



IACUC Guidance:	<b>TAMU-G-023</b>	Title:	<b>Guidelines on Recognizing Pain and Distress in Research Animals</b>	
		Location	Effective Date	Review By
		<b>College Station/Dallas/Galveston/Kingsville</b>	08/01/2023	7/31/2026
		<b>Houston</b>	09/01/2023	7/31/2026

**1. PURPOSE**

- 1.1. The purpose of this document is to help researchers recognize pain and distress in animals used for research, teaching, or other purposes at Texas A&M University.

**2. SCOPE**

- 2.1. This document does not list all signs of pain and distress; the common indicators are included.
- 2.2. Analgesia is discussed elsewhere. See TAMU-G-002.

**3. RESPONSIBILITY**

- 3.1. The **PI** should consult the **AV**, or designee regarding the plan for analgesia.
- 3.2. When uncertain if an animal is experiencing pain or distress, the **PI** should always seek the expert advice from the **AV**, or designee.
- 3.3. The **PI** must administer drugs as indicated in the approved protocol.

**4. DEFINITIONS AND/OR ACRONYMS**

- 4.1. **Analgesic:** Drug used to relieve pain.
- 4.2. **Anxiolytic:** Drug (chiefly) used to reduce anxiety.
- 4.3. **AV:** Attending Veterinarian. Individual designated by Texas A&M University to fulfil the regulatory role of AV. May also describe veterinary staff who report directly to, and have delegated authority from, the AV.
- 4.4. **BCS:** Body Condition Score. Visual assessment of the amount of fat/muscle covering the bones of an animal
- 4.5. **Cumulative Distress:** The concept that repeated procedures, or combinations of procedures, can combine to make the overall severity of distress greater.
- 4.6. **Distress:** The effect of stimuli that initiate adaptive responses that are not beneficial to the animal—thus, the animal’s response to stimuli interferes with its welfare and comfort.
- 4.7. **ERC:** Early Removal Criteria. Specific, predetermined indicators of pain and distress used to establish early study endpoints without loss of scientific quality.
- 4.8. **ERS:** Early Removal Score. A method of animal evaluation in which numerical values are assigned to specific clinical signs and/or behavioral observations.
- 4.9. **Experimental Endpoint:** Occurs when the scientific aims and objectives have been reached.
- 4.10. **Humane Endpoint:** The point at which pain or distress in an experimental animal is prevented, terminated, or relieved.
- 4.11. **IACUC:** Institutional Animal Care and Use Committee. Institutional body responsible for ensuring adherence to federal regulation and institutional policy relating to the care and use of animals in teaching, testing and research. Appointed by the Institutional Official.
- 4.12. **Morbidity:** State of disease or ill health.
- 4.13. **Moribundity:** State of dying/ approaching death.
- 4.14. **PI:** Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
- 4.15. **Refinement:** A change in some aspect of the experiment that results in a reduction or replacement of animals or in a reduction of any pain, stress or distress that animals may experience.
- 4.16. **Starey or Staring Coat:** A dry haircoat lacking in luster, usually carrying dandruff or scurf. May be caused by poor cutaneous circulation and lack of sebaceous secretion resulting from a general state of ill health; A

symptom noted in sick animals in which the hairs of the coat, instead of lying flat and being smooth and shiny, are standing up with the ends clear of each other and are dull and lustre-less.

## **5. GUIDELINES OR PROCEDURE**

### **5.1. Why Animal Welfare Matters**

- 5.1.1. Pain (and distress) cause changes to the animal's body systems and this can increase the variability in research data affecting research quality.
- 5.1.2. If an animal's welfare is seriously compromised, it may exceed the permitted end-points on the study, or die unexpectedly.
- 5.1.3. Ensuring good welfare implements refinement, and by avoiding the consequences of poor welfare, animal numbers may be reduced.
- 5.1.4. Poor welfare can complicate study management; e.g.: infections result in change in animal's behavior and alter food and water consumption.
- 5.1.5. Meet regulatory requirements to avoid unnecessary pain, suffering or distress.

