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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/or 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 the end of a study in relation to an individual animal based on one or a combination of physical (e.g., body weight), behavioral (e.g., grooming activity), or other signs of disease, diminished health/deterioration, and/or distress to determine when the study needs to end and an animal 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 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. <u>Guidelines</u>:

#### 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** (skeletally mature) **animals**.
- 3. A 10% decrease in baseline/initial body weight for growing (skeletally immature) animals: and/or in consultation with veterinary staff.
- 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 or growing 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. If a stable animal meets humane endpoint criteria, the Attending Veterinarian can make a clinical decision to maintain the animal until an amendment is approved.

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 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 or the veterinary care designee. 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. These pilot studies must be reflected in an approved protocol or amendment.

Some UC Davis facilities, such as nonhuman primate facilities (e.g., Primate Center, CNS), 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  $1 \text{ cm}^3$  of tumor growth to 1 g of body weight. For example, a tumor measuring  $2 \times 2 \times 2 \text{ cm}$  (or  $8 \text{ cm}^3$ ) 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.

The criteria for humane euthanasia of mice on protocols that have scientifically justified necrosis/ulceration of tumors will be any one of the following:

1. Necrosis/ulceration/scabbing greater than 2 cm in any direction.

2. Chronic discharge present (greater than 2 days).

3. Active bleeding or deep tissue exposure.

4. Animal attending to lesion (recurrent scratching/biting of tumor).

5. Any sign of pain or discomfort (hunched posture, painful response upon gentle tumor manipulation).

#### D. <u>Euthanasia</u>:

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 Principal Investigator (PI) or their designee to obtain permission to treat or euthanize the animal. If the veterinary staff is unable to contact the PI 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. The PI is responsible for ensuring their contact information as well as their alternate contact information is up to date on their animal care and use protocol. 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 "<u>Clinical Veterinarian</u> <u>Authority</u>".

## 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.
- NC3R's Humane Endpoints <u>https://www.nc3rs.org.uk/3rs-resources/humane-endpoints</u>
- United Kingdom Coordinating Committee on Cancer Research (UKCCCR). Guidelines for the Welfare of Animals in Experimental Neoplasia (2<sup>nd</sup> 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

	Cirk Cirk	BC 1 Mouse is emaciated • Skeletal structure extremely prominent; little or no flesh cover • Vertebrae distinctly segmented
Ş		BC 2 Mouse is under conditioned • Segmentation of vertebral column evident • Dorsal pelvic bones are readily palpable
P		BC 3 Mouse is well-conditioned • Vertebrae and dorsal pelvis not prominent; palpable with slight pressure
$\mathcal{O}$		BC 4 Mouse is over conditioned • Spine is a continuous column • Vertebrae palpable only with firm pressure
$\bigcirc$		BC 5 Mouse is obese • Mouse is smooth and bulky • Bone structure disappears under flesh and subcutaneous fat

## **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

#### BC 1



- 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 vertebricher over tail base.
    - Thick fat store over tail base, caudal vertebrae not palpable.

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







#### Attachment 3: Dog Body Condition Scores

# Nestlé PURINA BODY CONDITION SYSTEM Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.

Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.

TOO THIN

DEA

**OO HEAVY** 

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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 absorptionetry and deuterium axide dilution in client owned dogs. Compendium 2001; 23 (9A): 70 Laflamme 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

Call 1-800-222-VETS (8387), weekdays, 8:00 a.m. to 4:30 p.m. CT

🔀 Nestlé PURINA

Source: Nestle Purina Attachment 4: Cat Body Condition Scores

# 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.

Call 1-800-222-VETS (8387), weekdays, 8:00 a.m. to 4:30 p.m. CT



Source: Nestle Purina

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DEAI

OO HEAVY

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

		Ambulating	Right Lateral Viewed
1	<b>EMACIATED</b> – 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.	MA	Mon Back
1.	<ul> <li>VERY THIN – Hips, spinous processes, and ribs are prominent.</li> <li>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.</li> </ul>	MAR	NO WWW
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.	AR	Ker Car
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.	FR	<u>Nerror</u>
3	<b>OPTIMUM</b> – 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.	THR	12.1
3.	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.	UHR	
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.	HR	( Julie )
4.5	<b>OBESE</b> – 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. Bony contours smooth and poorly defined.	AAR	( Jahan )
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.	AAA	

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

#### Attachment 6: Horse Body Condition Scores



Source: University of California, Davis. Center for Equine Health Horse Report, July 2012.