Guidelines for Humane Endpoints in Animal Study Protocols

General
• Unrelieved pain and distress must be avoided whenever possible. When allowed, unrelieved pain and distress must be well justified and only continue as long as necessary for scientific reasons.
• Humane endpoints are required whenever experimental procedures cause pain, distress, or morbidity (signs of illness or disease) in animals.
• Humane endpoints are defined as points at which an animal’s pain or distress is ended or reduced by taking action as per the study protocol. Intervention includes euthanizing the animal, stopping a painful procedure, or alleviating the pain or distress with other appropriate measures. To the greatest extent possible, pre-emptive euthanasia should be advocated for any study in which an animal is likely to progress to a moribund state (impending death).
• Selection of humane endpoints require consideration of the scientific requirements of the study, the expected and possible adverse effects the research animals may experience, the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects.
• Humane endpoints have to be carefully set. Ending an experiment too early without meeting its objectives will waste animal’s lives.
• Effective use requires that properly qualified individuals perform observations of the research animals at appropriate time points.

Morbidity
• Morbidity is the condition of being sick or diseased and may indicate pain or distress.
• Examples of humane endpoints that indicate morbidity and potentially may predict pain or distress include:
  ▪ Hunched posture
  ▪ Rapid weight loss
  ▪ Diarrhea or constipation
  ▪ Vomiting
  ▪ Decreased food intake
  ▪ Severe or ulcerative dermatitis
  ▪ Rough hair coat, lack of grooming
  ▪ Sunken eyes
  ▪ Swollen limbs
  ▪ Nasal, ocular discharge
  ▪ Unsteady gait or lameness
  ▪ Lethargy, neurological signs
  ▪ Persistent recumbency
  ▪ Increased, decreased or labored respiration
  ▪ Coughing, nasal discharge
  ▪ Jaundice and/or anemia
  ▪ Bleeding from any orifice
- Pale mucous membranes (anemia)
- Self-induced trauma
- Hyperthermia or hypothermia
- Increased hormonal factors such as catecholamines, corticosteroids, prolactin, tumor necrosis factor, and interleukins
- Morton and Griffiths\(^6\) described the evaluation of five aspects of an animal's condition: body weight, physical appearance, measurable clinical signs (e.g. changes in heart rate, respiratory rate), changes in unprovoked behavior and response to external stimuli.
- Montgomery\(^5\) described clinical observations used in cancer research and toxicological studies. Parameters include changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces and locomotion.
- Ullman-Culler and Foltz\(^10\) described body scoring of rodents.

**Moribundity**

- The moribund condition is a clinically irreversible condition leading inevitably to death.
- While not the norm, the moribund animal may actually be past suffering. Therefore, it is important for intervention to occur as early as possible.
- Commonly used signs of moribundity include worsening degrees of the above signs of morbidity as well as the following:
  - Severely impaired mobility
  - Labored breathing and cyanosis (blue color to skin or mucous membranes)
  - Clinical dehydration and/or prolonged decrease food intake (more than 72 hours)
  - Severe, rapid weight loss (20% or more)
  - Muscle atrophy, lethargy, low body condition score
  - Lack of responsiveness to manual stimulation
  - Prolonged abnormal posture
  - Hematological or biochemical values indicating organ failure
  - Self-mutilation
  - Unconsciousness, unresponsiveness to external stimuli
  - Prolonged inability to eat or drink
  - Prolonged inability to urinate or defecate

**Endpoint Selection**

- It is not possible to have an exhaustive list of clinical signs that will occur in all situations. Endpoints may differ for each procedure, species and strain.
- Endpoints must be cardinal clinical signs that are observable and measurable.
- The selection of the particular endpoint should be made with the following considerations:
  - Appropriate observations of the animals
  - Assigning objective values to the observations
• Determine which observations are the most significant indicators of pain and distress in the specific circumstances of the study
• Determining which observation are the most significant predictors of further deterioration in the animal’s condition
• Meeting the scientific demands for an objectively measured and significant endpoint

Monitoring Animals
• All animals on any animal study are to be monitored daily by trained personnel.
• Personnel must understand what is normal for the species and strain, so animals should be viewed prior to procedures.
• The frequency of monitoring may need to be increased when animals develop clinical signs or if they are anticipated to develop clinical signs. Monitoring must also include weekends and holidays.
• Some species such as rodents, rabbits and livestock may not show many behavioral changes even when in severe pain.
• Animals should be initially viewed from a distance before approaching the cage, noting natural, undisturbed behavior and appearance. Then behavior and reaction to external stimulus (e.g. noise) should be evaluated. Animals can then be handled for clinical exam including weighing.
• Designated personnel should be notified as soon as animals show clinical signs. An assessment of the animals' condition should then be made and action taken according to the study protocol as soon as possible.
• Consideration should be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely.
• Dead animals must be promptly removed.
• Documentation of monitoring the animals is required.

