

Guidelines for Endpoints in Animal Study Proposals

Introduction

Endpoints are a part of every Animal Study Proposal (ASP) and the ACUC must address the endpoint for every animal on the study. It is ideal when the scientific aims and objectives of the study can be accomplished without adverse affects, pain or distress to the animal. However, this is not always possible and careful consideration must be given to 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. Where pain or distress is a necessary part of the study, a humane endpoint must always be used and is:

The IACUC approved earliest scientifically justified point at which pain or distress in an experimental animal can be prevented, terminated, or relieved, while meeting the scientific aims and objectives of the study.

The effective use of endpoints requires that properly trained and qualified individuals perform both general and study-specific observations of the research animals at appropriate time points. The assessment criteria and required response must be clearly defined, and the use of study-specific animal assessment records should be considered. Studies must be designed to minimize pain and/or distress. If pain or distress is unavoidable, then a scientific justification must be reviewed and approved by the ACUC and the earliest possible endpoint compatible with answering the scientific question must be employed. Such endpoints are preferable to death or moribundity since they minimize pain and distress.

When initiating a new set of experiments, the potential for pain and/or distress may be unknown and/or the nature and extent of resulting morbidity and/or moribundity and/or mortality cannot be anticipated. Therefore, smaller pilot studies may be useful as they can be instrumental to the development of an appropriate endpoint.

Finally, investigators performing studies that include pain or distress should throughout the studies try to refine the endpoint and the necessity for any morbidity and/or mortality.

Morbidity

Animal Study Proposals that include morbidity as an endpoint or that include animal procedures that have the potential to cause adverse sequella should address the following as they relate to the expected outcomes:

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 Griffiths⁶. 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 Montgomery⁵. 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, BC 1 through 5, as described by Ullman-Culler and Foltz¹⁰.
 - BC 1 - Mouse is emaciated – skeletal structure extremely prominent, little or no flesh cover, vertebrae distinctly segmented
 - BC 2 - Mouse is underconditioned – segmentation of vertebrate column evident, dorsal pelvic bones readily palpable
 - BC 3 - Mouse is well conditioned – vertebrae and dorsal pelvis are not prominent, palpable with slight pressure
 - BC 4 - Mouse is over conditioned – spine is a continuous column, vertebrae palpable only with firm pressure
 - BC 5 - Mouse is obese, mouse is smooth and bulky, bone structure disappears under flesh and subcutaneous fat
- b. The clinical signs, depending on severity, duration and response to appropriate therapy, that may constitute an endpoint include, but are not limited to:
- Rapid or progressive weight loss. Loss of body condition should also be considered and is especially useful in studies/animals where there is the potential for loss of body condition without loss of weight.
 - Debilitating diarrhea
 - Dehydration/reduced skin turgor
 - Edema
 - Sizable abdominal enlargement or ascites
 - Progressive dermatitis
 - Rough hair coat
 - Hunched posture
 - Lethargy or persistent recumbency
 - Coughing, labored breathing, nasal discharge
 - Jaundice, cyanosis, and/or pallor/anemia
 - Neurological signs
 - Bleeding from any orifice
 - Self-induced trauma
 - Any condition interfering with daily activities (e.g. eating or drinking, ambulation, or elimination)
 - Excessive or prolonged hyperthermia or hypothermia
 - For aquatic species additional signs can include scoliosis, emaciation, significant skin lesions and/or exposure of muscle or other tissue
- 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 should not exceed 20 mm in any one dimension; in an adult rat, a tumor should 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.

3) Tumors that interfere with eating or impair ambulation.

d. Considerations for aging studies:

1) Due to the effects of senescence, aging animals may naturally exhibit a number of clinical signs that would indicate significant morbidity in younger animals. Animals age at different rates so in addition to chronological age, factors such as genotype, background strain, and sex should be taken into consideration.

