The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) reviews all biomedical research studies involving laboratory animals. Humane endpoints are established in order to minimize pain or distress to experimental animals while still meeting the scientific objectives of the study and are part of the IACUC review (Guide, p 26). The IACUC has developed the following guideline to advise investigators in developing humane endpoints for animal use protocols.

This guideline discusses the following topics:
- Definitions
- Developing humane endpoints
- Monitoring frequency and criteria
- Moribund condition as an endpoint
- Scoring systems
- Humane endpoints for behavioral studies

DEFINITIONS

**Experimental endpoint**: terminal point of study that occurs when the scientific aims and objectives have been reached (Guide, p. 27)

**Humane endpoint**: the point at which pain or distress in an experimental animal is prevented, terminated, or relieved (Guide, p. 27)

**Morbidity**: a condition of being unhealthy or diseased

**Moribundity**: a severely debilitated clinical state that precedes imminent death (Toth 2000)

**Mortality**: death

DEVELOPING HUMANE ENDPOINTS

Humane endpoints should be selected based on their ability to accurately and reproducibly predict or indicate pain, distress, imminent deterioration, or death. **Specific** humane endpoints must be clearly defined in all animal protocols, particularly for all USDA Category D and E (Penn Category B and C) procedures. Selection of humane endpoints should involve consultation with program veterinarians in ULAR of OAW. Studies that commonly require special consideration for endpoints may include:

- **Tumor development**
- **Demelinating diseases**
- **Monoclonal antibody production**
- Animals with abnormal phenotypes
- Total body irradiation
- Toxicology studies
- Infectious diseases
- Vaccine challenge
- Pain and trauma modeling
- Organ or system failure
- Models of sepsis
- Models of cardiovascular shock

*Certain areas of research that are considered to have a high potential for producing pain and/or distress in laboratory animal species are specifically addressed in other Penn IACUC Guidelines.

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HUMANE ENDPOINTS FOR LABORATORY ANIMALS

To develop a humane endpoint, the researchers should describe the clinical progression that a particular animal or group of animals is likely to experience as a result of experimental manipulation or spontaneously occurring disease during the animals’ lifetimes. Research staff must be adequately trained in recognition of species-specific behaviors and, in particular, species-specific signs of pain, distress, and morbidity (see Appendix A).

The selection of appropriate humane endpoints requires a detailed knowledge of the impact of the procedure on the animal to allow for intervention before unpredicted distress or pain develops. 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 the veterinarian (Guide, p. 28). The IACUC may request a pilot study specifically related to endpoint determinations.

Investigators performing studies that include pain or distress should throughout the studies try to refine the endpoint and the necessity for any morbidity, moribundity, or mortality. The duration of these types of studies should be kept to a minimum. Before submission of a protocol, the research staff must ensure that the following have been determined and included:

- Development of both appropriate experimental and humane endpoints for the study
- Personnel responsible for determining that experimental and/or humane endpoints have been reached have been appropriately trained
- Description of current literature searches for alternatives for potentially painful or distressful procedures

MONITORING FREQUENCY AND CRITERIA

A detailed and descriptive plan for scheduled monitoring of research animals both before and after a procedure, including the provision of treatments and supportive care, must be included in the protocol submission. Investigators should be aware that as the potential for pain/distress in animals rises, there should be an increasing intensity of monitoring and frequency of observations performed. Various clinical signs, depending on severity and duration, may constitute an endpoint and/or consultation with a ULAR veterinarian.

All monitoring, assessments, scoring, weights, supportive care, and treatments must be documented in the individual animal record (for USDA-covered species) or other logbook or lab notebook maintained by the laboratory. These records must be available at all times for ULAR staff, IACUC members, or outside inspectors.

MORIBUND CONDITION AS AN ENDPOINT

While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there are studies that require moribund state or death as an endpoint. Procedures or experiments that are expected to produce a moribund state must be categorized as USDA Pain and Distress Category E (Penn Category C). The continuation of an experimental study to the point where an animal dies without the benefit of intervention or euthanasia (“death as an endpoint” study) is not acceptable without strong scientific justification.
HUMANE ENDPOINTS FOR LABORATORY ANIMALS

Various clinical signs may be indicative of a moribund state in laboratory animals and should be considered when developing endpoints. The following signs may be associated with a moribund state:

