University of Colorado Denver/Anschutz Medical Campus
Guidelines for Establishing Humane Endpoints in Animal Study Proposals

Introduction

Experimental studies may involve procedures that cause clinical signs or morbidity in the animals. 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. 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, and may provide better scientific data. It is suggested that in any given research animal study a quantifiable body or animal clinical health index be developed with a point scale that can be utilized to determine humane endpoints. Such indexes must be protocol-specific.

Morbidity

Animal Study Proposals that include morbidity as an endpoint or that include animal procedures that have the potential to cause adverse sequellae 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 (6). These are:

            body weight
            physical appearance
            measurable clinical signs
            unprovoked behavior
            response to external stimuli.

        2) Clinical observations used in cancer research and toxicological studies as described by Montgomery (5). Parameters include:

            changes in general appearance

skin and hair
eyes
nose
mouth
head
respiration
urine
feces
locomotion
(Table 4, *vida infra*).

3) Body condition scoring as described by Ullman-Culler and Foltz (10).

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

In developing a body scoring index, each of the clinical signs is given a point value and when a cumulative score, as set by the researcher, is reached the animal is removed from the study and euthanized. Each experiment would have its own scoring system developed by the researcher and reviewed and approved by the IACUC. These euthanasia points vary according to experiment but usually are a point at which further study on that animal would be inhumane, beyond the point at which relevant data would be obtained or that the condition would be irreversible and the animal very likely would progress to moribunity and death.

c. Additional signs in neoplasia studies that 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 must be euthanized.**

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 must be described. 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 must be described. 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 appear to 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. 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 propose death or moribundity as an endpoint include: infectious disease studies, drug and toxicity studies, and cancer research. **The UCD 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, head tucked into abdomen, 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 will be increased when animals exhibit the above or other signs of morbidity. Monitoring at night, 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 should be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely. Moribund and dead animals must be promptly removed from the cage.

d. Written records of monitoring of animals should be kept.

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

****** When a total score of three or more is reached, the PI is to be notified. 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:

1. Alternatives to Animal Testing on the Web (2004), Humane Endpoints Database. (http://apps1.jhsph.edu/altweb/humane/) Johns Hopkins Center for Alternatives to Animal Testing. Baltimore.

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

3. Hendriksen CFM and Morton DB, 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.

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

5. Montgomery CA (1990), Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. Cancer Bulletin 42:230-237.

6. Morton DB and Griffiths PHM (1985), Guidelines on the recognition of pain, distress and
discomfort in experimental animals and an hypothesis for assessment. Veterinary Record 116:431-43.

7. OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation (2000)

8. Stokes WS (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.

9. Toth (1997), The moribund state as an experimental endpoint. Contemp Top Lab Anim Sc 36:44-48.

10. Ullman-Culleré MH and Foltz CJ (1999), Body condition scoring: a rapid and accurate method for assessing health status of mice. Lab Anim Sc 49:319-323.

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

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

Tumor size references:

1. Bullard DE, Schold SC Jr, Bigner SH, Bigner DD (1981), Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell lines. J Neuropath Exp Neurol 40:410-427.

2. Hamm (1995), Proposed institutional animal care and use committee guidelines for death as an endpoint in rodent studies. Contemp Top Lab Anim Sc 34:69-71.

3. Sung C, Dedrick RL, Hall WA, Johnson PA, Youle RJ (1993), The spatial distribution of immunotoxins in solid tumors: assessment by quantitative autoradiography. Cancer Research 53: 2092-2099.

4. Tomayko MM and Reynolds CP (1989), Determination of subcutaneous tumor size in athymic (nude) mice. Cancer Chemother Pharmacol 24:148-154.

5. Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Stanbridge EJ, Weissman BE (1994), Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. Oncogene 9: 255-262.

Montgomery, C.A. Jr. (1990), Cancer Bulletin 42:230-237 and appeared in AWIC Newsletter, Spring 1995 6:4