

IACUC POLICIES, PROCEDURES, and GUIDELINES

HUMANE USE PAIN CLASSIFICATIONS (Pain Categories)

Purpose:

This document provides guidelines for the classification of animal use into the Humane Animal Use Categories used at the University of Kentucky. The intent of this document is to assist and provide guidance to investigators regarding appropriate humane use categorization. This policy document will also serve as a reference for members of the Institutional Animal Care and Use Committee (IACUC) regarding appropriate classification and protocol review requirements.

Responsibilities:

Humane Animal Use Categories (also referred to as Pain and Distress Classifications) are generally assigned prospectively and are based upon past experiences and anticipated results. Humane Animal Use Categories can be changed after the initiation of the study if unanticipated complications or effects occur resulting in a change in the level of pain or distress. The vast majority of cases, however, are properly classified initially during the protocol review process.

- The investigator provides the initial classification in the protocol submission. The investigator is likely the person most familiar with the proposed research project and the vertebrate animal procedures involved. The investigator should be able to provide a good initial assessment of potential pain and distress the animals will experience.
- The veterinarians have the responsibility of reviewing the initial classification and, if necessary and in consultation with the investigator, recommending a change in the humane use classification. The veterinarians' experience with the various laboratory animal species and how they react in similar instances adds a component that may not be known or recognized by the investigator. If the investigator and the veterinarian cannot come to a consensus on the pain classification, the recommendations of both parties will be presented to the IACUC.
- The final review of the Humane Animal Use Categories occurs at the level of the full IACUC. The IACUC membership includes a number of specifically designated members that bring a wide range of experiences to the evaluation. It is at this level where the full expertise of the investigator(s), the veterinarians, and the members of the IACUC can be integrated to provide the best evaluation of the appropriate Human Animal Use Categories.

- The University of Kentucky must submit an annual report to the United States Department of Agriculture listing the pain classification of all animals used in research, testing, and education. This annual report must be signed by either the Institutional Official or the President of the University of Kentucky.

General Guidelines:

The Humane Animal Use Categories used by the University of Kentucky IACUC are identical to those used by the United States Department of Agriculture (USDA) and defined in the Animal Welfare Regulations (9 CFR §2.36).

Humane Animal Use Category B: Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used.

Category B is generally restricted only to animals used only for breeding or production. Manipulations of the animals must be limited to routine husbandry and veterinary practices that are not directly related to any research and educational purpose. Mice that have tissue collected specifically for genotyping (tail snips) or phenotyping are classified in category C as this is a research procedure.

Humane Animal Use Category C: Animals used in teaching, research, experiments, or tests involving no pain, distress, or use of pain-relieving drugs.

Animals in category C are on research, testing, or educational protocols where they are not subjected to more than slight or momentary pain or distress. Routine procedures typically performed in human patients without anesthetics or analgesics (e.g., injections, tattooing, blood sampling) are included in this category along with ear punching, ear tagging, and tail-tip collection for genotyping prior to 21 days of age in mice. Euthanasia for tissue collection, terminal anesthesia and perfusion, behavioral observation protocols, positive reinforcement behavioral modifications, nutrition studies, etc. are the types of protocols where animals may be classified in humane animal use category C.

Humane Animal Use Category D: Animals used in teaching, research, surgery, or tests involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs are used. An essential component of any Category D protocol is the inclusion of an appropriate treatment (anesthesia, analgesia, euthanasia, etc.) to prevent, reduce, or eliminate the pain and distress.

Animals subjected to potentially painful or distressful procedures where an anesthetic, analgesic, or tranquilizer is used to alleviate the pain and distress are classified under pain category D. This category includes both survival surgical procedures as well as nonsurvival surgical procedures where the animal is maintained under a surgical plane of anesthesia for a set period of time. Category D may also be used in specific instances where onset of pain or distress leads to the euthanasia of the animal and in cases where acclimation of the animal to the restraint, procedure, or technique sufficiently reduces the distress that would have been present without the acclimation.

One must assume, lacking any contrary information, that if pain or distress would be anticipated in a human patient subjected to a similar procedure that an animal subjected to the procedure would experience a similar level of pain or distress. Consultation with the veterinarians regarding appropriate treatment regimens is encouraged to minimize both pain and distress to the animals and interference or complications with the research.

