



IACUC Guidance:	<b>TAMU-G-001</b>	Title:	<b>Guidelines on Choosing Appropriate Endpoints</b>
	Location	Effective Date	Review By
	<b>College Station/Dallas/Galveston/Kingsville</b>	10/01/2024	09/30/2027
	<b>Houston</b>	11/01/2024	09/30/2027

**1. PURPOSE**

- 1.1. Regulatory guidelines and humane considerations require that animal pain and distress be minimized in any experiment. This document provides guidance on the appropriate removal of animals from a study to minimize pain and distress without compromising the quality of scientific data.

**2. SCOPE**

- 2.1. Applies to vertebrate animals used for research, teaching, or other purposes under the oversight of the Texas A&M University IACUC.
- 2.2. Applies to experimental animals for which intervention and humane euthanasia is not possible due to study goals, as well as strains with a negative phenotype that progresses to death.
- 2.3. See TAMU-G-008 for signs of distress in fish species.
- 2.4. See TAMU-G-023 for guidelines on recognizing pain and distress in animals.

**3. RESPONSIBILITY**

- 3.1. The **PI**, who has precise knowledge of both the objectives of the study and the proposed model, should identify, explain, and include in the animal use protocol a study endpoint that is both humane and scientifically sound.
- 3.2. Together, the **PI, AV (or designee)** and the **IACUC** should determine humane endpoints before the start of the study, when possible.
- 3.3. The **IACUC** is responsible for protocol review with consideration of the description and rationale for anticipated or selected endpoints, as well as criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated.
- 3.4. **PIs** are responsible for developing, describing and following early removal criteria (ERC) in the approved protocol.
- 3.5. The **AV (or designee)** is authorized to provide necessary medical treatments or prevent pain and distress by humane euthanasia.
- 3.6. **Animal care and use program participants** are expected to adhere to the standards described in guidance documents unless an exception has been requested and approved by the IACUC.

**4. DEFINITIONS AND/OR ACRONYMS**

- 4.1. Centrally administered support service for animal research and teaching programs at Texas A&M University:
  - 4.1.1. **ARU**: Animal Resource Unit supports the College of Dentistry vivarium
  - 4.1.2. **CMP**: Comparative Medicine Program supports the Texas A&M College Station campus
  - 4.1.3. **PAR**: Program for Animal Resources supports the Institute of Biosciences and Technology vivarium
  - 4.1.4. **PRF**: Pharmaceutical Research Facility supports the Kingsville Pharmaceutical Science Facility vivarium
  - 4.1.5. **Sea Life**: The Sea Life Facility supports the Galveston campus
- 4.2. **BCS**: Body Condition Score. Visual assessment of the amount of fat/muscle covering the bones of an animal.
- 4.3. **Core Support**: Individuals performing professional activities such as husbandry or technical services as a function of a dedicated service organization.
- 4.4. **ERC**: Early Removal Criteria. Specific, predetermined indicators of pain and distress used to establish early study endpoints without loss of scientific quality.
- 4.5. **ERS**: Early Removal Score. A method of animal evaluation in which numerical values are assigned to specific clinical signs and/or behavioral observations.
- 4.6. **Experimental Endpoint**: Occurs when the scientific aims and objectives have been reached.

- 4.7. **Guidance:** Guidance documents are developed by the IACUC to provide procedural standards to the research community on the topics identified.
- 4.8. **Humane Endpoint:** The point at which pain or distress in an experimental animal is prevented, terminated, or relieved.
- 4.9. **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.
- 4.10. **Morbidity:** State of disease or ill health
- 4.11. **Morbidity:** state of dying/approaching death
- 4.12. **PI:** Principal Investigator. The individual who has ultimate administrative and programmatic responsibility for the design, execution, and management of a project utilizing vertebrate animals.
- 4.13. **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.
- 4.14. **Unanticipated or Adverse Event:** Any happening that is not consistent with routine expected outcomes that results in any unforeseen animal welfare issue-that impacts the health or safety of animals (unintended injury or illness, unrelieved pain or distress, death). May require reporting to federal regulators and accrediting bodies.
- 4.15. **USDA:** United States Department of Agriculture. USDA Animal Care, a unit under the Animal and Plant Health Inspection Service, administers the Animal Welfare Act (AWA) and associated Animal Welfare Act Regulations (AWAR).

