Institutional Animal Care and Use Committee		UNT Health
Title: Humane Endpoints		
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A. BACKGROUND INFORMATION

a. The purpose of this document is to provide guidelines for investigators to use when writing an Animal Use Protocol involving humane endpoints that reduce animal pain and distress, while still meeting research objectives when animals are used for biomedical research and teaching.

b. The Guide for the Care and Use of Laboratory Animals (the Guide) defines the difference between experimental endpoint and humane endpoint.

   i. Experimental Endpoint: "occurs when the scientific aims and objectives have been reached" (the Guide).

   ii. Humane Endpoint: "point at which pain or distress in an experimental animal is prevented, terminated, or relieved"(the Guide).

c. Rodents, rabbits, and swine all have an evolutionary tendency to conceal signs of pain and distress. Consistent, species-specific monitoring by trained personnel is essential to detect these early changes and ensure prompt veterinary evaluation and intervention, following humane endpoints and animal welfare standards.

B. RESPONSIBILITIES

a. It is the responsibility of all investigators using animals in research or teaching at our institution, the animal care staff and the IACUC to abide by and enforce these procedures.

b. Investigators and animal care staff are expected to:

   i. Establish humane end points to prevent animals suffering without intervention or death as an end point while still on study.

   ii. Minimize animal numbers within statistical constraints.

   iii. Monitor experimental animals exhibiting abnormal signs:

      1. A minimum of twice daily at least 6-8 hours apart during the work week.
      2. Once daily on weekends/holidays unless animals are expected to be in a morbid state.

   iv. Euthanize any animals found in a moribund state except when death is the endpoint as approved by the IACUC.

   v. In the event that DLAM staff discover a moribund animal, particularly during holidays or weekends, the emergency contact list will be used to promptly notify the PI and the designated individual authorized to make decisions on the PI's behalf. It is the responsibility of the PI and research team to respond in a timely manner (within 2hrs) before the animal is humanely euthanized by DLAM to prevent further suffering.

C. PROCEDURES

a. Euthanasia as an alternative to death as an endpoint:

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i. Legal, regulatory, and moral guidelines require that animal pain, distress, and suffering be minimized in any experiment. For these reasons, investigators are strongly encouraged to administer euthanasia, if experimental validity will not be compromised, in death-endpoint experiments prior to actual death of the animals. These objectives assume that investigators can differentiate between animals that are morbid (i.e., affected with disease or illness), and those that are moribund (i.e., in the state of dying).

ii. Investigators, DLAM staff and research personnel should be able to identify moribund animal models based on objective signs or symptoms of dying depending on experience with the animal model, professional judgment, and the experimental protocol. The combination of signs of symptoms indicating euthanasia may vary with experimental endpoint.

iii. The IACUC guidelines indicate that animals found moribund should receive euthanasia, but if experimental death itself is the required endpoint, the investigator may receive consideration for approval to conduct such studies by providing appropriate justification in the approved IACUC Protocol. Inconvenience or increased costs alone are not justifiable reasons, but the IACUC will otherwise, generally, accede to the scientific judgment of the investigator. Investigators are expected to make a good faith effort to justify their endpoints, or agree they can judge when to perform euthanasia on animals found moribund. Moreover, all investigators are expected to continue to monitor experimental animals at least daily (including weekends and holidays), to euthanize any animals which they judge should receive euthanasia, to use alternative endpoints to death when possible, and to minimize animal numbers within statistical constraints in general, but especially in death endpoint protocols.

b. The following shall be included in the protocol if death is an endpoint:

i. Written justification including:

a. Discussion of alternative endpoints
b. Literature citation
c. References to pertinent publications where appropriate

ii. Justification of the number of animals to be included

iii. Justification for the withholding of analgesics, if applicable

iv. At least twice daily monitoring once animals exhibit abnormal signs (at least 6-8 hours apart)

v. Maintenance of written records of monitoring

c. Suggested signs and symptoms for judging morbidity (disease/illness):

i. **Rodents:**

a. Rapid breathing rate
b. Breathing rate very slow, shallow, and labored
c. Rapid weight loss
d. Hunched posture
e. Dehydration- skin tent, sunken eyes
f. Hypo- or hyperthermia
g. Unkempt/rough coat, ulcerative dermatitis or infected tumors
h. Ocular/nasal discharge or staining
i. Anorexia (loss of appetite)
j. Diarrhea or constipation

