Introduction
Experimental studies may involve procedures that cause clinical symptoms or morbidity in the animals. The Animal Care and Use Committee (ACUC) must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, the expected and possible adverse effects the research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. 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. Studies should be designed to minimize pain and/or distress. 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 ACUC prior to the study. Such endpoints are preferable to death or moribundity since they minimize pain and distress. Efforts must be made to minimize pain and distress experienced by animals used in research.

Morbidity
Animal Study Proposals that include morbidity as an endpoint or that include animal procedures that have the potential to cause adverse sequelae should address the following:

1. Criteria that establish when the endpoint has been reached.
   a. There are several examples in the literature that might be considered, including:
      1) Evaluation of five aspects of an animal's condition as described by Morton and Griffiths. These are body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli.
      2) Clinical observations used in cancer research and toxicological studies as described by Montgomery. Parameters include changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces and locomotion (Table 1).
      3) Body condition scoring as described by Ullman-Culler and Foltz.
   b. The clinical signs, depending on severity and duration, that may constitute an endpoint include, but are not limited to: Rapid weight loss.
      • Diarrhea, if debilitating.
      • Progressive dermatitis.
      • Rough hair coat, hunched posture, lethargy or persistent recumbency.
      • Coughing, labored breathing, nasal discharge.
      • Jaundice and/or anemia.
      • Neurological signs.
      • Bleeding from any orifice.
      • Self-induced trauma.
      • Any condition interfering with eating or drinking (e.g. difficulty with ambulation).
      • Excessive or prolonged hyperthermia or hypothermia.
c. Additional signs in neoplasia studies that may constitute an endpoint include, but are not limited to: 1) A tumor burden greater than 10% body weight. In an adult mouse, a tumor may not exceed 20 mm in any one dimension; in an adult rat, a tumor may not exceed 40 mm in any one dimension. Formulas for calculating tumor size can be found in the literature (see tumor size references). 2) Tumors that ulcerate, become necrotic or infected.
d. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water.

2. A plan for monitoring the animals both before and after a change in any of the above aspects, providing care if appropriate, and increasing the level of monitoring. Monitoring or clinical care on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff.

3. Identification of personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia. Checklists or score sheets may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented.

Death or Moribundity
While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there are studies that require moribundity or mortality as an endpoint. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. Commonly used signs of moribundity include, but are not limited to: a) lack of responsiveness to manual stimulation; b) immobility; and/or c) an inability to eat or drink. In these studies, animals are permitted to die or become moribund, as a result of experimental procedures. In some cases, pain relieving measures are not used because such measures may compromise the experimental integrity of the study. Examples of research proposals that may have death or moribundity as an endpoint include: infectious disease studies, drug and toxicity studies, and cancer research. The following guidelines are suggested to assist the Animal Care and Use Committees in reviewing proposals with death or moribundity as endpoints.

Animal Study Proposals utilizing death or moribundity as an endpoint should contain the following information:
1. The scientific rationale for death or moribundity as an endpoint, including:
   a. What alternatives were considered, why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible.
   b. Why measures to relieve pain and/or distress cannot be utilized.
   c. Number of animals to be used and why this is the minimal number of animals required.
   d. Whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.

2. A plan for the following animal care and monitoring procedures:
   a. Animals involved in experiments that may lead to moribundity or death will be monitored 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, or abnormal breathing, difficulty with ambulation, decreased food or water intake, or self mutilation.
   b. The frequency of observation will be increased when animals exhibit the above or other signs of morbidity. Monitoring on weekends and holidays may require involvement of the investigative staff to supplement that provided by the animal care and veterinary staff. Designated personnel, including a veterinarian, should 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.
c. Consideration will 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.
d. Written records will be kept of monitoring.

**General endpoint 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

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