Colorado State University
Institutional Animal Care and Use Committee
Guidelines for Euthanasia of Rodents Based on Humane Criteria

Purpose

Although it is crucial to minimize the level of pain and distress experienced by laboratory animals, it is imperative that the scientific objectives of the study are achieved. The criteria that can serve as the basis for ending a test procedure sooner, to terminate or avoid pain and distress while still attaining study objectives, are referred to as humane endpoints. Ideal humane endpoints are those that can be identified before the onset of pain and distress.

General Humane Endpoints

The following are general humane endpoints for rodents that require euthanasia.

- The inability to reach food or water for more than 24 hours
- A 20% decrease in normal body weight
- A body condition score typically less than a 2 on a 5 point scale for adult animals
- An LAR[1] Behavior Score of D, or C for more than 24 hours (see Rodent Behavioral Score document below)
- Development of conditions that result in untreatable pain.

General observations for assessing pain and distress include change in body weight, external physical appearance, clinical signs (inability to reach food and water, lethargy or decreased mental alertness, labored breathing, inability to remain upright), changes in behavior, and responses to external stimuli. Animals should be frequently monitored early in the experiment. Those that are moribund should be euthanized. This requires that laboratory personnel carefully observe the animals for changes in health status, appearance, and behavior, and have knowledge of the treatment and procedures that the animals have undergone.

The LAR Rodent Behavioral Scoring system (see below) was developed to assist in identifying animals for humane endpoints. A Sample Animal Monitoring Scoring Sheet for recording use of the LAR Rodent Behavioral Scoring system is also at the end of this document.

During periods in which morbidity and mortality are expected to increase, animals must be evaluated a minimum of three times daily (every 8 hours). Those animals that are not expected to survive until the next scheduled evaluation should be humanely euthanized.

Humane endpoints will vary depending on the nature of the study and must be discussed with the veterinary staff. Identifying the initial signs that occur prior to a moribund state so that

[1] LAR = Lab Animal Resources

additional pain and suffering may be prevented should be developed. The LAR Behavioral Scoring is an example of the type of assessment for determining endpoints. More elaborate score sheets are an excellent resource for research purposes and data collection. They frequently overlap with the LAR system and the LAR scoring is recommended for IACUC purposes.

For studies with uncertain clinical effects (e.g. a new surgical procedure or testing a new infectious agent strain), pilot studies may be necessary to provide an opportunity to evaluate humane endpoints and assure the scientific objectives are met, before proceeding to large scale studies.

Death as an Endpoint

Death as an endpoint requires scientific justification and documentation that humane endpoints cannot be used. This may include the requirements of regulatory agencies (i.e. EPA, FDA, etc.)

Tumor burden

General guidelines above should be followed for rodents used in tumor studies. Additionally, euthanasia is indicated if:

- The tumor burden is greater than or equal to 10% of the animal's normal body weight
- The tumor exceeds 1.5 cm in size in any direction
- The animal's body condition score diminishes
- The tumor prevents ambulation or ability to reach food and water
- The tumor is severely ulcerated or abscessed, or
- The tumor is causing significant pain and distress.

Some studies of novel therapeutics may necessitate exceptions to some of the above indicators (e.g. tumor size). In such cases investigators must scientifically justify the exception to this normative criteria. Pilot studies may be required to establish individual humane endpoints for therapeutic cancer research.

Non-Experimental (Humane) Endpoints

As mice and rats age, additional clinical abnormalities may necessitate humane euthanasia. This is typically at the discretion of the veterinary staff. Some general guidelines for common conditions are:

- *Ulcerative dermatitis*: The lesions or scar tissue impede the animal's locomotion, or the animal is depressed or lethargic. The size of the lesion may also be considered as well as its response to therapy.
- *Prolapse:* Prolapse of the uterus or prepuce that results in dry necrotic tissue or if reduction is not possible. Rectal prolapse that exceed 5 mm or have dry necrotic tissue.
- *Neurologic Conditions:* Conditions such as hydrocephalus and prolonged seizures (more than 15 minutes). Torticollis which cannot be treated or if it does not respond to treatment.
- *Sick Rodent Posture:* Sick Rodent Posture is characterized by an LAR Behavior Score of D, or a score of C for more than 24 hours.

