Denver | Anschutz

IACUC
Institutional Animal Care and Use Committee

Subject:	**Guidelines for Establishing Humane Endpoints in Animal Study Proposals**
Source	Institutional Animal Care and Use Committee (IACUC)
Effective Date	08/10/2026
Replaces:	02/21/2020
Applies to:	Personnel involved in Research or Teaching involving animals.
Reference:	Animal Welfare Act; PHS Policy on Humane Care & Use of Laboratory Animals. Guide for the Care & Use of Laboratory Animals

Introduction

Experimental studies may involve procedures that cause clinical signs of illness in animals. The Institutional Animal Care and Use Committee (IACUC) must consider and approve the selection of the most appropriate humane 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, distress, and spontaneous death. If pain or distress is unavoidable, then a scientific justification and the humane endpoints for removing animals from the study must be approved by the IACUC prior to the start of any procedures. Such endpoints are preferable to death or moribundity (being near death) since they minimize pain and distress and may provide better scientific data. It is strongly recommended that in any given research animal study, a quantifiable clinical health index (e.g., a score sheet) be developed that can be utilized to determine humane endpoints. Such indexes may be protocol-specific.

Morbidity

Animal studies that include morbidity or that include animal procedures that have the potential to cause adverse sequelae should address the following:

1. Parameters: Criteria that establish when the endpoint has been reached.
    a. There are several examples in the literature that might be considered, including:
        i. Evaluation of five aspects of an animal's condition as described by Morton and Griffiths [(6)]. These are:
            - body weight
            - physical appearance
            - measurable clinical signs
            - unprovoked behavior
            - response to external stimuli
        ii. Clinical observations used in cancer research and toxicological studies as described by Montgomery [(5)] (Table 4, *vide infra*)
        Parameters include:
            - changes in general appearance
            - skin and hair

- eyes, nose, mouth
- head
- respiration
- urine, feces
- locomotion

iii. Body condition scoring (BCS) as described by Ullman-Culler and Foltz [10].

b. Clinical signs, depending on severity and duration, which 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
- significant self-induced trauma
- any condition interfering with eating or drinking (e.g., difficulty with ambulation)
- excessive or prolonged hyperthermia or hypothermia

Note: hypothermia of 34° C is predictive of mortality from bacterial pathogens.

In developing a scoring index, each of the clinical signs can be a point value and when a cumulative score is reached the animal must be removed from the study and often euthanized. The scoring system developed by the researcher is reviewed and approved by the IACUC. These euthanasia points vary according to experiment but are a point at which further study on that animal would be inhumane, beyond the point at which relevant data would be obtained or where the condition is irreversible and expected to progress to spontaneous death.

c. Additional signs in neoplasia studies that constitute an endpoint include, but are not limited to:

i. Adult mouse - tumors that exceed 20 mm in any one dimension in an adult mouse.

ii. Adult rat - tumors that exceed 40 mm in any one dimension.

- Formulas for calculating tumor size can be found in the literature (**see tumor size references**).

iii. Tumors that ulcerate, become necrotic or infected.

iv. A tumor burden greater than 10% of the animal's baseline body weight.

d. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water must be euthanized immediately.

2. Monitoring: 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 must be described in the protocol. 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. Responsible Party: Identification of personnel responsible for evaluation, record keeping, and persons responsible for euthanasia must be described. Checklists or score sheets may be

required by the IACUC to ensure 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 may 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:

1. Lack of responsiveness to manual stimulation
2. Immobility and/or an inability to eat or drink. In these studies, animals become moribund as a result of experimental procedures and may die. Examples of research proposals that may propose death or moribundity as an endpoint include infectious disease studies, drug and toxicity studies, and cancer research. **The CU Denver |Anschutz IACUC generally does not approve proposed studies with death as an endpoint.** The following guidelines will be used to assist the Animal Care and Use Committee in reviewing proposals with death or moribundity as endpoints.

Animal Study Proposals proposing 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 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, exudate around eyes and/or nose, skin lesions, abnormal breathing, difficulty with ambulation, decreased food or water intake, hydration and self-mutilation.
    b. The frequency of observation must be increased when animals exhibit the above or other signs of morbidity. Monitoring at night, weekends and holidays by researchers may be required to supplement monitoring provided by the animal care and veterinary staff.
    c. Written records of monitoring animals must be kept and accessible for IACUC review.

Example of score sheet for monitoring

Experimental treatment of a bacteria-induced, unilateral knee joint infection in mice.

Parameters to be monitored: body weight, physical appearance, and behavior.
A separate Score Sheet must be kept on each individual mouse.

Table 1-Body weight

Score	Observation
0	Normal
1	<10% weight loss
2	10-15% weight loss
3	>15% weight loss

Table 2-Physical appearance

Score	Observation
0	Normal
1	Lack of grooming
2	Rough hair coat, nasal/ocular discharge
3	Very rough coat, abnormal posture (head tucked into abdomen)

Table 3-Behavior

Score	Observation
0	Normal
1	Minor changes: limping, favoring inoculated leg
2	Abnormal: reduced mobility, inactive
3	Unsolicited vocalization, self-mutilation, restless or immobile

NOTE: When a score of 3 in any one category or a total of 6 is reached the animal must be euthanized.

Cancer and Toxicological Studies

Table 4-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, prolapse, 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 & 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

General Endpoint References

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