University Animal Care Committee Standard Operating Procedure		
Document No: 7.2	**Subject:** Humane Interventions (Mice)	
Date Issued: July 7, 2011	**Revision:** 3	**Page No:** 1

Location: Queen's University

Responsibility: Research personnel, Veterinary staff

Purpose: This Standard Operating Procedure (SOP) outlines the steps that must be taken to establish humane interventions and possible treatment for rodent research models. These interventions are to be determined and described on each Animal Use Protocol.

1. Introduction and Definitions: Humane interventions are clear criteria set a priori to define the point at which an experimental animal's pain and/or distress is terminated, minimized or reduced, by taking appropriate actions such as terminating a painful procedure, giving treatment to relieve pain and/or distress, or euthanizing the animal. Any actual or potential pain, distress, or discomfort should be minimized or alleviated by choosing the earliest intervention that is compatible with the scientific objectives of the research.

Weight loss percent and body condition score are two parameters used to define humane intervention points. The degree of weight loss and the body condition score at that point in time will determine whether intervention will be pursued, or whether the mouse will be euthanized. The sooner interventions are implemented the better the chance of the interventions being effective. Knowing when and how to apply humane interventions and/or endpoints will serve to improve the scientific quality of the research.

While the term study endpoint refers to the point at which the mice are removed from the study due to completion of the study, humane interventions are defined as actions or instructions including but not limited to:

- Adequate veterinary treatment, analgesia and/or supportive therapy to the animal(s).
- Termination of painful procedures.
- Removal of the animal(s) from the study.
- Modification of the experimental procedures to minimize the discomfort to the animal(s).
- Increasing the frequency of animal observations.
- Modification to the housing and husbandry practices to improve the comfort of the animal(s.)
- Euthanasia.

The role of the UACC is vital in establishing the structure to ensure that the earliest interventions consistent with producing reliable data are considered, identified, and used. Ensuring appropriate interventions involves the combined efforts of the PI, the veterinary care staff and the UACC to carry out the following instructions:

- Determine the humane interventions that are appropriate for the study.
- Ensure that humane intervention points are clearly defined in the Animal Use Protocol (AUP).
- Ensure all personnel responsible for making animal observations have been adequately trained to observe and recognize the interventions in the approved AUP.

The investigator should consider the following questions, to ensure that appropriate interventions will be in place:

- What are the scientific justifications for using the proposed endpoint?
- What is the expected time course for the animals, from initial treatment to first signs of pain/distress, to the death of the animal, based on previous information with the specific model under study?
- When are the effects to the animal expected to be the most severe?
- If the course of the disease and expected signs of the adverse effects are unknown, could an initial (pilot) study, under close observation by the investigator and/or veterinary staff, answer these questions?
- Has a checklist of observations, on which the interventions will be based, been established?
- Who will monitor the animals (identify all responsible) and keep records?
- Has a clear chain for reporting observations been established?
- What will be the frequency of animal observations: a) during the course of the study; and b) during critical times for the animals?
- Do the investigators, animal care and technical staff have the training and expertise to monitor the animals adequately?
- What provisions have been made to deal with any animals that show unexpectedly severe signs and symptoms?
- For toxicological studies, has existing toxicological data been evaluated?

2. Materials:

- Scale
- Weight Loss chart
- Body Condition Scoring chart
- Logbook
- 0.9% sodium chloride
- Sterile needles (26-27g)
- Sterile 1ml syringes
- Ensure or Boost ***check with PI to confirm use, could alter microbiome***
- Small petri dishes
- Moist chow
- Meloxicam injectable

3. Procedures

- *Establishing Intervention and Final Endpoints:*
    - Review literature and perform web-based searches of established models and alternative methods. Implement the alternatives whenever possible.
    - Consult with veterinary care staff on study refinements designed to minimize pain and distress.

- Schedule regular animal observations at an appropriate frequency to ensure early detection of signs of pain and discomfort.
- Increase the frequency of observations and measurements in response to a decline in the animal's condition and during pre-determined critical periods during the study.
- Keep records of all observations including specific measurements or data (e.g., body weight).

