fort Worth, TX, USA.
✉e-mail: Christina.Aguilar@unthsc.edu;

Karina.Garcia@unthsc.edu;
Nathalie.Sumien@unthsc.edu

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2. USDA, Animal Welfare Act and Animal Welfare Regulations. https://www.aphis.usda.gov/animal_welfare/downloads/bluebook-ac-awa.pdf



The Numbers Game

Are the IACUC's processes regarding animal numbers and triennial renewals acceptable?

This scenario involves two tracks of acquiring animals: via a vendor; and with an in-house breeding colony.

While it is upon the investigator to maintain and keep track of animals ordered, bred, and used, it would be beneficial to have a guideline in place that clarifies how the institution counts animals “used”. This would require some consultation and discussion between the IACUC and facility staff who track animal usage within the facilities to ensure the policy reflects how animals are counted and reported in the census. The guideline could also include examples of acceptable justifications for increasing animal numbers when needed.

While having guidance in place is good practice, it would be even more beneficial to have sections in the institution's protocol form where the Principal Investigator (PI) can list the number of animals still housed in the facilities at the time of the renewal. It would also be helpful to have questions or sections specific to protocols that request approval for in-house breeding. For such breeding protocols, the PI should account for the breeders, the number of offspring expected per litter, the number of offspring that will be used for research, and provide justification for any animals that may be bred but not used for procedures (such as animals culled out due to genetics). Managing these numbers can be tricky for investigators; however institutions require a specific number to be listed in the protocol for approval. Breeding issues such as smaller than expected litters, poor genetic outcome, or unexpected mortality could all be factors

in why an investigator would request more animals. But in this scenario, it appears that the PI was not aware of the facility's practice to count the animals “leftover” from one renewal to the next. In my opinion, the institution's process in this scenario does count the same animal twice. The process is not necessarily “wrong” though, if it reflects the total number of animals needed over the next three years. In this case, the PI should be aware that this is how animals are counted, and make necessary adjustments to his request at the time of renewal. If more mice are needed before the next renewal, then an amendment should be submitted with justification for the increase. In this scenario, clearly the PI does not understand how animal numbers are counted, and there should be a policy and a standard operating procedure (SOP) in place to avoid confusion in the future.

Are these processes aligned with federal mandates?

The Animal Welfare Act regulations state, “A rationale for involving animals and for the appropriateness of the species and numbers of animals to be used¹.” PHS Policy states, “identification of the species and approximate number of animals to be used²”. OLAW's position on animal numbers is that PHS policy implies that a system be in place to track and monitor animal usage³. The institution is in line with the regulations as there is a mechanism in place to track the animal numbers approved by the IACUC. It's the tracking system of the institution that should be made clear to the PIs so that they can be more accurate in the requests for total number of animals at the time of triennial renewal⁴.

At what point are animals considered “used”?

Any animal used in procedures for the purposes of research should be considered “used”. In cases where animals are acquired from a vendor, it could be beneficial for researchers to place orders when ready to move forward with procedures that will involve those animals. Animals used specifically for breeding from the in-house breeding colony should be counted in the overall total number of animals requested. Offspring from the breeding colony should be counted as used whenever they experience an approved procedure, even if it is just an identification procedure or a genotyping method. Multiple amendments for one protocol to increase animal numbers in short spans of time could indicate a problem in colony management or unexpected outcomes from procedures with animals acquired from vendors. In any case, scientific justification should be provided when researchers seek approval for an increase in animal numbers within the three years of protocol approval. □

Richelle Scales ✉
University of California, Berkeley, Berkeley, CA, USA.
✉e-mail: rscales@berkeley.edu

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