
DEFINITIONS

Euthanasia - 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.

Acceptable method - As defined by the American Veterinary Medical Association (AVMA), an acceptable method of euthanasia renders an animal unconscious and insensitive to pain and distress as quickly as possible, followed by cessation of all respiratory and circulatory functions and brain activity.

Acceptable method with Conditions - These methods are considered “Acceptable” (see above) only if specific conditions are met, as indicated in the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. For example, neonatal rodents and reptiles may be resistant to the effects of CO₂ inhalation – therefore, a secondary physical method to confirm death is required. On the other hand, the preparation for some physical methods (e.g., decapitation) may cause stress in the animals, so administration of a sedative or anesthesia is required. Once the specified conditions are met, these methods are considered fully acceptable under the Guidelines.

POLICY

To prevent/alleviate animal suffering, all laboratory animals must be euthanized in a timely manner, either as described in the approved protocol according to established time points, or as soon as necessary if established criteria or humane endpoints are reached. While every effort will be made to reach a member of the study team in the event that an animal requires early or unscheduled euthanasia due to unrelieved pain/distress, the IACUC has empowered the Attending Veterinarian to immediately euthanize any animal that is found near death or suffering from intractable pain. Researchers are advised to ensure that animal care staff can always reach someone with authority to deal with sick or injured animals.

All IACUC protocols (see Section 15 of IACUC Protocol) must include a description of the method(s) that will be used to euthanize animals as well as a description of how death will be confirmed. The method(s) to be used must be consistent with the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

Since rodents can be particularly resistant to euthanasia by standard methods such as inhalation of CO₂ or gas anesthesia, IACUC policy requires that an approved secondary physical method of euthanasia (e.g. decapitation, cervical dislocation, exsanguination or removal of vital organs) be employed prior to carcass disposal in ALL rodent species. Failure to employ both the primary and secondary methods in all animals as described in the protocol will be treated as serious protocol non-compliance.
NOTE: Unintended recovery of animals after apparent death from CO₂ or other euthanasia agents constitutes serious regulatory noncompliance. All incidents involving unintended recovery of euthanized animals on protocols funded by the Public Health Service / National Science Foundation are reported to the Office of Laboratory Animal Welfare at NIH.

COMMON METHODS OF EUTHANASIA

- Inhalation of CO₂ - must follow the Standard Operating Procedure for CO₂ euthanasia.
- Inhalation of anesthesia gas– acceptable with conditions for rodents and other small animals (< 7 kg). Typically used as part of a two-step process with a secondary physical method of euthanasia such as decapitation or cervical dislocation.
- Immersion agents – e.g. MS 222/Tricaine. Acceptable for aquatic species, usually in connection with a secondary physical method.
- Cervical Dislocation – acceptable for small birds, mice and immature rats. Requires training and should be performed under anesthesia unless specifically approved by the IACUC.
- Decapitation – acceptable for rodents and small rabbits. Requires training; anesthesia recommended unless justified in the IACUC protocol and approved by the IACUC. Guillotines must be sharpened and adjusted frequently to ensure proper performance. The IACUC Administrator or representative must observe one euthanasia without anesthesia performed by the person responsible for training lab personnel. Any person performing a physical method of euthanasia (i.e. decapitation, cervical dislocation) on an un-anesthetized animal must be properly trained.
- Injectable barbiturate agents – e.g. sodium pentobarbital, Euthasol®, Eutha 6®, Fatal Plus® - acceptable for most species.
- Perfusion/tissue harvesting - any animal undergoing perfusion/tissue harvesting must be under a surgical plane of anesthesia before any incisions are made. A surgical plane of anesthesia must be maintained until the heart stops. Any person performing euthanasia by perfusion must be properly trained. Perfusion/tissue harvesting is considered non-survival surgery. The Surgical Procedures section of the IACUC protocol must be completed and approved by the IACUC.

CONFIRMATION OF DEATH IN EUTHANIZED ANIMALS

All research personnel must receive adequate and appropriate training in all methods of euthanasia employed in the laboratory; they must also be trained to evaluate vital signs in the species used to confirm death in the animals. A profoundly anesthetized or severely ill animal can appear dead upon cursory examination; measurements such as lack of movement or visible lack of breathing are not precise enough to declare that a euthanized animal is dead.
CONFIRMATION OF DEATH – GENERAL ASSESSMENT

The following assessment criteria apply to all species and for all methods of euthanasia. For specific information about confirming death in rodents and ectothermic vertebrates, please see below.

- Heart beat: must be assessed for five minutes or more. The best assessment is through direct palpation of either the pulse in the carotid or femoral artery or direct cardiac palpation. If there is any question, the thorax should be opened, the heart exposed and viewed directly or palpated to confirm lack of activity. Arterial pulse of smaller species may be 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 confirm death.
- 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 unresponsive to light. Some drugs and experimental agents (e.g., anticholinergics such as atropine) can prevent pupillary reactivity or otherwise affect this neurological response.
- Respiratory pattern: Profoundly anesthetized animals may exhibit shallow and irregular breathing patterns that may be confused for lack of spontaneous breathing. Thus, lack of spontaneous breathing should not be used as sole criteria for confirming euthanasia.

SPECIAL CONSIDERATIONS FOR RODENTS

Rodents, especially neonates, are particularly resistant to euthanasia by overdose of inhaled agents such as CO₂ or even injectable agents; for this reason, the IACUC requires a secondary physical method of euthanasia FOR ALL RODENTS after the animal is profoundly anesthetized, prior to carcass disposal. Acceptable secondary methods for adult and neonatal rodents include: decapitation, perfusion of a histological fixative via the major blood vessels, pneumothorax by opening the thorax, complete severing of the spine just below the base of the skull using a dorsal approach, or cervical dislocation for animals under 200g.

CONFIRMATION OF DEATH IN ECTOTHERMIC VERTEBRATES

Additional care must be taken to ensure death following euthanasia in ectothermic vertebrates such as fish, reptiles and amphibians. Such animals may normally exhibit very low heart rates, and the heart and brain are very tolerant to hypoxia; many ectotherms can voluntarily hold their breath for an hour or more. Absence of heart rate and/or breathing will not necessarily provide confirmation of death in these animals; secondary methods of euthanasia, which include but are not limited to removal of the heart or decapitation, must be conducted for ectothermic vertebrates.