IACUC Guideline

MONITORING AND HUMANE ENDPOINTS FOR LABORATORY ANIMALS

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
- Overview of humane endpoints
- Monitoring frequency, criteria, and documentation
- Developing humane endpoints
- Scoring systems

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. Humane endpoints function as a refinement to experimental endpoints and provide investigators with an effective way to achieve their research goals while maximizing animal welfare. (Guide, p. 27; Laboratory Animal Medicine, p. 1662).

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

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

**Mortality:** death

OVERVIEW OF 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 Penn Category B and C (USDA Category D and E) procedures. Selection of humane endpoints should involve consultation with program veterinarians in ULAR or OAW. Studies that commonly require special consideration for endpoints include:

- Tumor development*
- Demyelinating 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
- Behavior studies
- Models of sepsis
- Models of cardiovascular shock

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*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. See these other Guidelines for specific humane endpoint recommendations for these areas of research.

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). Documentation of subtle differences from an animal’s baseline behavioral profile is critical in recognizing signs of distress and/or discomfort. Determining earlier study endpoints or modifying experimental procedures can be effective ways to minimize pain and/or distress. 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).

The selection of appropriate humane endpoints requires a detailed knowledge of the impact of the procedure on the animal and of expected outcomes. Appropriate humane endpoints should be selected to minimize or prevent unnecessary pain or distress. 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. If any unexpected adverse events or outcomes occur, the IACUC and ULAR facility veterinarian should be notified, and the protocol should be amended promptly to describe these and refine the endpoints. 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 appropriate monitoring plan
- Development of both appropriate experimental and humane endpoints for the study
- Personnel responsible for evaluating animals for experimental and/or humane endpoints are listed on the protocol and have been appropriately trained
- Description of current literature searches for alternatives for potentially painful or distressful procedures

MONITORING FREQUENCY, CRITERIA, AND DOCUMENTATION

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.

Note that even if a procedure itself is not expected to cause distress, if the substance being administered is expected to lead to the development of clinical signs of pain/distress, then the
monitoring plan should include the entire duration of those signs rather than describing the monitoring only during the administration itself. For example, an intraperitoneal injection of lipopolysaccharide or streptozotocin is not considered painful/distressful, but because the substance being injected is expected to cause clinical signs (sepsis or diabetes mellitus, respectively), a monitoring plan and humane endpoints are needed for the duration of the study.

Complete documentation of all monitoring and assessments of animals is required. All monitoring, assessments, scoring, weights, supportive care, and treatments that are listed in the protocol must be documented in the individual animal record (for USDA-covered species) or other laboratory logbook or lab notebook (for non-USDA-covered species). These records must be available for inspection by ULAR or OAW staff, IACUC members, and outside inspectors.

DEVELOPING HUMANE ENDPOINTS

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 Penn Category C (USDA Pain and Distress Category E)). 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.

Various clinical signs should be considered when developing endpoints. The following is a list of clinical signs that would commonly warrant immediate removal from the study:

- Intractable seizures
- Labored breathing, respiratory distress, or cyanosis
- Hematologic or biochemical parameters indicative of severe compromise incompatible with life
- Uncontrolled bleeding
- Inability to ambulate or maintain upright position
- Marked dehydration (skin tent duration greater than 3 seconds)
- Weight loss in excess of 20% from baseline body weight (or weight reduced by 20% compared to age-matched controls, if studying juveniles)
- 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
- Unconsciousness with no response to external stimuli

Note that not all of these are relevant to all studies or species; the appropriate endpoints should be selected based on the proposed model, as well as consultation with a ULAR or OAW veterinarian. Humane endpoints for USDA-covered species in particular should be carefully considered together with your ULAR veterinarian.

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. 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. A ULAR or OAW veterinarian can assist in development of an appropriate scoring system.
### 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>Rodent</td>
<td>Decreased activity; excessive licking and scratching; self-mutilation; 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); altered facial appearance (Grimace scale)</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; increased grooming behavior (excessive licking and scratching); reduced movement and rearing behavior</td>
<td>Excessive salivation; hunched posture; altered facial appearance (Grimace scale)</td>
<td>Rapid, shallow respiration; decreased food/water consumption; reduced fecal output</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 rough/ungroomed; hunched or stretched/arched 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>Huddled body posture; altered facial expression (grimace, clenched teeth)</td>
<td>Decreased food/water consumption</td>
</tr>
<tr>
<td>Pig</td>
<td>Decreased activity; increased aggression; anti-social behavior, lethargy; unable/unwilling to rise; teeth grinding; rubbing or shaking affected area</td>
<td>Abnormal posture (hunched back), stiff body movements, “tiptoe” walking pattern</td>
<td>Vocalization (squealing/grunting) when painful area palpated; decreased food/water consumption</td>
</tr>
<tr>
<td>Sheep</td>
<td>Lethargy; inappetance; unable/unwilling to rise; teeth grinding; lip curling; licking painful area</td>
<td>Abnormal posture; reluctant to move; altered facial appearance (Sheep Pain Facial Expression Scale)</td>
<td>Increased respiration rate; decreased rumen motility</td>
</tr>
<tr>
<td>Fish</td>
<td>Erratic swimming (may include attempts to jump from the water); aggression; flashing (rubbing against tank side)</td>
<td>Rapid opercular movements (increased respiratory rate); clamped fins; altered color</td>
<td>Decreased food consumption (usually the first sign)</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
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BC 5

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

**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 in Rodents**

<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>Normal</td>
<td></td>
</tr>
<tr>
<td>1. &lt; 10 percent weight loss</td>
<td></td>
</tr>
<tr>
<td>2. 10-20% 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</strong></td>
<td></td>
</tr>
<tr>
<td>Body condition score &gt;3</td>
<td></td>
</tr>
<tr>
<td>BCS &gt;2 and &lt; 3</td>
<td></td>
</tr>
<tr>
<td>BCS 1.5-2</td>
<td></td>
</tr>
<tr>
<td>BCS of less than 1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Physical Appearance</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Lack of grooming</td>
<td></td>
</tr>
<tr>
<td>Rough coat, nasal/ocular discharge</td>
<td></td>
</tr>
<tr>
<td>Very rough coat, abnormal posture</td>
<td></td>
</tr>
<tr>
<td><strong>Measurable Clinical Signs</strong></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Small changes of potential significance</td>
<td></td>
</tr>
<tr>
<td>Body temp change of 1-2°C, cardiac and respiratory rates ↑ up to 30%</td>
<td></td>
</tr>
<tr>
<td>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>Normal</td>
<td></td>
</tr>
<tr>
<td>Minor changes</td>
<td></td>
</tr>
<tr>
<td>Abnormal, reduced mobility, decreased alertness, inactive</td>
<td></td>
</tr>
<tr>
<td>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>Normal</td>
<td></td>
</tr>
<tr>
<td>Minor depression/exaggeration of response</td>
<td></td>
</tr>
<tr>
<td>Moderately abnormal responses</td>
<td></td>
</tr>
<tr>
<td>Violent reactions, or comatose</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** This scoring template should be modified as needed 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 immediate removal from study.
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CITED REFERENCES


OTHER REFERENCES


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