



## **DEFINITION AND GUIDELINES FOR HUMANE ENDPOINTS FOR STUDY ANIMALS**

These guidelines are meant to assist PIs in their choice of humane endpoints in animal studies and assist them in making decisions about *early termination* of an animal's enrollment in a study (which typically involves euthanasia of an animal before the study is finished). Ultimately, use of humane endpoints should help to minimize the degree of pain or distress experienced by research animals.

The Guide (8<sup>th</sup> Ed) notes that: “The *experimental endpoint* of a study occurs when the scientific aims and objectives have been reached. The *humane endpoint* is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved.” It is sometimes difficult and challenging to balance these two objectives. The PI, who has precise knowledge of both the objectives of the study and the proposed model, must identify, explain and include in the animal use protocol study endpoints that are both humane and scientifically sound. The IACUC and the veterinarian must agree and approve of the humane endpoints that are proposed.

The use of humane endpoints is a refinement that provides an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress. While all studies should employ endpoints that are humane, special consideration must be given to studies that involve tumor models, infectious disease, vaccine challenges, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, models of organ failure and cardiovascular shock. It is strongly advised that the PI consult with the veterinarian on the issue of humane endpoints in the planning stages of a project involving those types of procedures.

Both USDA regulations and the Guide note that investigators must be qualified by training and experience to accomplish experimental manipulations in a humane manner. All personnel who will be working with animals must be trained to recognize the signs of pain and distress in animals. Observations of experimental animals must be of adequate frequency depending on the severity of the animal's condition (sometimes once/day is adequate and at other times 3x/day is required). Weekends and holidays should not be overlooked. Investigators should request veterinary advice and support if uncertain or unclear about whether an animal is experiencing pain or distress.

**Death as an Endpoint:** The IACUC strongly recommends that PIs NOT use death as an experimental endpoint, and will require extensive scientific justification for the use of such. A refinement would be to use the “moribund condition” (or earlier clinical signs as discussed below) as a humane endpoint trigger to euthanize an animal before the study is over. Moribund is defined as: *being in the state of dying : approaching death*. Clinical signs should be observed as predictors of lethality, before the animal becomes moribund if possible, to enable essential data collection while minimizing animal suffering. A combination of 2 or more intermediate clinical signs (*see below*), or even one severe sign, should be enough to warrant early intervention and possibly euthanasia of an animal (see Table 1 below, for reference of clinical signs). Please refer to IACUC guidelines for solid tumor studies for additional information specifically related to animals in tumor studies.

**Euthanasia Decision:** If the PI or designee cannot be located in a reasonable length of time, the veterinarian will use their best clinical judgment regarding whether or not euthanize an animal. The PI or designee(s) will usually discuss such a decision with the veterinary staff - *however, it is noted that, by law, the veterinarian has the final authority in this matter if there is disagreement.*

### **Signs for Evaluating the Moribund Condition**

The following guidelines can be used as objective parameters of a moribund condition. An animal might exhibit one of these signs without being considered moribund, but two or more are usually indicative. Sometimes one severe sign may be sufficient to conclude that the animal is near death/dying. Such signs should constitute the basis for developing humane endpoints for a study.

1. Impaired ambulation (unable to reach food and water easily) whether due to a large mass or poor body or neurological condition (weakness).
2. Evidence of muscle atrophy or other signs of body emaciation.
  - rapid weight loss (15-20% within a few days)
  - extended period of weight loss
  - Note that due to tumor growth, an animal's body weight is not always proportionate.
3. Obvious signs of illness including but not restricted to:
  - central nervous system signs (head tilting, hydrocephalus, tremors, seizures, circling, paresis)
  - chronic diarrhea or constipation
  - rough haircoat, hunched or rounded posture, distended abdomen
  - dyspnea or cyanotic (obvious difficulty breathing which may occur with metastatic models which seed the lungs or due to infection)
  - paleness (indicating anemia, this most easily visualized in nude animals)
  - marked discolored urine
  - ocular discharge
  - prolonged inappetence
4. Paralysis
5. Inability to remain upright
6. Frank bleeding from any orifice
7. Trauma
8. Non-healing or extensive skin lesions/wounds/ulcerations
9. Distended urinary bladder with difficulty expressing
10. Distended abdomen due to ascites or neoplasia



**TABLE 1: Clinical Signs of Advancing Toward the Moribund Condition** *(These may be used to develop humane endpoints in a study protocol)*

Minor	Ocular discharge - in rodents
	Ruffled, dull, or unkempt fur/hair coat
	Weight loss of less than 10%
Intermediate	Ataxia - uncoordinated movements
	Dehydration of 5-10%; visualized by an animal being smaller than cage mates, shriveled/ shrunken appearance
	Hunched back - protective posture or painful stance; distended abdomen; constipation
	Lethargy - no energy reserves
	CNS signs: tremors; head tilt
	Impaired ambulation
	Weight loss of 10 - 20%
Severe	Anorexia - inability to eat or drink
	Dehydration of >10% this may be consistent with diarrhea (bedding pasted around anus)
	Anemia evidenced by pale mucous membranes
	Marked discolored urine
	Diarrhea (bedding pasted around anus)
	Cyanotic - inadequate oxygenation, blue - tinged mucous membranes; difficulty breathing
	Hypothermia - cold to the touch
	Moribund - lack of a righting reflex
	Emaciation: weight loss of >20%; muscle wasting
	Painful lesion(s) or conditions
	CNS signs: hydrocephalus, seizures, circling
	Frank bleeding from any orifice
Paresis/Paralysis - inability to ambulate.	