Euthanasia

If the veterinary staff has examined an animal and determined that it will not survive until the next scheduled examination, every attempt will be made to contact the principle investigator to obtain permission to euthanize the animal. If the veterinary staff is unable to contact the PI, the animal will be euthanized and the PI contacted.

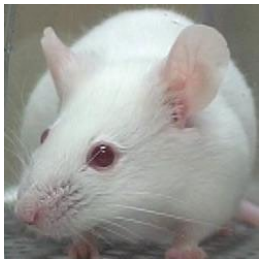
References

- ILAR Journal V41(2) 2000.
- Ullman-Cullere MH, Foltz CJ. Body condition scoring: a rapid and accurate method for assessing health status in mice. Lab Anim. Sci. 1999 Jun;49(3):319-23.
- Hickman, DL, Swan M. 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. 2010. 49(2):155-159.
- Stasiak K, D Maul, E French, PW Hellyer, S VandeWoude. Species specific assessment of pain in laboratory animals. J. Am. Assoc. Lab Anim. Sci. 2003. 42(4):13-20. +
- Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS. Coding of facial expressions of pain in the laboratory mouse. Nat. Methods. 2010 Jun;7(6):447-9.
- LAR Behavioral Scoring available at https://vprnet.research.colostate.edu/lar/

Colorado State University Laboratory Animal Resources

Rodent Behavioral Scoring

A



- Normal, healthy rodent
- Awake when person is in room (except hamsters)
- Well-groomed (clean, smooth coat)
- Explores environment or observes
- Builds/repairs nest
- Normal posture and gate with flat back and no limping, hopping, staggering, or dragging/carrying of limbs
- Readily eats food and drinks water
- Facial expressions: normal with no bulging to cheeks or face, whiskers and ears forward and alert

Note: Normal behavior may be accompanied by conditions which should be reported (e.g. skin trauma, eye lesions).

B

- Rodent with slight or subtle behavioral changes
- May not groom adequately and coat may be a little oily or rough
- May have porphyrin staining on the coat indicating stress (rats)
- Eating and drinking adequately, but less active than normal
- May rest in "hunched" position (but this is transitory)
- Less interest in environment, other animals or people
- May have gait abnormalities or other signs of trauma/illness (e.g. nasal or ocular discharge, rectal prolapse)
- Facial expressions: mild cheek and facial bulge, eyes squinting, ears and whiskers may be held closer to head

Note: This behavior may be the result of experimental manipulation or illness

C

- Rodent definitely not acting normally
- Reluctant to move
- Rests upright with the back hunched (sick rodent posture)
- Back stays hunched when animal moves
- Animal will move if prodded with finger
- Not eating or drinking normally
- Poorly groomed (haircoat is oily, dirty and stands on end)
- Facial expressions: cheek and facial bulge is significant, eyes squinting or closed, ears and whiskers may be held close to head
- Notify veterinary staff immediately

Note: There should be a time limit in the protocol for animals at Score C, otherwise veterinary services will determine if the animals should be euthanized.

D

- Rodent is MORIBUND (near death and will not recover)
- Animal is either hunched in sick rodent posture or lying on its side
- Only sign of life is breathing which is shallow and either slow or rapid
- Eyes sunken or closed
- If prodded, animal will only respond minimally
- May feel cool to touch
- PI should be notified that euthanasia must be performed
- Notify veterinary staff immediately

Photos: Coding of facial expressions of pain in the laboratory mouse. Langford DJ et al. Nat Methods. (2010)

BC 1

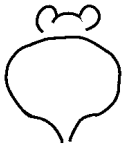
Mouse is emaciated.
- ***Skeletal structure extremely prominent; little or no flesh cover.***
- ***Vertebrae distinctly segmented.***

BC 2

Mouse is underconditioned.
- ***Segmentation of vertebral column evident.***
- ***Dorsal pelvic bones are readily palpable.***

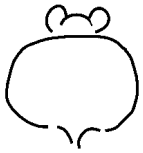
BC 3

Mouse is well-conditioned.
- ***Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.***



BC 4

Mouse is overconditioned.
- ***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.***

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

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.

Sample Animal Monitoring Score Sheet (using LAR Rodent Behavioral Scoring System)

					A or B	C or D	
Name:	Date	Animal ID	Weight	CSU IACUC Daily Behavioral Score for rodents (A-D)	If noted as an A or B continue daily monitoring	If noted as a C, increase monitoring to 3 x/day. If noted as a D, Euthanize immediately	Weight loss