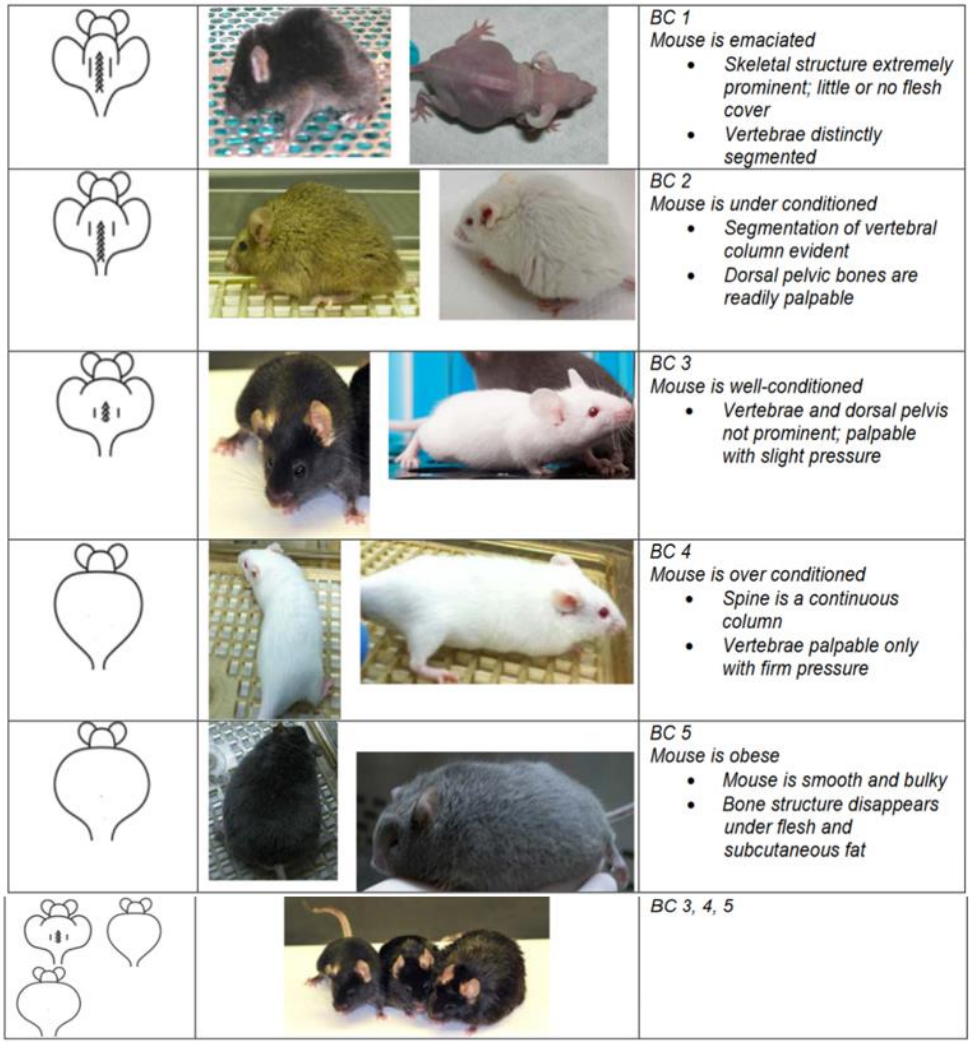
- *Recommended General Intervention and Final Endpoints:*
  - Weight loss exceeding 15% of baseline bodyweight. For young animals, failure to maintain normal weight gain within 15% of age-matched control animals.
    - All mice are to be weighed prior to having any manipulations done (baseline weight). The starting weight should be written on the animal identification card.
    - Mice that will be used in any studies should be weighed weekly until the study is initiated.
    - All weights should be recorded in a logbook that is kept accessible and recorded on the back of the cage card. A copy of the weight-loss chart and body condition chart should be kept in the logbook.
    - If surgical procedures are done, the mice are weighed daily in the post-operative period, for 3-5 days. If weight loss is noted during this period, the mouse should be supplemented with 0.9% NaCl SC 1ml twice daily and Ensure, Boost or moist chow. Depending on the surgical protocol the mice may already be on meloxicam for post-operative analgesia. The meloxicam could be continued as required. Supportive care is continued until weight returns to pre-surgical level. The body condition score is also assessed daily during this period. If weight loss persists such that it surpasses 15% and body condition score reaches '2', the mice will be euthanized.
    - Once mice are started on study, what they experience and how their health progresses will determine the frequency of weighing. Initially the mice should be weighed weekly and then progress to two-three times weekly. Body condition should also be assessed each time they are weighed.
    - *Nearing 10% weight loss, lab personnel should recognize the probable need for intervention.* As soon as weight loss is noted supportive care is initiated. It is not necessary to wait until 15% weight loss occurs. The weight chart should be reviewed to see what percent of weight loss has occurred. The standard weight loss endpoint percent is 15%. In some cases, protocols may have received UACC protocol approval to go to 20% but the progression to either degree of weight loss requires immediate intervention to lessen the risk of losing valuable study mice.
    - Supportive care includes 0.9% NaCl SC twice daily until the weight loss stops and the weight is regained. Additional supportive care may include Ensure or Boost and/or moist chow. Supportive care continues until the weight stabilizes and is regained. The sooner it is initiated, the more likely the mice are to regain the weight.
    - If weight loss continues and/or the body condition score reaches '2', the mice will be euthanized.
    - In long term studies the mice may experience significant weight gain. It will be necessary to review and readjust the weight loss percentage monthly as well as monitor body condition score closely.

