GUIDELINES FOR HUMANE ENDPOINTS IN ANIMAL STUDIES

Table of Contents

1. Background

According to the Guide for the Care and Use of Laboratory Animals (8th edition), “the use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelieved or severe animal pain and distress, including death.” The humane endpoint is defined as “the point at which pain or distress in an experimental animals is prevented, terminated or relieved.” Preemptive euthanasia can help prevent unnecessary pain and distress and also provides advantages to the researcher, including easier collection of tissues and blood for postmortem analysis, as well as eliminating the collection of non-useful data that may show severe and unexpected physiologic derangements.

2. Purpose

The purpose of this guideline is to assist researchers in selecting appropriate humane endpoints when submitting an Animal Use Protocol (AUP) to the Animal Care and Use Committee (ACUC). Researchers should euthanize animals at the earliest possible stage of illness, while still allowing the investigator to collect the necessary data.

3. Guidelines for Humane Endpoints

Animals showing pain or distress that is not justified in the AUP and approved by the ACUC MUST be euthanized or must be treated in consultation with the Office of Laboratory Animal Care (OLAC) veterinary staff to avoid unapproved pain and distress. If treatment would invalidate experimental results, the animals must be euthanized, and a revision to the AUP

describing the unrelieved pain and distress must be approved by the ACUC before continuing the experiment.

When creating humane endpoints, researchers should consider the following parameters:

Parameter	What to look for
General Appearance	Dehydration (sunken eyes, skin tenting), decreased body weight or loss of body condition, missing anatomy, abnormal posture, hypothermia, fractured appendage, swelling, tissue masses, prolapse, paraphimosis (prolapsed penis)
Fur/Skin	Urine stain, pallor, redness, cyanosis, icterus, wound, sore, abscess, ulcer, alopecia, ruffled fur
Eyes	Exophthalmos, ptosis, reddened eye, lacrimation, discharge, opacity
Nose, Mouth, and Head	Head tilt, 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, paralysis, paresis

4. Moribund Animals

Moribundity is defined as “the clinically irreversible condition leading inevitably to death.” If the moribund state is to be used as an endpoint, the AUP must consider possible alternatives, why measures cannot be taken to relieve pain and distress, and why animals cannot be euthanized at an earlier state. All moribund animals must be euthanized unless death as an endpoint is well-justified and approved in the AUP. If moribundity is approved as an endpoint and animals show signs of pain/distress, but have not reached the moribund state, animals must be

monitored at least once daily (including weekends and holidays) with appropriate documentation (see Appendix A), or more often depending on the model and progression of disease.

Moribund animals are defined as the following:

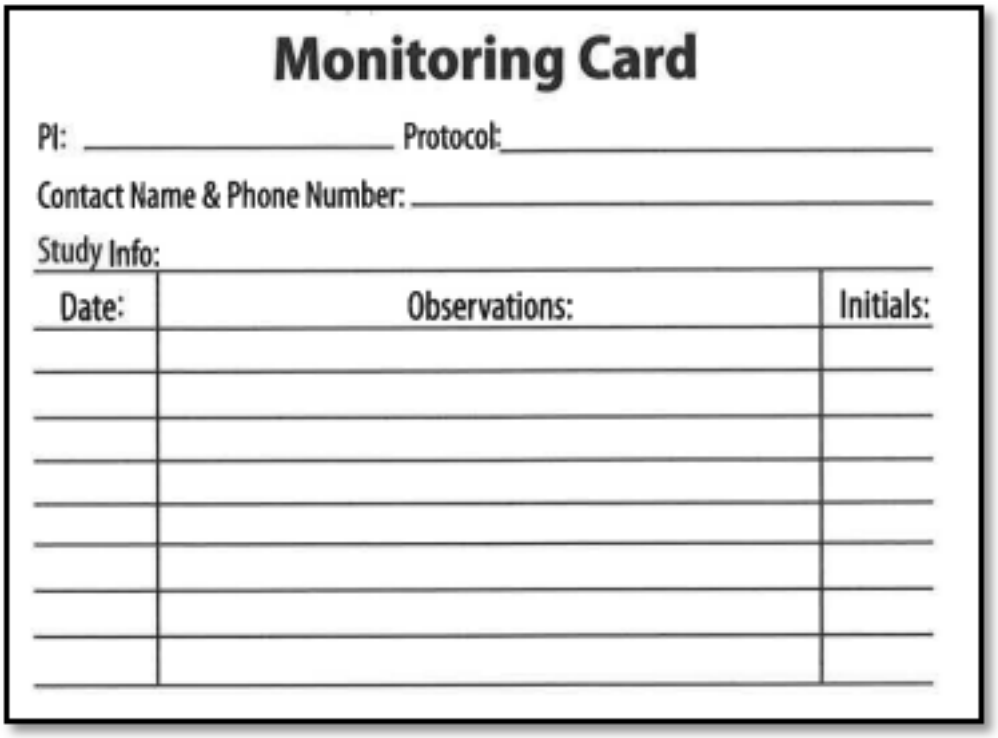
- Inability to right itself or lie in a sternal position
- Inability to eat or drink or any condition that interferes with the ability to eat or drink
- Inappetance or severe dehydration (prolonged skin tenting) greater than 48 hours
- Respiratory distress, agonal breathing, cyanosis
- Paralysis/paresis
- Uncontrollable hemorrhage
- Unrelievable, progressive hypothermia
- Unresponsiveness to stimuli
- BCS < 2/5 (see Appendix B for mice and Appendix C for rats); or Irreversible weight loss (≥15%) compared to normal control animals