## 5. GUIDELINES OR PROCEDURE

- 5.1. Early Removal Criteria
  - 5.1.1. ERC should be developed by the PI based on pain and distress anticipated due to the animal model or experimental procedures outlined in the animal use protocol.
  - 5.1.2. The establishment of early endpoints for intervention in a study that has the potential to cause pain or distress is an example of refinement.
  - 5.1.3. Common clinical signs, such as lethargy or weight loss, are often used as ERC.
    - 5.1.3.1. Body condition score can be more useful than weight loss alone.
    - 5.1.3.2. Decisions should not be based on a single criterion.
  - 5.1.4. Clinical presentations or parameters that are specific to the project must also be included as ERC.
    - 5.1.4.1. Generic criteria may not be appropriate.
    - 5.1.4.2. A literature search may show a predictive parameter. PIs are encouraged to seek the services of a TAMU reference librarian or the USDA National Agricultural Library Animal Welfare Information Center (AWIC) for assistance with literature searches.
  - 5.1.5. Unanticipated or Adverse Events
    - 5.1.5.1. Well-planned experiments with clearly delineated scientific and humane endpoints will help to ensure that a contingency plan is in place for problems that may arise during the study.
    - 5.1.5.2. It is understood that adverse effects may occur that were not anticipated at the study onset. ERC should be updated to reflect these changes via an amendment. See TAMU-G-015 for guidelines on reporting unanticipated events.
- 5.2. Early Removal Scores
  - 5.2.1. Humane intervention points are predetermined and based on the total numerical score assigned to each established criterion.
  - 5.2.2. Numerous scoring systems are already established and published in scientific literature, e.g. AWO-O-023 Liver Disease Scoring Sheet.
  - 5.2.3. Consult CMP/ARU/PAR/PRF for guidance in establishing a new scoring system.
- 5.3. ERC/ERS Monitoring Timepoints

- 5.3.1. In order to implement the ERC or ERS, animals on the study should be monitored at appropriate frequencies, including starting or baseline monitoring with increasing monitoring post-procedurally, to permit timely termination of the experiment once the endpoint has been reached. This may require weekly, daily or even hourly observations.
- 5.4. Death (or moribundity) as an Experimental Endpoint
  - 5.4.1. Protocols incorporating the use of death or moribundity as an endpoint will receive additional IACUC review and approval may be granted on a case-by-case basis (where scientific justification includes the specific information gained in the period between morbidity and death).
  - 5.4.2. These protocols must contain:
    - 5.4.2.1. A description of palliative therapies, including non-drug therapies (e.g., soft bedding, heating pads, easier access to food or water, alternate food types) or a robust scientific justification as to why they are being withheld.
      - 5.4.2.1.1. CAUTION: Use of heat lamps and non-thermoregulating electric heating pads can result in severe burns or hyperthermia in animals. The use of safer equipment such as a circulating water blanket or isothermic pad is recommended, where possible.
    - 5.4.2.2. A detailed description and timeline of clinical signs and/or behavioral abnormalities that are anticipated to occur prior to death.
    - 5.4.2.3. The frequency and intervals at which the animals will be monitored by personnel skilled in recognizing these clinical signs and/or behavioral abnormalities.
    - 5.4.2.4. A description of the records that will be maintained of animal observations and the personnel responsible for the observation, including the name(s) of the individual(s) who will be responsible for the monitoring, or an indication that this monitoring will be conducted by core support husbandry staff.
  - 5.4.3. The cages of the specific animals that are subject to death or moribundity as an experimental endpoint must be labeled to indicate that death or moribundity of the animal is expected, and the approximate time frame in which this will occur.
  - 5.4.4. Animals must be monitored at least daily (including weekends and holidays). Animals may need to be monitored more frequently depending on the type of study.
    - 5.4.4.1. Records documenting the monitoring of these animals must be maintained and be available for review during IACUC inspections.
    - 5.4.4.2. Consideration should be given to moving animals to individual cages when their condition deteriorates to the point that injury from cage mates is possible. See TAMU-G-027
    - 5.4.4.3. Dead animals must be removed from cages as soon as possible.
  - 5.4.5. Note that at any time, CMP/ARU/PAR/PRF veterinarians are authorized to provide necessary medical treatments, diagnostic tests or perform euthanasia.
- 5.5. Alternative Endpoints for Common Models. While all studies should employ endpoints that are humane, studies that commonly require special consideration include those that involve tumor models, infectious diseases, vaccine challenge, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, organ or system failure, and models of cardiovascular shock.
  - 5.5.1. For Infectious Disease Animal Models
    - 5.5.1.1. Hypothermia – Monitoring body temperature should be part of the study design utilizing an infectious disease animal model. Lowered body temperature, hypothermia, has been shown to be an accurate indicator of a deteriorating condition in animals. A significant decrease (>4-6°C) has been correlated with death and has been shown to be useful as an endpoint in numerous infectious disease models including influenza and bacterial septicemia. There are both noninvasive and invasive means of measuring temperature in small laboratory animals including

