



Policy 357

Humane Endpoints

Responsible Official:	Research Administration
Administering Division/Department:	Research Compliance and Regulatory Affairs / IACUC
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357.1 Purpose:

This policy outlines the requirements for developing and defining humane endpoints for all animals placed on an Emory IACUC protocol. A humane endpoint is defined in the 8th edition of *The Guide for the Care and Use of Laboratory Animals* as “the point at which pain or distress in an experimental animal is prevented, terminated, or relieved.” These are different from experimental endpoints, which occur when scientific objectives have been met, but the two can be closely linked. The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress, including death, and should be relevant and reliable. 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. The final determination of humane endpoints should involve the PI, veterinarian and the IACUC, and should be defined, when possible, prior to the start of the study.

357.2 Definitions

Moribund: A severely debilitated state that precedes imminent death. The point at which an animal is in the state of dying. This is frequently seen as an inability to right; hypothermia, unresponsive, unable to walk or some combination thereof.¹⁴

Experimental endpoint: This occurs when the scientific aims and objectives have been reached.

Humane endpoint: The point at which pain and distress is prevented, terminated, or relieved in an experimental animal.

Cumulative endpoints: Endpoints which may warrant consideration for animals held long-term and for animals that have multiple scientific experiences.¹¹

Embryo: Means an egg-laying vertebrate animal at any stage of development prior to hatching.

Hatching: Means to bring forth offspring from the egg.

357.3 Default Endpoints:

Although not comprehensive, the following criteria may be considered standard, default endpoints for “low- risk” animal models. Low-risk animal models are considered to be those which would not generally be felt to have pain and distress under normal conditions, but may experience untoward, potentially severe, effects unpredictably. For models in which animals are expected to have pain and distress, these default endpoints may not generally be sufficient and additional refinement of the endpoints on a case-by-case basis may be required.

- Loss of greater than 25% of body weight from baseline weight when assigned to the protocol, concomitant with a declining body condition score.
 - **NOTE:** A growth nomogram must be used to adjust the basal weight for growing animals.
- Major organ failure or medical conditions unresponsive to treatment.
- Decreased body condition score (score less than 2 on a species-specific scale of 1-5, guidelines located in Appendix F) and that does not improve with nutritional support.



- Surgical complications unresponsive to immediate intervention.
- Non-rodent animals, other than rabbits, that have complete anorexia for 4 days or are unable to consume sufficient nutrients without assistance for 7 days. An animal affected by inappetence will receive supportive care and monitoring by veterinary and investigative staff until endpoints have been determined.
- Tumor arising either spontaneously or from experimental means with a burden meeting endpoint based on the Emory IACUC Policy 304 Tumor Burden Scoring.
- Self-directed trauma that is nonresponsive to behavioral or medical management.
- Excessive or prolonged hyperthermia or hypothermia.
- Significant pain that cannot be alleviated by analgesics.
- Other signs judged by experienced veterinary staff to be indicative of a moribund condition.
- Clinical or behavioral signs resulting in acute unrelievable stress, significant chronic stress or distress and unresponsive to appropriate intervention.
- In the case of rodents, the following abnormalities persisting for 24 hours, and for rabbits persisting for 48 hours would warrant euthanasia:
 - Lethargy, inactivity or hyper-activity
 - Increased, decreased, or labored breathing
 - Hunched posture
 - Piloerection/matted fur
 - Signs of dehydration
 - Debilitating diarrhea or constipation
 - Abnormal spontaneous coloration such as jaundice, pallor, or cyanosis
 - Suppressed response, lack of response, or abnormal vocalization to handling and stimulation
 - Anorexia or significant hyporexia concomitant with weight loss
 - One or more unresolving skin ulcers, or progressive dermatitis
- In the case of reptiles, the following endpoints would warrant euthanasia:
 - Loss of 25% of body weight from baseline weight when assigned to the protocol
 - Decreased body condition score (when able to be performed on a given reptile species) and that does not improve with nutritional support
 - Major organ failure or medical conditions unresponsive to treatment
 - Surgical complications unresponsive to immediate intervention
 - Clinical or behavioral signs resulting in acute unrelievable stress, significant chronic stress or distress, and unresponsive to appropriate intervention
 - Physiologically inappropriate inactivity or severe lethargy persisting for 48 hours and unresponsive to treatment

