Guidelines for Humane Endpoints in Animal Study Protocols

1. Background

   a. Regulation
   As first defined by Russell and Burch (1959), research with animals should be guided by the “Three Rs”: Reduce (animal numbers); Replace (animals with other models); Refine (procedures to decrease pain and distress). The Guide (2011) states “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.” Further, The Guide mandates that “The PI…should identify, explain, and include in the animal use protocol, a study endpoint that is both humane and scientifically sound.” Humane endpoints are, therefore, special considerations for the IACUC to examine and approve, and should be addressed in Section E of your application.

   b. Humane Endpoints
   When allowed, unrelieved pain and distress must be well justified and only continue as long as necessary for scientific reasons.
   • Humane endpoints are defined as the point at which pain or distress is prevented, terminated or relieved. Humane endpoints provide an alternative to experimental endpoints that result in unrelieved or severe pain and distress, including death. Action is taken as per the study protocol and 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.
   • Death as an endpoint is generally unacceptable and must be fully justified and approved by the IACUC

2. Policy

   a. Humane endpoints must be established whenever experimental procedures cause or potentially can cause pain, distress, or morbidity (signs of illness or disease) in animals. Humane endpoints have to be carefully set in consultation with the Attending Veterinarian. Ending an experiment too early without meeting its objectives will waste animal’s lives.
   A non-inclusive list follows:
   • Infectious disease
   • Tumor burden
   • Experimental manipulation
   • Unusual phenotypes
   • Vaccine challenge
   • Antibody production

   b. A plan outlining specific criteria, 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 and intervention as designated in the study protocol and possibly score sheet.
3. Methodology

We encourage the use of this set of guidelines as a resource and/or template for the preparation of protocols. Note, however, that endpoints, scoring criteria, and justifications should address the specific needs of the protocol section E, in consultation with the Attending Veterinarian.

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 should be, ideally, 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

Examples of Humane Endpoints - General

- The following are examples:
  - Hunched posture
  - Weight loss
  - Diarrhea or constipation
  - Inability to urinate
  - Vomiting
  - Decreased food intake
  - Dehydration
  - Muscle atrophy
  - Low body condition score
  - Lack of responsiveness to manual stimuli
  - Prolonged abnormal posture
  - Hematological or biochemical values indicating organ failure
  - Self-mutilation
  - Unconsciousness
  - Severe or ulcerative dermatitis
  - Rough hair coat, lack of grooming
  - Swollen limbs
  - Nasal, ocular discharge
  - Unsteady gait or lameness
  - Lethargy, neurological signs
  - Persistent recumbency
  - Increased, decreased or labored respiration and cyanosis (blue color to skin or mucous membranes)
  - 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 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 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 described body scoring of rodents.

Humane endpoints can be classified based on the type of parameter:
- Appearance/Behavior (posture, activity, response to handling)
- Physiological changes (weight loss, respiration, dehydration)
- Biomedical changes (anemia, corticosteroids, insulin, glucose)

Examples of Endpoints - 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

- 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.
  - Animals will 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.

- 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
  - Fin and skin condition
  - Mucus production
  - Color change
  - Increased or decreased respiratory rate
  - Posture in the tank
  - Social interaction
  - Increased or decreased activity
  - Avoidance to mechanical prod or light beam
Monitoring Animals

- All animals on a study are to be monitored daily at appropriate time points by properly qualified individuals. 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. The unexpected death of any animal other than fish or amphibians must be reported to the facility veterinarian, IACUC or AV.
- Documentation of monitoring the animals is required. When scoring sheets are not required, documentation is made on the room census sheet or other room log.

Score Sheets

- Score sheets help to ensure that appropriate observations are made, consistently interpreted, and properly documented.
- Score sheets are not required when relatively minor pain or distress may occur such as antibody production using other-than Complete Freund’s Adjuvant or low grade infection that causes no or very minor symptomology. However, investigators must be prepared to develop and implement score sheets if conditions change.
- Prior to the start of the experiment, a decision about when to implement a score sheet must be reached. Its implementation should be before the onset of pain and distress or as soon as possible thereafter.
- Current score sheets must be kept in the animal facility.
- Score sheets may need to be specific for each experimental procedure, each species and even each strain.
- Score sheets list the selected endpoints.
- Endpoints and score sheets should be modified as the study progresses until the list is relevant to the assessment of suffering.
- Guidance notes on how to record humane endpoints and necessary action should be located on the bottom of the score sheet.
- Signs should be recorded as present (+) or absent (-) or unsure (+/-) 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 must be identified which would indicate intervention (euthanasia, treatment and/or removal from the study). This threshold represents an ethical balance between the anticipated benefits of the research and the degree of animal pain and distress (as indicated by the humane endpoints).
- Score sheets will be periodically reviewed by the IACUC.
Examples of Response Variables and Scoring System

- **Body Weight**
  - < 10% weight loss = 1
  - 10 – 20% weight loss = 2
  - 20% weight loss = 3

- **Physical appearance**
  - Normal = 0
  - Lack of grooming = 1
  - Rough coat, nasal/ocular discharge = 2
  - Very rough coat = 3

- **Behavior**
  - Normal = 0
  - Minor changes = 1
  - Inactive = 2
  - Unresponsive = 3

- **Breathing:** R = Rapid; S = Shallow; L = Labored; N = Normal

- **Body Condition Score:** 0 = Normal; 3 = Emaciated

- 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.

**Example of Pain Score Sheet.** (see following page) Evaluation criteria must apply to the experiment. Not all criteria fit every situation.

<table>
<thead>
<tr>
<th>Date of Study:</th>
<th>Animal Number:</th>
<th>Starting Weight (g):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
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<tr>
<td>Weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% weight change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight score</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Physical Appearance**

- Grooming
- Ulceration

**Behavior**

- Activity
- Hunched Posture
- Breathing
- Dehydration
- Tremors
- Circling
- Body Condition Score

**Total score**

- Nothing Abnormal
- Number of animals euthanized/died

**Initials**
Example Scoring Criteria – must include cumulative score action

Score 0 to 3 with 0 = normal

- Weight loss: <10% = 1; 10-20% = 2; >20% = 3
- Grooming: Lack of grooming = 1; rough coat, nasal discharge = 2; very rough coat = 3
- Ulceration: Loss of hair, skin intact = 1; ulceration size of a dime or less = 2; ulceration larger than a dime = 3
- Activity: Normal = 0; minor changes = 1; inactive = 2; unresponsive = 3
- Breathing: R= Rapid; S = Shallow; L = Labored; N = Normal; present = 2
- Dehydration, Tremors, Circling: present = 2
- Body Condition Score: Normal = 0; Emaciated = 3
- Monitor animals once a day. Once Total Score is 3, monitor twice a day.
- Euthanize when weight loss is greater than 20% or when cumulative score of 6 or greater.

References:


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


http://www.olis.oecd.org/olis/2000doc.nsf/4f7ad21b1a6085c125696f005d8ee7c125692700623b74c12569bb005aa3d5/$FILE/00087372.pdf


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