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UC Davis Institutional Animal Care and Use Committee (IACUC)

Title: Humane Endpoints for Laboratory Animals

I. <u>Purpose</u>:

The purpose of this document is to provide guidelines for selecting an endpoint that minimizes animal pain and/or distress, while still meeting research objectives, when animals are used for biomedical research, teaching, and testing. These guidelines are provided to assist investigators in fulfilling their ethical responsibilities to minimize animal pain and/or distress. Investigators are expected to consult with Campus Veterinary Services and/or the veterinary care designee noted on their approved protocol to assist with developing project specific humane endpoints and for additional information or questions. In this document "endpoint" refers to one or a combination of physical (e.g., body weight), behavioral (e.g., grooming activity), or other signs of disease or distress that are used typically during a longitudinal experimental procedure in which animal health may deteriorate (e.g., inoculation with an infectious agent), and to decide when an intervention will be terminated or an animal may be euthanized.

II. <u>Background</u>:

Although it is crucial to minimize the level of pain and distress experienced by laboratory animals, it is also important that the scientific objectives of experimental studies are achieved. The criteria that provide the basis for terminating experimental procedures in order to minimize or alleviate any actual or potential pain, distress, or discomfort is made by choosing the earliest endpoint that is compatible with the scientific objectives; these criteria are referred to as humane endpoints. Selection of such endpoints by the investigator involves consultation with Campus Veterinary Services and/or the veterinary care designee, and the endpoints chosen must be approved by the IACUC. For additional reference material, see below.

The principles of humane endpoints apply to all species. Humane endpoints for species or specific projects that may not be covered in this document are determined on a case-by-case basis in consultation with Campus Veterinary Services or the veterinary care designee.

III. Guidelines:

A. <u>General Humane Endpoints:</u>

The following are general humane endpoints that require euthanasia:

- 1. The persistent inability to reach food or water for >12 hours.
- 2. A 20% decrease in baseline/initial body weight for adult animals.
- 3. A 10% decrease in baseline/initial body weight for growing animals.
- 4. A Body Condition Score (attachments 1-6) less than a 2 on a 5-point scale or less than a 3 on a 9-point scale **for adult animals.**
- 5. Development of conditions that result in significant pain that cannot be alleviated by analgesics.

Prior approval by the IACUC is required if an investigator wishes to maintain an animal on study when endpoints meet the above criteria.

General observations for assessing pain and distress include change in body weight, external physical appearance, clinical signs (e.g., inability to reach food and water, lethargy or decreased mental alertness, labored breathing, inability to remain upright), significant changes in behavior, and responses to external stimuli. Sick animals should be identified as early as possible prior to a moribund state (e.g., near death). Laboratory personnel must carefully observe the animals for changes in health status, appearance, and behavior, and have knowledge of the animal treatment(s) and procedures that are part of the approved IACUC protocol. Animals should be weighed and the weight documented on a frequency previously determined from the approved protocol to help ensure animals do not exceed 20% weight loss. Ideally, initial weights should be collected as a basis for comparison prior to any experimental manipulation including compound administration and surgery.

During periods when morbidity and mortality are expected to increase, animals must be evaluated a minimum of two times daily (at least 6 hours apart including an AM and PM observation during the vivarium's lights-on cycle). 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. Protocols may include more specific criteria. Investigators requesting departures from these standard endpoints must discuss the preferred options with Campus Veterinary Services or the veterinary care designee. Identifying the initial signs that occur prior to a moribund state in order to avoid additional pain and suffering is key to developing humane

endpoints. Criteria with a scoring system provides an excellent, objective method for identifying the appropriate time for euthanasia, and can be developed with the assistance of Campus Veterinary Services or the veterinary care designee for individual projects. Objective criteria are best when they can be uniformly applied by a variety of personnel. A Body Condition Score (BCS) is one example of the type of assessment for inclusion in such a scoring system. The attached references are general guidelines for Body Condition Score assessment. Training on BCS recognition is available by Campus Veterinary Services. Should an animal appear ill or unthrifty Campus Veterinary Services or the veterinary care designee must be contacted for further assessment.

Pilot studies will provide an opportunity to evaluate humane endpoints and assure the scientific objectives are met before proceeding to large scale projects.

Some UC Davis facilities, such as non-human primate facilities, have more specific criteria and guidelines for euthanasia that must be approved by the IACUC prior to implementation.

