Jerald Silverman, DVM, Column Coordinator

Amending a protocol to import and breed knockout mice

Dr. Paulie O'Rourke was elated when a colleague at another university developed a knockout mouse that was important to O'Rourke's research. She quickly requested that the Import-Export Coordinator at Great Eastern University import for her two breeding pairs of the knockout mice. Because O'Rourke's protocol was approved for 150 mice and only 50 had been used to date, the coordinator began the import process. When the breeding animals arrived and were entered into a five-week quarantine period, O'Rourke submitted a minor protocol amendment requesting permission to breed the new mice to generate sufficient animals to complete her studies. The amendment stated that other than a small number of animals to be used specifically for breeding, the total number of animals needed for experimentation would remain unchanged because she would do the exact same experiments previously approved by the IACUC, substituting the knockout mice for the previously approved strains.

When the IACUC administrator received the amendment request, she was not sure whether to process it as a minor or major amendment. She asked Larry Covelli, the IACUC chairman, for his opinion. "Larry," she said, "is breeding these mice considered a new procedure, so that I have to process this request as a major amendment, or is it so insignificant that it's a minor amendment?"

"You know," said Covelli, "I don't remember if we had to face this question in the past, but my guess is that it's a minor amendment. But on the other hand, if these new mice have some phenotypic peculiarities that affect their health or even just their breeding, maybe it's a major amendment. And now that I'm thinking about it, what would happen to these mice if we put this through as a major amendment and we find out they have some phenotypic abnormality that creates questions about their use in O'Rourke's research and then, for some reason, the IACUC doesn't approve the protocol? How come we imported these mice before the IACUC saw the amendment?"

"We imported them because the IACUC approved using mice," said the administrator. "We don't ask what color they are or how many tails they have. They're mice."

O'Rourke believes she followed proper procedure, and even the IACUC is unsure whether or not there is a problem. What is your opinion?

RESPONSE

Approval before import

John A. Salig, MS, RLATG, CPIA

According to the Animal Welfare Act and Regulations (AWARs)[1] and the PHS *Policy on Humane Care and Use of Laboratory Animals* (PHS *Policy*)[2], IACUCs are required to review and approve significant changes regarding the use of animals in ongoing activities described in an IACUC-approved animal study proposal[3]. The NIH interprets significant changes to mean those that have the potential to substantially and directly affect the health and well-being of the experimental animals and must therefore be reviewed and approved by the IACUC. The PHS *Policy* and AWARs do not indicate that changing the strain of the mouse model is a significant change. But the IACUC may be concerned if the creation of this knockout mouse (which can be considered a transgenic strain by the NIH Office of Biotechnology Activities) requires ABSL-2, ABSL-3 or ABSL-4 containment. I would hope that if this were the case, either the Importing Coordinator or the sending facility would make note of it.

I believe that O'Rourke was in error for failing to obtain IACUC permission before importing these mice. According to PHS *Policy*[3], minor changes not deemed significant, which are reviewed and approved by the delegated reviewer(s), should be reported to the IACUC at its next regular meeting. The key word here is "approved." Looking for permission after initiating the transfer process is not requesting permission, but rather asking forgiveness.

I would think that one of the requirements involved in importing animals from another facility would be prior review and permission by the IACUC. In that case, the Import Coordinator would also be at fault for not checking.

Perhaps it is time for this IACUC review its Standard Operating Procedures for importing of animals into its facilities.

1. Animal Welfare Act and Regulations. 9 CFR.
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* (US Department of Health and Human Services, Washington, DC, 1986, amended 2002).
3. Office of Laboratory Animal Welfare. *OER Revised Guidance Regarding IACUC Approval of Changes in Personnel Involved in Animal Activities.* Notice OD-03-046. (US National Institutes of Health, Washington, DC, 6 June 2003).

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RESPONSE

New strains, strain-free

Cheryl Cheney, BA, BS, ALM, CPIA, Lesley Kline, BS & Christopher Wilber, BA

IACUCs are responsible for ensuring that animals selected for a procedure are of an appropriate species[1]. Strain specificity is another matter entirely; no regulations or guidelines require IACUC review or approval of intraspecific variants[2]. The *Guide for the Care and Use of Laboratory Animals* (the *Guide*) endorses the recording of animal strain details and highlights various program elements that may require strain-specific adjustments (e.g., humane endpoints and husbandry)[3], but in the absence of any institution-specific policies on the matter, great flexibility in strain acquisition is afforded to investigators.

Therefore, it seems reasonable for O'Rourke to believe that a protocol amendment was only necessary insofar as she planned to introduce a new procedure (breeding) and a small increase in overall animal quantities to accommodate it. There are also valid reasons why O'Rourke may have delayed amendment submission until after the mice arrived. Because the mouse is of a newly developed strain, the source laboratory may not have many spare founders available, and, if the relevant gene is of broad research interest, demand could easily overwhelm supply. Mice from a noncommercial source must remain in quarantine for 5 weeks at Great Eastern University (GEU); hence, there was adequate time to prepare and process an amendment after their confirmed arrival. Without assurance that the mice would be sent, filing an amendment beforehand may have meant wasted time for both the investigator and the IACUC.