### **5.2. In order to recognize pain, distress and suffering, the ability to recognize the positive signs of health and good welfare are required. This includes:**

- 5.2.1. An initial assessment of the animal without disturbance, during a time of day when most active (or with attention to the current light cycle, if applicable).
  - 5.2.1.1. Familiarize the animals with people
  - 5.2.1.2. Use remote video if available and feasible
- 5.2.2. An examination of the animal
  - 5.2.2.1. Looking at each body area
  - 5.2.2.2. Comparing animals side by side
  - 5.2.2.3. Assessing behavior
  - 5.2.2.4. Assessing response to handling
  - 5.2.2.5. Assessing response to the administration of an anxiolytic/analgesic, per approved protocol and/or in conjunction with the AV, or designee.

### **5.3. Examples of Potential Causes of Stress in Laboratory Animals**

#### **5.3.1. Husbandry Practices**

- 5.3.1.1. Inappropriate or variable temperature, humidity, ventilation, or illumination
- 5.3.1.2. Inappropriate cage or enclosure size
- 5.3.1.3. Noise
- 5.3.1.4. Too infrequent change in bedding or removal of waste
- 5.3.1.5. Stale food or dirty water
- 5.3.1.6. Denial of positive social stimulation
- 5.3.1.7. Maternal deprivation
- 5.3.1.8. Social intimidation or abuse by companions
- 5.3.1.9. Unprofessional behaviors or practices
- 5.3.1.10. Unenriched environments

#### **5.3.2. Experimental Design**

- 5.3.2.1. Food and water deprivation
- 5.3.2.2. Inadequate caging
- 5.3.2.3. Poor or inappropriate technique
- 5.3.2.4. Failure to adapt or handle animals
- 5.3.2.5. Restraint
- 5.3.2.6. Social deprivation
- 5.3.2.7. Frequent changes in procedures or personnel

### **5.4. Scoresheets**

- 5.4.1. Give scores to different abnormalities, using carefully defined criteria, to track the progress of an animal’s welfare, e.g. TAMU-F-025 Liver Disease Score Sheet
- 5.4.2. Check scores against the study protocol to see what signs of poor welfare were anticipated, and what actions should be taken.
- 5.4.3. Useful in determining humane endpoints of a study (See TAMU-G-001)

5.5. Common Indicators of Pain

Demeanor/ Attitude	Quiet, depressed, apprehensive, fearful, anxious, restless, irritable
Behavior	Reduction in activity, interaction, exploratory behavior, grooming, food and water intake
Posture	Tensing up, resists handling, protects painful area, turning head towards pain source, aggressive, hypersensitive, unresponsive
Vocalization	Squeal, bark, groan, grunt, whimper, whine, growl, etc. especially when handled
Systemic changes	Increased heart and respiratory rates, increased temperature, muscle tremors, dilated pupils, changes in white cell counts, acute phase reactant proteins and stress hormones
Response to analgesia	Improvement of condition with therapy, lessening of signs of pain

5.6. Common Signs of Localized Pain

Region	Signs of pain
Head, ear, throat, mouth	Rubbing, shaking, head tilt, head pressing or holding, “stiff-neck posture”, self-mutilation, depression, and reluctance to swallow, eat, drink or move
Ophthalmic	Reluctance to move, scratching, rubbing, squinting (blepharospasm), discharge, redness
Orthopedic	Abnormal posture, abnormal gait, reluctance to move, guarding, licking, biting, self-mutilation
Abdominal	Protecting painful area (guarding), abnormal posture, unwillingness to stand, licking or looking at area, vomiting, anorexia
Thoracic	Change in respiratory rate and pattern (abdominal breathing, short shallow breaths), reluctance to move, pain on chest compression, anxiety, cyanosis (blue)
Perineal	Scotching, biting, licking, self-mutilation