B. <u>Death as an Endpoint</u>:

If an animal must be allowed to die without intervention in order to answer a scientific question, this is considered "death as an endpoint". Death as an endpoint is not typically necessary for research protocols but may be required in some situations, including acute toxicity testing, assessment of virulence of pathogens, and neutralization tests for toxins.

Death as an endpoint requires scientific justification, IACUC approval, and documentation in the protocol that the above humane endpoints cannot be used. Such justifications may include reference to the requirements of regulatory agencies (e.g., EPA, FDA).

C. <u>Tumor Burden</u>:

General guidelines regarding tumor burden should be followed. Euthanasia is indicated if one or more of the following criteria are met: the total tumor burden is ≥10% of the animal's normal body weight; any single tumor measuring >2 cm in size in any direction for mice or >4 cm for rats; a diminished Body Condition Score of <2/5 or <3/9; the tumor is preventing normal ambulation or the ability to reach food and water; the tumor appears ulcerated, necrotic or infected; or the tumor is causing significant pain and distress. Certain tumor therapies under investigation may result in an expected progression of tumor necrosis, ulceration,

and/or healing; this must be addressed in the approved IACUC protocol. If the tumor is infected the clinical veterinary service will provide additional recommendations.

Measuring the mass of a tumor *in vivo* typically entails equating 1 cm 3 of tumor growth to 1 g of body weight. For example, a tumor measuring 2 x 2 x 2 cm (or 8 cm 3) is equivalent to a tumor mass of 8 g. If a mouse weighs 30 g, a tumor burden of 8 g is >10% of the animal's normal body weight and therefore meets a humane endpoint.

D. Euthanasia:

If the veterinary staff has examined an animal and determined that it will not survive until the next scheduled examination, a reasonable effort will be made to contact the Principle Investigator (P.I.) or their designee to obtain permission to treat or euthanize the animal. If the veterinary staff is unable to contact the P.I. or designee, the veterinary staff is authorized to euthanize the animal.

It is important for investigators and their designees to promptly respond to all veterinary communications. If immediate euthanasia is not indicated and an animal is deemed stable by the clinical veterinarian(s), then a plan for further monitoring or intervention (e.g., implementing analgesics, antibiotics, diagnostics) as needed will be instituted. See IACUC Policy-47 "Clinical Veterinarian Authority".

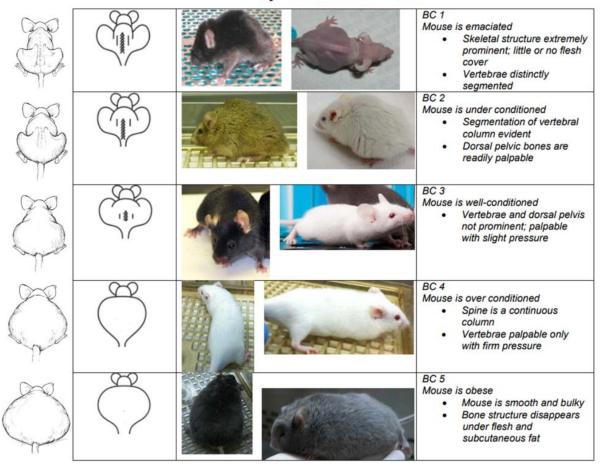
IV. <u>Resources</u>:

- AAALAC International Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation
 - https://www.aaalac.org/pub/?id=E9017C90-F2B6-83CE-4F2D-FD20B73803D1
- 2. Association of Primate Veterinarians' Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research: JAALAS V59(1) 2020
- 3. Humane Endpoints for Animals Used in Biomedical Research and Testing: ILAR Journal V41 (2) 2000.
- 4. NC3R's Humane Endpoints https://www.nc3rs.org.uk/3rs-resources/humane-endpoints
- United Kingdom Coordinating Committee on Cancer Research (UKCCCR). Guidelines for the Welfare of Animals in Experimental Neoplasia (2nd ed). Br J Cancer 77:1-10, 1998.
- 6. Toth, LA. 2018. Identifying and Implementing Endpoints for Geriatric Mice. Comp. Med. 8(6): 439

- 7. Clark, TS, LM Pandolfo, CM Marshall, AK Mitra, and JM Schech, (2018). Body Condition Scoring for Adult Zebrafish. JAALAS 57(6):698-702.
- 8. IACUC Policy-47 "Clinical Veterinarian Authority" https://research.ucdavis.edu/wp-content/uploads/IACUC-47.pdf

Attachment 1: Mouse Body Condition Scores

Body Condition Score Chart



Sources: Burkholder et al, 2012 Health Evaluation of Experimental Laboratory Mice, Cur Protoc Mouse Biol, Vol 2 pp. 145-165; UCSF IACUC; Journal of the American Association for Laboratory Animal Science 1999

Attachment 2: Rat Body Condition Scores



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.

