Guidelines for Humane Endpoints in Animal Study Proposals

Introduction: Endpoints are a critical component of every Animal Study Proposal (ASP) and must be reviewed by the IC Animal Care and Use Committee (ACUC). Not only does consideration of endpoints help ensure consideration for the welfare of research animals, but IC ACUC consideration of endpoints is also required by both the *Guide for the Care and Use of Laboratory Animals* (*Guide*) and the U.S. Government Principles for Utilization and Care of Vertebrate Animals in Testing, Research, and Training.(1, 2) Every ASP should include humane endpoints and experimental endpoints, which are defined as follows:

- Experimental endpoint: The experimental endpoint is the time at which the experiment will end due to completion of the scientific aims and objectives.
- Humane endpoint: A humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. This may sometimes supersede the experimental endpoint.

While the scientific aims and the study objectives can ideally be accomplished before the onset of pain, distress, or other adverse effects to the animal, this is not always possible. Therefore, it is, imperative that ASPs describe humane endpoints that will lead to the end of the study and prevention of unnecessary pain or distress. Well-defined humane endpoints are, in all situations, preferable to death or moribundity since they minimize pain and distress and are considered refinements. The ACUC, Principal Investigator (PI), and veterinarian should work together to develop endpoints as ASPs are developed and before they are approved. These endpoints may work off existing literature for specific models,(3-25) may consider cumulative/lifetime use,(26-28) or may be developed based on some of the criteria below. Considerations may include:

- Scientific requirements/objectives of the study
- Expected and/or possible adverse effects that the research animals may experience (i.e., pain, distress, illness, etc.) due to the study procedures
- Probable time course and progression of those adverse effects
- Earliest, most predictive indicators of present or impending adverse effects
- Expected or possible adverse phenotypes due to animal species, strain, or model
- The use of serial imaging or biomarkers that might permit the detection of experimental endpoints that precede the development of significant clinical signs.

It is also important to consider that while some endpoints may be expected or planned based on the experimental model being used, there may always be unexpected or spontaneous non-experimentally related conditions. These conditions may arise in animals during the conduct of research and may be unrelated to the research being conducted but still result in unnecessary pain or distress. Conditions may be specific, such as a spontaneous tumor, or may be more of a general deterioration of health/quality of life. No matter what the reason or the condition, animal welfare and experimental results might be impacted significantly and therefore the unexpected conditions must be addressed appropriately. ASPs should, therefore, include humane endpoints beyond those driven by the study itself. For example, a cancer study may require endpoints for tumor size but should also define endpoints that may develop outside of tumor growth.

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, frequency, 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. Consultation with veterinary staff should also be considered and can be an important step to identifying humane endpoints. In some situations, veterinary treatment may be warranted(29) but, in others, these animals must be euthanized to prevent unnecessary pain or distress or unnecessary death.

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 may not be easily anticipated. Therefore, while it is important to recognize the potential limitations of pilot studies, smaller pilot studies may be instrumental and sometimes required by ACUCs to contribute to the development of an appropriate endpoint, determine appropriate animal numbers, determining the need for interventions, or even determining the appropriateness of the chosen species.(30-32) ACUCs may require additional communication between the laboratory and the ACUC to approve larger-scale studies and develop the best humane endpoints. In some animal models, conditions that impact animal welfare can be expected to continue or recur. If not already present, these should be included in a modification to the ASP as expected resultant effects to alert animal care staff and specify appropriate additional animal care and/or euthanasia.

Investigators performing studies that include pain or distress should re-assess the necessity for morbidity, moribundity, or mortality throughout the studies and refine the endpoints whenever possible. Post-approval monitoring(33) is another tool the ACUC can use to determine if endpoints are being used as intended and if further refinements are possible.

Guidance on Developing Humane Endpoint Criteria:

Morbidity is defined as suffering from a disease or medical condition or a state of unhealthiness. It is too vague to be a specific endpoint in an ASP. However, clinical signs of morbidity may be used by the ACUC, PI, and veterinarian to define endpoints.