the use of infrared temperature scanners, tympanic infrared thermometers or implanted thermistor microchips.

- 5.5.1.2. Acute Phase Proteins (APPs) – Increases in serum levels of cytokines and acute phase proteins can be used as predictors of severity and outcome. Elevations in cytokines can be transient; however, APPs rise in response to increased cytokine production and high levels of major APPs correlate well with the presence and severity of infectious disease.
- 5.5.1.3. Physiologic and Behavioral Changes – These include, but are not limited to, weight loss (10-20%), decreased activity, and inappetence/anorexia. These clinical signs are typically secondary to the effects of alterations in cytokine levels. Establishing an observational checklist of these deviations may be helpful in identifying the expected progression through these deviations and can be used to determine criteria indicative of an endpoint.
- 5.5.2. Alternative Endpoints for Hematopoietic/Lymphatic Cancer Research
  - 5.5.2.1. Increases in circulating tumor cells can forecast the onset of clinical signs; however, when human leukemia cells are engrafted into immunodeficient mice, the number of circulating cancer cells is less predictive. In the absence of reliable laboratory-based assays, animals with leukemia/lymphoma should be observed for early clinical signs such as anemia, weight loss (>20%) or impaired respiration. An evaluation system is recommended to assess the severity of the disease. This system would be based on a combination of physiologic, biochemical and hematologic parameters.
- 5.5.3. Alternatives for Vaccine Potency Testing
  - 5.5.3.1. In-vitro serologic testing – To replace the challenge procedure, in vitro serologic testing has been used to assess antibody responses of animals after immunization. For example, a good correlation has been shown between the titer of antibodies induced and the level of protection after challenge has been confirmed for toxoid and some of the clostridial vaccines.
- 5.5.4. Alternatives for Median Lethal Dose (LD50) Test
  - 5.5.4.1. Up-and-Down Procedure – Dosing is performed in individual animals in a staircase fashion. The next dose depends on the results from the previous animal, i.e. if the animal survived, the dose is higher and if the animal died, the next dose is lower. Additionally, there is a revised version of this procedure which provides additional guidance on how this procedure can be utilized to reduce the number of animals as compared to the traditional LD50 test.
- 5.5.5. Animals with Tumor Burdens
  - 5.5.5.1. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting research objectives.
  - 5.5.5.2. Suggested using ERC listed in 5.5.
  - 5.5.5.3. Progression to moribundity is impacted by choice of implantation. Choose a site that will have minimal impact on adjacent normal structures. The muscle distention caused by intramuscular implantation is considered painful, as are tumors which involve the eye. Ideally, tumors should be placed so that their growth will not interfere with normal mobility and functions such as eating, drinking, defecation and urination. Most preferred site is subcutaneous or intradermal implantation in the flank.
  - 5.5.5.4. Frequency of monitoring needs will vary by expected growth for the type tumor, aggressiveness of the tumor and metastatic potential.
- 5.5.6. Novel Studies
  - 5.5.6.1. When novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC, and veterinarian. See TAMU-G-030.