Key IACUC personnel at GEU were undecided on the significance of the changes O'Rourke proposed for her protocol. OLAW expects IACUCs to assess the necessity for a breeding colony and how it will be maintained[4], yet adding breeding to a protocol could justifiably be treated as a minor amendment because it is generally free of pain or distress. However, Covelli worries that the disabled gene(s) of the knockout mice may have a detrimental effect on animal welfare that might warrant full committee review. This seems unlikely to be the case if the recommendations of the *Guide* were followed; the veterinarian that authorized the shipment of the animals should have already investigated whether they had any special husbandry needs[3]. Presumably, if specific accommodations (e.g., special diet) were required to ensure animal well-being, the investigator would have been advised to contact the IACUC for guidance prior to importation. In any case, "[i]t is prudent for an IACUC to develop a policy on the kinds of changes that are considered significant in order to avoid ambiguity"[4]; OLAW Frequently Asked Question number D.9 (ref. 5) may be used as a starting point when drafting such a policy.

To avoid similar confusion in the future, the GEU IACUC may wish to adopt policies to require strain-specific details in protocols, inclusive of nomenclature and any special measures required to accommodate specific phenotypes (e.g., husbandry modifications or specific euthanasia criteria)[4]; to treat strain addition as significant only if inherent morbidity is expected to manifest; to permit strain import from noncommercial sources prior to IACUC approval only if no inherent morbidity is expected; to introduce mechanisms to communicate strain approvals to procurement staff; and to clearly communicate policy and procedure updates to all relevant personnel.

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. Carbone, L. Justification for the use of animals. in *The IACUC Handbook* 2nd edn. (eds.

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:

The Public Health Service *Policy on Humane Care and Use of Laboratory Animals* (PHS *Policy*; section IV.B.7) requires the IACUC to "review and approve, require modifications in (to secure approval) or withhold approval of proposed significant changes regarding the use of animals in ongoing activities"[1]. In this scenario, breeding activities were not included in the original approved animal study proposal. This is a significant change requiring IACUC review and approval to ensure that the standards of care and animal well-being are maintained[2].

In addition, information on the phenotype should be provided to the IACUC in the proposal modification. A description of any clinical conditions that require special support or known morbidities that require intervention to minimize pain and distress should be included[3].

If a knockout mouse strain was produced specifically in direct response to this principal investigator on a PHS-supported study, then the PHS *Policy* (sections IV.A.1 and IV.C.1) requires the producing institution to have an approved Animal Welfare Assurance with OLAW and the activity to be approved by the institution's IACUC[1].

Local institutional policies may require IACUC approval before an investigator orders new strains of animals or imports them from a new collaborator or other source. This determination is left to the discretion of the IACUC to most appropriately meet institutional needs[2].

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. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals–Frequently Asked Questions.* (US Department of Health and Human Services, Washington, DC, 2006, revised 2011). <http://grants.nih.gov/grants/olaw/faqs.htm#proto_9>
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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Silverman, J., Suckow, M.A. & Murthy, S.) (CRC Press, Boca Raton, FL, 2007).
3. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
4. Applied Research Ethics National Association, Office of Laboratory Animal Welfare, National Institutes of Health. *Institutional Animal Care and Use Committee Guidebook* 2nd edn. 95 (US National Institutes of Health, Washington, DC, 2002, reprinted 2008).
5. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals—Frequently Asked Questions.* Protocol Review, Question No. D-9. (US Department of Health and Human Services, Washington, DC, 2006; revised 2011). <http://grants.nih.gov/grants/olaw/faqs.htm#proto_9>

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RESPONSE

Significant concerns?

Todd A. Jackson, DVM, DACLAM

The concerns of the IACUC chair and administrator revolve around the issue of what constitutes significant changes to a previously approved protocol. Significant changes require IACUC review and approval prior to implementation. In this scenario, three specific questions are asked: Is the addition of breeding procedures to a protocol a significant change? Is a change in the rodent stock or strain to be used significant? Does the procurement of animals from a collaborator at another institution rather than from a commercial vendor or local collaborator constitute a significant change?

OLAW, with the concurrence of USDA, has stated that IACUCs have some discretion in defining what they consider to be significant changes and in establishing mechanisms to determine significance on a case-by-case basis[1]. OLAW has also stated that examples of significant changes include, but are not limited to, changes in the objectives of a study; from non-survival to survival surgery; resulting in greater discomfort or in a greater degree of invasiveness; in the species or in approximate number of animals used; in Principal Investigator; in anesthetic agent(s) or the use or withholding of analgesics; in the method of euthanasia; and in the duration, frequency, or number of procedures performed on an animal.

The IACUC chair also raised concerns about whether the new knockout mice could have phenotypic abnormalities that negatively affect their well-being. That is a valid concern whenever new genetically modified animals (GMAs) are produced. According to the *Guide for the Care and Use of Laboratory Animals*, "When the initial characterization of a GMA reveals a condition that negatively affects animal well-being, this should be reported to the IACUC"[2]. By carefully monitoring GMAs and reporting health concerns, the IACUC and the veterinary staff may help identify proactive measures to minimize or alleviate associated pain or distress.

The IACUC chair and administrator should use this opportunity to discuss their concerns with the entire committee and to set clear guidelines as to what the IACUC will consider significant versus minor changes to protocols. Commonly, the addition of breeding procedures is considered significant, but a change in stock or strain and the procurement of mice from a new collaborator are not. By those standards, O'Rourke has acted appropriately in obtaining the mice through Great Eastern's Import Coordinator, by having the mice quarantined on arrival and presumably tested for subclinical pathogens before contact with other research mice, and by submitting a protocol amendment for IACUC approval before breeding the new mice. The IACUC should also ensure that appropriate training and reporting mechanisms are implemented to ensure the notification of GMA-related health issues and the determination of appropriate palliative measures and humane endpoints whenever compromised GMAs are identified.

1. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals –Frequently Asked Questions* (US Department of Health and Human Services, Washington, DC, 2006; revised 2011). <http://grants.nih.gov/grants/olaw/faqs.htm#proto_9>
2. 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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