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, testing or teaching. 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:

- The Animal Welfare Act Regulations (9CFR 2.C.2.36.b.5)
- 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). Therefore, CSU IACUC asks that Pain Category information be provided by the PI in IACUC protocols, prior to initiating research, so that CSU remains in good standing with all applicable agencies and sponsors.

III. Ethical Principles
CSU IACUC believes that the proper use of animals includes the avoidance or minimization of discomfort, distress and pain. 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.

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. Protocol Preparation
In completing an animal protocol, the PI needs to provide the IACUC with a full understanding of how a proposed procedure might impact an animal. Assigning a USDA Pain Category is just one piece of information that must be provided, but the PI must also share with the IACUC any and all manifestations of altered behavior or altered clinical status that either will or might possibly arise because of the procedure.

Below we have provided examples of types of procedures and their appropriate designation as Pain Category B, C, D and E, as a guide to the PI. Procedures that are 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 indicate the nature of their indecision to the IACUC or the Attending Veterinarian (AV) and/or delegate(s) for their guidance.

**Pain Category B:**
Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.
- Animals being bred or housed, without any research manipulation, prior to either euthanasia (culling) or transfer to a research or teaching protocol.

**Pain Category C:**
Animals upon which teaching, research, experiments, or tests were conducted involving no more than momentary pain and/or distress,
- Handling, weighing, observing free behavior in animals in teaching or research activities
- Peripheral injections, fluid collections (in modest amounts) 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.), or blood/fluid collection.
- Tail snips in mice and rats\(^1\)

**Pain Category D:**
Animals upon which experiments, teaching, research, surgery or tests were conducted involving accompanying pain or distress, and for which appropriate preventative anesthetic, analgesic, or tranquilizing drugs were used.
- Survival or non-survival surgery
- Laparoscopy or needle biopsy
- Exposure (cut-down) of blood vessel for catheter placement
- Exsanguination, perfusion, cervical dislocation, or thoracotomy with anesthesia

\(^1\) 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 E:**
Animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals, and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. Disease inoculation that may result in clinical symptoms for which an animal must either be treated with analgesia/anesthesia or euthanized are also included here.

(An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be 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) are assigned USDA pain category C or E by CSU IACUC.

- Pain Category C: Clinical symptoms 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 symptoms.
- Pain Category E: Clinical symptoms experienced after inoculation, tumor induction, or infection but no clinical treatment is given for pain relief, to prevent or alleviate symptoms.

The IACUC understands that it may not be possible to predict the incidence of unexpected deaths due to experimental manipulations, and asks PIs to prepare accordingly by proactively assigning a certain percentage of the total inoculated animals as Pain Category E. The PI may provide documentation to demonstrate the percentage of total inoculated animals should be assigned as Pain Category C vs. E. 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/)