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Interpreting guidance on significant changes

The newly released *Guidance on Significant Changes to Animal Activities*¹ is intended to expedite aspects of research with laboratory animals. For some situations, however, the intent of the change may not be as obvious as anticipated. For example, the new guidance states that a request for an increase in the previously approved number of animals “may be handled administratively, according to an existing IACUC-reviewed and approved policy without additional consultation or notification.” Yet, when the details become known, what was originally thought to be a clear statement may not be clear, as is illustrated by a question that arose at Great Eastern University.

Dr. Jack Schwartz, a prominent obesity expert, conducted a study using C57Bl/6 mice in which he found that suppressed expression of the gene *nol* by small interfering RNA resulted in a statistically significant increase in high fat diet-induced obesity. The mice also had an unanticipated significant increase in the incidence of spontaneous mammary adenocarcinomas. Schwartz hypothesized that *nol* might be

functioning as a tumor suppressor gene, and therefore, blocking its activity resulted in a greater cancer incidence. He submitted a protocol modification request to add 50 BALB/c mice to his study to determine if the unexpected cancer finding was unique to the C57Bl/6 background or if the results could be reproduced in BALB/c mice. Because the IACUC had a policy in place to allow administrative approval of a request for additional animals, the IACUC administrator approved his request, reasoning that there were no new procedures being requested and that BALB/c and C57Bl/6 mice were both of the genus *Mus*.

One month later, Schwartz submitted another protocol modification request, this time for the addition of 50 C57Bl/6 mice in which a signaling gene in the *nol* pathway was knocked out. There were no other changes to the experiment. This time, the IACUC administrator wondered if the requested modification was in the spirit of the new regulatory guidance, which appears to have been developed to address a need for additional animals to complete an

IACUC-approved experiment. She thought that studying the knocked-out signaling gene was a different experiment than the original one, which was specifically focused on the gene *nol*. She believed that the new request for 50 more mice should be reviewed by the IACUC for its “societal value,” its potential to advance knowledge and its harm:benefit analysis. In her opinion, the proposed use of the additional animals constituted a different experiment even though no new procedures were being proposed.

Was the IACUC administrator right in approving the request for the BALB/c mice, or does a change in genetic background warrant IACUC review? Did she make the right decision to have the request for the C57Bl/6 knockout mice reviewed by the IACUC? Did the administrator overstep her authority?

1. National Institutes of Health. Guidance on significant changes to animal activities. Notice NOT-OD-14-126. (National Institutes of Health, Washington, DC, 26 August 2014).

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RESPONSE

Consequences of flawed IACUC policies

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The good news is that Great Eastern’s IACUC does have an approved policy in place for increases in animal numbers, as required by new regulatory guidance¹. But the facts of this scenario suggest that the policy provides insufficient guidance for the IACUC administrator’s decision-making. OLAW held a webinar² in August 2014 that listed some of the considerations for developing a policy and made clear that this would not be a simple task. The former, generally accepted

custom that permitted some increase in animal numbers, typically on a percentage basis, was problematic (e.g., what is 10% of 5 approved animals?). The new guidance requires a policy that determines parameters for the number of additional animals that falls within the “approximate number of animals to be used”³.

Changes in study objectives require full committee review or designated member review¹. Schwartz’s incidental discovery of spontaneous tumors apparently led him to want to test an entirely new hypothesis—with additional mice of a different strain (BALB/c). A new protocol is appropriate here, with a review of the literature for similar research and alternatives for this new objective. A comprehensive policy should cover this situation and should mandate consultation with the attending

veterinarian or the IACUC chair for requests not explicitly covered by the policy. A request for additional animals that includes any other deviations from the original protocol should be reviewed by another qualified member of the IACUC. Even a seemingly minor change, such as strain, may call for different husbandry requirements. Certainly a change from an obesity study to a tumor formation study requires a different set of monitoring criteria and humane endpoints.

