ASSIGNING PAIN AND DISTRESS CATEGORIES TO ACUC PROTOCOLS

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1. Background

Federal regulations require that procedures involving vertebrate animals used in research and teaching be categorized according to their potential for causing pain or distress to animal subjects. Use of live vertebrate animals in research and teaching must be described in an Animal Use Protocol (AUP) that is reviewed and approved by the Animal Care and Use Committee (ACUC). ACUC is responsible for ensuring that investigators have avoided or minimized discomfort, distress and pain to animals, that appropriate alternatives to any procedures that may cause more than slight or momentary pain or distress have been considered, and that the PI has consulted with an Office of Laboratory Animal Care (OLAC) veterinarian in the planning of the procedures.

2. Purpose

The purpose of this guideline is to assist researchers in selecting the appropriate pain and distress category when submitting an AUP to the ACUC. All vertebrate animal procedures used in research or teaching must be assigned a pain and distress category. This guideline provides definitions and examples (see Appendix 1) of these pain and distress levels.

3. Definitions

Pain: An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

Painful procedure: 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.

Distress: An aversive, negative state in which coping and adaptation processes fail to return an organism to physiological and/or psychological homeostasis.

Category B: Animals being held, bred, or conditioned for use in teaching, experiments, research or surgery, but not yet used for such purposes.

Category C: Animals that are subject to procedures that cause no pain or distress, or procedures that cause only momentary or slight pain or distress and do not require the use of pain-relieving drugs.

Examples: Routine injections, other examples (see Appendix 1).

Category D: Animals subjected to potentially painful or stressful procedures that are alleviated by appropriate anesthetics, analgesics, tranquilizers, removal from study, euthanasia or other approved methods.

Examples: All surgeries, other examples of pain in excess of that caused by injections or other minor procedures (see Appendix 1).

Category E: Animals subjected to potentially painful or stressful procedures that are not alleviated by anesthetics, analgesics, tranquilizers or other approved methods. Withholding anesthesia/analgesia must be scientifically justified in writing and approved by the ACUC.

Examples: Exposure of animals to external stressors (e.g., forced aggression, predator odor), other examples (see Appendix 1).

4. **References**

- Animal Welfare Act, 7 USC Ch. 54, Section 2143. Standards and certification process for humane handling, care, treatment, and transportation of animals.

- Animal Welfare Regulations, 9 CFR, Chap 1, Sec §2.36.

- Carstens E., Moberg G. P. (2000). Recognizing pain and distress in laboratory animals. ILAR J. 41, 62–71.

- National Research Council. 2009. Recognition and Alleviation of Pain in Laboratory Animals. Washington, DC: The National Academies Press. https://doi.org/10.17226/12526.

- National Research Council. 2008. Recognition and Alleviation of Distress in Laboratory Animals. Washington, DC: The National Academies Press. https://doi.org/10.17226/11931.

- U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 2016. Animal Care Policy Manual. Policy 11. Painful and Distressful Procedures. https://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf

- U.S. Department of Agriculture, Animal and Plant Health Inspection Service. 2013. *Animal Welfare Inspection Guide*, Appendix A.

https://www.aphis.usda.gov/animal_welfare/downloads/Animal-Care-Inspection-Guide.pdf

