Purpose: In accordance with federal regulations, the Institutional Animal Care and Use Committee (IACUC) reviews all biomedical research studies involving laboratory animals, including information about humane endpoints for the animals in the studies. This guideline discusses the following topics:

- Developing humane end points
- Humane endpoints for behavioral studies
- Moribund condition as a humane end point
- Monitoring frequency
- Scoring systems
- Euthanasia

Definitions

Euthanasia: the act of inducing humane death in an animal

Experimental endpoint: terminal point of study that occurs when the scientific aims and objectives have been reached (Guide 2011)

Humane end point: point at which pain or distress in an experimental animal is prevented, terminated, or relieved (Guide 2011)

Moribund: severely debilitated clinical state that precedes imminent death (Toth 2000)

Developing Humane Endpoints

Humane endpoints should be selected based on their ability to accurately and reproducibly predict or indicate pain and/or distress, imminent deterioration, or death. It is required that SPECIFIC humane endpoints be clearly defined in all animal protocols, and particularly for all UPENN Pain Category B and C procedures. Humane endpoints should be determined in consultation with ULAR veterinarians.

Studies that commonly require special consideration for endpoints may include:

- tumor development **
- infectious disease
- vaccine challenge
- pain and trauma modeling
- monoclonal antibodies production **
- assessment of toxicological effects
- organ or systemic failure
- models of cardiovascular shock
- demyelinating diseases**
- generation of animals with abnormal phenotypes **

**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 UPENN IACUC Guidelines.

Approved 8-23-2011
IACUC Guideline
Humane Intervention and Endpoints for Laboratory Animal Species

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’ lifetime. Research staff must be adequately trained in recognition of species-specific behaviors and, in particular, species-specific signs of pain, distress, and morbidity (see Table 1).

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 2011)

Please note: the IACUC may request a pilot study specifically related to endpoint determinations.

The duration of biomedical studies involving pain and distress should be kept to a minimum. Before submission of a protocol, the research staff should ensure that the following have been determined and included:

(i) development of both appropriate experimental AND humane endpoints for the study;
(ii) assignment of the appropriately trained person(s) responsible for determining that an experimental and/or a humane endpoint has been reached;
(iii) description of current literature searches for alternatives for any/all potentially painful/distressful procedures.

Humane Endpoints in Behavioral Studies

In all behavior studies and tests, proposed procedures for monitoring, record keeping, and humane intervention must 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/discomfort that may be minimized either through an earlier endpoint determination or by modifying experimental procedures. Subtle changes detected in the animal’s demeanor or its 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 promptly notify the ULAR veterinary staff so that the animal can be more fully evaluated. (NRC 2003)

Moribund Condition as a Humane Endpoint

Procedures or experiments that are expected to produce a moribund state must be categorized as UPENN Pain Category C. These types of studies will be reviewed by the full IACUC and must have scientific justification. 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 are indicative of a moribund condition in laboratory animals. If any of these signs are noted, prompt consultation with the ULAR veterinary staff or euthanasia must occur. The following signs can quickly lead to a moribund state and should be considered when developing endpoints:

- Any condition interfering with eating or drinking (e.g. difficulty with ambulation)
- Inability to remain upright
- Rapid weight loss or net weight loss of more than 20% of the body weight

Approved 8-23-2011
IACUC Guideline
Humane Intervention and Endpoints for Laboratory Animal Species

- Prolonged inappetance
- Evidence of muscle atrophy/marked loss of body condition
- Diarrhea, if debilitating or constipation
- Markedly discolored urine, polyuria or anuria
- Rough hair coat, hunched posture, lethargy or persistent recumbency
- Central nervous system disturbance - head tilt, seizures, tremors, circling, paresis
- Lack of physical or mental alertness
- Coughing, labored breathing, nasal discharge, or respiratory distress
- Jaundice and/or anemia (paleness)
- Unexplained/uncontrolled bleeding from any site on the body
- Excessive or prolonged hyperthermia or hypothermia
- Marked dehydration

Monitoring Frequency
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.

Scoring Systems (example provided in Table 2)
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, should facilitate the implementation of timely euthanasia before the onset of clinically overt signs of moribundity. (Toth 2000)

Scoring systems are one way in which humane endpoints can be defined and implemented. The attached example of a scoring system is based upon routine observations. In this example, a score is assigned to each variable, 0 (normal or mild) to 3 (severe change/variation from normal). 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. The example in this document should be modified for specific species and designed to fit each protocol and animal model.

Euthanasia
Timely euthanasia can improve research and scientific validity by eliminating distress and improving animal well-being, alleviating unnecessary animal suffering, while potentially enhancing the integrity and quality of samples to be collected. (Stokes 2000)

Animals must be euthanized in accordance with the approved protocol, based upon the current American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, or as recommended by the ULAR veterinary staff.

Approved 8-23-2011
### TABLE 1. 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><strong>Rodents</strong></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)</td>
<td>Rapid, shallow respiration; decreased food/water consumption; tremors</td>
</tr>
<tr>
<td><strong>Rabbit</strong></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><strong>Dog</strong></td>
<td>Excessive licking; increased aggression; increased 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><strong>Cat</strong></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><strong>Nonhuman Primate</strong></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>
TABLE 2. 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 Temperature change of 1-2°C, cardiac and respiratory rates increased up to 30 percent</td>
<td></td>
</tr>
<tr>
<td>3 Temperature change of &gt; 2°C, cardiac and respiratory rates increased up to 50 percent, 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>
</tbody>
</table>

**This representative scoring template should be modified for specific species and designed to fit each protocol and animal model.**

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Representative Body Condition Scoring (BCS) charts for rodents

NOTE: BCS should be extrapolated to the particular species approved in your IACUC protocol.

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.

BC 5
Mouse is obese.
- Mouse is smooth and bulky.
- Bone structure disappears under flesh and subcutaneous fat.

A “+” or a “-” can be added to the body condition score if additional increments are necessary (i.e. ..., 2, 3, ...).


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.

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.

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 Vol 49 No 2 pg 155-159.
IACUC Guideline
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References