Weight Loss Chart

Starting weight (g)	Weight at 10% weight loss (g)	Weight at 15% weight loss (g)	Weight at 20% weight loss (g)
18g	16.2g	15.30g	14.40g
19	17.1	16.15	15.20
20	18	17.00	16.00
21	18.9	17.85	16.80
22	19.8	18.70	17.60
23	20.7	19.55	18.40
24	21.6	20.40	19.20
25	22.5	21.25	20.00
26	23.4	22.10	20.80
27	24.3	22.95	21.60
28	25.2	23.80	22.40
29	26.1	24.65	23.20
30	27	25.50	24.00
31	27.9	26.35	24.80
32	28.2	27.20	25.60
33	29.7	28.05	26.40
34	30.6	28.95	27.20
35	31.5	29.75	28.00
36	32.4	30.60	28.80
37	33.3	31.50	29.60
38	34.2	32.30	30.40
39	35.1	33.15	31.20
40	36	34.00	32.00
41	36.9	34.85	32.80
42	37.8	35.70	33.60
43	38.7	36.55	34.40
44	39.6	37.40	35.20
45	40.5	38.25	38.00
46	41.4	39.10	36.80
47	42.3	39.95	37.60
48	43.2	40.80	38.40
49	44.1	41.65	39.20
50	45	42.50	40.00
51	45.9	43.35	40.80
52	46.8	44.20	47.60
53	47.7	45.05	42.40
54	48.6	45.90	43.20
55	49.5	46.75	44.00

- Body condition score (BCS) less than 2.
- Uncontrolled seizures.
- Impaired mobility which interferes with normal eating, drinking, ambulating or grooming.
- No or weak response to external stimuli.
- Hypothermia.
- Mass that is ulcerated, necrotic or impairing normal function (e.g., eating, drinking) or exceeding acceptable size endpoints:
  - Mice: $2cm^3$ or 10% of the baseline bodyweight
- Pale eyes and/or extremities and/or mucous membranes.
- Uncontrolled hemorrhaging.
- Self-mutilation.
- Specific organ failure assessed by physical examination and, where possible, ancillary tests (hematology, biochemistry, imagery, etc.).
- Respiratory distress: labored breathing, increased or decreased respiratory rate, cyanosis.
- Hunched posture, lethargy, and lack of grooming.
- Incoordination, paralysis.
- Abnormal vocalizations.

Body Condition Score Chart



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

References:

1. Guidelines for Assessing the Health and Condition of Mice. Charmaine J. Foltz and Mollie Ullman- Cullere. *Lab Animal.* April 1999. Vol 28, No. 4, pps. 28-32.
2. http://www.ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf
3. Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice Lab Animal Science, Vol 49 (3) 319-323, 1999

Revised: October 20, 2015 / February 28, 2019 / October 22/2020