Colorado State University
Institutional Animal Care and Use Committee
(IACUC)

Guidelines on Pain Categories

I. Purpose
Colorado State University (CSU) is committed to protecting the welfare of animals used in research and teaching programs on campus and by campus-affiliated researchers. The university community depends on the Institutional Animal Care and Use Committee (IACUC) to provide ethical oversight of CSU’s Animal Care and Use Program (ACUP) and the animal facilities. Of the utmost importance to the IACUC is that we minimize the pain and/or distress experienced by animals used for research, teaching, or testing. The intent of this guideline is to assist “Principal Investigators” (PIs) in protocol preparation, and specifically, in assigning to specific animal activities the appropriate U.S. Department of Agriculture (USDA) pain and distress categories.

II. Federal Regulations
Federal regulations and guidelines upon which the CSU guidelines are founded include:

- Animal Welfare Regulations (9CFR Part 2 Subpart C §2.36)
- PHS Policy on Humane Care and Use of Animals
- Guide for the Care and Use of Laboratory Animals
- US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

CSU is required to report annually to the USDA how many animals, and which species, were used at each of four “pain categories” (B, C, D, and E)\(^1\). Therefore, CSU IACUC includes pain category information in IACUC protocols, prior to initiating research.

III. Ethical Principles
CSU IACUC is responsible for appropriate use of animals, which includes reduction of pain or distress. The determination of when pain or distress is felt by an animal can be subjective, but such bias can be overcome by:

1. Using scoring systems that assess multiple, relevant aspects of an animal’s behavior and clinical status, and
2. Making an underlying assumption that any procedure that would cause discomfort, distress or pain in a human would cause similar responses in an animal (US Government Principle IV).

\(^1\) USDA Annual Report

Original and Revisions: 5/25/15, 12/16/14, 3/22/16, 11/19/19, 4/27/21
Procedures that cause more than momentary pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Under no circumstances should surgical procedures be performed on unanesthetized animals or animals that have been paralyzed by chemical agents. Animals that would otherwise suffer moderate-severe or chronic pain or distress, which cannot be relieved, must be humanely euthanized as soon as the researcher/caretaker is aware of their distress, unless an exception is approved by the IACUC.

IV. IACUC Protocol Preparation
In an IACUC protocol, the PI needs to explain how a proposed procedure might impact the animal. Assigning a pain category is just one piece of information, but the PI must also share with the IACUC any manifestations of altered behavior or clinical status that will or might arise due to the procedure.

Below are examples of procedures types and their appropriate designation as pain category B, C, D and E, as general guidelines. Procedures likely to fall into pain categories D/E must be planned in consultation with a veterinarian. This guide is not exhaustive; if a PI is unsure of how to categorize a procedure, they should consult the Attending Veterinarian (AV) or AV delegate(s) for guidance.

**Pain Category B:** Defined as breeding animals or holding animals that will be used in research, teaching, or testing but have not yet been used for such purposes.

- Breeding or housing animals without any research manipulation, prior to euthanasia (culling) or transfer to a research or teaching protocol.

**Pain Category C:** Defined as performing research, teaching, or testing procedures on animals that involve no more than momentary pain and/or distress.

- Handling, weighing, observing free behavior in animals in teaching or research activities
- Peripheral injections, fluid collections, or catheter placements
- Ear-punch of rodents
- Dietary studies that do not alter clinical health
- Routine agricultural procedures associated with livestock husbandry
- Live-trapping and marking
- Positive-reward behavioral training
- Chemical immobilization of animals for imaging (e.g. MRI, CT scans, etc.), blood/fluid collection
- Tail snips in mice and rats

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2 No more than 5 mm of total tissue may be taken. Tail snip is performed with a sterile surgical blade or a sharp pair of scissors. Scissors should be initially sterile and wiped with ethanol between animals. Bleeding must be controlled using pressure, silver nitrate, Kwikstop, or other coagulating agent.
**Pain Category D:** Defined as performing research, teaching, or testing procedures on animals that may involve accompanying pain or distress, and for which appropriate preventative anesthetic, analgesic, or tranquilizing drugs may be needed.

- Survival or non-survival surgery
- Laparoscopy or needle biopsy
- Exposure (cut-down) of blood vessel for catheter placement

**Pain Category E:** Defined as performing research, teaching, or testing procedures on animals that may involve accompanying pain or distress, and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the results. *An explanation of the procedures producing pain or distress on these animals and the reasons such drugs are not used is attached to the USDA annual report.*

- Application of noxious stimulation, trauma or shock from which an animal cannot escape or which an animal cannot avoid
- Exposure to extreme environmental conditions (e.g., hypoxic chambers, extreme temperature or humidity conditions without acclimation)
- Paralysis or prolonged restraint of a conscious animal
- Breeding of a genetically engineered phenotype that causes pain/distress that will not be alleviated

**V. Considerations for Progressive Disease Studies**

Progressive Disease Studies (e.g. infectious disease studies) can be difficult to assess ahead of time and some considerations are provided below.

- Pain Category C: Clinical signs will not be experienced after inoculation, tumor induction, or infection because the endpoint of the study will occur prior to the animal experiencing any clinical signs.
- Pain Category D: Clinical signs will be experienced but will be relieved by analgesics or euthanasia as soon as clinical signs are noted-this should be accompanied by appropriate monitoring intervals based on the disease progression.
- Pain Category E: Clinical signs are experienced after inoculation, tumor induction, or infection but no clinical treatments are administered for scientific reasons-this must be fully justified in the protocol and endpoint criteria beyond the initial clinical signs must be described.

The IACUC understands that it may not be possible to predict the incidence of unexpected deaths/clinical illness due to experimental manipulations, and asks PIs to document when these occur and report them in the Annual Reviews so that the USDA document can be accurately prepared.
Further information on how the IACUC handles Progressive Disease Studies can be found in the Policy on Observation of Animals in Progressive Disease Models.

VI. Additional Resources

- [https://www.aclam.org/Content/files/files/Public/Active/position_painanddistress.pdf](https://www.aclam.org/Content/files/files/Public/Active/position_painanddistress.pdf)
- [https://www.research.colostate.edu/ricro/iacuc/policies-and-guidelines/](https://www.research.colostate.edu/ricro/iacuc/policies-and-guidelines/)