Part I - Euthanasia as an Alternative to Death as an Endpoint in Rodents

1. Background Information
   a. Legal, regulatory, and moral guidelines require that animal pain, distress, and suffering be minimized in any experiment. For these reasons, investigators are strongly encouraged to administer euthanasia in death-end-point experiments prior to actual death of the animals - if experimental validity will not be compromised. These objectives assume that investigators can differentiate between animals that are morbid (i.e., affected with disease or illness), and those that are moribund (i.e., in the state of dying).
   b. The IACUC believes that an investigator can judge and should perform euthanasia on moribund rodents based on objective signs or symptoms of dying depending on experience with the animal model, professional judgment, and the experimental protocol. The combination of signs of symptoms indicating euthanasia may vary with experimental end point.
   c. The IACUC guidelines indicate that animals found moribund should receive euthanasia, but if experimental death itself is the required end point, the investigator may receive consideration for approval to conduct such studies by providing appropriate written justification in a memorandum at the time the Animal Protocol Form is submitted to the Committee. Inconvenience or increased costs alone are not justifiable reasons, but the IACUC will otherwise; generally, accede to the scientific judgment of the investigator. Investigators are expected to make a good faith effort to justify their end points, or agree they can judge when to perform euthanasia on animals found moribund in a particular protocol. Moreover, all investigators are expected to continue to monitor experimental animals at least daily (including weekends and holidays), to euthanize any animals which they judge should receive euthanasia, to use alternative end points to death when possible, and to minimize animal numbers within statistical constraints in general, but especially in death-end-point protocols.

2. Responsibilities:
   a. All investigators are expected to:
      i. Use alternative end points when possible.
      ii. Minimize animal numbers within statistical constraints.
      iii. Have experimental animals monitored at least twice daily, i.e., early morning and late afternoon, during the work week. On weekends and holidays, animals will be monitored on a once daily basis unless animals are expected to be in a morbid state.
      iv. Euthanize any animals found in a moribund state except when death is the end point
as approved by the UTA IACUC.

b. If death itself is the required end point of the study, the investigator may receive approval to conduct such studies by providing appropriate justification in the written protocol. Inconvenience or increased costs will not be used as reasons for justification. Investigators will be expected to make a good faith effort to justify the end points.

3. Suggested Signs and Symptoms for Judging Morbidity (disease/illness) in Rodents
   i. rapid breathing rate
   ii. breathing rate very slow, shallow, and labored
   iii. rapid weight loss
   iv. hunched posture
   v. hypo- or hyperthermia
   vi. ulcerative dermatitis or infected tumors
   vii. anorexia (loss of appetite)
   viii. diarrhea or constipation

4. Suggested Signs and Symptoms for Judging the Moribund Condition (state of dying) in Rodents. Signs and symptoms of morbidity will be observed plus:
   a. impaired ambulation (unable to easily reach food or water)
   b. evidence of muscle atrophy or other signs of emaciation (body weight is not always appropriate, especially since tumors may artificially increase body weight)
   c. any obvious illness including such signs as lethargy (drowsiness, aversion to activity, lack of physical or mental alertness), prolonged anorexia, bleeding, difficulty breathing, central nervous.
   d. Inability to remain upright
   e. Approved methods of euthanasia:
      i. Carbon Dioxide: Carbon dioxide is acceptable for euthanasia in appropriate species. Compressed CO2 gas cylinders are the only recommended source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (e.g., antacids) is unacceptable.
      ii. No inhalant Pharmaceutical agents: The use of injectable euthanasia agents (Pentobarbitol sodium, MS 222, Potassium chloride) is the most rapid and reliable method of performing euthanasia. It is the most desirable method when it can be performed without causing fear or distress in the animal. It is of utmost importance that personnel performing this technique are trained and knowledgeable in the proper use of these agents and their use in the appropriate species.
Part II – Euthanasia by Cervical Dislocation or Decapitation (Complies with the 2013 Report of the AVMA Panel on Euthanasia recommendations on euthanasia by cervical dislocation or decapitation.)

1. Cervical Dislocation
   a. This method of euthanasia shall only be used in poultry, small birds, mice, rats weighing <200g, and Rabbits weighing < 1kg.
   b. Cervical dislocation may be used unconditionally in the above species if the animal is anesthetized first. Without prior anesthetization, this method may be only used when scientifically justified by the user and approved by the IACUC. Prior use by the investigator shall not be deemed as scientific justification.
   c. If the IACUC approves this method for use without prior anesthesia, the UTA Attending Veterinarian shall observe the personnel performing the cervical dislocation to ensure that they have properly trained. The Attending Veterinarian shall then submit an approval memo to be included in the protocol file.

2. Decapitation
   a. This method of euthanasia shall only be used in laboratory rodents, rabbits weighing <1kg, birds, fish, amphibians, and reptiles.
   b. Decapitation may be used unconditionally in the above species if the animal if anesthetized.
   c. The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine. (See separate Guillotine SOP.) Without prior anesthetization, this method may only be used when scientifically justified by the user and approved by the IACUC. Prior use by the investigator shall not be deemed as scientific justification.
   d. If the IACUC approves this method for use without prior anesthesia, the UTA Attending Veterinarian shall observe the personnel performing the decapitation to ensure that they have been properly trained. The Attending Veterinarian shall then submit an approval memo to be included in the protocol file.