357.4 Special Considerations:

The current edition of *"The Guide"* (page 27) indicates that "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 cardio-vascular shock". These models often cannot utilize common default humane endpoint guidelines and therefore should be developed on a case-by-case basis in conjunction with the IACUC and veterinarians. Appendices 1-6 provide sample humane endpoint guidelines for a number of common model systems. Note also that the IACUC has separate policies for determining humane endpoints for [tumor models](#) and [MPTP treatment](#) as detailed in the appendix material below.

357.5 Protocol components:

The following items must be included when outlining humane endpoints in the IACUC protocol:

357.5.1 Definition of endpoints. Endpoints are thresholds that, when reached, require a response. This could entail removal from the study until the condition had adequately improved, clinical treatment sufficient to allow experimentation to continue, or more commonly euthanasia. When novel studies are proposed and information on a procedure's effects on animals is limited or unavailable, or humane endpoints cannot be identified or defined, a pilot study may be recommended or required by the IACUC. Pilot studies should evaluate several parameters to determine which parameters can best be used as humane endpoints and they offer the advantage of helping to determine the time course and nature of adverse effects on an animal.



357.5.2 Assessment criteria. These are the parameters measured during a study that will be indicative of an animal's general health and well-being or clinical condition, or that will indicate if an animal is experiencing pain or stress (either acute, or chronic). Examples include, but are not limited to, measuring body weight, food and water consumption, body temperature, tumor size, imaging findings, blood chemistry or hematology abnormalities, or assessing the ability to ambulate.

357.5.3 Scoring systems. It may be difficult to assess the impact of certain procedures on an animal since the biological sequela of an experimental paradigm may be complex and are confounded by variations in responses by different species. Therefore, a score sheet system may be beneficial during a pilot study, and potentially throughout an experimental project. A score sheet system provides an objective format for deciding which observations are important, ensures that specific observations are not overlooked, and provides a blueprint for training individuals involved in the research project. A score sheet system allows each member of the research team to make and judge observations in a uniform manner. Score sheets should include the parameters that will be assessed for every animal, the different conditions that may be observed for each parameter, and the total score(s) that are criteria for euthanasia. Numerous scoring systems have been established, validated, and published in scientific literature, some of which are included in Appendices 1-6. Investigators may seek to establish new scoring systems to aid in the determination of humane endpoints or may seek initial training, in this case EU DAR or EPC veterinarians and the IACUC can be consulted.

357.5.4 Frequency of monitoring. This states the regularity, often number of times per day and per week, that responsible personnel will observe the animal or measure the parameters identified as assessment criteria. Monitoring requirements may change through the course of a study as a condition worsens over time or with experimental manipulation

357.5.5 Required response. This is the intervention that must occur when the defined endpoint(s) has been reached. The intervention must be timely, or as early as possible, and will typically be medical treatment or euthanasia and may be performed by research or veterinary staff in compliance with pre-set arrangements in the IACUC- approved protocol and sometimes on other circumstances.

357.6 Other special considerations:

357.6.1 Aging studies. Senescent animals may naturally exhibit several clinical signs that would indicate significant morbidity in younger animals. Aging animals may also experience certain benign ailments at an increased incidence. Genotype, background strain, chronological age, and sex should all be considered in the process of developing endpoints in these studies. Thus, for lifespan studies, where clinical signs of morbidity associated with aging are expected and necessary for the scientific aims of the study, the endpoint of the study should be as objectively described as possible by the investigator. For individual aged rodents, published studies have shown that serial decreases in temperature and decreasing body weight over several weeks correlate with imminent death,^{15,17} thus these represent endpoints that can be utilized by investigative staff.

