



Dartmouth College

## Animal Care and Use Program

### Institutional Animal Care and Use Committee

#### IACUC Guideline

**Title:** Guidelines for Establishing Humane Endpoints in Animal Study Proposals

**Introduction:** Experimental studies may involve procedures that cause clinical symptoms of morbidity in animals. Some of these may then be required to lead to moribundity or death as an endpoint. The Institutional Animal Care and Use Committee (IACUC) 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. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease, if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity as endpoints 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 those that include animal procedures that have the potential to cause adverse sequella should address the following:

- Criteria that establish when the endpoint has been reached. There are several examples in the literature that might be considered, including:
  - Evaluation of five aspects of an animal's condition as described by Morton and Griffiths.<sup>1</sup> These are body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli.
  - Clinical observations used in cancer research and toxicological studies as described by Montgomery.<sup>2</sup> Parameters include changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces and locomotion
- 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.

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<sup>1</sup> Morton and Griffiths (1985), Veterinary Record 116:431-43.

<sup>2</sup> Montgomery (1990), Cancer Bulletin 42:230-237.  
and appeared in AWIC Newsletter, Spring 1995 6:4

- Self-induced trauma.
  - Any condition interfering with eating or drinking (e.g. difficulty with ambulation).
  - Excessive or prolonged hyperthermia or hypothermia.
- Additional signs in neoplasia studies that may constitute an endpoint include, but are not limited to:
  - A tumor burden greater than 10% body weight, and in an adult mouse, a mean tumor diameter exceeding 1.5 cm or in an adult rat, a mean tumor diameter exceeding 40 mm. Formulas for calculating tumor size can be found in the literature (see tumor size ref.).
  - Tumors that ulcerate, become necrotic or infected.
  - Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water.
- 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.
- Identification of personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia. Checklists/ 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. 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. It should be understood that it is difficult to get approval for death as an endpoint without strong scientific justification. The IACUC typically requires that the PI determine if an earlier endpoint could substitute for allowing the animal to die. The following guidelines are suggested to assist the IACUC in reviewing proposals with death or moribundity as endpoints.

### **Animal Study Proposals utilizing death or moribundity as an endpoint should contain the following information:**

- The scientific rationale for death or moribundity as an endpoint, including:
  - What alternatives were considered, why morbidity as an endpoint cannot be used, and how alternatives will be used whenever possible.
  - Why pain relieving measures 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.
- A plan for the following animal care and monitoring procedures:
  - 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.
  - The frequency of observation will be increased when animals exhibit the above or other signs of moribundity. 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.

- 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.
- Written records will be kept of monitoring.

**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.

Hendriksen and Morton, ed. (1998), *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands. Laboratory Animals Ltd, by Royal Society of Medicine Press Limited, London, England.

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

Toth (1997), *Contemporary Topics* 36:44-48.

Stokes (1999) *Humane Endpoints in Animal Experiments for Laboratory Animals Used in Toxicity Testing* Proceedings of the 3rd World Congress on Alternatives and Animal use in the Life Sciences, 31 August - 2 September 1999, Bologna, Italy.

United Kingdom Co-ordinating Committee on Cancer Research (1997), *UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia*, 2nd ed. London, England.

**Tumor size references:**

- Bullard et al. (1981), *J. Neuropath. Exp. Neurol.* 40:410-427.  
 Tomayko and Reynolds (1989), *Cancer Chemother. Pharmacol.* 24:148-154.  
 Sung et al. (1993), *Cancer Research* 53: 2092-2099.  
 Welch et al. (1994), *Oncogene* 9: 255-262.  
 Hamm (1995), *Contemporary Topics* 34:69-71.

**Table 1**

Selected Clinical Observations Used in Cancer Research and Toxicological Studies

Parameter	What to look for
General Appearance	Dehydration, decreased body weight, missing anatomy, abnormal posture, hypothermia, fractured appendage, swelling, tissue masses, prolapsed, paraphimosis
Skin and Fur	Discoloration, urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur
Eyes	Exophthalmos, microphthalmia, ptosis, reddened eye, lacrimation, discharge opacity
Nose, Mouth, and Head	Head tilted, nasal discharge, malocclusion, salivation
Respiration	Sneezing, dyspnea, tachypnea, rales
Urine	Discoloration, blood in urine, polyuria, anuria
Feces	Discoloration, blood in the feces, softness/diarrhea

Locomotor

Hyperactivity, hyperactivity, coma, ataxia, circling, muscle, tremors