Source: Journal of the American Association for Lab Animal Science 2010

🔀 Nestlé PURINA BODY CONDITION SYSTEM Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss TOO THIN Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass. Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist and abdominal tuck. Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident. Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from side. Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent. Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present. Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distention may be present. Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention. The BODY CONDITION SYSTEM was developed at the Nestlé Purina Pet Care Center and has been validated as documented in the following publications: Mawby D, Bartges JW, Moyers T, et. al. Comparison of body fat estimates by dual-energy x-ray absorptiometry and deuterium oxide dilution in client owned dogs. Compendium 2001; 23 (9A): 70 Lafformse DP Development and Validation of a Body Condition Score System for Dogs. Canine Practice July/August 1997; 22:10-15 Kealy, et. al. Effects of Diet Restriction on Life Span and Age-Related Changes in Dogs. JAVMA 2002; 220-1315-1320 Nestlé PURINA Call 1-800-222-VETS (8387), weekdays, 8:00 a.m. to 4:30 p.m. CT

Source: Nestle Purina

🔀 Nestlé PURINA **BODY CONDITION SYSTEM** Ribs visible on shorthaired cats; no palpable fat; severe abdominal tuck; lumbar vertebrae and wings of ilia easily palpated. Ribs easily visible on shorthaired cats; lumbar vertebrae obvious with minimal muscle mass; pronounced abdominal tuck; no palpable fat. Ribs easily palpable with minimal fat covering; lumbar vertebrae obvious; obvious waist behind ribs; minimal abdominal fat. Ribs palpable with minimal fat covering; noticeable waist behind ribs; slight abdominal tuck; abdominal fat pad absent. Well-proportioned; observe waist behind ribs; ribs palpable with slight fat covering; abdominal fat pad minimal. Ribs palpable with slight excess fat covering; waist and abdominal fat pad distinguishable but not obvious; abdominal tuck absent. Ribs not easily palpated with moderate fat covering; waist poorly discernible; obvious rounding of abdomen; moderate abdominal fat pad. Ribs not palpable with excess fat covering; waist absent; obvious rounding of abdomen with prominent abdominal fat pad; fat deposits present over lumbar area. Ribs not palpable under heavy fat cover; heavy fat deposits over lumbar area, face and limbs; distention of abdomen with no waist; extensive abdominal fat deposits. 🔀 Nestlé PURINA Call 1-800-222-VETS (8387), weekdays, 8:00 a.m. to 4:30 p.m. CT

Source: Nestle Purina

Attachment 5: Non-Human Primate Body Condition Scores

Body Condition Scoring of Nonhuman Primates Using Macaca mulatta as a Model

		Ambulating	Right Lateral Viewed
1	EMACIATED — Very prominent hip bones (easily palpable and likely visible), prominent facial bones, spinous processes and ribs. Minimal to no muscle mass is palpable over ileum or ischium. Anus may be recessed between ischial callosities. Body is very angular, no subcutaneous fat layer to smooth out prominences.	Mi	from Back
1.5	VERY THIN — Hips, spinous processes, and ribs are prominent. Facial bones may be prominent. There is very little muscle present over the hips and back. Anus may be recessed between ischial callosities. Body is angular, no subcutaneous fat to smooth out prominences.	FM	
2	THIN — Very minimal fat reserves, prominent hip bones and spinous processes. Hips, spinous processes and ribs are easily palpable with only a small amount of muscle mass over hips and lumbar region.	FIN	Mary .
2.5	LEAN — Overlying muscle gives hips and spine a more firm feel. Hip bones and spinous processes are readily palpable, but not prominent. Body is less angular because there is a thin layer of subcutaneous fat.	Fil	RECO
3	OPTIMUM — Hip bones, ribs and spinous processes are palpable with gentle pressure but generally not visible. Well developed muscle mass and subcutaneous fat layer gives spine and hips smooth but firm feel. No abdominal, axillary or inguinal fat pads.	MR	12. h. w.
3.5	SLIGHTLY OVERWEIGHT — Hip bones and spinous processes palpable with firm pressure but are not visible. Bony prominences smooth. Rib contours are smooth and only palpable with firm pressure. Small abdominal fat pad may be present.	STA	
4	HEAVY — Bony contours are smooth and less well defined. Hip bones, spinous processes and ribs may be difficult to palpate due to more abundant subcutaneous fat layer. May have fat deposits starting to accumulate in the axillary, inguinal or abdominal areas.	THE	The bar
4.5	OBESE — This animal will often have prominent fat pads in the inguinal, axillary or abdominal region. Abdomen will be pendulous when animal sitting or ambulating. Hip bones and spinous processes difficult to palpate. Bory contours smooth and poorly defined.	FAN	(John)
5	GROSSLY OBESE — Obvious, large fat deposits in the abdominal, inguinal and axillary regions. Abdominal palpation is very difficult due to large amount of mesenteric fat. Pronounced fat deposits may alter posture/ambulation. Hip bones, rib contours and spinous processes only palpable with deep palpation.	MA	