- Unconsciousness with no response to external stimuli
- Intractable seizures
- Labored breathing, respiratory distress, or cyanosis
- Hematologic or biochemical parameters indicative of organ failure incompatible with life
- Uncontrolled bleeding
- Inability to ambulate or maintain upright position
- Prolonged inappetance and/or marked dehydration
- Evidence of muscle atrophy or marked loss of body condition (see Appendix B)
- Chronic or debilitating vomiting, diarrhea or constipation
- Excessive or prolonged hyperthermia or hypothermia
- Inability to eat or drink

SCORING SYSTEMS

Professional and clinical judgments are essential for the evaluation of an animal’s well-being and are critical to the ultimate decision of euthanasia for humane reasons. As well, objective data-based approaches to predicting imminent death, when developed for specific experimental models, can facilitate the implementation of timely euthanasia before the onset of clinically overt signs of a moribund state (Toth 2000). Scoring systems are one way in which humane endpoints can be defined and implemented. The attached example of a scoring system (see Appendix C) is based upon routine observations. Scoring systems may be developed or modified for specific species and designed to fit individual protocols or animal models.

HUMANE ENDPOINTS IN BEHAVIORAL STUDIES

In behavior studies and tests with the potential for pain and distress, proposed procedures for monitoring, record keeping, and humane interventions should be described in the protocol. A baseline behavioral profile of an animal should be established if changes in behavior are going to be used to monitor the animal for distress. An understanding of the species-typical behavior of the animals used in awake, behaving experiments is critical for adequately assessing the animal for signs of stress and/or discomfort that may be minimized either through an earlier endpoint determination or by modifying experimental procedures. Subtle changes detected in the animal’s demeanor, willingness to work in a study, or sudden changes in performance on behavioral tasks may be the first indicators of a health problem that should be investigated. If such changes are noted, the researcher should consult with ULAR veterinary staff so that the animal can be more fully evaluated (NRC 2003, p. 31-32).
CITED REFERENCES


OTHER REFERENCES


Tulane University Institutional Animal Care & Use Committee. “Policy of Humane Experimental Endpoints in Rodent Research”


Appendix A: Indicators of Pain in Several Common Laboratory Animals (NRC 2003).

<table>
<thead>
<tr>
<th>Species</th>
<th>General Behavior</th>
<th>Appearance</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodents</td>
<td>Decreased activity; excessive licking and scratching; self-mutilization; may be unusually aggressive; abnormal locomotion (stumbling, falling); writhing; does not make nest; hiding</td>
<td>Piloerection; rough/stained haircoat; abnormal stance or arched back; porphyrin staining (rats)</td>
<td>Rapid, shallow respiration; decreased food/water consumption; tremors</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Head pressing; teeth grinding; may become more aggressive; increased vocalizations; excessive licking and scratching; reluctant to locomote</td>
<td>Excessive salivation; hunched posture</td>
<td>Rapid, shallow respiration; decreased food/water consumption</td>
</tr>
<tr>
<td>Dog</td>
<td>Excessive licking; increased aggression; increased vocalizations, inclusive of whimpering, howling, and growling; excessive licking and scratching; self-mutilation</td>
<td>Stiff body movements; reluctant to move; trembling; guarding</td>
<td>Decreased food/water consumption; increased respiration rate/panting</td>
</tr>
<tr>
<td>Cat</td>
<td>Hiding; increased vocalizations, inclusive of growling and hissing; excessive licking; increased aggression</td>
<td>Stiff body movements; reluctant to move; haircoat appear rough, ungroomed; hunched posture; irritable tail twitching; flattened ears</td>
<td>Decreased food/water consumption</td>
</tr>
<tr>
<td>Nonhuman Primate</td>
<td>Increased aggression or depression; self-mutilation; often a dramatic change in routine behavior (e.g., locomotion is decreased); rubbing or picking at painful location</td>
<td>Stiff body movements; reluctant to move; huddled body posture</td>
<td>Decreased food/water consumption</td>
</tr>
</tbody>
</table>
Appendix B: Representative Body Condition Scoring (BCS) charts for rodents

**MICE**

**BC 1**  
Mouse is emaciated  
- Skeletal structure extremely prominent, little or no flesh cover  
- Vertebrae distinctly segmented

**BC 2**  
Mouse is under-conditioned  
- Segmentation of vertebral column evident  
- Dorsal pelvic bones are readily palpable

**BC 3**  
Mouse is well-conditioned  
- Vertebrae and dorsal pelvis not prominent, palpable with slight pressure

**BC 4**  
Mouse is over-conditioned  
- Spine is a continuous column  
- Vertebrae palpable only with firm pressure

**RATS**

**BC 1**  
Rat is emaciated  
- Segmentation of vertebral column prominent if not visible  
- Little or no flesh cover over dorsal pelvis, pins prominent if not visible  
- Segmentation of caudal vertebrae prominent