Establishing a Plan
• A plan is required to follow animal care and monitoring procedures.
• The plan must identify personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and intervention.
• The plan includes score sheet or checklists and intervention as designated in the study protocol.

Score sheets
• Score sheets or checklists are helpful in ensuring that appropriate observations are made, consistently interpreted, and properly documented.
• Score sheets may need to be specific for each experimental procedure, each species and even each strain.
• Score sheets list the selected endpoints.
• Endpoints should be modified as the study progresses until the list is relevant to the assessment of suffering.
• Signs should be recorded as present (+) or absent (-) or a degree (0 – 3 representing normal or to severe). By convention, negative signs indicate normality.
• A cumulative rating may be obtained by adding the score for each category. An increase may indicate deviation from normal which can be interpreted as an indication of increasing pain and distress.
• A threshold should be identified which would indicate intervention.

Examples of Endpoints for Specific Studies
• Infectious disease studies
  ▪ Hypothermia of 34°C (predictive of mortality from bacterial pathogens)
  ▪ Ruffled coat
  ▪ Weight loss
  ▪ Ocular discharge
  ▪ Lethargy
  ▪ Hunched posture
  ▪ Ataxia
  ▪ Tremor
  ▪ Cyanosis

• Example plan for chronic infectious disease in mice
  ▪ Any negative effects with respect to animal condition are to be recorded on the score sheets and reported to the PI.
  ▪ Animals will be observed twice daily.
  ▪ Hands-on monitoring will be conducted weekly.
  ▪ Animals on study are to be weighed weekly.
  ▪ The frequency of observations will be increased to twice daily and hands-on monitoring including weights will be increased to twice weekly once any clinical signs are noted.
  ▪ Score sheets will be kept in the animal facility.
  ▪ Foot inoculation – foot swelling will be measured with a caliper weekly. When foot swelling is 4 or more mm in diameter, the animal with be euthanized.
  ▪ Skin inoculation – skin lesions will be measured weekly. When the lesion reaches 10 mm in total diameter (sum of two measurements at 90 degrees to each other), the animal will be euthanized.

<table>
<thead>
<tr>
<th>Body weight</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 10% weight loss</td>
</tr>
<tr>
<td>2</td>
<td>10 – 20% weight loss</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 20% weight loss</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical appearance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Lack of grooming</td>
</tr>
</tbody>
</table>
2 | Rough coat, nasal/ocular discharge
3 | Very rough coat, abnormal posture

**Behavior**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Minor changes; limping, favoring inoculated limb</td>
</tr>
<tr>
<td>2</td>
<td>Abnormal; reduced mobility, inactive</td>
</tr>
<tr>
<td>3</td>
<td>Unsolicited vocalization, self-mutilation, restless or immobile</td>
</tr>
</tbody>
</table>

- When a total score of three or more is reached, the PI is to be notified. When a score of 3 in any one category or a total of 6 is reached, the animal will be euthanized.

- Examples of endpoints for fish
  - Slower than normal rate of gain (weight loss is extremely slow for fish in sub-optimal environment or health)
  - Change in feeding activity or consumption
  - Physical appearance
  - Fin and skin condition
  - Mucus production
  - Color change
  - Increased or decreased respiratory rate
  - Posture in the tank
  - Social interaction
  - Hyper or hypoactivity
  - Avoidance to mechanical prod or light beam

**References:**


4. Institute for Laboratory Animal Research Journal (2000), Humane Endpoints for Animals Used in Biomedical Research and Testing. 41: No. 2


12. Netherlands Centre Alternatives to Animal Use http://www.vet.uu.nl/nca/documents/humane_endpoints

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Revised –