5.7. Chronic Pain

- 5.7.1. Can be harder to recognize, because its onset is slow and intensity likely not constant.
- 5.7.2. Clinical Signs Associated with Acute vs Chronic Pain

Acute Pain	Chronic Pain
Decreased food and water intake	Decreased appetite
Acute weight loss	Chronic weight loss, poor body condition
Protecting (guarding) painful area, teeth grinding in some species	Alterations in urinary and bowel activities
Vocalizing, especially on palpating painful area or following movement	Behavioral changes e.g. aggression, withdrawn, hypersensitive, unresponsive
Licking, biting, scratching, or shaking affected area, flinch	Self-mutilation
Restlessness (e.g., pacing, repeated lying down and getting up); increased respiratory rate	Increased tear production, porphyrin staining around eyes (particularly in rats)
Lack of or reduced mobility, lethargic	Reduced activity, limping; lethargy
Failure to groom, ruffled fur; sweating	Lack of grooming



Abnormal posture, hunched up	Decreased litter size
Separation from group, hiding	Separation from group
Depression, lack of inquisitiveness	

5.8. Species-Typical Signs of Pain

5.8.1. Although a comprehensive description of species-specific signs has not been produced, the following notes and comments might be helpful.

5.8.2.

Dogs	Cats (generally only show signs when very ill)
Quiet, inattentive, recumbent, apprehensive, aggressive	Quiet, apprehensive, cowering, hiding, aggressive
Flinch, stiff, reluctant to move, lameness, abnormal posture, trembling, attempts to escape	Flinch, reluctant to move, recumbent, stiff, abnormal posture, vocalize, growl, cry, hissing, spitting
Bite, scratch, shake or lick/ guard painful site	Licking of painful area, limping, guarding
Lack of appetite, fast breathing, panting; increased body temperature	Lack of appetite, failure to groom
Whimper, howl, growl	Inappropriate elimination, or not moving away from excrement
Inappropriate elimination, or not moving away from excrement	Open mouth breathing

5.8.3.

Rabbits	
Behavior	Inactive, hunched, hiding, withdrawn, aggressive, vocalize, increased activity, licking, scratching or biting affected site, self-mutilation, guarding
Attitude	Apprehensive, anxious
Clinical Signs	Increased respiration, lack of appetite, immobility, salivation, teeth grinding, struggling on handling, increased body temperature, poor fecal production, squinting, pale eyes (if albino)

5.8.4.

Rats and Mice	Guinea Pigs
Behavioral changes, struggling, biting, vocalization, depression, nonresponsive	Vocalization, attempts to escape, stampede, reluctance to move, altered gait
Poor body condition, wasting, dehydration, decreased growth and reproduction, sunken eyes, eyelids closed	Timid, apprehensive, passive, unresponsive
Fast and labored breathing, shallow infrequent respiration, chattering (mice), sneezing (rats), porphyrin staining around eyes and nose, pale or bluish extremities	Cold, bluish extremities
Piloerection, decreased grooming, dirty hair coat, scratching	Decreased food and water consumption, anorexia
Hunched, crouched, recumbent; stretching, back arching or abdominal pressing	
Lack of appetite, no feces, pica, cannibalism	
Excessive licking, scratching, self-mutilation, ataxia	

5.8.5.

Sheep and Goats	Pigs
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Decreased appetite, decreased rumen sounds, depressed, dull, increased respiration, grunting, lameness, tearing, straining, agitation, resent handling, aversion to handler, reduced exploratory behavior	Reluctance to stand or move, hiding, separation, loss of appetite, piloerection
Change in posture, repeated lying down and standing up, reluctance to move, separation for others	Squealing on palpation of painful area, changes in vocalization
Teeth grinding, apprehension, decreased response, head carried low, ears down or flat, separation	Aggression, change in demeanor, social behavior
Vocalization	Changes in gait, posture, respiration, temperature, change in skin color

5.8.6.

