UNIVERSITY OF TEXAS AT ARLINGTON

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

EUTHANASIA AND HUMANE ENDPOINTS SOP

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 University of Arlington (UTA) Institutional Animal Care and Use Committee (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 endpoint.
   C. The IACUC guidelines indicate that animals found moribund should receive euthanasia. Endpoints other than death must always be considered and should be used whenever the research objective can be attained with non-lethal endpoints. Use of death as an endpoint is discouraged and must be justified in writing in proposals and its use must be approved by the IACUC prior to beginning a study.
   D. Investigators are expected to make a good faith effort to justify their endpoints 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 animals according to a detailed plan described in the IACUC protocol, to euthanize any animals which they judge should receive euthanasia, to use alternative endpoints to death when possible, and to minimize animal numbers within statistical constraints in general, but especially in death-endpoint protocols.

2. Responsibilities – All investigators are expected to:
   A. Use alternative endpoints when possible.
   B. Minimize animal numbers within statistical constraints.
   C. Describe detailed post-procedure monitoring plan for animals in IACUC protocol.
   D. Euthanize any animals found in a moribund state except when death is the endpoint as approved by the UTA IACUC.
   E. Describe detailed criteria for humane endpoints in IACUC protocol. (See Part II)

F. Monitor for 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
   ix. ataxia

G. Monitor for Signs and Symptoms for Judging Moribund Condition (state of dying) in Rodents. Signs and symptoms of morbidity will be observed plus:
   i. evidence of muscle atrophy or other signs of emaciation (body weight is not always appropriate, especially since tumors may artificially increase body weight)
   ii. any obvious illness including such signs as lethargy (drowsiness, aversion to activity, lack of physical or mental alertness), prolonged anorexia, bleeding, and difficulty breathing.
   iii. inability to remain upright and impaired ambulation (unable to easily reach food or water)

H. Investigators must use methods of euthanasia described in the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition or provide justification to the IACUC and obtain approval to use a method that is not in the AVMA Guidelines.

3. Alternatives to LD50

A. Regulatory agencies, including the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA), highly discourage the use of conventional LD50 testing and recommend alternatives be used that greatly reduce the number of animals and when possible, use signs of morbidity (e.g. hypothermia following venom exposure or septicemia) rather than mortality as the endpoint. The preferred alternative protocol is the Up-and-Down Procedure (UDP). Other methods include the Limit Test, Fixed Dose Procedure (FDP), Acute Toxic Class (ATC) method, Dose-Probing Test, and Pyramiding Test.
   i. The Up-and-Down Procedure (UDP), also referred to as the staircase method, involves dosing one animal at a time, beginning with the anticipated LD50 or one step below. Each subsequent animal is then given a higher or lower dose depending upon the previous animal's survival or probable survival. The total number of animals used is typically 6-10. The EPA provides free software to assist in calculating the recommended sequential doses, estimated LD50 and confidence intervals. When using humane endpoints with UDP, it may be possible to incorporate survival assays such as Kaplan–Meier, when duration of survival is needed in addition to mortality.

ii. The Limit Test examines the effects of a single dose on 5 to 10 animals.
iii. The Fixed Dose Procedure (FDP) is similar to the Limit Test. However, depending upon the results of the first dose, a second or third dosage may be tested in subsequent groups.
iv. The Acute Toxic Class (ATC) Method utilizes a similar step procedure but includes three fixed starting doses and only three animals per dose. Depending on the morbidity or mortality observed, additional steps may be necessary.
v. In the Dose-Probing Test, three widely spaced dosages are each tested on one or two animals.
vi. In the Pyramiding Test, two animals are given increasing doses on alternate days until a specific limit is reached or morbidity is observed.

Part II – Criteria for Euthanasia of Animals (Humane Endpoints)

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. Central nervous system 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 in less than 1 week or chronic gradual, but 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 UTA Animal Care Facility (ACF) staff / AV required).
Q. 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 presence 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, unrelenting 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 UTA ACF staff / AV, in consultation with the investigator, as to whether or not it is appropriate to attempt to relieve apparent pain through the use of these agents. Use of these agents can often confound data interpretation since many of these agents may produce effects in blood parameters, food/water consumption, appearance, mobility, neurologic measurements, etc.

Part III - Euthanasia by Cervical Dislocation or Decapitation *(Complies with the American Veterinary Medical Assocation of (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition recommendations on euthanasia by cervical dislocation or decapitation.)*

1. Cervical Dislocation
   A. This method of euthanasia can be used in mice and in rats weighing <200g.
   B. Cervical dislocation may be used unconditionally in the above-mentioned species if the animal is first anesthetized. Without prior anesthetization, this method may be only used when scientifically justified by the user and approved by the UTA IACUC. Prior use of this method of euthanasia by the investigator shall not be deemed as scientific justification.
   C. If the UTA IACUC approves this method for use without prior anesthesia, at the discretion of the UTA IACUC or the UTA Attending Veterinarian (AV), the UTA AV shall observe the personnel performing the cervical dislocation to ensure that they have a sufficient level of proficiency to perform the procedure.

2. Decapitation
   A. Decapitation may be used unconditionally in laboratory rodents if the animal is anesthetized.
   B. 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 IACUC Guillotine Maintenance SOP.) Without prior anesthetization, this method may only be used when scientifically justified by the user and approved by the UTA IACUC. Prior use of this method of euthanasia by the investigator shall not be deemed as scientific justification.
   C. Decapitation using scissors or sharp blades is acceptable with conditions for altricial neonates. Some rodent neonates, whether altricial or precocial, may have tissue mass that is too large for scissors, so appropriate decapitation tools should be selected. Consultation with UTA AV is recommended before including this method in an IACUC protocol.
   D. If the UTA IACUC approves this method for use without prior anesthesia, at the discretion of the UTA IACUC or the UTA AV, the UTA AV shall observe the personnel performing the decapitation to ensure that they have a sufficient level of proficiency to perform the procedure.

3. Justification
   A. Acceptable scientific justification for cervical dislocation or decapitation 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 IACUC protocol to test for significant differences between physical methods (i.e., gas inhalation [carbon dioxide or isoflurane] or injectable euthanasia agent overdose). The results of the pilot study would then be reviewed by the UTA IACUC before granting final approval to use physical methods of euthanasia.
      ii. Results of a literature review must be submitted with the IACUC protocol.
      iii. The review should demonstrate that the AVMA approved methods would not work in the specific study being reviewed.

References:

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition

Relevant IACUC Policies/Procedures:

Assessment of Pain and Distress
Veterinarian Notification of Animal Welfare Issues
Anesthesia in Laboratory Animals
Tumor Scoring (Scoring Endpoints in Tumor Studies in Rats and Mice)
Guillotine Maintenance
Reporting and Processing Animal Care and Use Concerns and Incidents
Reporting Adverse Events