357.6.2 Studies involving neonates. Scoring methods and identified humane endpoints are often systemically determined from the study of young adult rodents. Consequently, studies utilizing neonatal or growing animals may require special humane endpoints. Body weights may be utilized as a humane endpoint. If young animals with an expected growth curve are not gaining weight, then those animals are declining in weight over time, despite the appearance of a stable weight measurement. When working with young rodents, the body weights should be compared to an age-matched control of the same strain. For sepsis studies utilizing neonatal rodents, scoring systems with observation-based parameters that correlated with morbidity and mortality have been published.^{2,3}

357.7 Death as an endpoint:

The use of death as an endpoint is generally discouraged. However, it is understood that in some special circumstances it is necessary or unavoidable and thus will be considered on a case-by-case basis. If an animal must be allowed to die without intervention in order to answer a scientific question, then adequate scientific justification, documentation that outlined early humane endpoints or stress or pain-relieving drugs cannot be used, detailed plans for monitoring and supportive care including frequency of monitoring and record- keeping practices, and final IACUC approval are required.

357.7.1 Scientific rationale for death as an endpoint must include at a minimum:



- What alternatives were considered, why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible.
- Why measures to relieve pain and/or distress cannot be utilized.
- The number of animals that will be allowed to reach death as an experimental endpoint and justification for it being the minimum number necessary to achieve scientific objectives.
- Whether animals can be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.

357.8 Veterinary Oversight:

The Attending Veterinarian (AV) has the responsibility for oversight of the health and welfare of animals used for research. The AV and designated veterinary staff can intervene and have the authority to euthanize any animal in the interest of animal welfare regardless of approved humane endpoints. Note however, that a DAR/EPC veterinarian can determine that euthanasia is not required for an animal that meets the above criteria, provided that the animal is under veterinary care and the veterinarian determines that the animal is likely to respond to treatment. All attempts will be made to reach mutual agreement with the PI and research staff whenever possible when these decisions must be made.

357.9 Unexpected hatching of embryos at or after 80% of development (e.g., day 17 for chickens).

Unexpected hatchlings or embryos will be euthanized according to the current edition of the AVMA Guidelines for the Euthanasia of Animals and adopted by Emory as Policy 377.

357.9.1 If there is unexpected hatching, the researcher must contact the Division of Animal Resources (DAR) immediately providing your name, contact phone number, and the location of the hatchlings. During normal business hours call 404-727-3248. During non-business hours call 404-727-6111 and police dispatch will contact the veterinary staff.

357.10 Related IACUC Policies

- IACUC Policy 363: MPTP Guidelines:
http://www.iacuc.emory.edu/documents/MPTP_guidelines.pdf
- IACUC Policy 304- Tumor Burden Scoring Guidelines:
http://www.iacuc.emory.edu/documents/tumor_burden_scoring.pdf

357.11 References

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List of Appendices

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Contact Information

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Appendix 1: Example humane endpoint guidelines for Transplant studies:

Renal Transplant Studies: Euthanasia will be performed if renal function ceases or becomes impaired such as a creatinine of greater than 8.0mg/dl or a BUN of greater than 100mg/dl sustained for 3 days, or if a creatinine greater than 4.0mg/dl or a BUN of greater than 80mg/dl sustained for more than 5 days associated with decreased activity of the animal or severe anemia with a hematocrit of less than 20 for greater than a week or if in the opinion of the



attending veterinarian or PI the animal is experiencing substantial, unrelievable pain or illness. In addition, the IACUC guideline for endpoints will be used for these studies.

Additional reference values may be considered for allo- and xeno- transplantation as shown in the table below:

Allo-transplant	Xeno-transplant
two consecutive serum creatinine levels > 5.0mg/dl or two consecutive serum BUN levels >100mg/dl	two consecutive serum creatinine levels > 5.0mg/dl or two consecutive serum BUN levels >120mg/dl

Appendix 2: Example humane endpoint guidelines for rodent hind limb ischemia:

1. Immediate euthanasia for animals with necrosis extending beyond the foot.
2. Euthanasia of animals that have performed self-mutilation.
3. Euthanasia seven days post-surgery for animals with necrosis extending beyond the toes.
4. Euthanasia of animals with toe necrosis that fail to recover functionality of the limb 10 days post-surgery noting that rodents typically lose ankle flexion after surgery, but recover mobility over 4-10 days.
5. Functionality will be assessed by the ability to bend the ankle.

