University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

Pain and Distress Class Categorization

Policy: All vertebrate animals used for research or teaching, including pilot and/or internally funded research, must be assigned to a USDA pain and distress category [B, C, D, or E (formerly 0, I, II, or III correspondingly)]. This policy provides definitions and examples of the pain and distress categorization utilized by the University of Louisville IACUC. This policy is intended to serve as a resource for Principal Investigators, as well as IACUC reviewers, in writing or reviewing *Proposals*. Specific examples of procedures that fall into pain and distress categories B, C, D, and E are provided in Appendix 1 below. Appendix 1 is not all-encompassing, and some experimental procedures may not be listed or clearly divided into categories here. Principal Investigators are encouraged to consult with the Attending Veterinarian or IACUC Chair in these instances.

Rationale: All USDA-regulated animals used for research or teaching must be assigned into appropriate pain and distress class categories[1]. Additionally, many institutions classify other animals in a similar fashion. In general, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals[1,2]. Federal regulations require that investigators demonstrate to the IACUC that "alternatives to procedures that may cause more than momentary pain or distress to the animals" have been considered[3,4].

Procedures, Guidelines, and Exceptions:

1. All animals must be categorized as Pain and Distress Category B, C, D, or E within the IACUC *Proposal* under the "Experimental Group" section. Examples of common procedures and their appropriate designations is provided in Appendix 1. The IACUC ultimately determines pain categories on a case-by-case basis.
2. Each experimental group should be listed under the highest pain and distress category that will apply to the animal at any time while the animal is listed on the procotol. Multiple pain categories will not be assigned to one experimental group.
3. Scientific justification and IACUC approval is required for withholding pain-relieving agents. Animals exhibiting signs of pain, discomfort or distress are expected to recieve appropriate relief unless otherwise approved by the IACUC. Category E *Proposals* must also detail the incompatibility of pain/distress relief with the goals of the research.
4. Principal Investigators must provide a description of their consideration of alternatives to painful and/or distressful procedures for *Proposals* containing Category D or E experimental groups for IACUC review. This should be detailed in the "Consideration of Alternatives" section of the *Proposal* and should include the methods and sources used to determine that suitable alternatives were unavailable.

Appendix 1:

Category B	**Category C**	**Category D**	**Category E**
Animals will be acquired/held, but not used or manipulated in any way.	Animals will experience no pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents.	There is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Note: Appropriate endpoints may qualify procedures for Class II.	Animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents.
Examples:	**Examples:**	**Examples:**	**Examples:**
1. Animals being housed without any research manipulation, prior to euthanasia or transfer to another protocol 2. Observation of animal behavior in the wild without manipulating the animal or it's environment	1. Breeding protocols 2. Holding or weighing animals in teaching, outreach or research activities 3. Observation of animal behavior in the lab 4. Rodent ear punching 5. Tail snips in mice ≤ 21 days old 6. Peripheral Injections, blood collection or catheter implantation 7. Feed studies, which do not result in clinical health problems 8. Positive reward training or research 9. AVMA approved euthanasia procedures 10. Euthanasia of breeding animals or unused offspring	1. Survival surgery or painful procedures with anesthesia and appropriate analgesia 2. Non-survival surgical procedures 3. Induction of cancer/tumors with appropriate endpoints 4. Footpad injections with adequate analgesia 5. Use of adjuvants with adequate analgesia 6. Laparoscopy or needle biopsies with adequate analgesia 7. Induced infections or antibody production with adequate supportive care, treatment, or endpoints 8. Tattooing with adequate analgesia 9. Food restriction that reduces the animals weight by no more than 20% with adequate endpoints 10. Tail snips in mice > 21 days old with adequate anesthesia and analgesia 11. Genetically engineered phenotype that causes pain or distress that will be	1. Exposure to agents or radiation levels that cause serious illness, such as LPS administration without analgesia, Dextran Sulfate Sodium (DSS)-induced colitis without analgesia, TNBS (2,4,6-trinitrobenzene sulfonic acid)-induced colitis without analgesia or palliative treatment intervention. 2. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation after clinical symptoms are evident without medical relief 3. Ocular or skin irritancy testing without adequate analgesia 4. Prolonged food or water deprivation or restriction for the purpose of inducing cachexia or dehydration 5. Application of noxious stimuli such as electrical shock

		alleviated 12. Inhalant exposure to chemicals (except pharmaceutical anesthetic agents used with a calibrated vaporizer for anesthesia) with adequate supportive care, treatment, removal criteria from chamber or exposure, and/or adequate endpoints 13. Exposure to agents or radiation levels that do not cause serious illness and have analgesic administration and/or palliative treatment intervention.	that the animal cannot avoid/escape 6. Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes 7. Exposure to extreme or stressful environmental conditions 8. Use of Complete Freund's Adjuvant without analgesia 9. Genetically engineered phenotype that causes pain or distress that will not be alleviated 10. Inhalant exposure to chemicals (except pharmaceutical anesthetic agents used with a calibrated vaporizer for anesthesia) where adequate supportive care, treatment, removal criteria from chamber or exposure, and/or adequate endpoints cannot be provided without interfering with study objectives

References:

1. APHIS/USDA Policy 11 & Policy 12. https://www.ncbi.nlm.nih.gov/books/NBK99537/
2. Interagency Research Animal Committee (IRAC). 1985. *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*.
3. Animal Welfare Act, Public Law 89-544, as amended by P.L. 91-579, P.L. 94-279, P.L. 99-198, P.L. 101-624
4. National Institutes of Health. 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. NIH Office of Laboratory Animal Welfare, Bethesda