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:

Table 1: Examples of Clinical Observation-Based Endpoints

Location/Category	Possible Endpoints
General signs	• Rapid or progressive weight loss • Anorexia • Debilitating diarrhea • Dehydration (reduced skin turgor, sunken eyes) • Lethargy or persistent recumbency • Bleeding from any orifice • Condition interfering with normal ambulation especially if it causes an inability to reach food or water • Abnormal posture • Hypothermia • Swelling or edema • Rapid muscle loss (emaciation) • Significant reduction in body condition score(34-36)

Location/Category	Possible Endpoints
Skin and fur	• Jaundice/icterus • Urine stains • Pallor or cyanosis • Redness • Ruffled fur • Abscess formation • Wounding or injury
Eyes	• Exophthalmos • Ptosis • Discoloration – icterus, pallor, hemorrhage • Corneal ulceration
Respiration	• Dyspnea • Tachypnea • Gasping
Feces/urine	• Blood in urine/stool • Significant and persistent diarrhea or polyuria • Significant or persistent constipation or anuria
Neurologic	• Seizures • Paralysis • Paresis • Circling • Head tilt • Blindness • Hydrocephalus

Neoplasia	• Unexpected tumor formation unrelated to study
	• A tumor burden greater than 10% body weight.
	• In adult mice/rats the longest tumor diameter or estimated tumor volume can be considered for endpoints.
	  ○ Subcutaneous flank tumor should not exceed 20 mm in any one dimension (mice) or 40 mm in any one dimension (rats).
	  ○ Tumor volume estimated at 2-3 cm^3 (mice) or 4-5 cm^3 (rats) using the ellipsoid volume formula
	• Tumors that ulcerate and/or become necrotic and/or infected.
	• Tumors that interfere with their ability to perform bodily functions, significantly impairs gait, or impairs an animal's ability to obtain food or water.
	• As with other models, literature reviews can provide valuable insight.(4, 37, 38)

Location/Category	Possible Endpoints
Aging studies	• Special considerations may apply. 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 increased incidence. Genotype, background strain, chronological age, and sex should therefore be considered in the process of developing endpoints in these studies. • Literature suggests that in aging rodents there are changes in serial measurements of temperature and body weight that correlate with imminent death • As with other models, literature reviews can provide valuable insight.(39-45)

Guidance for Monitoring Requirements:

To help identify any of the defined endpoints, a plan for monitoring the animals during the study must be defined in the ASP for review by the ACUC.(27-29) Laboratories and animal facilities are expected to monitor at a frequency dependent on the study details and potential outcomes, expected to provide care if appropriate, and should plan on increasing the level of monitoring as necessary. 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.

The ASP should identify the personnel responsible for monitoring, evaluation, record keeping, and euthanasia. Checklists or score sheets may be helpful in ensuring appropriate observations are made, consistently interpreted, and properly documented. In addition, personnel responsible for monitoring and evaluation should communicate with veterinary staff and PIs whenever needed.

Importantly, the humane endpoint observations and interventions do not need to remain static. Instead, they can change over time based on input from the technicians, laboratory staff, and veterinarians observing animals, working together with the ACUC.(46)

Guidance on Endpoints Progressing to 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. The use of a pilot study can be used to help guide both the laboratory and ACUC to refine the study and determine the necessity of progressing to death or moribundity as an endpoint. Commonly used signs of moribundity include, but are not limited to:

- lack of responsiveness to manual stimulation;
- immobility; and/or
- 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 graft vs. host disease. However, even in these types of studies, humane endpoints before death should be considered by the Principal Investigator, ACUC, and veterinarian. The following guidelines are suggested to assist the ACUCs in reviewing proposals with death or moribundity as endpoints:

Required Information for ASPs Utilizing Death or Moribundity as an Endpoint:

a. The scientific rationale for death or moribundity as an endpoint, including:
    i. What alternatives were considered, why clinical signs predicting death cannot be used instead of moribundity/death, and how alternatives will be used whenever possible.
    ii. Why measures to relieve pain and/or distress cannot be utilized.
    iii. 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.
    iv. Whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.
b. The PI, working with the ACUC and veterinary staff, should develop a plan for animal care and monitoring procedures for animals on these studies. This may include:
    i. Animals involved in experiments that may lead to moribundity or death must be monitored at least daily (including weekends and holidays) by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for at least clinical signs as outlined in Table 1.
    ii. Animals developing these clinical signs may require more frequent observation, for example several times daily, including on weekends and holidays.
    iii. 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 by the veterinarian and/or laboratory staff should be made as soon as possible, and a plan of action for euthanasia and/or treatment established.
    iv. 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.
    v. Records of monitoring frequency and observations will be kept.

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