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Restraint and distress in wild mice

The question at the research team meeting was how to get blood samples every 20 minutes for 2 hours from wild-caught mice, *Mus musculus*, to carry out glucose tolerance tests. The issues of capturing and housing the mice had already been resolved with the IACUC office and the animal facility personnel, but now the researchers were writing the actual IACUC protocol. Even with the help of Laboratory Animal Center veterinarians, the group was having trouble with the details of the blood sampling procedure. One suggestion was to quickly catch the mouse by the tail and lance the tail vein, but this was considered to be too stressful for a wild animal. Other suggestions included placing an intravenous catheter in the tail vein or keeping the animal for the full 2 hours in a commercial

mouse restrainer. Eventually, they settled on a method used by a colleague at a different school. The mouse would have the end of its tail taped to a table for 2 hours, but it would otherwise be free to move. At first nobody believed the technique would work, but a video of a wild mouse restrained that way, along with the colleague's glucose data from blood sampling, convinced the group that the animals would settle down sufficiently to allow the researchers to obtain a drop of blood by nicking a tail vein with a 23-gauge needle.

When the protocol finally reached the IACUC, the committee was not quite sure how to handle it. The mouse would be restrained for nearly 2 hours, but should this be considered prolonged restraint? The veterinarian who pre-reviewed the

protocol did not think so, but some members did, while other members felt it was not restraint at all. The last group said that their committee did not categorize a rat on a tether as being restrained and that the mouse having only its tail restrained fit into the same category. Then there was a related question: if the committee were to agree that the study included prolonged restraint, would it be a USDA category E study (unalleviated pain or distress due to experimental needs) or, as some members believed, a category C study (no pain or distress)?

Do you think the restraint method planned for the mice should be considered prolonged restraint? If approved by the IACUC as prolonged restraint, should the study be category E or category C?

RESPONSE

Perhaps a pilot study

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Wild-caught mice are subject to USDA regulation. The *Guide for the Care and Use of Laboratory Animals* (the *Guide*)¹, the Public Health Service *Policy on Humane Care and Use of Laboratory Animals*² and the Animal Welfare Act and Regulations³ ask generally that alternatives to painful or distressful procedures be considered. Here this is addressed in the IACUC's question of whether to categorize the study in column C or column E. The *Guide* provides more specific guidance on this subject, recommending that alternatives to physical restraint be considered and that physical restraint be the minimum necessary to accomplish scientific goals—both of which have been addressed by the IACUC. The regulations are silent, however, on any specific time

limit and put the burden of defining “prolonged restraint” on the IACUC.

Any time an animal's movement is restricted, there is the potential for stress or distress, which needs to be addressed. The research group provided the IACUC with a video of a wild mouse undergoing the same procedure in a colleague's lab and with data showing the mouse had normal glucose levels as evidence that the restraint procedure was not stressful to the animal, but no information was provided regarding prior acclimatization of the mouse to the restraint procedure before testing. Such acclimatization could have affected both behavior during restraint and glucose levels. Contacting the principal investigator to clarify this point may be one way to address the concern before the IACUC is ready to approve the use of animals for this study. The IACUC might also suggest that the investigator do a pilot study measuring either serum glucose or fecal cortisol levels for a pre-determined time.

Once the IACUC has observed or

reviewed any further requested information, it may be comfortable assigning the animals used to the appropriate column in the USDA annual report. As the USDA report is a report of events that have already occurred, the IACUC might choose to assign the animals to a column provisionally for the protocol and reevaluate after the experiments or the pilot study is complete.

1. Institute for Laboratory Animal Research. *Guide for the Care and Use of Laboratory Animals* 29–30 (National Academies Press, Washington, DC, 2011).
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* Section IV.C.1.a: Review of PHS-Conducted or Supported Research Projects. (US Department of Health and Human Services, Washington, DC, 1986; amended 2002).
3. Code of Federal Regulations. Title 9, Chapter 1, Subchapter A—Animal Welfare: Part 2 Regulations. §2.31(d)(i) and (ii).

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