FOM IACUC GUIDELINE
HUMANE INTERVENTION AND ENDPOINTS FOR LABORATORY ANIMAL SPECIES

Purpose: The Faculty of Medicine Institutional Animal Care and Use Committee (FOM IACUC) reviews all biomedical research studies involving laboratory animals, including information about humane endpoints for the animals in the studies. This guideline discusses the following topics:

- Developing humane end points
- Humane endpoints for behavioral studies
- Moribund condition as a humane end point
- Monitoring frequency
- Scoring systems
- Euthanasia

Definitions

Euthanasia: the act of inducing humane death in an animal
Experimental endpoint: terminal point of study that occurs when the scientific aims and objectives have been reached (Guide 2011)
Humane end point: point at which pain or distress in an experimental animal is prevented, terminated, or relieved (Guide 2011)
Moribund: severely debilitated clinical state that precedes imminent death (Toth 2000)

Developing Humane Endpoints

Humane endpoints should be selected based on their ability to accurately and reproducibly predict or indicate pain and/or distress, imminent deterioration, or death. It is required that SPECIFIC humane endpoints be clearly defined in all animal protocols, and particularly for all Pain Category C and D procedures. Humane endpoints should be determined in consultation with Attending Veterinarian.

Studies that commonly require special consideration for endpoints may include:

- tumor development **
- infectious disease
- vaccine challenge
- pain and trauma modeling
- monoclonal antibodies production **
- assessment of toxicological effects
- organ or systemic failure
- models of cardiovascular shock
- demyelinating diseases**
- generation of animals with abnormal phenotypes **

**Certain areas of research that are considered to have a high potential for producing pain and/or distress in laboratory animal species are specifically addressed in other FOM IACUC Guidelines.

To develop a humane endpoint, the researchers should describe the clinical progression that a particular animal or group of animals is likely to experience as a result of experimental manipulation or spontaneously occurring disease during the animals' lifetime. Research staff must be adequately trained in recognition of species-specific behaviors and, in particular, species-specific signs of pain, distress, and morbidity (see Table 1).

The selection of appropriate humane endpoints requires a detailed knowledge of the impact of the procedure on the animal to allow for intervention before unpredicted distress or pain develops. "When novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC, and the veterinarian."(Guide 2011)
Please note: the IACUC may request a pilot study specifically related to endpoint determinations.

The duration of biomedical studies involving pain and distress should be kept to a minimum. Before submission of a protocol, the research staff should ensure that the following have been determined and included:

(i) development of both appropriate experimental AND humane endpoints for the study;
(ii) assignment of the appropriately trained person(s) responsible for determining that an experimental and/or a humane endpoint has been reached;
(iii) description of current literature searches for alternatives for any/all potentially painful/distressful procedures.

Humane Endpoints in Behavioral Studies

In all behavior studies and tests, proposed procedures for monitoring, record keeping, and humane interventions must be described in the protocol. A baseline behavioral profile of an animal should be established if changes in behavior are going to be used to monitor the animal for distress. An understanding of the species-typical behavior of the animals used in awake, behaving experiments is critical for adequately assessing the animal for signs of stress/discomfort that may be minimized either through an earlier endpoint determination or by modifying experimental procedures. Subtle changes detected in the animal's demeanor or its willingness to work in a study or sudden changes in performance on behavioral tasks may be the first indicators of a health problem that should be investigated. If such changes are noted, the researcher should promptly notify the veterinary staff so that the animal can be more fully evaluated (NRC 2003).

Moribund Condition as a Humane Endpoint

Procedures or experiments that are expected to produce a moribund state must be categorized as Pain Category D. These types of studies will be reviewed by the full FOM IACUC and must have scientific justification. The continuation of an experimental study to the point where an animal dies without the benefit of intervention or euthanasia ("death as an endpoint" study) is not acceptable without strong scientific justification.

Various clinical signs are indicative of a moribund condition in laboratory animals. If any of these signs are noted, prompt consultation with the veterinary staff or euthanasia must occur. The following signs can quickly lead to a moribund state and should be considered when developing endpoints:

- Any condition interfering with eating or drinking (e.g. difficulty with ambulation)
- Inability to remain upright
- Rapid weight loss or net weight loss of more than 20% of the body weight
- Prolonged inappetance
- Evidence of muscle atrophy/marked loss of body condition
- Diarrhea, if debilitating or constipation
- Markedly discolored urine, polyuria or anuria
- Rough hair coat, hunched posture, lethargy or persistent recumbency
- Central nervous system disturbance - head tilt, seizures, tremors, circling, paresis
- Lack of physical or mental alertness
- Coughing, labored breathing, nasal discharge, or respiratory distress
- Jaundice and/or anemia (paleness)

- Unexplained/uncontrolled bleeding from any site on the body
- Excessive or prolonged hyperthermia or hypothermia
- Conclusive evidence that untreatable organ failure has occurred with signs associated with the failure of the organ system
- Marked dehydration

Monitoring Frequency

A detailed and descriptive plan for scheduled monitoring of research animals both before and after a procedure, including the provision of treatments and supportive care, must be be included in the protocol submission. Investigators should be aware that as the potential for pain/distress in animals rises, there should be an increasing intensity of monitoring and frequency of observations performed.