- https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b17_zebrafish.pdf

Appendix 1 - Examples of Pain and Distress Categories

Category B	Category C	Category D	Category E
Examples	**Examples**	**Examples**	**Examples**
1. Animals being bred or housed, without any research manipulation, prior to euthanasia or transfer to another protocol	1. Holding or weighing animals in teaching, outreach or research activities 2. Observation of animal behavior in the lab 3. Ear notching or ear punching of rodents, injectable microchipping, point or automated tattooing 4. Tail snips in mice ≤ 21 days old 5. Research procedures that involve no more than slight or momentary pain or distress (e.g., drawing blood, standard peripheral injection, peripheral catheter placement, gastric gavage). 6. Feed studies or special diets/water that do not result in clinical health problems, pre-anesthetic fasting up to 16 hours 7. Positive reward training or research 8. Exposure to alterations in environmental conditions (not extreme) with appropriate conditioning and microenvironment 9. Unknown genetically engineered phenotype or known phenotypes 10. that do not elicit pain or distress 11. Imaging/Laser procedures 12. Non-invasive or low-impact behavioral studies (e.g., open field, mazes, conditioned place preference,	1. Survival surgery 2. Non-survival surgical procedures 3. Laparoscopy or needle biopsies 4. Blood/fluid collection procedures requiring anesthesia (e.g., retro-orbital blood collection, CSF tap) 5. Non-standard injections: footpad injections; retro-orbital injections; injections of irritating compounds that cause pain or distress that will be alleviated; repeated injections 6. Exposure of blood vessels for catheter implantation 7. Induced infections or antibody production, excluding ascites if pain or distress is alleviated 8. Tattooing that takes longer than a moment 9. Exposure of skin to UV light to induce sunburn if pain or distress is alleviated 10. Tail snips in mice > 21 days old 11. Non-surgical research procedures expected to cause more than slight or momentary pain or distress that is alleviated via analgesia/ anesthesia, tranquilizers, removal from procedure, euthanasia or other approved method 12. Food Scheduling > 24hrs 13. Water scheduling alleviated by provision of additional water or	1. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation after clinical symptoms are evident without medical relief/euthanasia or require death as an endpoint 2. Ocular or skin irritancy testing 3. Food or water deprivation beyond that necessary for ordinary pre-anesthetic preparation 4. Application of noxious stimuli such as electrical shock that the animal cannot avoid/escape 5. Any procedures for which needed analgesics, tranquilizers, sedatives, anesthetics, removal from procedure or euthanasia must be withheld for justifiable study purposes 6. Exposure to extreme environmental conditions 7. Induction of radiation sickness 8. Sepsis models 9. Footpad injections 10. Paralysis or immobilization of a conscious animal 11. Genetically engineered phenotype that causes pain or distress that will not be alleviated 12. Forced exercise (e.g., swimming, treadmill protocols). 13. Behavioral tests that require inducing pain or distress (e.g., forced

	rotarod, food/fluid preference), includes voluntary locomotion encouraged by researcher and allodynia/hyperalgesia tests (e.g. hot plate, tail flick, Von Frey). 13. Nonsurgical embryo collection 14. Routine agricultural husbandry procedures approved by the ACUC in a protocol or SOP 15. Live trapping 16. AVMA approved euthanasia procedures 17. Perfusion post-euthanasia (heart is no longer beating) for tissue collection 18. Zebrafish procedures on stages 3-7dpf	fluids if signs of dehydration 14. Irradiation with reconstitution (for lethal levels), monitoring and supportive care 15. Genetically engineered phenotype that causes pain or distress that will be alleviated 16. Anesthetize and release (i.e., for blood sampling) of wildlife 17. Tumor induction/ implantation if relief of pain/distress 19. Exsanguination with anesthesia (heart is still beating) 20. Perfusion with anesthesia (heart is still beating) for tissue collection	aggression sleep deprivation, inescapable electric shock) 14. Euthanasia by procedures not approved by the AVMA 15. Antibody production models requiring ascites 16. Field studies in which an animal's natural environment is modified and the animal is exposed to stimuli that they cannot avoid/escape, and/or is severe enough to cause pain or distress (e.g., prey in predator/prey studies, death as an endpoint)

Appendix 2 – Clinical Signs of Pain/Distress by Species

Species	Behavior	Appearance
Rodents	Decreased activity; excessive licking/scratching; self-mutilation; avoidance or aggression; abnormal locomotion (stumbling); writhing; no nest building; reduced eating/ drinking; aversion toward conspecifics	Ungroomed; piloerection; rough or stained hair coat; abnormal stance or hunched back; recumbent; porphyrin staining (“red tears” in rats); rapid, shallow respirations
Rabbits	Head pressing; bruxism; aggressive or avoidance; increased vocalizations (e.g., squeals, cries); reluctant to ambulate; self-mutilation	May not show large change; hyper salivation; hunched posture; rapid, shallow respirations
Fish	Improper buoyancy; lethargy; surface breathing	Opercular flaring; sloughed mucus; clamped fins; petechiation or hemorrhage; change in body color; scale loss; whirling
Xenopus	Buoyancy problems- reluctance to dive; slow to respond; swim upside down or circle	Excess skin shedding; petechial and ecchymosis of integument; cloudy eyes; sunken, hour-glass shape of the coelomic cavity or large, distended coelomic cavity
Reptiles	Weakness or lethargy	Incomplete shedding, including retained spectacles; discoloration
Birds	Inappetence; Altered gait or posture; lethargy	Wasting (decreased pectoral muscles); ruffled feathers; rapid open mouth breathing (panting); dull eyes