ii. **Rabbits:**
    a. Rapid breathing rate, open mouth breathing or slow, shallow breathing
    b. Lethargic, depressed posture
    c. Teeth grinding (can indicate pain)
    d. Rapid weight loss
    e. Anorexia/ decreased water intake
    f. Dehydration (sunken eyes)
    g. Neurological signs such as head tilt, ataxia, circling and /or seizures
    h. Gastrointestinal signs such as decreased feces, excessive diarrhea, bloating, pain, and distention
    i. Ocular/nasal discharge
    j. Unkempt/rough coat, hair loss, wounds, dermatitis
    k. Urogenital signs such as straining to urinate, hematuria, genital discharge

iii. **Pigs:**
    a. Rapid breathing rate with signs of abdominal effort when breathing, grunting
    b. Lethargic, reluctance to rise, abnormal posture
    c. Rapid weight loss or poor gain
    d. Anorexia/ decreased water intake
    e. Dehydration- skin tenting, dry mucosa
    f. Respiratory signs such as coughing, nasal discharge
    g. Neurological signs such as incoordination, paddling, head tilt, seizures
    h. Gastrointestinal signs such as diarrhea, constipation, bloating, and vocalizing when touched
    i. Skin and coat conditions such as mange, lice, tail/ear biting, wounds, skin lesions
    j. Urogenital signs such as straining, hematuria, genital discharge, swelling

d. Suggested signs and symptoms for judging moribund condition (state of dying). Signs and symptoms of morbidity will be observed plus:
    i. Impaired ambulation (unable to easily reach food or water)
    ii. Evidence of muscle atrophy or other signs of emaciation (body weight is not always appropriate, especially since tumors may artificially increase body weight)
    iii. Any obvious illness including such signs as lethargy (drowsiness, aversion to activity, lack of physical or mental alertness), prolonged anorexia, bleeding, difficulty breathing, central nervous.
    iv. Inability to remain upright

e. **Criteria for euthanasia:**
    i. When an animal meets any of the following criteria, it should be considered for euthanasia:
        a. Rapid weight loss (animals are weighed a minimum of twice per week) of 10-15% or greater body weight
        b. Debilitating diarrhea
        c. Labored breathing
        d. Lethargy
        e. Persistent recumbence
        f. Significantly abnormal neurological signs
        g. Bleeding from any orifice
        h. Self-induced trauma

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i. Impaired mobility
j. Difficulty obtaining food and water
k. Inability to maintain normal body temperature
l. Clinical signs of pain that do not respond to analgesics
m. Tumor greater than 2 cm (measured in any direction)
n. Necrotic or ulcerated tumors
o. Animal refuse to eat or drink for a significant length of time

f. Special Considerations for Experimental Autoimmune Encephalitis (EAE) model:

i. The EAE Model is used to study multiple sclerosis (MS). It is used to study the immunogenetic and histopathological features of the disease. It is induced by immunizing animals with self-antigens from basic myelin protein, triggering an autoimmune inflammatory reaction in the central nervous system tissue. This impact often presents a progressive, ascending paralysis.

ii. Because of the variability in onset and presentation and potential animal welfare implications, close monitoring and provision of supportive care is necessary for EAE animals.

iii. Scoring System:

a. Body condition score (BCS) must be used to evaluate the general physical condition of EAE animals and used to compliment the functional score. The standard rodent BCS assigns a score ranging from 1-5 based upon body fat and muscle mass over caudal dorsum, where a score of "1" indicates emaciation and a score of "5" indicates a grossly obese animal. The ideal body condition is represented by a score of 3. (See Body Condition Scoring (BCS) System for Rodents Attachment)

b. Clinical signs associated with the progression of ascending paralysis in EAE model are graded using a six-stage scale of 0-5, with "0" indicating a clinical normal animal and "5" indicating paralysis of all four limbs (quadriplegia).