Scoring Systems (example provided in Table 2)

Professional and clinical judgments are essential for the evaluation of an animal's well being, and are critical to the ultimate decision of euthanasia for humane reasons. As well, objective data-based approaches to predicting imminent death, when developed for specific experimental models, should facilitate the implementation of timely euthanasia before the onset of clinically overt signs of moribundity (Toth 2000).

Scoring systems are one way in which humane endpoints can be defined and implemented. The attached example of a scoring system is based upon routine observations. In this example, a score is assigned to each variable, 0 (normal or mild) to 3 (severe change/variation from normal). The cumulative score gives an indication of the likelihood that the animal is experiencing pain or distress. Humane endpoints can be established based on these criteria. A total score of >5 or a score of 3 in any one variable, regardless of the total score should warrant mandatory evaluation/decision by a veterinarian or humane euthanasia. The example in this document should be modified for specific species and designed to fit each protocol and animal model.

Euthanasia

Timely euthanasia can improve research and scientific validity by eliminating distress and improving animal well-being, alleviating unnecessary animal suffering, while potentially enhancing the integrity and quality of samples to be collected (Stokes 2000).

Animals must be euthanized in accordance with the approved protocol, based upon the current American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, or as recommended by the Attending Veterinarian.

TABLE 1. Indicators of Pain in Several Common Laboratory Animals [a] (NRC 2003)

Species	General Behavior	Appearance	Other
Rodents	Decreased activity; excessive licking and scratching; self-mutilation; may be unusually aggressive; abnormal locomotion (stumbling, falling); writhing; does not make nest; hiding	Piloerection; rough/stained haircoat; abnormal stance or arched back; porphyrin staining (rats)	Rapid, shallow respiration; decreased food/water consumption; tremors
Rabbit	Head pressing; teeth grinding; may become more aggressive; increased vocalizations; excessive licking and scratching; reluctant to locomote	Excessive salivation; hunched posture	Rapid, shallow respiration; decreased food/water consumption
Dog	Excessive licking; increased aggression; increased vocalizations, inclusive of whimpering, howling, and growling; excessive licking and scratching; selfmutilation	Stiff body movements; reluctant to move; trembling; guarding	Decreased food/water consumption; increased respiration rate/panting
Cat	Hiding; increased vocalizations, inclusive of growling and hissing; excessive licking; increased aggression	Stiff body movements; reluctant to move; haircoat appear rough, ungroomed; hunched posture; irritable tail twitching; flattened ears	Decreased food/water consumption
Nonhuman Primate	Increased aggression or depression; selfmutilation; often a dramatic change in routine behavior (e.g., locomotion is decreased); rubbing or picking at painful location	Stiff body movements; reluctant to move; huddled body posture	Decreased food/water

a No single observation is sufficiently reliable to indicate pain; rather several signs, taken in the context of the animal's situation, should be evaluated. The signs of pain may vary with the type of procedure (e.g., orthopedic versus abdominal pain).

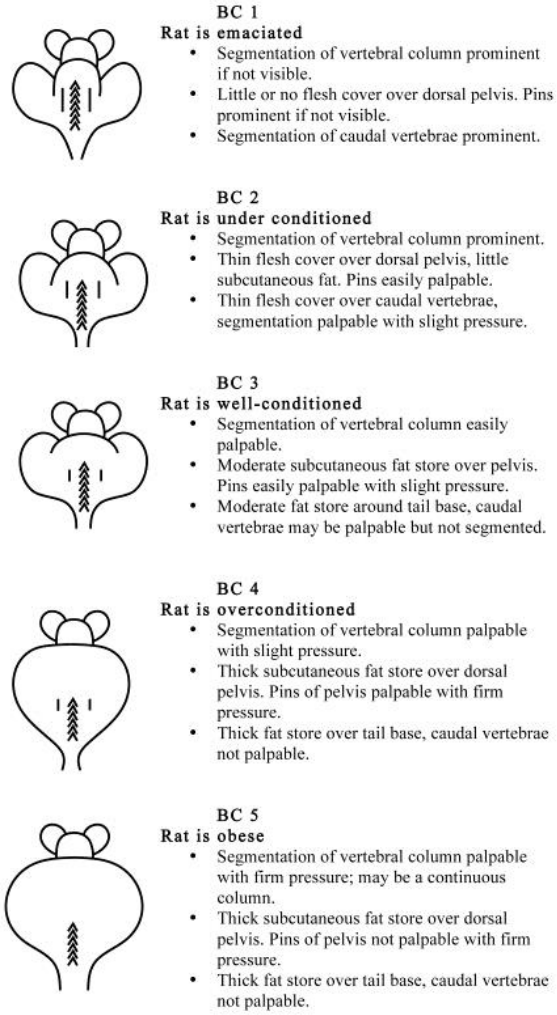
Table 2 : Representative Scoring System ** for Determining Humane Endpoints