5. References

- Franco NH, Correia-Neves M, Olsson IA. (2012) How "humane" is your endpoint? Refining the science-driven approach for termination of animal studies of chronic infection. PLoS Pathogens. 8(1):e1002399. doi: 10.1371/journal.ppat.1002399.
- Guidelines for Endpoints in Animal Study Proposals, 2005 [cited May 5, 2015]. Available at http://oacu.od.nih.gov/ARAC/documents/ASP_Endpoints.pdf
- Hawkins P. (2002) Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. Lab Anim. 36(4):378-95.
- Hickman DL and Swan M. (2010) Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease. J Am Assoc Lab Anim Sci. 49(2):155-9.
- Montgomery CA. (1990) Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. Cancer Bulletin. 42:230-237.
- Ullman-Culleré MH, Foltz CJ. (1999) Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Anim Sci. 49(3): 319-23.

Call 3-VETS if there is an animal emergency
510-643-8387

Appendix A – Monitoring Cage Card Example



Monitoring Card

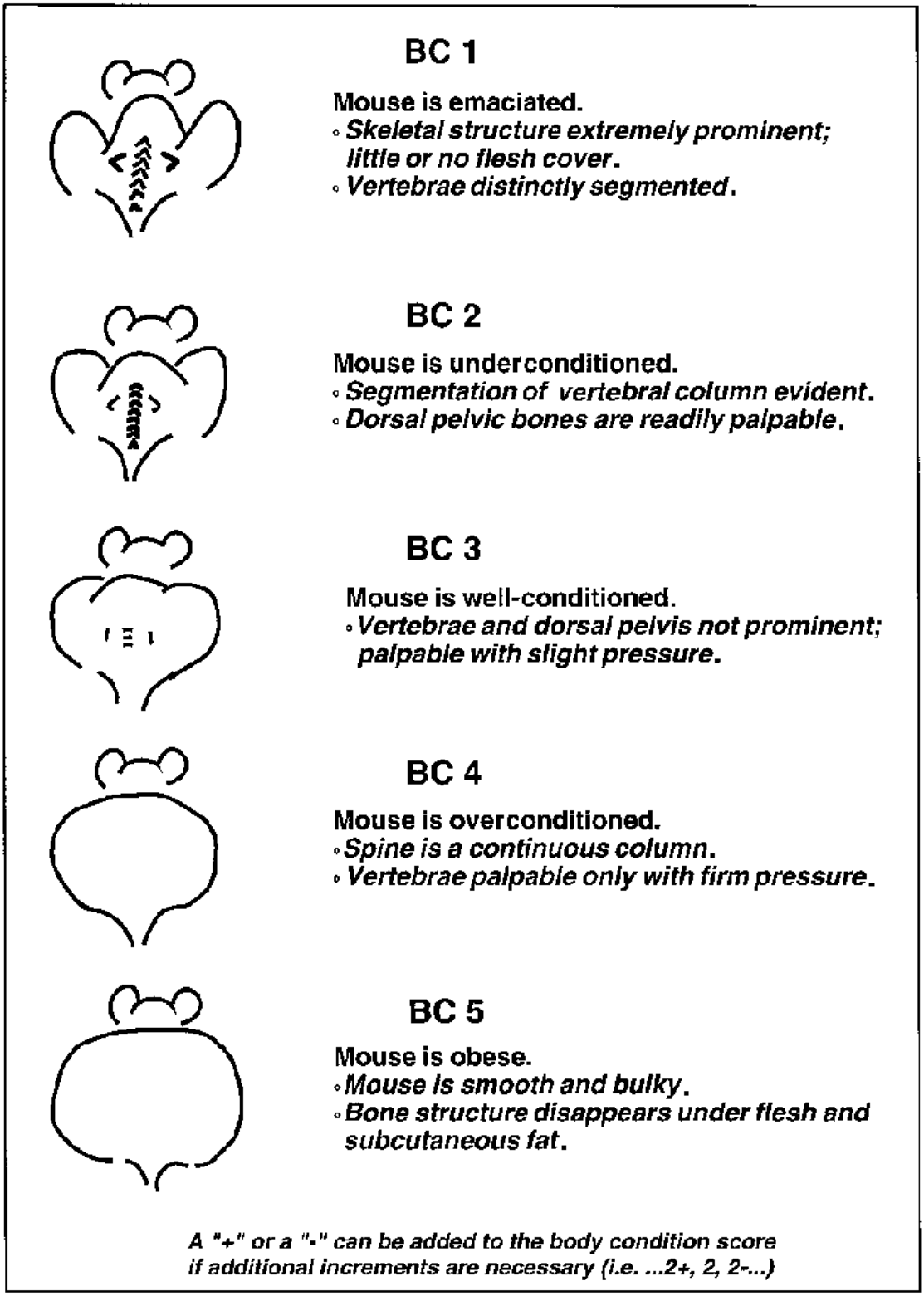
PI: _____ Protocol: _____

Contact Name & Phone Number: _____

Study Info: _____

Date:	Observations:	Initials:

Appendix B - Mouse Body Condition Scoring



(Courtesy of Ullman-Culleré MH et al.)

Appendix C - Rat Body Condition Scoring

BC 1
Rat is emaciated
- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

BC 2
Rat is under conditioned
- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.

BC 3
Rat is well-conditioned
- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.

BC 4
Rat is overconditioned
- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

BC 5
Rat is obese
- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.

(Courtesy of Hickman DL et al.)