A. EAE Score = 1, 2 (Flaccid Tail, Hind Limb Weakness):

1. When clinical signs are expected to begin, laboratory staff must begin monitoring at least once daily, including weekends and holidays. Daily monitoring must include overall activity, EAE score, and hydration status. Animals with increased skin turgor (skin tenting) must receive 1 cc of 0.9% saline subcutaneously once daily.
2. Weight and body conditions score must be recorded at least twice weekly.
3. Provisions for increased food and water access must be made. Water bottles with long sipper tubes. Pelleted food placed on floor of cage.
4. Moist chow or diet gels are recommended and must be placed in cage if animals have a BSC of 2 or have lost >10% of their initial body weight.

B. EAE Score – 3 (Hind Limb Paralysis, Urinary Incontinence):

1. All monitoring described above must continue.
2. Animals must be monitored daily for dermatitis, urine scald, penile prolapse (males), or tail lesions. The appearance of any of these conditions must be reported to DLAM Staff.

3. The bladder must be palpated for bladder atony (inability to urinate). Signs of atony include a large bladder, urine staining of fur, and/or distended abdomen.
4. Once bladder atony is identified, affected animals require twice daily, manual expression to evacuate urine (8-12-hour interval).
5. Place animals on ALPHA-dri bedding.
6. Moist chow or diet gels must be placed in the cage.

C. EAE Score = 4 (Forelimb Weakness with Hind Limb Paralysis):
1. All monitoring described above must continue.
2. Wight and BCS must be recorded daily.
3. Animals that maintain a score of 4 for more than 24 hours must be euthanized, unless described and justified in the IACUC approved protocol. These animals may require additional fluid, nutritional and heat support

D. EAE Score = 5 (Paralyzed Front and Hind Limbs):
1. Animals exhibiting these signs must be euthanized no later than the end of the day.
2. Animals with additional evidence of morbidity (recumbent, poorly responsive or failure to right, abnormal breathing) must be euthanized immediately.

c. Other functional scoring systems may be utilized if clearly defined in the IACUC-approved protocol, and posted in the animal room to be referenced by DLAM staff.

iv. Record Keeping:
a. A log must be maintained by laboratory personnel for all animals beginning at the time of inoculation. The dated log must include all relevant monitoring information including animal weight, BCS, EAE score, hydration and bladder status, treatments administered, and personnel initials. The log or a current copy must be kept in the animal housing room, and accessible to DLAM staff at all times.

