Euthanasia of Research Animals Guidelines

I. Introduction:

Euthanasia is the act of inducing humane death in an animal by a method that induces rapid loss of consciousness and death with a minimum of pain, discomfort or distress.

Animal welfare regulations require that the Institutional Animal Care and Use Committee (IACUC) approve the euthanasia method for research animals. The proposed method of euthanasia and the criteria that will be used to assess pain and distress in animals that need to be euthanized ahead of schedule must be described in detail in the IACUC Animal Care and Use Protocol. Additionally, a plan for responding to calls from the Attending Veterinarian or Department of Animal Research Services (DARS) personnel about sick or injured animals must be described.

It is the responsibility of the Principal Investigator to ensure that each member of the study team is properly trained and familiar with an established course of action in the event that an animal requires euthanasia to alleviate pain, distress or discomfort. At least one member of the study team must be available locally at all times to euthanize any animal exhibiting symptoms consistent with pain, distress and/or discomfort. Principal Investigators are primarily responsible for the euthanasia of all animals purchased or bred under their approved protocols. DARS staff will only euthanize animals after contacting laboratory staff, unless immediate euthanasia is required to relieve acute animal suffering.

II. Ensuring Euthanasia of Laboratory Animals:

Most experimental animal use protocols involve the euthanasia of study animals at some predetermined end point. The table below summarizes the American Veterinary Medical Association's (AVMA) recommendations on humane euthanasia methods. Successful application of the methods outlined above requires that personnel be adequately trained in performing the various techniques **and** that personnel be competent in confirming that death has occurred.

A profoundly anesthetized or severely ill animal can easily appear dead upon cursory examination; one cannot rely solely on imprecise measures such as lack of movement and lack of visible breathing to declare an animal dead. Personnel who are trained to recognize cessation of vital signs in the species being euthanized must confirm the death of an animal. In addition, multiple assessment parameters should be employed. Rodents, especially neonates, are particularly resistant to euthanasia by overdose of inhaled agents such as CO2; for this reason, the IACUC requires that a secondary physical method of euthanasia (e.g. cervical dislocation or decapitation) be performed after the animal is profoundly anesthetized, prior to carcass disposal.

- **NOTE: Unintended recovery of animals after apparent death from CO2 or other inhalant euthanasia constitutes serious noncompliance with the PHS Policy and unacceptable deviation from the provisions of the Guide for the Care and Use of**

Laboratory Animals.

III. Euthanasia Criteria:

Most experimental animal use protocols involve the euthanasia of study animals at a predetermined end point when the animals are clinically healthy. However, in the event animals become ill or debilitated, either as a result of spontaneous disease or as a result of research, the criteria below must be utilized in the decision to provide euthanasia. Fulfillment of one criterion can constitute grounds for euthanasia. Exceptions are permitted only if approved by the IACUC 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).

Animals experiencing one or more of the criteria listed below must be euthanized. If DARS personnel identify such animals, an attempt will be made to contact the Principal Investigator or an alternate responsible person. If a responsible person cannot be located, the Attending Veterinarian will authorize euthanasia of severely debilitated or moribund animals.

1. **Weight loss:** loss of >20 percent of body weight (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, muscle wasting, and/or body condition scoring.
2. **Change in appetite:** complete anorexia for 24 hours in small rodents, up to 5 days in large animals; partial anorexia (less than 50% of caloric requirement) for 3 days in rodents, 7 days in large animals.
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:** anorexia, lethargy, reluctance to move, hypothermia with little likelihood to recover (assuming that the animal has fully recovered from anesthesia).
5. **Infection:** infection involving any organ system (either overt or indicated by increased body temperature or WBC parameters) that fails to respond to antibiotic therapy within an appropriate time and is accompanied by systemic signs of illness.
6. **Organ dysfunction/failure:** signs of severe organ system dysfunction nonresponsive to treatment, or with a poor prognosis as determined by a UCM veterinarian:
    - **Respiratory:** dyspnea and cyanosis unresponsive to appropriate medical therapy.
    - **Cardiovascular:** acute blood loss resulting in hematocrit below 20% or severe chronic anemia (Hct < 15%).
    - **Gastrointestinal:** severe vomiting or diarrhea (duration greater than 24 hours, unresponsive to medical therapy), obstruction, intussusception.

- **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 or fracture 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.

7. **Tumor growth:** Solid tumors that exceed 10 percent of normal body weight in rodents (i.e. 1 cm3 = 1 gm); appear necrotic due to overgrowing its blood supply, or tumor growth that impedes an animal's ability to ingest food, water or ability to move about its cage and remain clean and dry.
8. **Uncontrollable pain/distress:** animals showing signs of pain and/or distress that is not responsive to analgesics/anesthetics, or as determined by a UCM veterinarian.