Variable		Score
Body Weight Changes		
0	Normal	
1	< 10 percent weight loss	
2	10-15 ercent weight loss	
3	>20 percent weight loss	
Body Condition Score (see diagram for details)		
0	Body condition score >3	
1	BCS >2 and <3	
2	BCS>1 and <2	
3	BCS of 1 or less	
Physical Appearance		
0	Normal	
1	Lack of grooming	
2	Rough coat, nasal/ocular discharge	
3	Very rough coat, abnormal posture, enlarged pupils	
Measurable Clinical Signs		
0	Normal	
1	Small changes of potential signifance	
2	Temperature change of 1-2^{0}C, cardiac and respiratory rates increased up to 30 percent	
3	Temperature change of >2^{0}C, cardiac and respiratory rates increased up to 50 percent, or markedly reduced	
Unprovoked Behaviour		
0	Normal	
1	Minor changes	
2	Abnormal, reduced mobility, decreased alertness, inactive	
3	Unsolicited vocalizations, self mutilation, either very restless or immobile	
Behavioural Responses to External Stimuli		
0	Normal	
1	Minor depression/exaggeration of response	
2	Moderately abnormal responses	
3	Violent reactions, or comatose	
	TOTAL:	

** This representative scoring template should be modified for specific species and designed to fit each protocol and animal model.

Representative Body Condition Scoring (BCS) charts for rodents

NOTE: BCS should be extrapolated to the particular species approved in IACUC protocol



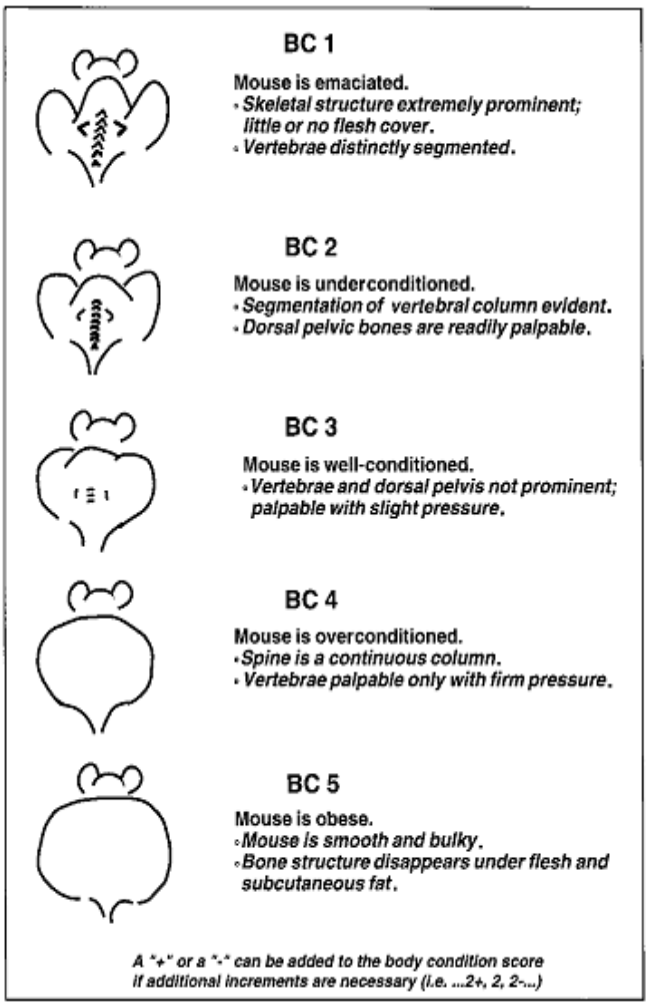
BC 1
Rat is emaciated
- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

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.



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-...)

Figure 1 **Figure 2**

Figure 1: Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease, Debra L. Hickman and Melissa Swan. J Am Assoc Lab Anim Sci. Mar 2010; 49(2): 155–159. Published online Mar 2010.

Figure 2: **Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice**. Mollie H. Ullman-Culleré1 and Charmaine J. Foltz. Laboratory Animal Science, Copyright 1999 by the American Association for Laboratory Animal Science. Vol 49, No 3 June 1999.

