1. Introduction
Studies should be designed to minimize pain and/or distress and to prevent spontaneous death. If pain or distress is unavoidable, then a scientific justification and the humane endpoints for removing animals from the study or for their euthanasia must be approved by the IACUC prior to the study. Such endpoints are preferable to death or moribundity since they minimize pain and distress. It is suggested that in any given research animal study a quantifiable body or animal clinical health index be used. The effective use of endpoints requires that properly qualified individuals perform both general and study-specific observations of the research animals at appropriate time points.

If an animal must be allowed to die without intervention in order to answer a scientific question, this is considered "death as an endpoint". Death as an endpoint is not typically necessary for research protocols but may be required in some situations, including acute toxicity testing, assessment of virulence of pathogens, and neutralization tests for toxins.

2. Purpose
The purpose of this document is to provide guidelines for selecting an endpoint that reduces animal pain and/or distress, while still meeting research objectives when animals are used for biomedical research, teaching, and testing. Important concepts to know when writing a protocol:

- In this document "endpoint" refers to one or a combination of physical (e.g., body weight), behavioral (e.g., grooming activity) or other signs of disease or distress that are used, typically during a longitudinal experimental procedure in which animal health may deteriorate (e.g., inoculation with an infectious agent), to decide when an intervention will be terminated or an animal may be euthanized to minimize pain or distress.
- The criteria that provide the basis for terminating experimental procedures in order to minimize or alleviate any actual or potential pain, distress, or discomfort is made by choosing the earliest endpoint that is compatible with the scientific objectives; these criteria are referred to as humane endpoints. Selection of such endpoints by the investigator involves consultation with the Institutional Veterinarian, and the endpoints chosen must be approved by the IACUC. For additional reference material, the ILAR Journal, volume 41, is devoted to this topic.
- The principles of humane endpoints apply to all species. Humane endpoints for species or specific projects that may not be covered in this document are determined on a case-by-case basis in consultation with the Institutional Veterinarian.

3. Procedures (Follow A and B when writing the protocol and C once approved)
   A. Humane endpoints must be described in the protocol for studies with expected morbidity or mortality and approved by the IACUC. When writing a protocol:
      - Consult the Institutional Veterinarian and review this document for guidance (See sections below).
      - Choose the earliest endpoint that is compatible with the scientific objectives.
• Use pilot studies when possible to provide an opportunity to evaluate humane endpoints and assure the scientific objectives are met, before proceeding to large scale studies.
• A scoring system can be used. Consult the veterinarian and describe the scoring system to be used in the protocol.
• Proper approval from the IACUC is required if an investigator wishes to maintain an animal on study once the animal is moribund and exhibits clear criteria requiring euthanasia. Obtaining such approval requires scientific justification.
• Death as an endpoint requires scientific justification and documentation in the IACUC protocol that the above humane endpoints cannot be used.

B. If death or moribundity will be used as an endpoint, include in the justification when writing the protocol:
• What alternatives were considered?
• Why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible.
• Why measures to relieve pain and/or distress cannot be utilized.
• Number of animals to be used and why this is the minimal number of animals required.
• Whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.
• What statistical or other methods were used to determine the number of animals required to achieve valid results?
• A plan for animal care and monitoring procedures:
  ✓ Animals involved in experiments that may lead to moribundity or death must be monitored at least twice daily by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for at least the following: abnormal posture, rough hair coat, head tucked into abdomen, exudate around eyes and/or nose, skin lesions, abnormal breathing, difficulty with ambulation, decreased food or water intake, dehydration and self-mutilation.
  ✓ Indication of who will conduct the monitoring
  ✓ The frequency of observation will be increased when animals exhibit the above or other signs of morbidity. Monitoring at night, weekends and holidays must be provided by the investigative staff one or more times a day over and above care provided by the animal care and veterinary staff. The Institutional Veterinarian must be notified as soon as animals show signs of disease. An assessment of the animals’ condition should be made as soon as possible and a plan of action established.
  ✓ Consideration should be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals in the cage is likely and/or compromised access to food/water. Moribund and dead animals must be promptly removed from the cage.
  ✓ Written records of monitoring of animals should be kept.