2) For lifespan studies, where clinical signs of morbidity associated with aging are expected and necessary for the scientific aims and objectives of the study, the endpoint of the study should be as objectively described as possible by the investigators. Recent information suggests that in aging rodents there are changes in serial measurements of temperature, respiratory patterns, and bodyweight that correlate with imminent death. However, these changes may not be predicative at the individual animal level. Where more subjective endpoints such as deterioration in general health or quality of life are used, the assessment will rely on a veterinarian's observation and judgment in consultation with the Principal Investigator as to when the endpoint has been reached.

e. Unexpected experimentally induced conditions: When performing a study and an unanticipated adverse affect occurs due to the experimental manipulation (morbidity, moribundity, mortality, pain or more than momentary distress) the animals must be immediately euthanized or treated at the first overt signs of adverse affects to avoid IACUC unapproved pain and distress. If standard veterinary treatment would affect the experimental results, the animals will be euthanized unless withholding treatment for the condition(s) is specifically approved on the ASP. An animal study proposal amendment must be submitted to obtain IACUC approval to continue the experiment on to an endpoint which will include the previously unanticipated pain or distress.

f. Unexpected /spontaneous non-experimentally related conditions: Conditions may arise in breeders or other "normal" animals or during the conduct of research that are unexpected and unrelated to the research being conducted. Conditions may be specific, such as a spontaneous tumor, or may be more of a general deterioration of health/quality of life. These conditions can still have a significant impact on animal welfare and experimental results and must be addressed appropriately. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water must be euthanized. In less severe cases that may include pain or distress, the unexpected/unrelated condition should be assessed for the impact on animal welfare and experimental results. If it impacts experimental results, the animal should be euthanized. If it does not affect the experimental results (this would also be the case with a breeder or a normal untreated animal), standard veterinary treatment must be provided. If standard veterinary treatment would affect the experimental results, the animals should be euthanized unless withholding treatment for the condition(s) is specifically approved on the ASP. If the condition worsens following treatment, the animal should be euthanized dependent on veterinary judgment and with Principal Investigator consultation.

In some animal models, such as specific phenotypes in Genetically Modified Animals (GMAs), conditions that impact animal welfare can be expected to continue or reoccur. These should be included in the ASP as “expected resultant affects” to alert animal care staff and specify appropriate additional animal care and/or euthanasia.

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 as necessary, should be in place. 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. Unless otherwise specified in the ASP, monitoring and taking the appropriate actions is the responsibility of the investigative staff, including on weekends and holidays.
3. Identify the 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. The number of animals that will be allowed to reach morbidity/death and justification for it being the minimum necessary to achieve the scientific objectives.
 - 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 at least 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, exudates 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. A system should be in place where designated personnel, including a veterinarian, are notified when 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.

Imaging/Biomarkers

The use of serial imaging or biomarkers may permit the detection of experimental endpoints that precede the development of significant clinical signs, and consideration should be given to their use especially in studies that would otherwise result in morbidity, morbidity, or mortality.

General endpoint references:

1. Alternatives to Animal Testing on the Web (2004), Humane Endpoints Database. (<http://altweb.jhsph.edu/humane-endpoints.htm>) 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) [http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono\(2000\)7&doclanguage=en](http://www.oecd.org/officialdocuments/displaydocumentpdf?cote=env/jm/mono(2000)7&doclanguage=en)
8. Netherlands Centre Alternatives to Animal Use http://www.vet.uu.nl/nca/documents/humane_endpoints
9. Ray MA, et al (2010) Identification of Markers for Imminent Death in Mice Used in Longevity and Aging Research. JAALAS 48(3):282-288
10. Recognition and Alleviation of Pain In Laboratory Animals; NRC 2009
11. 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.

12. Toth (1997), The moribund state as an experimental endpoint. *Contemp Top Lab Anim Sc* 36:44-48.
13. 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.
14. 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:

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.
6. Workman P, et al; Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer* (2010) 102, 1555-1577

Approved by ARAC 10/9/96

Reapproved - 2/10/99

Revised - 3/8/00, 1/12/05, 11/14/07, 05/11/11

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, 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, 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,

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