3. Justification
   a. Acceptable scientific justification may be accomplished by one of the following methods:
      i. A small pilot study consisting of 6 – 10 animals per group may be incorporated into the protocol to test for significant differences between physical methods (i.e. cervical dislocation or decapitation) or acceptable methods (i.e., gas inhalation [carbon dioxide or isoflurane] or barbiturate overdose). The results of the pilot study would then be reviewed by the IACUC before granting final approval to use physical methods of euthanasia.
      ii. Results of a literature review must be submitted with the protocol.
iii. The review should demonstrate that the AVMA approved methods would not work in the specific study being reviewed.

iv. The IACUC may consider an ongoing study as justified if the investigator has provided strong justification that terminating the use of cervical dislocation or decapitation without anesthesia would severely affect the study.

b. Unacceptable justification for continuing to use cervical dislocation or decapitation would include:

i. The study is ongoing and the procedures cannot change midstream without compromising the results; this method of euthanasia has been performed for years. Prior data collection would be made useless. The IACUC would respond to any of these by asking the investigator to perform a pilot study as outlined above.

ii. Colleagues at other institutions are using these methods and they are “industry standard.” Since the AVMA’s recommendations are fairly recent, different institutions are at varying stages of implementing them.

iii. Current grant requests do not cover a pilot study and no funds are available to perform it. The IACUC is sensitive to this issue. However, the IACUC is charged with making sure the University is in compliance with all applicable guidelines and regulations. One suggestion would be to share the cost of the pilot study with several colleagues within or outside the University. The results of the study should be attached to any similar protocol submitted as justification. Another suggestion would be to contact the Office of Grants & Contract Services for grants that may be available for this purpose. Since many institutions are affected, publications in a peer reviewed journal would be highly recommended.

Part III – Criteria for Euthanasia of Animals

1. Guidelines: When an animal meets any of the following criteria, it should be considered for euthanasia:

a. Prostration – Animal is consistently unwilling/unable to stand.

b. Paralysis – Unwilling/unable to use limbs. Positive controls on neurotoxicology studies should be handled on an individual case basis.

c. CNS disorders such as head tilt, incoordination, ataxia, tremors, spasticity, seizures, circling, or paresis. Positive controls on neurotoxicology studied should be handled on an individual case basis.

d. Severe weight loss/emaciation – Animal has not consumed an appreciable amount of food for a time sufficient to produce substantial weight loss (acute loss of 20-25% body weight less than 1 week or continuous decline in body weight), and/or cannot be encouraged to eat by dietary changes (when permitted).

e. Labored breathing – Animal appears to have difficulty breathing.

f. Persistent coughing, wheezing and respiratory distress which cannot be resolved by therapy.

g. Unhealthy appearance such as rough coat, hunched posture, and distended abdomen, especially if prolonged (more than three days), which cannot be resolved by therapy.

h. Diarrhea, especially if prolonged (more than three days), leading to emaciations and/or
debilitation, which cannot be resolved by therapy.

i. Prolonged or intense diuresis leading to emaciation.

j. Prolonged bleeding from natural orifices.

k. Microbial infections interfering with a study which cannot be resolved by therapy.

l. Gross abdominal distension.

m. Maimed/broken limbs – Any extensive self-mutilation or obviously broken limb, which is unlikely to readily heal and/or affects the animal’s ability to feed or drink normally.

n. Prolapsed tissues – Animal has obviously prolapsed, necrotic tissue (genital, rectal, etc.)

o. Persistent, self-induced trauma.

p. Clinical signs of suspected infectious disease requiring necropsy for diagnosis (consultation with staff veterinarian required.)

q. Large ulcerated mass – Most animals are euthanized if masses are apparent. For chronic toxicology studies only: Since masses open/drain, regress in size, and/or because certain animals can accommodate them in a relatively normal manner, it is necessary to rely on experience and good judgment when deciding whether or not to euthanize an animal as a result of the presences of one or more masses. In general, if the mass severely restricts the animal’s ability to eat, drink, eliminate waste, breathe, or move, if the mass becomes widely necrotic or ruptures and body fluid loss is excessive, or if there is a large mass around the head, the animal should be euthanized.

r. Comatose/pale/cold to the touch.

s. Other- Any obvious, unreleenting condition which appears to produce pain which cannot be alleviated due to protocol requirements. Since many study protocols and/or regulatory agency guidelines do not specify when/if analgesic/anesthetic agents can be used, it must be the decision of the staff veterinarian, in consultation with the PI, as to whether or not it is appropriate to attempt to relieve apparent pain through the use of these agents. Their use can often confound data interpretation since many of these agents may produce effects in blood parameters, food/water consumption, appearance, mobility, neurologic measurements, etc.

References:
Chuck Montgomery, JAVMA, Vol 191, No.10, November 15, 1987