Laurentian University
Université Laurentienne

Standard Operating Procedure

Euthanasia Guideline Attachment Revised Dec 2019

Purpose: Animals are normally euthanized at the end of a study for the purpose of sample collection or post-mortem examination. Animals may be euthanized because they are experiencing pain or distress. Euthanasia is defined as a pain-free or stress-free death.

Policy: The ACC has approved certain methods for humanely killing animals that meet the definition of euthanasia. See Euthanasia Guidelines and Endpoints SOP. The appropriateness of the method may vary from species to species. These guidelines are adapted from the report of the American Veterinary Medical Association Panel on Euthanasia, J Am Vet Med Assoc 218:669-696, 2001.

Responsibility: Research Team and Technologists

Investigators or technicians who require advice or assistance on proper techniques of euthanasia may contact the animal facility director.

CRITERIA FOR EUTHANASIA

Euthanasia of animals is expected if animals demonstrate the conditions listed below, whether the animal has been manipulated or not. Additional criteria may be specified on the Animal Use Protocol (AUP). Fulfillment of one criterion can constitute grounds for euthanasia. **Exceptions** are permitted only if approved by the ACC as part of the protocol review process (i.e. the clinical signs listed below are expected as part of the experiment and appropriate measures are taken to minimize pain or discomfort in the animals).

1. **Weight loss**: loss of 20-25% (depending on attitude, weight recorded at time of arrival, and age: growing animals may not lose weight, but may not gain normally) or if not measured, characterized by cachexia and muscle wasting.
2. **Inappetance**: complete anorexia for 24 hours in small rodents, partial anorexia (less than 50% of caloric requirement) for 3 days in small rodents.
3. **Weakness/inability to obtain feed or water**: Inability or extreme reluctance to stand which persists for 24 hours, assuming that the animal has recovered from anesthesia.
4. **Moribund state**: depression coupled with body temperature below 99 F, or non-responsive to stimulation, assuming that the animal has recovered from anesthesia.

5. **Infection**: infection involving any organ system (either overt or indicated by increased body temperature or white blood cell parameters) which fails to respond to antibiotic therapy within an appropriate time and is accompanied by systemic signs of illness.
6. **Signs of severe organ system dysfunction non-responsive to treatment, or with a poor prognosis as determined by the university veterinarian**:

   e.g.

   Respiratory: dyspnea, cyanosis.
   Cardiovascular: blood loss or anemia resulting in hematocrit below 20%; one transfusion may be performed.
   Gastrointestinal: severe vomiting or diarrhea, obstruction, intussusception; peritonitis, evisceration (immediate euthanasia required).
   Urogenital: renal failure characterized by elevated BUN, creatinine or uroperitoneum.
   Nervous: CNS depression, seizures, paralysis of one or more extremities; pain unresponsive to analgesic therapy.
   Musculoskeletal: muscle damage, bone injury, locomotor deficits, etc. resulting in inability to use the limb, unless anticipated as part of the study.
   Integumentary: Non-healing wounds, repeated self-trauma, second or third-degree heating pad burns, ulcerated tumours

7. **Tumours which reach a size that restricts movement and normal activities or that are ulcerated.**

Some recommended endpoints for tumours are:

- the tumour 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 tumour mass);
- ulceration/infection of the tumour site;
- invasion of surrounding tissues by a localized tumour; o persistent self-induced trauma.

Tumour burden should not exceed 5% of the animal's normal body weight for routine tumour passage or 10% for animals involved in therapeutic experiments (10% typically represents a subcutaneous flank tumour diameter of 17mm in a 25g mouse or 35mm in a 250g rat).

ANIMAL ASSESSMENTS

The general physical condition of the animal is an important factor in effectively following the progression of wellness based on Body condition scores (BCS) It is important to note that treatments designed to affect tumour growth (such as chemotherapeutics) which are often part of tumour load studies, can lead to weight loss and poor body condition. Thus, the BCS becomes an important assessment tool in the tumour load experiments.

Rodents must be euthanized if:

- The body condition score is 1/5
- The body condition score is 2/5 and the mouse has decreased activity/responsiveness
- The tumour affects the rodent's gait or normal posture, ability to eat, urinate, or defecate (independent of the size of the tumour)
- veterinarian determines that the animal should be euthanized for humane concerns

General clinical signs should be assessed. Any evidence of lethargy or other change in behavior, change in ambulation, diarrhea, neurological signs (e.g. circling, head tilt) or increased respiratory effort need to be reported to the veterinary staff.

The known biology and effects of any individual tumour model will be described in the AUP, including expected clinical signs, anticipated morbidity/mortality, interventions for the relief of pain and suffering, and objective criteria for the assessment of humane endpoints.

Any animal which is found to be at protocol endpoint or which meets the guidelines for end stage illness must be euthanized.

SURGERY TO CORRECT EXPERIMENTAL COMPLICATIONS

Only one major surgical procedure (involving entry of abdomen or thorax or removal of tumour) may be performed per animal, unless indicated on an approved protocol. Therefore, major surgery intended to correct complications arising after a major experimental procedure is not permitted without prior approval. In such cases, euthanasia must be performed. Procedures such as repair of dehiscences and wound cleaning/debridement for treatment of infection may be performed if considered minor and following notification and approval of the university veterinarian.

Appendix C: Representative Scoring System for Determining Humane Endpoints Variable

Body Weight Changes

1. 0 Normal
2. 1 < 10 percent weight loss
3. 2 10-15 percent weight loss
4. 3 > 20 percent weight loss

Body Condition Score (see diagram for details)

1. 0 Body condition score >3
2. 1 BCS>2and<3
3. 2 BCS >1 and <2
4. 3 BCSof1orless

Physical Appearance

1. 0 Normal
2. 1 Lack of grooming
3. 2 Rough coat, nasal/ocular discharge
4. 3 Very rough coat, abnormal posture, enlarged pupils

Measurable Clinical Signs

1. 0 Normal
2. 1 Small changes of potential significance
3. Body temperature up 1 – 2 degrees and cardiac/ respiratory rate increase by 20 %
4. Body temperature up 1 – 2 degrees and cardiac/respiratory rate increased by 50 % or markedly decreased.

Unprovoked Behaviour

1. 0 Normal
2. 1 Minor changes
3. 2 Abnormal, reduced mobility, decreased alertness, inactive
4. 3 Unsolicited vocalizations, self-mutilation, either very restless or immobile

Behavioural Responses to External Stimuli

1. 0 Normal
2. 1 Minor depression/exaggeration of response
3. 2 Moderately abnormal responses
4. 3 Violent reactions, or comatose

Score *Note: This scoring template (refer to body condition scoring template SOP) should be modified for specific species and designed to each protocol and animal model . In this example, a score is assigned to each variable, 0(normal or mild) to 3 (severe). 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.*