5.5.7. Suggested matching of ERC to Clinical Sign

Clinical Sign - Category	Clinical Sign - Detail	ERC/Criteria for Moribundity
Changes in general appearance	Abnormal Posture, Discoloration of Feces, Discoloration of Fur, Discoloration of Urine, Lack of Grooming, Nasal Discharge, Ocular Discharge, Prostration, Rough Coat, Swelling	Impaired mobility of lesions interfering with eating, drinking or ambulation. Icterus (jaundice; yellow color to skin). Markedly discolored urine, polyuria, or anuria if prolonged (>3days). Impaired mobility or lesions interfering with eating, drinking or ambulation. Paralysis. Persistent lateral recumbency. <b>BCS*</b> of <2. <b>ERS**</b> of >or=3
Changes in appetite or weight	Decreased appetite, Increased Appetite, Weight Loss: <10%, <20% (provide scientific justification)	Extended period of weight loss progressing to emaciation with <b>BCS*</b> of <2. Rapid weight loss of >20% in 1 week. <b>ERS**</b> of >or=3.
Changes in respiration	Labored, Rapid, Slow, Shallow, Sneezing, Wheezing	Persistent cough, rales, wheezing, nasal discharge. Respiratory distress (dyspnea) or cyanosis
Changes in behavior/neurologic status/musculoskeletal	Agitation, Depression, Hyperactivity, Hypoactivity, lethargy, Spasticity, Tremors, Impaired Ambulation, Muscle Atrophy, Paralysis [Limb or other (describe)]	Central nervous system signs (head tilt, tremors, spasticity, seizures, circling or paresis) with anorexia and hindering of animals' ability to obtain food/water. Impaired mobility or lesions interfering with eating, drinking or ambulation.
Tumors, skin conditions	List % of total body weight or size. Justify tumor burdens exceeding 10% Total Body Weight. Tumor necrosis (<1cm mice, <2cm rats) with ulceration and dry scab within 24 hours.	Tumors that are >10% and <20% of animal's original body weight (provide justification for tumor burdens that will exceed 10% of total body weight). Tumors >2cm in mice or >4cm in rats. Ulcerated tumors that are moist and not healing (>1cm mice, >2cm rats). Extensive necrotic tissue or skin ulceration with >10% body surface affected. Persistent self-induced trauma. Tumors significantly impede ambulation and/or cause significant pain or distress. Tumors hinder ability to obtain food/water.
Other Clinical Signs	Bleeding, Blindness, Coma, Constipation, Diarrhea, Hyperthermia, Hypothermia, Infection, Self-Induced Trauma, Other [provide description]	Icterus (jaundice; yellow color to skin). Markedly discolored urine, polyuria, or anuria if prolonged (>3days). Persistent self-induced trauma. <b>BCS*</b> of <2. <b>ERS**</b> of >or=3.
Injury or Mortality for Captured Wildlife	Address historical animal injury and mortality rates associated with your capture and animal handling methods and methods taken to eliminate such injuries, etc.	Describe your plan for providing veterinary care or humane euthanasia for animals that experience a serious injury or illness.
	<b>*Body Condition Score (BCS)</b>	<b>**Early Removal or Moribundity Score (ERS)</b>
	1 – Emaciated 2 – Under-conditioned 3 – Well-conditioned 4 – Over-conditioned	1 – Normal appearance 2 – Decreased mobility, weight loss, lack of grooming



	5 - Obese	3 – Extreme lethargy, body weight loss of >20%, unresolved dehydration >=10% 4 – Non-responsive to stimulation
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**6. RECORDS**

- 6.1. Research records, including records of monitoring described within this document, must be maintained consistent with Texas A&M University Standard Administrative Procedures (SAPS) 15.99.03.M1.03. and 29.01.03.M0.01.

**7. EXCEPTIONS**

- 7.1. The PI may request an exception to the above standards by describing the departure in the AUP
- 7.2. For programmatic exceptions, the facility director or manager may submit a request for the exception using TAMU-F-013.