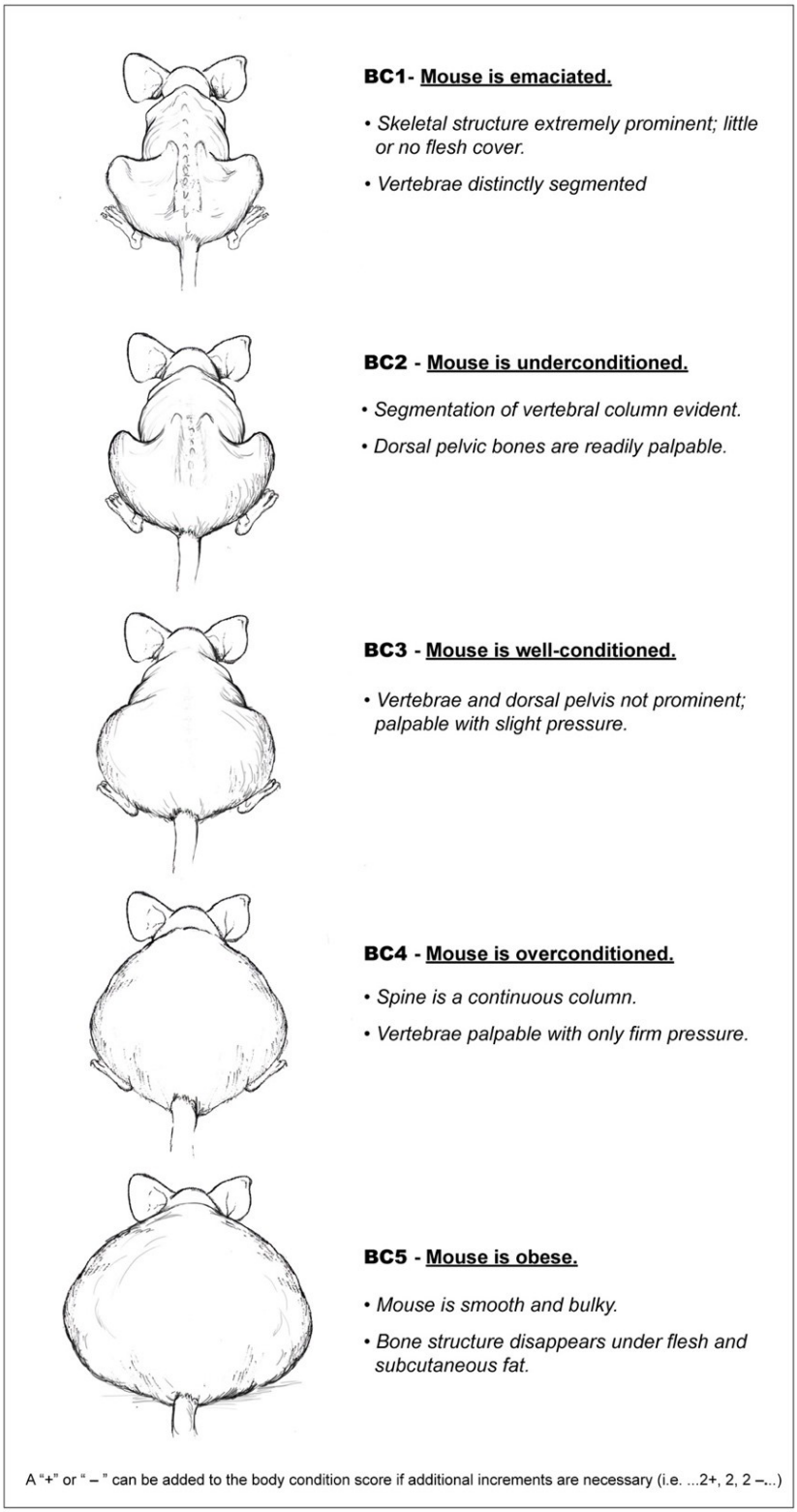
v. Humane Endpoints:
a. Specific endpoints for EAE that are study and presentation specific must be described and justified in the IACUC protocol.
b. Animals that lose >20% of their baseline weight or have a BCS of 1 must be euthanized by the end of the day.
c. Certain EAE models have a well-documented, chronic, relapsing/remitting course characterized by the development of disease with intervals of recovery. In models with this presentation, it may be appropriate to permit a higher degree of weight loss with the expectation that animals will recover both function and body condition in time. This exception to the 20% endpoint, and any additional supportive measures required during the period of high weight loss, must be discussed with the Attending Veterinarian, and must be described and justified in the IACUC approved protocol. This exception does not apply to models with a progressive course that lack a period of remission.
d. Animals exhibiting an EAE Score of 4 for greater than 24 hours must be euthanized. Unless described and justified in the IACUC approved protocol.

e. Animals exhibiting an EAE Score of 5 must be euthanized by the end of the day.
f. Animals with additional evidence of morbidity (recumbent, poorly responsive or failure to right, abnormal breathing) must be euthanized immediately.

D. REFERENCES AND ATTACHMENTS

a. Institute of Laboratory Animal Resources (2011). Guide for the Care and Use of Laboratory Animals. National Academies Press, Washington, D.C.
b. Animal Welfare Act, Public Law 89-544 as amended; codified at 7 U.S.C. 2131-2159.
c. PHS Policy on Humane Care and Use of Laboratory Animals, NIH, Office of the Director, Revised 2015.
d. Office of Animal Care and Use, Animal Research Advisory Committee, National Institutes of Health. Guidelines for Endpoints in Animal Study Proposals.
e. Wolfensohn, Sarah, et al. "Reducing suffering in experimental autoimmune encephalomyelitis (EAE)." *Journal of pharmacological and toxicological methods* 67.3 (2013): 169-176.
f. Palle, Pushpalatha, et al. "The more the merrier? Scoring, statistics and animal welfare in experimental autoimmune encephalomyelitis." *Laboratory animals* 50.6 (2016): 427-432.

g. Body Condition Scoring (BCS) System for Rodents Attachment
h. Example Animal Monitoring Sheet



Body condition scoring is a quick and easy methodology that is useful in assessing animal health. It is particularly helpful when body weight might not reflect body condition (e.g. presence of tumors, ascites, organomegaly, pregnancy). Simply run your finger over the sacral area and score the animal according to the chart.

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Animal ID	Date	Time	Initials

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