GUIDELINES FOR SELECTING APPROPRIATE ENDPOINTS IN SPECIFIC AREAS OF BIOMEDICAL RESEARCH AND TESTING

For some specific areas of biomedical research and testing, more detailed guidelines for selecting an appropriate endpoint are provided in this section. Endpoint guidelines for animals used in monoclonal antibody production, cancer research, toxicology, infectious disease studies, and pain research are included. However, these are not the only areas where specific guidelines can be developed using the expertise of the Attending Veterinarian and the oversight of the IACUC.

A. Monoclonal Antibody Production in Rodents

Guideline: That as long as rodents continue to be used for monoclonal antibody production, the following endpoints be established:

- the increase in body weight due to the accumulation of ascites fluid in the abdomen and/or tumor growth should not produce pain and/or distress to the animal;
- depending on the condition of the mouse, a maximum of two taps of the ascites fluid are allowed, with the second tap being a terminal procedure. Ascites fluid taps should be done under general anesthesia.

B. Cancer Research

Guideline: For all cancer research in animal models, endpoints should be established that minimize the potential for pain and/or distress in the animals. Some recommended endpoints are:

- the tumor mass should not proceed to the point where it significantly interferes with normal bodily functions, or causes pain or distress due to its location (solid tumors);
- weight loss exceeding 20% of the body weight of a similar normal animal (taking into account the tumor mass);
- ulceration/infection of the tumor site;
- invasion of surrounding tissues by a localized tumor;
- persistent self-induced trauma.

C. Toxicological Studies and Toxicity Testing

i. Acute toxicity testing

Guideline: Before a protocol that includes safety/efficacy/toxicity testing with death as an endpoint for regulatory purposes can be accepted by the institution's animal care committee, there must be clear, written documentation obtained by the investigator from the appropriate regulatory agency that the proposed test is a necessary part of the submission for licensing/approval. The investigator must also demonstrate to the animal care committee that an alternative in vitro test will not be acceptable to the regulatory agency, and that this testing has not been previously done elsewhere.

Guidelines on Acceptable Testing Standards: Toxicity tests should be done according to the guidelines of the Organization for Economic Cooperation and Development (OECD), International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Health Canada (HC), or US Food and Drug Administration (FDA), using the minimum number of animals possible, and with all possible consideration for the relief of animal pain and/or distress.

ii. Chronic toxicity studies and studies in aging

Guideline: Before a protocol that requires holding animals to an age close to or beyond the median survival age specific to the species or strain (e.g., chronic toxicity studies, carcinogenicity testing, or aging studies) is approved by the institution's animal care committee, the investigator in collaboration with the veterinary staff must establish the endpoint criteria for euthanasia of the animals, the persons responsible for monitoring the animals' condition, and the authority of the persons who will make the decision to euthanize.

D. Pain Research

Because it is an inherent aspect of studies of pain in humans and animals that some pain must be produced, such experiments raise special ethical concerns. This difficulty was recognized early, as the study of pain became a more distinct discipline. The following guiding principles have been extracted from the literature cited in the accompanying discussion in this section.

Guidelines:

- the animals should be exposed to the minimal pain necessary for the purposes of the experiment;
- the duration of the pain must be as short as possible and the number of animals involved kept to a minimum;
- threshold levels of pain stimuli rather that supra-threshold levels should be used whenever possible;
- if models of acute pain, or acute pain tests are being used, where the pain is not terminated by the animal's reaction, but may extend beyond the time necessary to obtain results, the pain should be terminated as quickly as possible;
- tests other than avoidance tests are strongly discouraged;
- animal models experiencing chronic pain should be provided with adequate analgesia at all times. Exceptions to this should be restricted to those times justified to the institutional animal care committee by the investigator with evidence that the analgesics will interfere with the aims of the investigation.

E. Infectious Disease Studies, Vaccine Trials, etc.

Guideline: For all infectious disease research, including virulence tests in animal models, endpoints should be established that minimize the potential for pain and/or distress in the animals.

Some studies in infectious disease (e.g., tests to establish the virulence of an infectious organism) are still being conducted with mortality as the proposed endpoint (also referred to as the Rodent Protection Test). The use of PD50 (Protective Dose 50) tests in mice may be required when anti-infective studies are done.

References

1. **Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease,** Debra L. Hickman and Melissa Swan. J Am Assoc Lab Anim Sci. Mar 2010; 49(2): 155–159. Published online Mar 2010.
2. **Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice**. Mollie H. Ullman-Culleré1 and Charmaine J. Foltz. Laboratory Animal Science, Copyright 1999 by the American Association for Laboratory Animal Science. Vol 49, No 3 June 1999.
3. University of Pennsylvania IACUC Guideline, Humane Intervention and Endpoints for Laboratory Animal Species. http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/iacucguideline-humaneendpoints-8%2023%2011.pdf.
4. National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals. Washington(DC): National Academies Press (US) 2009.