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

- 8.1. References
  - 8.1.1. Alternatives to Animal Testing on the Web (2004), Humane Endpoints Database. (http://altweb.jhsph.edu/) Johns Hopkins Center for Alternatives to Animal Testing. Baltimore.
  - 8.1.2. Wallace, J. Humane Endpoints and Cancer Research. ILAR 41(2), 2000.
  - 8.1.3. Rispin, et al. Alternative Methods for the Median Lethal Dose (LD50) Test: The Up-and-Down Procedure for Acute Oral Toxicity. ILAR 43(4), 2002.
  - 8.1.4. Hendriksen, CFM and Steen, B. Refinement of Vaccine Potency Testing with the Use of Humane Endpoints. ILAR 41(2), 2000.
  - 8.1.5. Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals, 8th Edition. National Academies Press, 2011.
  - 8.1.6. Olfert, ED and Godson, DL. Humane Endpoints for Infectious Disease Animal Models. ILAR 41(2), 2000.
  - 8.1.7. HCCA. "Humane Endpoints" a Useful metric in Assessing Health of Research Animals. November 2018.
  - 8.1.8. Brayton, C, N. Forbes, J. Watson. Department of Molecular & Comparative Pathobiology Johns Hopkins University School of Medicine. Baltimore MD USA. 2015
- 8.2. Resources
  - 8.2.1. For more information on establishing ERC/ERS, please contact:
    - 8.2.1.1. CMP: at (979) 845-7433
    - 8.2.1.2. PAR: at (713) 677-7471
    - 8.2.1.3. PRF: at (361) 221-0770
    - 8.2.1.4. Sea Life Facility: at (409) 740-4574
- 8.3. USDA National Agricultural Library Animal Welfare Information Center ([AWIC](#))
- 8.4. [TAMU Libraries](#)
- 8.5. Texas A&M University SAPs:
  - 8.5.1. [15.99.03.M1.03](#) The Responsible Stewardship of Research Data
  - 8.5.2. [29.01.03.M0.01](#) Security of Electronic Information Resources
- 8.6. [IACUC/AWO Referenced Documents](#): (requires TAMU NetID authentication)
  - 8.6.1. AWO-O-023 Liver Disease Score Sheet
  - 8.6.2. TAMU-F-013 Request for Programmatic Exception from Animal Welfare Standards
  - 8.6.3. TAMU-G-008 Guidelines on Working with Zebrafish
  - 8.6.4. TAMU-G-015 Guidelines for Reporting Unanticipated or Adverse Events and Protocol Drift
  - 8.6.5. TAMU-G-023 Guidelines on Recognizing Pain and Distress in Research Animals
  - 8.6.6. TAMU-G-027 Guidelines on Environmental Enrichment and Single Housing of Social Biomedical Species Housed Indoors





8.6.7. TAMU-G-030 Guidelines for Pilot Studies

8.7. Acknowledgements

8.7.1. This document was partially adapted using materials obtained from the University of Arizona and the Ohio State University.

**9. HISTORY**

Effective Date	Version #	Description
10/17/2019	000	College Station/Galveston: New Document
3/19/2020	001	College Station/Galveston: Additional guidance related to tumor production/burden; 5.3.2.1.1 new subpoint
4/30/2020	002	Houston/Kingsville: New format and content; replaced IBT-203.02, IBT-204.02. Reviewed and approved via email.
5/19/2020	003	Dallas: New format and content; replaced CD-203.01, CD-204.01.
01/03/2022	004	College Station/Galveston: Renewal; updated definitions, references; Reviewed and approved via email.
02/01/2022	005	Houston/Kingsville: Renewal; updated definitions, references; Reviewed and approved via email.
02/15/2022	006	Dallas: Renewal; updated definitions, references
03/24/2022	007	College Station/Dallas/Galveston: Merging of Dallas animal care and use program with College Station/Galveston
10/20/2022	008	College Station/Dallas/Galveston/Kingsville: Merging of Kingsville animal care and use program with College Station/Dallas/Galveston.
10/01/2024	009	College Station/Dallas/Galveston/Kingsville: Renewal; updated title, scope, definitions and resources sections; modified procedure section to include consultation with AWIC, expansion of monitoring timepoints; addition of records and exceptions sections.
11/01/2024	010	Houston: Renewal; updated title, scope, definitions and resources sections; modified procedure section to include consultation with AWIC, expansion of monitoring timepoints; addition of records and exceptions sections.