IV. Acceptable Methods (mice, rats, guinea pigs, hamsters):

CO_2 inhalation is the most common, acceptable method of euthanasia for mice, rats, guinea pigs and hamsters. A few important aspects of this procedure are:

1. Neonatal rodents (up to 10 days of age) are resistant to the effects of CO_2. In these situations, decapitation or freezing is an acceptable method of euthanasia.
2. Species should not be mixed. The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments.
3. Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide as it allows the inflow of gas to the induction chamber to be controlled. Dry ice is not acceptable. "Either USP Grade A (medical) or Grade B (industrial) carbon dioxide may be considered acceptable as they each provide a minimum purity for carbon dioxide of 99.0%."[2]
4. Without pre-charging the chamber, place the animal(s) in the chamber and introduce 100% carbon dioxide. A fill rate of about 10% to 30% of the chamber volume per minute with carbon dioxide, added to the existing air in the chamber should be appropriate to achieve a balanced gas mixture to fulfill the objective of rapid unconsciousness with minimal distress to the animals.[3] (Example for a 10-liter volume chamber, use a flow rate of 1 to 3 liter(s) per minute.) Sudden exposure of conscious animals to carbon dioxide concentrations of 70% or greater has been shown to be distressful.[1]
5. Expected time to unconsciousness is usually within 2 to 3 minutes.[4] Observe each rodent for lack of respiration and faded eye color. Maintain CO_2 flow for a minimum of 1 minute after respiration ceases. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO_2. If

unconsciousness has not yet occurred within 2 to 3 minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO_2 supply, and/or leaks. Appropriate CO_2 concentrations and exposure times will prevent unintended recovery.

6. An accepted and common practice is to group animals for euthanasia. The process of grouping animals immediately prior to euthanasia should provide each individual animal with the ability to make normal postural adjustments. Alternatively, animals should be euthanized in their home cage whenever possible. When euthanizing successive groups of animals using the same cage/container, the euthanizing container should be cleaned between uses to remove the potential distress secondary to remaining pheromones, etc.[1] Alternatively, a new/unused container should be used with each group

V. Assessment Parameters for Confirmation of Death:

1. **Heart beat:** must be assessed for a minute or more. The best assessment is through direct palpation of either the pulse in the carotid or femoral artery of a large animal or direct cardiac palpation. If there is any question, the thorax should be opened, the heart exposed, viewed directly and its mechanical activity observed and palpated. Arterial pulse of smaller species such as mice and rats is difficult to palpate, so direct inspection of cardiac mechanical activity is necessary. Lack of electrical activity of the heart as determined by ECG (provided
that the leads are correctly connected) may also be utilized to document euthanasia.
2. **Pupillary response to light:** Shine a bright light into the eyes of the animal. A constriction (narrowing) of the pupil indicates a neurological response. Upon death, the pupils will become dilated and fail to constrict in response to light. Investigators should be aware that some drugs and experimental agents (e.g., anticholinergics such as atropine) can prevent pupillary reactivity or otherwise affect this neurological response.
3. **Respiratory pattern:** Profoundly anesthetized animals may exhibit shallow and irregular breathing patterns that may be confused with lack of spontaneous breathing. Thus, lack of spontaneous breathing should not be used as the sole criterion for confirming euthanasia.

VI. Additional Recommended Practices to Ensure Death in Rodents:

The criteria listed above (assessment of heart beat, papillary light response and respiratory pattern) may be difficult to apply to rodents due to their small size; consequently, there is a risk of animals recovering after they are presumed dead, particularly following the use of inhalant agents. The following additional steps must be taken to ensure that animals are properly euthanized.

- Animals must be rechecked for lack of vital signs 5-10 minutes after the administration of the euthanasia agent (e.g., C02, anesthetic overdose).
- A physical method of euthanasia after an animal is rendered unconscious and insensitive to pain. Examples include:
    - Exsanguination – i.e., great vessels severed, cardiac perfusion, removal of vital organs.

- Incision of the chest cavity to produce a pneumothorax (collapsed lung) and cessation of respiration.
- Decapitation or cervical dislocation.

VII. Who to Contact:

For more detailed information and training in acceptable euthanasia methods, please contact DARS at 209-228-4189.

VIII. References:

1. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf
2. OLAW seminar on the Use of Non-Pharmaceutical-Grade Chemicals and Other Substances in Research with Animals, March 1, 2012. http://grants.nih.gov/grants/olaw/120301_seminar_transcript.pdf
3. Danneman PJ, Stein S, Walshaw SO. Humane and practical implications of using carbon dioxide mixed with oxygen for anesthesia or euthanasia of rats. Lab Anim Sci 1997, 47:376-385.
4. Neil L, Weary DM. Behavioral responses of the rats to gradual-fill carbon dioxide euthanasia and reduced oxygen concentrations. Applied Animal Behavior Science 100 (2006) 295-308.

IX. Useful Resources:

- Conlee KM, et al. Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. Lab Animals 39:137-161, 2005.
- Klaunberg BA, O'Malley J, Clark T, Davis JA. Euthanasia of Mouse Fetuses and Neonates. Contemporary Top Lab Anim Sc 2004, 43:(5) 29-34.
- Report of the ACLAM Task Force on Rodent Euthanasia, August 2005.
- Pritchett-Corning KR. Euthanasia of neonatal rats with carbon dioxide. JALAS 2009, 48 (1), 23-27.
- Wong D, Makowska IJ, Weary DM. Rat aversion to isoflurane versus carbon dioxide. Biology letters, 2013, 9 (1).
- McIntyre AR, Drummond RA, Riedel ER, Lipman NS. Automated mouse euthanasia in an individually ventilated caging system: System development and assessment. JALAS 2007, 46 (2), 65-73.