Similarly, unless Schwartz had the foresight to add to the second protocol the 50 additional C57Bl/6 mice as a second potential experiment (contingent on the experimental results of the first request), that request requires a third protocol, or at least an amendment to the second protocol. In both cases, assuming the work is funded, Schwartz may also need to seek approval

A word from OLAW

In response to the questions posed in this scenario, the Office of Laboratory Animal Welfare (OLAW) offers the following guidance:

The Public Health Service *Policy on Humane Care and Use of Laboratory Animals* (PHS Policy; IV.B.7)¹ requires that the IACUC “review and approve, require modifications in (to secure approval), or withhold approval of proposed significant changes regarding the use of animals in ongoing activities.” Notice NOT-OD-14-126, *Guidance on Significant Changes to Animal Activities*², was released by NIH with the concurrence of USDA, APHIS, Animal Care and allows IACUCs the flexibility to meet the PHS Policy requirement through the approval of institutional policies regarding some significant changes. The proposed changes specified in these policies may then be incorporated into research projects without the need for full committee or designated member review.

The scenario described in this column is addressed in paragraph 3 of NOT-OD-14-126: “A significant change that may be handled administratively according to an existing IACUC-reviewed and -approved policy without additional consultation or notification is an increase in previously approved animal numbers”².

NOT-OD-14-126 permits IACUCs to employ administrative handling of requests to increase previously approved animal numbers. Several qualifications must be met to utilize this guidance.

First, the IACUC must have an approved policy specifying the conditions under which animal numbers may be increased by administrative handling. Such a policy must clearly define the limits of the policy and whether an increase is allowed as a percentage, an exact number or a number relative to the original number approved (e.g., a 10% increase in rodents will be permitted as long as the study objectives remain unchanged)³.

Second, the IACUC must authorize who may administratively handle the increase (e.g., IACUC office, IACUC administrator, IACUC chair, attending veterinarian).

Third, the policy must include a provision for incorporating the change into the approved animal activity documentation (e.g., a change will be submitted to the IACUC office during the next working day and will be added to the approved protocol within 2 working days by the IACUC office staff).

Fourth, the policy must be compliant with NOT-OD-14-126 (e.g., modifications requesting a change in species or study objectives or impacting animal welfare must be reviewed by the IACUC and may not be administratively handled).

Fifth, the policy must address whether the original rationale for the number of animals to be used supports the requested change and require an expanded explanation if the original rationale does not³.

And finally, the policy may be species-specific, allowing a certain amount of flexibility with one species and a different amount for another³.

When an IACUC implements NOT-OD-14-126, approval occurs at the time the IACUC approves the institutional policy. Administrative handling is not approval, rather it is a procedure during which qualified person(s) authorized by the IACUC verify that the requested modification is within the scope of the IACUC-approved policy and appropriate in the specific situation.

An institution may choose to include stipulations for consultation with the attending veterinarian, IACUC chair or IACUC member(s) in its institutional policy, although this is not required.

In this scenario, the details of the IACUC’s policy are not evident. If a comprehensive policy is in place and the requested change addresses the required conditions in the policy, then administrative handling is appropriate. If conditions are not met, the change must be referred to the IACUC for full committee or designated member review. Person(s) administratively handling significant change requests always have the authority to refer a decision to the IACUC for review. The IACUC administrator did not overstep her authority in deciding to refer the request for addition of knockout mice to the IACUC for review.

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. National Institutes of Health. *Guidance on significant changes to animal activities. Notice NOT-OD-14-126.* (National Institutes of Health, Washington, DC, 26 August 2014).
3. Office of Laboratory Animal Welfare. *Special Seminar: Guidance on significant changes to animal activities.* (21 August 2014). <http://grants.nih.gov/grants/olaw/educational_resources.htm#a_08212014>

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from the funding agency to move in this new direction.