Appendix 3: Example humane endpoint guidelines for rodent total body irradiation or bone marrow transplantation^{9,10}

Scoring system

Posture

- 0—normal body posture
- 1—slightly hunched posture
- 2—moderately hunched posture
- 3—severely hunched posture

Eye Appearance*

- 0—eyes are greater than 75% open
- 1—eyes are 50-75% open
- 2—eyes are 25-50% open
- 3—eyes are less than 25% open

*Mice must first be assessed for congenital malformations such as small eyes (microphthalmia) or no eyes (anophthalmia) in order to accurately use this grading scale.

Activity Level

- 0—moves around the cage normally and is very active
- 1—slightly reduced activity or a mild gait abnormality
- 2—moving very slowly or has a severely altered gait
- 3—does not move at all, reluctant to move, does not take more than 3 or 4 steps

Humane Endpoint Criteria

- Mice with a score of 8 or 9 are considered to have met humane endpoint criteria and are to be euthanized.
- Mice with a score of 7:
 - Should be euthanized if they can be used to meet experimental endpoints.



- If mice are part of a study that is evaluating therapeutics or have had bone marrow reconstitution, monitoring must be increased to a minimum of twice daily.
- If mice have 25% weight loss, their score increases to 8, or there is no improvement within 48 hours, mice are to be euthanized.

Appendix 4: Example rodent amyotrophic lateral sclerosis (ALS) and ascending neurologic disease models scoring system^{1,5,13}

Scoring System

- 0— (pre-symptomatic) full extension of hind legs away from lateral midline when mouse is suspended by its tail, and mouse can hold this for two seconds, suspended two to three times.
- 1— abnormal splay, collapse or partial collapse of leg extension towards lateral midline (weakness) or trembling of hind legs during tail suspension.
- 2— (paresis onset) - toes curl under at least twice when walking 12 inches, or any part of the foot is dragging along the bottom of the cage or table. Mouse able to right itself from both sides.
- 3— (paralysis): rigid paralysis or minimal joint movement, foot not being used for generating forward motion. Mouse able to right itself from both sides.
- 4— (humane endpoint): rigid in hindlimbs with no forward motion. Mouse cannot right itself within 30 s after being placed on either side.

Humane Endpoint Criteria

- An animal with a score of 4 is considered to have met humane endpoint criteria and is to be euthanized.
 - If at any point an animal cannot reach food made accessible on the cage floor or water, regardless of score, it is considered to have met humane endpoint criteria and is to be euthanized.

Appendix 5: Example mouse sepsis scoring systems^{2,3,4,7,8,12}

Two-parameter Sepsis Scoring system³:

Activity, response to stimulus

- 0— Mouse is performing any prior to stimulus: Climbing, running, fighting. Normal spontaneous walking. Mouse displays normal reaction in response to stimulus.
- 1— Slightly suppressed activity prior to stimulus. Spontaneous walking but slower than normal. Mouse moves forward in response to stimulus but slower than normal.
- 2— Moderately suppressed activity. No spontaneous walking observed prior to stimulus. Mouse moves at least two steps forward in response to stimulus but much slower than normal.
- 3— No activity. No spontaneous walking observed prior to stimulus. Mouse moves less than 2 steps forward in response to touch. Mouse may experience tremors in response to touch.