Humane Animal Use Category E: Animals used in teaching, experiments, research, surgery, or tests involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be provided to and approved by the IACUC. This approved explanation must be attached to the annual report submitted to the USDA.

Category E animal use protocols are controversial and receive increased IACUC, USDA, and PHS scrutiny. The basic premise of a category E animal use protocol is that while you can relieve the anticipated pain and distress you have chosen not to do so because it would interfere with the proposed research and invalidate the results. The scientific or regulatory justification for withholding the analgesics, anesthetics, tranquilizers, or other treatments designed to relieve pain and distress must be clearly stated in the animal use protocol and approved by the IACUC. Scientific justification must be specific, addressing why each potential class of drugs that might be effective at reducing the pain and distress would likely interfere with the research objectives. If a regulatory justification is used, the specific regulation must be referenced in the justification. Category E projects present an explicit responsibility on the researcher to explore alternative methods before proceeding with the study.

Examples of potential Category E procedures include studies of pain and inflammation, toxicity or virulence testing where death is used as an end-point (LD₅₀), the use of noxious stimuli (including electric shock) where escape is impossible, the induction of aggressive behavior leading to self-mutilation or fighting, food and water deprivation beyond that necessary for routine pre-surgical preparation, whole body irradiation, and paralysis of a conscious animal.

Alternatives to Painful or Distressful Procedures:

The Animal Welfare Act (AWA) regulations require principal investigators to consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals (Humane Animal Use Categories D and E) and provide a written narrative of the methods used and sources consulted to determine the availability of alternatives, including refinements, reductions, and replacements.

“Alternatives or alternative methods are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use in pursuit of the minimization of animal pain and distress consistent with the goals of the research. These include methods that use non-animal systems or less sentient animal species to partially or fully replace animals (for example, the use of an *in vitro* or insect model to replace a mammalian model), methods that reduce the number of animals to the minimum required to obtain scientifically valid data, and methods that refine animal use by lessening or eliminating pain or distress and, thereby, enhancing animal well-being. Potential alternatives that do not allow the attainment of the goals of the research are not, by definition, alternatives.”⁴

The use of a database search is generally the most effective and efficient method for demonstrating compliance with the requirement to consider alternatives to painful/distressful procedures. Conferences, colloquia, subject expert consultants, or other sources may provide relevant and up-to date information regarding alternatives in lieu of, or in addition to, a database search. In all cases, it is the IACUC’s responsibility to assess that a reasonable and good faith effort was made to determine the availability of alternatives or alternative methods. If a bona fide alternative method (one that could be used to accomplish the goals of the animal use proposal) is identified, the written narrative must justify why this alternative was not used.

Additional Assistance:

The veterinarians of the Division of Laboratory Animal Resources (DLAR) are available to assist investigators in determining the appropriate Humane Animal Use Category and to provide guidance and advice regarding appropriate anesthetics, analgesics, and tranquilizers. For more information and veterinarian contact information see the DLAR website (<http://www.research.uky.edu/dlar/index.html>).

Developing an effective and efficient set of key words, search strategies, and databases to identify alternatives to painful/distressful procedures may be complicated and difficult. Assistance in developing an appropriate and efficient database search for alternatives may be obtained from the reference librarian most familiar with the research subject area (<http://www.uky.edu/Libraries/allguides.php>).

References:

1. Animal Welfare Act, United States Code, Title 7, Chapter 54, Sections 2131-2159.
2. Animal Welfare Regulations, Code of Federal Regulations, Title 9, Chapter 1, Subchapter A, Parts 1-4.
3. United State Department of Agriculture, Animal Care Policy Manual, Policy 11 – Painful Procedures, Issue Date: April 14, 1997.
4. United State Department of Agriculture, Animal Care Policy Manual, Policy 12 – Consideration of Alternatives to Painful/Distressful Procedures, Issue Date: June 21, 2000.
5. Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 1996.
6. Public Health Service Policy on Humane Care and Use of Laboratory Animals, Office of Laboratory Animal Welfare, National Institutes of Health, Public Health Service, 2002.

Approved and Adopted by the
Institutional Animal Care and Use Committee
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