In addition to the evidently flawed policy, we would see these errors as arising from a communication problem: when there is even a glimmer of doubt, staff should consult with others on interpretations of regulations and decision-making. The attending veterinarian and IACUC chair

must be easily accessible and must welcome such inquiries in order to facilitate reliable, consensus-based team decisions.

The intent of the new guidance is to allow researchers to do their work with as little hindrance as possible. The IACUC should develop policies that support this intent without compromising compliance. Articulating conditions (examples are always

helpful) where the IACUC administrator can make confident decisions on increasing animal numbers will avoid the problems in this scenario.

1. National Institutes of Health. *Guidance on significant changes to animal activities. Notice NOT-OD-14-126.* (National Institutes of Health, Washington, DC, 26 August 2014).



- Office of Laboratory Animal Welfare. Special Seminar: Guidance on significant changes to animal activities. (21 August 2014). <http://grants.nih.gov/grants/olaw/educational_resources.htm#a_08212014>
- Public Health Service. *Policy on Humane Care and Use of Laboratory Animals IV, D, 1, a* (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).

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RESPONSE

Administrator actions appropriate

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The *Guidance on Significant Changes to Animal Activities*¹, indicates that administrative approval for increasing animal numbers on an approved protocol utilizing the same procedures does not violate either the Public Health Service *Policy on Humane Care and Use of Laboratory Animals* or the guidance itself. In this case, however, the main purpose of Schwartz’s experiment must be considered; siRNA suppression of the gene *nol* not only produced the expected outcome of a high incidence of diet-induced obesity but also resulted in an increase in spontaneous mammary adenocarcinomas. Schwartz hypothesized that *nol* might serve as a tumor suppressor and planned to confirm this by repeating the experiment using another mouse strain, BALB/c. This repetition using a different strain could help to confirm that the phenotype was likely due to *nol* and not a random, spontaneous mutation within the C57BL/6 genome unrelated to *nol*. Genetic drift within strains has occurred in the past, such as the drift that created the two substrains C3H/HeJ and C3H/HeN and their very different responses to endotoxin². It is important to trace back and identify the mutation when these shifts occur. By itself, switching from one mouse strain to another does not violate the guidance provided because the animals used are all of the species *Mus musculus* and the aim of the modification was to ensure that the phenotype of interest was not due to factors other than *nol*. Administrative

approval was sufficient for this initial request for BALB/c mice.

Schwartz later requested 50 C57BL/6 mice in which a signaling gene in the *nol* pathway was knocked out. The IACUC administrator believed, quite correctly, that the genetic modification might result in a whole new phenotype. The use of small interfering RNA is temporary and can be discontinued, reversing the blockade on target gene (i.e., *nol*) expression. This is different from knocking out a signaling gene in the *nol* pathway; the resulting phenotype would be expressed constitutively. Because of this key difference, an IACUC review is required. The *Guide for the Care and Use of Laboratory Animals* states, “Regardless of whether genetic manipulation is targeted or random, the phenotype that initially results is often unpredictable and may lead to expected or unexpected outcomes that affect the animal’s well-being or survival at any stage of life”³. From our point of view, the IACUC Administrator made the right decision, because genetically modified animals usually require a more global evaluation to identify any possible phenotypic changes resulting from the genetic alternation. Under the new regulatory guidance¹, changes that cause or have the potential to cause a negative effect on animal welfare either directly or indirectly must be considered as significant changes. Large changes in phenotype could definitely impact the welfare of the animal. Thus, at the very least, an IACUC-approved pilot study would be preferred to investigate this knockout strain for any possible spontaneous lesions that may impact animal welfare.

