

Guidelines for Endpoints in Animal Study Proposals

Introduction

Study endpoints are a part of every Animal Study Proposal (ASP) and the Animal Care and Use Committee (ACUC) must address the specific endpoints for every study. It is ideal when the scientific aims and the study objectives can be accomplished without adverse effects, 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;
- Expected and/or possible adverse effects that the research animals may experience (pain, distress, illness, etc.);
- Probable time course and progression of those adverse effects;
- 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 be used and approved by the ACUC.¹ A humane endpoint is the 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. Such endpoints are preferable to death or moribundity since they minimize pain and distress and may be considered refinements.

The effective implementation of endpoints requires properly trained and qualified individuals to perform both general and study-specific observations on research animals at appropriate time points. The assessment criteria and required response from the PI or designee must be clearly defined within the ASP, and the use of study-specific animal assessment records should be considered. Studies must be designed to minimize pain and/or 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 and determining animal numbers.

Finally, investigators performing studies that include pain or distress should re-assess the necessity for any morbidity, moribundity, or mortality throughout the studies and refine the endpoints whenever possible. Post-approval monitoring is another tool the ACUC can use to determine if endpoints are being used as intended and if further refinements are possible.

Morbidity

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

1. Criteria to identify the humane endpoint:
 - a. There are several examples in the literature that might be considered for most species, 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 include: changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces and locomotion (Table 1).

3) Body condition (BC) scoring can be applied to most species, including zebrafish², BC 1 through 5.^{3, 14}

BC 1 - Emaciated – skeletal structure extremely prominent, little or no flesh/muscle mass, vertebrae distinctly segmented

BC 2 – Under-conditioned – segmentation of vertebrate column evident, dorsal pelvic bones readily palpable, reduced muscle mass

BC 3 - Well conditioned – vertebrae and dorsal pelvis are not prominent/visible, palpable with slight pressure

BC 4 - Over conditioned – spine is a continuous column, vertebrae palpable only with firm pressure

BC 5 - Obese, contour 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 in most species include, but are not limited to:

Rapid or progressive weight loss. Young or growing animals should have weight assessed by using growth charts typical for their species/strain, such that lack of weight gain is considered. 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.

Anorexia (lack or loss of appetite) or failure to drink

Debilitating diarrhea

Dehydration/reduced skin turgor

Edema

Sizable abdominal enlargement or ascites

Progressive dermatitis

Rough hair coat/unkept appearance

Hunched posture

Lethargy or persistent recumbency

Loss of a righting reflex

Coughing, labored breathing, nasal discharge Jaundice, cyanosis, and/or pallor/anemia

Neurological signs

Seizures

Paresis / paralysis

Circling or head tilt

Blindness

Bleeding from any orifice

Self-induced trauma

Any condition interfering with daily activities (e.g. eating or drinking, nest-building, 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

For zebra fish - complete loss of equilibrium, complete cessation of movement with no response to external stimuli (moribund) and respiratory distress (excessive operculum

movement or gasping for air).

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 literature¹⁸⁻²⁰⁻²⁴.
- 2) Tumors that ulcerate, become necrotic or infected.
- 3) Tumors that interfere with eating or impair ambulation.

Table 1. Examples of Clinical Observation-Based Endpoints

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, coma, ataxia, circling, muscle, tremors,

Montgomery, C.A. Jr. (1990), *Cancer Bulletin* 42:230-237

d. Considerations for aging studies:

- 1) Senescent animals may naturally exhibit several clinical signs that would indicate significant morbidity in younger animals. Aging animals may also experience certain benign ailments at an increasing incidence. Genotype, background strain, chronological age, and sex should therefore be considered in the process of developing endpoints in these studies.
- 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, and bodyweight that correlate with imminent death^{12,15,17}. 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. Additional parameters to consider in evaluation of larger species could include complete blood counts, blood chemistries, urinalysis and other minimally invasive techniques to evaluate organ function.

- e. Unexpected experimentally induced conditions: When performing a study and an unanticipated adverse effect occurs due to the experimental manipulation (morbidity, moribundity, mortality, pain or more than momentary distress), the animals must be immediately euthanized or treated to avoid ACUC 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 in the ASP. If unexpected experimental conditions are determined necessary for the study, then an animal study proposal amendment must be submitted to obtain ACUC approval to continue the experiment with 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 the condition 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 in 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. If not already present, these should be included in a modification to 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 moribundity/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; abnormal breathing, difficulty with ambulation, decreased food or water intake, lack of response to stimulation, or self- mutilation.
 - b. The frequency of observation will be increased when animals exhibit the above described 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 frequency and observations.

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, moribundity, or mortality.

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Approved by ARAC 10/09/96

Reapproved - 02/10/99

Revised - 03/08/00; 01/12/05; 11/14/07; 05/11/11; 04/10/13; 03/04/16; 04/24/19