C. Once you have IACUC approval and start your project:
• Notify the veterinarian when animals exhibit signs of pain and distress or otherwise become moribund.
• For studies in which morbidity or death is not expected this document should be used as guidance in consultation with the veterinarian when animals exhibit signs of pain and distress.
• If the study is approved with expected morbidity or death as an endpoint, investigators must monitor animals at least daily, including weekends and holidays, and keep detailed records of any observations and treatments.
• During periods in which morbidity and mortality are expected to increase, animals must be evaluated by the investigator a minimum of two times daily (every 8-12 hours). Those animals that are not expected to survive until the next scheduled evaluation should be humanely euthanized.
• At all times during the study, the well-being of the research animals must be balanced against the requirements of the study.

4. **Humane Endpoints for Studies with Expected Morbidity or Mortality**

In most studies, animals must be humanely euthanized if they experience unrelieved pain or distress, based on the euthanasia criteria described below and in accordance with the AVMA Guidance on Euthanasia, 2007 and the UCB SOP #6 Euthanasia.

The following are general humane endpoints that require euthanasia.
- The inability to reach food or water for more than 24 hours.
- A 20% decrease in normal body weight.
- A Body Condition Score typically > 5 point (see Section 7: Assessing Signs of Pain and Distress, below, for explanation of these scales). There are other body condition scales that can be used.
- Development of conditions that result in significant pain that cannot be alleviated by analgesics.

General observations for assessing pain and distress include change in body weight, external physical appearance, clinical signs (e.g., inability to reach food and water, lethargy or decreased mental alertness, labored breathing, inability to remain upright), significant changes in behavior, and responses to external stimuli. As a general rule sick animals should be identified as early as possible prior to a moribund state. Laboratory personnel must carefully observe the animals for changes in health status, appearance, and behavior, and have knowledge of the treatment and procedures that the animals have undergone.

Humane endpoints will vary depending on the nature of the study. Protocols may include more specific criteria. Investigators must discuss this with the Institutional Veterinarian. Identifying the initial signs that occur prior to a moribund state in order to avoid additional pain and suffering is key to developing humane endpoints. Criteria with a scoring system provide an excellent, objective method for identifying the appropriate time for euthanasia, and can be developed with the assistance of the Institutional Veterinarian for individual projects.

If the veterinarian has examined an animal and determined that it will not survive until the next scheduled examination, a reasonable attempt will be made to contact the Principal Investigator (P.I.) to obtain permission to euthanize the animal. If the veterinary staff is unable to contact the P.I., the veterinarian will euthanize the animal.

Death as an endpoint requires scientific justification and documentation in the IACUC protocol that the criteria for euthanasia cannot be used. Such justifications may include reference to the requirements of regulatory agencies. Studies with death as an endpoint (also known as survival duration studies, LD50 etc.) are not approved by the UCB Institutional Animal Care and Use Committee unless this endpoint is scientifically justified in the animal use protocol.
Humane Endpoints/ Criteria for euthanasia:

- **Weight loss**
  - In adult animals, loss of >20 percent of body weight compared to the pre-study weight or to age-matched controls
  - In growing animals, or in animals whose body weight has not been recorded, or in tumor studies, weight loss will be assessed by body condition.
  - **Decrease in appetite**
    - Complete anorexia for 24 hours in small rodents, up to 5 days in large animals;
    - Partial anorexia (less than 50% of caloric requirement) for 3 days in rodents, 7 days in large animals.

- **Weakness/Inability to obtain feed or water**
  - Inability or extreme reluctance to stand which persists for 24 hours (assuming that the animal has recovered from anesthesia).

- **Moribund state**
  - An animal found to be in a state of dying characterized by severe depression, nonambulatory, complete anorexia and hypothermia with little likelihood of recovery.

- **Infection**
  - Infection (either overt or indicated by abnormal body temperature or WBC parameters) which fails to respond to therapy within an appropriate time.

- **Tumor growth**
  - Solid tumors that exceed 10 percent of normal body weight. Assuming 1 cm³ = 1 gm, the formula for the weight of the tumor is $\frac{4}{3}\pi r^3$. i.e. for a 25 g mouse, a tumor 1.7 cm in diameter = 2.57 gm = maximum allowable size. For tumors not perfectly round, use the average radius.
  - Loss of body condition indicating that tumor growth is being supported by body and/or metabolic reserves.
  - Tumor growth that impedes an animal's ability to ingest food or water and other normal bodily functions or its ability to move about its cage and remain clean and dry.
  - Tumors that appear to be causing the animal pain or distress that cannot be relieved with analgesics or other palliative measures.
  - Evidence of tumor necrosis or ulceration indicating the tumor has outgrown its blood supply.
  - Evidence of organ dysfunction and/or failure from either primary or metastasized tumors.