The IACUC administrator asked for a protocol review of the request for C57BL/6 knockout mice for ‘societal value,’ potential to advance knowledge and harm:benefit analysis. We support this reasoning because it seems that Schwartz was originally attempting to confirm the *nol* effect (i.e., unexpected phenotype of mammary carcinoma) on multiple strains. It now seems, however, that Schwartz’s reason for knocking out the signaling gene in the *nol* pathway is to investigate the gene’s putative role as a tumor suppressor. This is a much different line of scientific inquiry than obesity research, his original objective. Therefore, the IACUC should weigh in on the study objective, study design and animal numbers needed. “Changes in study

objectives” are clearly delineated under item 1.e. of the guidance document as constituting significant changes that require IACUC approval¹. Additionally, when reviewing the protocol, the research facility has the duty to verify that the researcher and staff members all have proper training⁴. Thus, the IACUC should weigh the credentials of the investigator. Does Schwartz, an obesity researcher, have the necessary scientific background and training to pursue this new line of inquiry? Is additional expertise needed? Also, because Schwartz was focused on obesity research when writing the original protocol, he may not have picked up on any known information regarding *nol* in terms of tumors and tumor suppression. A new protocol would require a new literature review to ensure that animals would not be wasted on repeating research that has already been published⁴. Therefore, we agree that the IACUC administrator made the right decision in requiring a new protocol for the addition of the C57BL/6 knockout mice.

- National Institutes of Health. Guidance on significant changes to animal activities. Notice NOT-OD-14-126. (National Institutes of Health, Washington, DC, 26 August 2014).
- Qureshi, S.T. *et al.* Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J. Exp. Med.* **189**, 615-625 (1999).
- Institution for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 8th edn. (National Academies Press, Washington, DC, 2011).
- Animal Welfare Act Regulations. 9 CFR. Chapter 1, Subpart C.

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RESPONSE

One wrong, one right

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The ability to grant administrative approval of a request to add more animals of an approved species to a protocol reduces the IACUC’s burden, allowing the IACUC to focus on more substantive animal use requests and program issues, and also benefits the investigator by accelerating

approval. The intent of the administrative approval option is to streamline the approval process for practical reasons, such as finishing an approved experiments if, for example, biological variability was greater than expected, age-related animal loss occurred or breeding results were poor. This option was not intended for use when the additional animals are needed to expand or change the original objectives or when the model or experimental design is modified. These types of changes should have a scientific rationale that should be evaluated by the IACUC.

This scenario illustrates the complexities in determining whether approval of protocol modification requests requires deliberation over their rationale and impact. To make this determination, the IACUC must ask whether the animals are being used for new experiments or to complete approved experiments.

Schwartz first requested approval to use an additional 50 mice of a different background strain (BALB/c), and this request received administrative approval. Because the *nol*

studies using C57BL/6 mice resulted in an unanticipated incidence of adenocarcinomas, we presume that studying adenocarcinoma incidence was not included in the original project scope. Thus, on one hand, the addition of 50 *Mus musculus* mice to the project seems to qualify for administrative approval because both C57BL/6 and BALB/c mice are *Mus musculus*. On the other hand, the focus on the role of the gene *nol* in adenocarcinomas seems to be a new study objective, and the use of a different genetic background represents a broadening of the project scope, because the potential for background influence on *nol*-induced cancer incidence is now being tested. In other words, the protocol modification request represents a change in the research objectives and, therefore, should have been reviewed by the IACUC. Although the IACUC administrator's rationale for approval was not necessarily flawed, the request shows how the natural progression of experimentation can introduce unexpected nuances (e.g., genotypic differences) and expanded project scopes, which might not be evident when the

request is considered literally and out of the context of what has already been approved by the IACUC.

Schwartz later requested approval to use an additional 50 C57BL/6 mice (same background as approved in the original protocol) in which a signaling gene in the *nol* pathway was knocked out. Assuming that the project scope expansion had been addressed during approval of his first request to use additional animals, this request represents no change in the species, experimental activities or overall project objectives, but the knockout mouse is likely to be physiologically different, which represents a change in approach to creating the experimental model (i.e., switching from exogenous to endogenous *nol* suppression) and thus constitutes a need for IACUC review. Therefore, we concur with the IACUC administrator's assessment that the change in approach "constituted a different experiment even though no new procedures were being proposed".

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