Eyes

- 0— Open
- 1— Partially closed
- 2— Half-closed
- 3— Mostly or completely closed



Murine Sepsis Scoring System (MSS)⁴

Appearance

- 1— Coat is smooth
- 2— Patches of hair with piloerection
- 3— Majority of back hair has piloerection
- 4— Piloerection may or may not be present, mouse appears “puffy”
- 5— Piloerection may or may not be present, mouse appears emaciated

Level of consciousness

- 0— Mouse is active
- 1— Mouse is active but avoids standing upright
- 2— Mouse activity is noticeably slowed. The mouse is still ambulant.
- 3— Activity is impaired. Mouse only moves when provoked, movements have a tremor
- 4— Activity severely impaired. Mouse remains stationary when provoked, with possible tremor

Activity

- 1— Normal amount of activity. Mouse is displaying any of the following activities: eating, drinking, climbing, running, fighting
- 2— Slightly suppressed activity. Mouse is moving around bottom of cage
- 3— Suppressed activity. Mouse is stationary with occasional investigative movements
- 4— No activity. Mouse is stationary
- 5— No activity. Mouse experiencing tremors, particularly in the hind legs

Response to stimulus

- 1— Mouse responds immediately to auditory stimulus or touch
- 2— Slow or no response to auditory stimulus; strong response to touch (moves to escape)
- 3— No response to auditory stimulus; moderate response to touch (moves a few steps)
- 4— No response to auditory stimulus; mild response to touch (no locomotion)
- 5— No response to auditory stimulus. Little or no response to touch. Cannot right itself if pushed over

Eyes

- 1— Open
- 2— Eyes not fully open, possibly with secretions
- 3— Eyes at least half closed, possibly with secretions
- 4— Eyes half closed or more, possibly with secretions
- 5— Eyes closed or milky

Respiratory rate

- 1— Normal, rapid mouse respiration
- 2— Slightly decreased respiration (rate not quantifiable by eye)
- 3— Moderately reduced respiration (rate at the upper range of quantifying by eye)
- 4— Severely reduced respiration (rate easily countable by eye, 0.5 s between breaths)
- 5— Extremely reduced respiration (>1 s between breaths)

Respiration quality

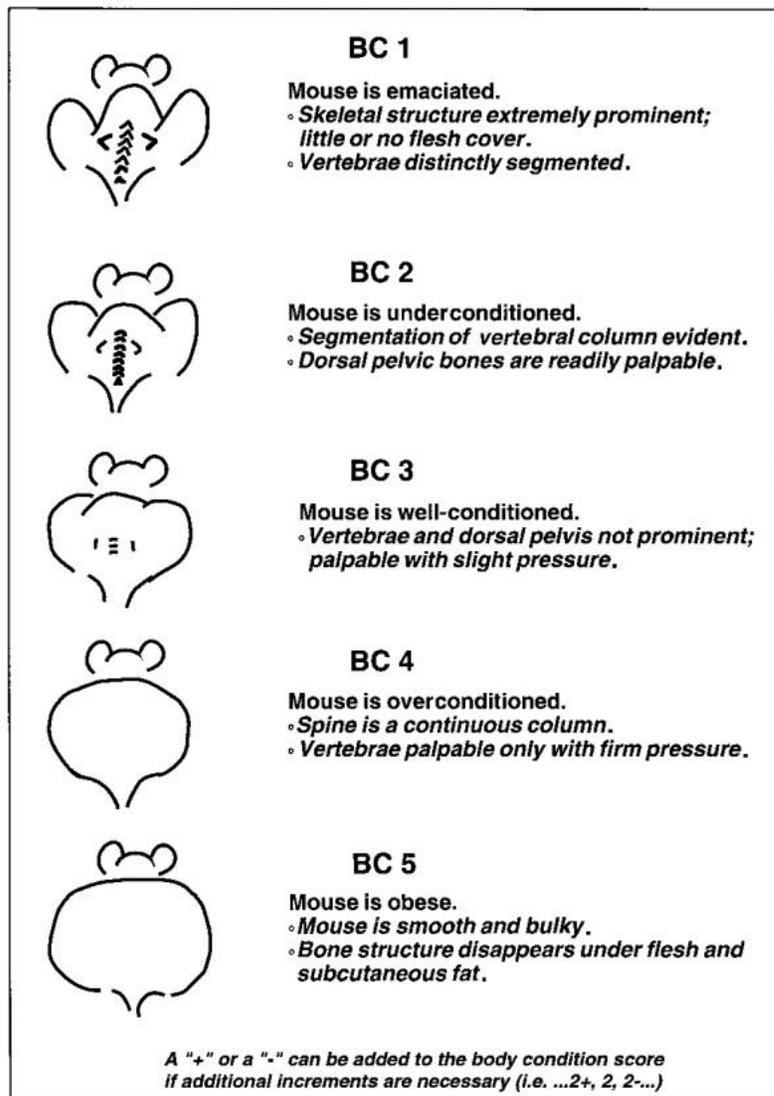
- 1— Normal
- 2— Brief periods of labored breathing
- 3— Labored, no gasping
- 4— Labored with intermittent gasps
- 5— Gasping