Cattle	Horses
Dull, depressed; head held low. Reduced overall activity. Change in posture or gait.	Reluctance to be handled, restlessness, anxiety, depression. Change in posture or gait.
Licking, kicking offending area. Tail flicking. Restlessness.	Bruxism, sweating, tail switching
Decreased appetite, weight loss; decreased milk production, decreased rumination.	Interrupted feeding; look, bite or kick at offending area
Grunting, bruxism, bellowing.	Walk in circles, roll, and injure self.
Shallow respiration.	Increased respiration and pulse

5.8.7.

Birds/Poultry	Reptiles/Amphibians
Vocalization, excessive movement including increased head movements.	Flinching/muscle contraction, abnormal postures, lameness, decrease in avoidance movement
Droopy wings, reduced perching, immobility	Aggression
Abnormal posture or gait.	Inappetence, lethargy, weight loss
Increased heart and respiratory rates	Increased respiratory rate
	Guarding or rubbing affected area
	Skin darkening

5.8.8.

Fish	
Can be difficult to determine. Responses to harmful stress include an increased ventilatory pattern with excessive movement of fins.	Physical Appearance: eye, fin, skin condition; mucus production, color change
Unprovoked behavior: position in the water column, changes in social interactions (direct attacks, domination of choice tank locations, schooling, and social isolation), hyper- or hypoactivity	Measurable Clinical Signs: feed consumption, respiratory rate, posture in water column
Provoked behavior: feeding activity, threat response, avoidance reactions, lack of response to external stimulation	Other: Abnormal movements such as flashing (erratic swimming) or scraping the body, unexpected jumping or escape behavior

**6. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION**

6.1. References

- 6.1.1. National Research Council, Recognition and Alleviation of Pain and Distress in Laboratory Animals, National Academy Press, Washington, DC, 1993.
- 6.1.2. Staisak KL, Maul D, French E, Hellyer PW, Vandewoude S. Species-specific assessment of pain in laboratory animals. *Contemp. Top. Lab. Anim. Sci.* 42 (4): 13-20, 2003.
- 6.1.3. [Canadian Council on Animal Care](#). 2005. Guidelines on: the care and use of fish in research, teaching and testing.
- 6.1.4. Carstens E, Moberg GP. Recognizing pain and distress in laboratory animals. *ILAR J.* 41(2): 62-71, 2000.
- 6.1.5. Tang, G., Seume, N., Häger, C. *et al.* Comparing distress of mouse models for liver damage. *Sci Rep* **10**, 19814 (2020). <https://doi.org/10.1038/s41598-020-76391-w>

6.2. Resources:

- 6.2.1. [Mouse Grimace Scale](#)
- 6.2.2. [Rabbit Grimace Scale](#)
- 6.2.3. [Rat Grimace Scale](#)
- 6.2.4. [National Centre for the Replacement Refinement & Reduction of Animals in Research \(NC3Rs\) 3Rs Resource Library](#)

6.3. [IACUC/AWO Referenced Documents](#): (requires TAMU NetID authentication)

- 6.3.1. TAMU-G-001 Guidelines for Choosing Appropriate Endpoints
- 6.3.2. TAMU-G-002 Guidelines on the use of Anesthesia and Analgesia
- 6.3.3. AWO-O-023 Liver Disease Score Sheet
- 6.3.4. AWO-O-072 AUP Tool – ERC Worksheet

6.4. Acknowledgements

- 6.4.1. This document was partially adapted using materials from Boston and Johns Hopkins Universities and the University of Iowa.

**7. HISTORY**

Effective Date	Version #	Description
10/17/2019	000	College Station/Galveston: New document
05/25/2020	001	Houston/Kingsville: New document. Reviewed and approved via email.
06/16/2020	002	Dallas: New Document
03/24/2022	003	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	004	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.
08/01/2023	005	College Station/Dallas/Galveston/Kingsville: Renewal; minor modification to definitions and resources, additional examples of species-typical signs of pain
09/01/2023	006	Houston: Renewal; minor modification to definitions and resources, additional examples of species-typical signs of pain. Reviewed and approved via TEAMS.