- **Unrelieved pain/distress**
  - Signs of significant pain and/or distress which are unresponsive to analgesics/anesthetics, or as determined by the UCB veterinarian.

- **Organ dysfunction/failure**
  - Signs of severe organ system dysfunction non-responsive to treatment, or with a poor prognosis as determined by a UCB veterinarian. Examples include but are not limited to:
    - **Respiratory:** labored breathing and cyanosis unresponsive to appropriate medical therapy.
    - **Cardiovascular:** acute blood loss resulting in shock or severe anemia; cardiac failure.
    - **Gastrointestinal:** severe vomiting, diarrhea; rectal prolapse or intestinal obstruction.
    - **Urogenital:** renal failure characterized by elevated BUN or creatinine; urinary tract obstruction; ruptured bladder; uroperitoneum; vaginal, uterine, or penile prolapse.
    - **Nervous:** CNS depression; seizures; paralysis of one or more extremities; neurological conditions which impede an animal's ability to ingest food or water or its ability to move about its cage and remain clean and dry inhibiting eating and drinking.
    - **Musculoskeletal:** muscle damage or bone fracture resulting in inability to use the limb or severe pain.
    - **Integument:** non-healing wounds or severe burns covering more than 10% of the body; repeated self-trauma.
Humane Endpoints/ Criteria for euthanasia (Continued):

- **Cancer and Toxicological Studies**
  
  Selected Clinical Observations Used in Cancer Research and Toxicological Studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>What to look for</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Appearance/Dehydration</td>
<td>decreased body weight, missing anatomy, abnormal posture, hypothermia, fractured</td>
</tr>
<tr>
<td></td>
<td>appendage, swelling, tissue masses, prolapse, paraphimosis</td>
</tr>
<tr>
<td>Skin and fur Discoloration</td>
<td>urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer,</td>
</tr>
<tr>
<td></td>
<td>alopecia, ruffled fur</td>
</tr>
<tr>
<td>Eyes</td>
<td>Exophthalmos, microphthalmia, ptosis, reddened eye, lacrimation, discharge,</td>
</tr>
<tr>
<td></td>
<td>opacity</td>
</tr>
<tr>
<td>Nose, Mouth &amp; Head</td>
<td>Head tilted, nasal discharge, malocclusion, salivation, Respiration, Sneezing,</td>
</tr>
<tr>
<td></td>
<td>dyspnea, tachypnea, rales</td>
</tr>
<tr>
<td>Urine Discoloration</td>
<td>blood in urine, polyuria, anuria</td>
</tr>
<tr>
<td>Feces Discoloration</td>
<td>blood in the feces, softness/diarrhea</td>
</tr>
<tr>
<td>Locomotor/Hyperactivity</td>
<td>hyperactivity, coma, ataxia, circling, muscle, tremors</td>
</tr>
</tbody>
</table>
5. **Assessing Signs of Pain and Distress**

To assess the possibility of pain and distress objectively, it is helpful to assign values to various observations of animal condition and behavior. An example of a scoring system is presented below. Based on observations, a score is assigned to each variable, 0 (normal or mild) to 3 (severe changes 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 criteria such as:

- the total score (e.g. a total score > 5), or
- a score of 3 in any one variable, regardless of the total score.

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

**TOTAL**
References
ILAR Journal V41(2) 2000.

http://web.research.colostate.edu/ACP/PDF_Docs/AALAS%20body%20condition%20scoring.pdf

http://safetyservices.ucdavis.edu/IACUC/policies/humane-endpoints

http://ko.cwru.edu/docs/rodentcondition.pdf

http://web.research.colostate.edu/ACP/PDF_Docs/AALAS%20body%20condition%20scoring.pdf


http://www.nal.usda.gov/awic/newsletters/v6n1/6n1offer.htm


ILAR website: Recognizing Pain in Animals.
http://dels.nas.edu/animal_pain/index.shtml

See also the IACUC Policy for Euthanasia of Research Animals.

General endpoint references:

Canadian Council on Animal Care (1998), Guidelines on: Choosing an appropriate endpoint in experiments using animals for research, teaching and testing. Ottawa, Canada.

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


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