Institutional Animal Care and
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INSTITUTIONAL ANIMAL CARE AND USE POLICY
Guidelines for Assigning Procedures and Animals to USDA Pain and Distress Categories
Approval Date: December 5, 2022

Purpose

The purpose of this policy is to help writers and reviewers of animal protocols with assigning procedures and animals to United States Department of Agriculture (USDA) pain and distress categories.

A painful procedure is defined as "any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure is applied, that is, pain in excess of that caused by injections or other minor procedures."

When writing an animal protocol for a study, the principal investigator (PI) and other contributors should review the definitions and examples of USDA pain and distress categories below. They must then assign each procedure in the protocol to the appropriate category. They must also assign each animal to the highest category (E > D > C) it will be subjected to. Furthermore, they are obligated to

i) avoid or minimize discomfort, distress, and pain to the animals in the study;
ii) consider appropriate alternatives to any procedure that may cause more than slight or momentary pain or distress (categories D and E); and
iii) consult with the Attending Veterinarian in the planning of procedures for USDA-regulated species.

The Institutional Animal Care and Use Committee (IACUC) is responsible for ensuring that PIs have met these obligations and will accept or modify the proposed categories during its review of the protocol.

Categories

Category C procedures are those that do not cause more than momentary or slight pain or distress or that do not require the use of pain-relieving drugs or non-pharmacological support measures.

Category D procedures are potentially painful procedures for which anesthetics, analgesics, tranquilizers, or other pharmacological or non-pharmacological support measures will be used. The key distinction is that animals are given appropriate anesthesia or pain-relief measures to limit their pain and distress as much as possible.

Category E procedures are those that are painful or stressful and are used without the use of anesthetics, analgesics, tranquilizers, or other pharmacological or non-pharmacological support measures. Withholding of pain- or distress-relieving measures must be scientifically justified in writing and approved by the IACUC.

Category E studies are given particular scrutiny because the IACUC must be satisfied that less-painful or stressful alternatives are not available or that less-painful or -stressful endpoints cannot reasonably be used. By law, the institution must submit a report to the USDA annually. This report summarizes each category E procedure together with a scientific justification for its use. Generally, the justification provided by the researcher in the animal protocol is used for this purpose. Once submitted to the USDA, this information will be available to the public. Therefore, it is important that information related to category E procedures is complete and accurate.

Search for Alternatives to Category D & E Procedures

The Animal Welfare Act and Regulations require PIs to consider alternatives, including refinements, reductions, and replacements, to category D and E procedures. A written narrative of the methods used and sources consulted to determine the availability of alternatives must be provided with the initial protocol submission and with any subsequent amendment that adds or modifies a category D or E procedure.

The recommended method for identifying alternatives is searching dedicated databases, such as those listed at Altweb at Johns Hopkins, or the Animal Welfare Information Center at the National Agricultural Library (AWIC) . Under some circumstances, the use of conferences, consultants (statisticians, subject-matter experts, etc.), or other sources may be justified in addition to database searches. Non-database sources must also be documented in the protocol submission, e.g., by listing a consultant's name and qualifications and the date and content of the consultation.

If the search for alternatives identifies a less-painful or -distressful method that would accomplish the goals of the proposed animal use, the IACUC requires an explanation for why the alternative cannot be used.

Additional searches for alternatives are not required at the time of a protocol's first and second annual review. However, the PI must search for alternatives at least once every three years, consistent with the triennial *de-novo*-review requirements of the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

References:

1. Animal Welfare Regulations (9CFR, Chapter 1, Subchapter A, Part 2, Subpart C, Section 2.36)
2. Public Health Service Policy on Humane Care and Use of Laboratory Animals
3. USDA Animal Care Policy #11
4. USDA Animal Care Policy #12

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Table 1 – USDA Pain and Distress Categories with Example Procedures

Category C	Category D	Category E
Animals are subjected to procedures that cause no or only momentary or slight pain or distress and that do not require the use of pain- or distress-relieving drugs or measures.	Animals are subjected to potentially painful or distressful procedures for which they receive appropriate anesthetics, analgesics, tranquilizers, or other (pharmacological or non-pharmacological) support measures.	Animals are subjected to potentially painful or distressful procedures that are **NOT** relieved with anesthetics, analgesics, tranquilizers, or other (pharmacological or non-pharmacological) support measures. Withholding anesthesia, analgesia, or support measures must be scientifically justified in writing and approved by the IACUC.
Example Category C	**Example Category D**	**Example Category E**
1. Handling or weighing animals 2. Collection of tissues for genotyping: ear punching of rodents, mouse tail biopsies (< 2 mm) at ≤ 21 days of age, blood collection, swab collection 3. Most animal behavior studies, such as: - Observation and activity monitoring - Active place avoidance - Positive-reward conditioning or research - Strength and climbing tests - Rotorod and balance beams - Object recognition or marble burying - Running wheel - Food or fluid preference - Open-field, mazes, and other tests that allow exploration and movement to preferred locations 4. Peripheral injections, blood collection, or percutaneous catheter implantation 5. Food- or water-controlled studies that do not result in clinical health problems. 6. Dosing routes of administration - Subcutaneous injections - Intramuscular injections - Intravenous injections - Intraperitoneal injections - Oral Gavage 7. Anesthesia or sedation for restraint during a painless or only momentarily-painful procedure, such as blood collection, percutaneous catheter placement, tattooing 8. AVMA-approved euthanasia procedures, including anesthesia followed by perfusion 9. Device restraint with acclimation	1. Surgery (survival or non-survival) 2. Tumor production 3. Retro-orbital blood collection 4. Exposure of blood vessels for catheter implantation 5. Induced infections or antibody production (ascites method) 6. Genetically engineered phenotypes that cause pain or distress that will be alleviated 7. Tail snips with mice > 21 days old 8. Aversive or restrictive behavioral studies of limited duration, such as: - Forced Swim Test - Device restraint without acclimation 9. Laparoscopy or needle biopsies	1. Toxicological or microbiological testing, cancer research, or infectious disease research that requires continuation without medical relief after clinical symptoms are evident or that requires death as an endpoint 2. Any procedure for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes 3. Exposure to extreme environmental conditions 4. Genetically engineered phenotypes that cause pain or distress that will not be alleviated 5. Aversive or restrictive behavioral studies of extended duration, such as: - Device restraint without acclimation - Inescapable foot shock - Chronic variable stress paradigms 6. Injury models without pain and distress relief, such as: - Traumatic brain injury - Stroke