**BC 2**  
Rat is under-conditioned  
- Segmentation of vertebral column prominent  
- Thin flesh cover over dorsal pelvis, little subcutaneous fat, pins easily palpable  
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure

**BC 3**  
Rat is well-conditioned  
- Segmentation of vertebral column easily palpable  
- Moderate subcutaneous fat store over pelvis, pins easily palpable with slight pressure  
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented

**BC 4**  
Rat is over-conditioned  
- Segmentation of vertebral column palpable with slight pressure  
- Thick subcutaneous fat store over dorsal pelvis, pins of pelvis palpable with firm pressure  
- Thick fat store over tail base, caudal vertebrae not palpable
HUMANE ENDPOINTS FOR LABORATORY ANIMALS

**BC 5**

**Mouse is obese**
- Mouse is smooth and bulky
- Bone structure disappears under flesh and subcutaneous fat

**BC 5**

**Rat is obese**
- Segmentation of vertebral column palpable with firm pressure, may be a continuous column
- Thick subcutaneous fat store over dorsal pelvis, pins of pelvis not palpable with firm pressure
- Thick fat store over tail base, caudal vertebrae not palpable


Adapted from: Hickman D, Swan M. 2010. Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease, JAALAS 49(2) 155-159

*Note: BCS should be extrapolated to the particular species approved in your IACUC protocol*
### Appendix C: Representative Scoring System for Determining Humane Endpoints

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight Changes</strong></td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 &lt; 10 percent weight loss</td>
<td></td>
</tr>
<tr>
<td>2 10-15 percent weight loss</td>
<td></td>
</tr>
<tr>
<td>3 &gt; 20 percent weight loss</td>
<td></td>
</tr>
<tr>
<td><strong>Body Condition Score (see diagram for details)</strong></td>
<td></td>
</tr>
<tr>
<td>0 Body condition score &gt;3</td>
<td></td>
</tr>
<tr>
<td>1 BCS &gt;2 and &lt; 3</td>
<td></td>
</tr>
<tr>
<td>2 BCS &gt;1 and &lt;2</td>
<td></td>
</tr>
<tr>
<td>3 BCS of 1 or less</td>
<td></td>
</tr>
<tr>
<td><strong>Physical Appearance</strong></td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Lack of grooming</td>
<td></td>
</tr>
<tr>
<td>2 Rough coat, nasal/ocular discharge</td>
<td></td>
</tr>
<tr>
<td>3 Very rough coat, abnormal posture, enlarged pupils</td>
<td></td>
</tr>
<tr>
<td><strong>Measurable Clinical Signs</strong></td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Small changes of potential significance</td>
<td></td>
</tr>
<tr>
<td>2 Body temp change of 1-2°C, cardiac and respiratory rates ↑ up to 30%</td>
<td></td>
</tr>
<tr>
<td>3 Body temp change of &gt; 2°C, cardiac and respiratory rates ↑ up to 50%, or markedly reduced</td>
<td></td>
</tr>
<tr>
<td><strong>Unprovoked Behavior</strong></td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Minor changes</td>
<td></td>
</tr>
<tr>
<td>2 Abnormal, reduced mobility, decreased alertness, inactive</td>
<td></td>
</tr>
<tr>
<td>3 Unsolicited vocalizations, self mutilation, either very restless or immobile</td>
<td></td>
</tr>
<tr>
<td><strong>Behavioral Responses to External Stimuli</strong></td>
<td></td>
</tr>
<tr>
<td>0 Normal</td>
<td></td>
</tr>
<tr>
<td>1 Minor depression/exaggeration of response</td>
<td></td>
</tr>
<tr>
<td>2 Moderately abnormal responses</td>
<td></td>
</tr>
<tr>
<td>3 Violent reactions, or comatose</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL:</strong></td>
<td></td>
</tr>
</tbody>
</table>

Note: This scoring template should be modified for specific species and designed to fit each protocol and animal model. In this example, a score is assigned to each variable, 0 (normal or mild) to 3 (severe). The cumulative score gives an indication of the likelihood that the animal is experiencing pain or distress. Humane endpoints can be established based on these criteria. A total score of >5 or a score of 3 in any one variable, regardless of the total score should warrant mandatory evaluation/decision by a veterinarian or humane euthanasia.