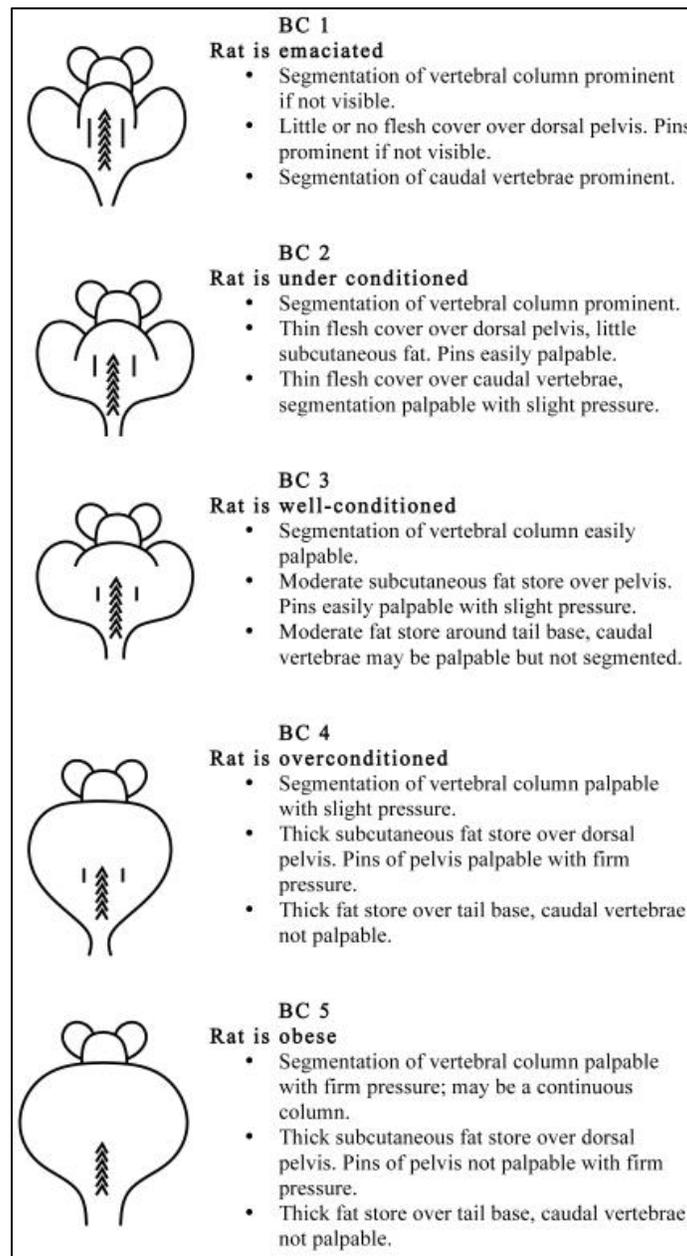
Humane endpoint criteria

- Two-parameter scoring system: Mice will be euthanized when they are awarded a combined activity-response to stimulus and eyes scores of greater than or equal to 5. Stimulus defined as observer placing 2 fingers at the mouse's tail base and gently pulling the mouse back 1 inch to observe for a forward response to the stimulus.
- Murine Sepsis Score (MSS): Mice with a score of 10 are highly likely to die within two hours and should be humanely euthanized.

Appendix 6: Species-specific body condition score diagrams -Mouse and Rat



Mouse body condition score diagram¹⁶



Rat body condition score diagram⁶