Source: Journal of the American Association of Laboratory Animal Science 2012

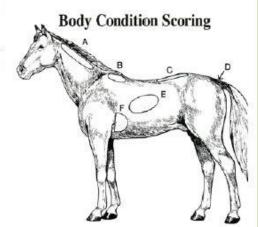
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Henneke Body Condition Scoring System

Body condition, or the measure of fat cover, can be evaluated by visual appraisal and palpation. A scoring system in horses uses six areas of the body to assign scores of 1 (extremely emaciated) to 9 (obese). The six areas are: (A) along the neck, (B) withers, (C) crease down back, (D) tailhead, (E) ribs, and (F) behind the shoulder. Note that a long hair coat or a winter hair coat may "hide" skeletal protrusions or fat deposits. Thus, it is best to evaluate the animal from several different angles and palpate (feel) the appropriate areas of the body to determine the proper body condition score.

A score between 5 and 7 is considered ideal for healthy horses. Horses scoring in the 1 and 2 category should be evaluated further for causes such as medical conditions, dental problems, or the lack of proper nutrition. Individual body condition scores (1-9) are as follows:

- 1— Poor. Animal extremely emaciated; spinous processes, ribs, tailhead, hip joints and lower pelvic bones projecting prominently; bone structure of withers, shoulders and back easily noticeable; no fatty tissue can be felt.
- 2— Very Thin. Animal emaciated; slight fat covering over base of spinous processes; transverse processes of lumbar vertebrae feel rounded; spinous processes, ribs, tailhead, hip joints and lower pelvic bones prominent; withers, shoulders and back structure faintly discernible.



- 3— Thin. Fat buildup about halfway on spinous processes; transverse processes cannot be felt; slight fat cover over ribs; spinous processes and ribs easily discernible; tailhead prominent, but individual vertebrae cannot be identified visually; hip joints appear rounded but easily discernible; lower pelvic bones not distinguishable; withers, shoulders and neck accentuated.
- 4— Moderately Thin. Slight ridge along back; faint outline of ribs discernible; tailhead prominence depends on conformation, fat can be felt around it; hip joints not discernible; withers, shoulders and neck not obviously thin.
- 5— Moderate. Back is flat (no crease or ridge); ribs not visually distinguishable but easily felt; fat around tailhead beginning to feel spongy; withers appear rounded over spinous processes; shoulders and neck blend smoothly into body.
- 6— Moderately Fleshy. May have slight crease down back; fat over ribs spongy; fat around tailhead soft; fat beginning to be deposited along the side of withers, behind shoulders and along sides of neck.
- 7— Fleshy. May have crease down back; individual ribs can e felt, but noticeable filling between ribs with fat; fat around tailhead soft; fat deposited along withers, behind shoulders and along neck.
- 8— Fat. Crease down back; difficult to feel ribs; fat around tailhead very soft; area along withers filled with fat; area behind shoulder filled with fat; noticeable thickening of neck; fat deposited along inner thighs.
- 9— Extremely Fat. Obvious crease down back; patchy fat appearing over ribs; bulging fat around tailhead, along withers, behind shoulders and along neck; fat along inner thighs may rub together; flank filled with fat.

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