Euthanasia Policy

General Policy

All euthanasia procedures performed within animal facilities are to be consistent with the recommendations in the *2013 Report of the AVMA Panel on Euthanasia.* Requests to perform euthanasia using techniques other than those in that document must be approved by the IACUC and may require monitoring by the veterinarian.

Euthanasia may be performed as required by protocol study endpoints, to relieve pain/distress from experimental manipulations or spontaneous conditions, and as appropriate in other situations deemed necessary by a veterinarian. Animals that are experiencing undue pain/distress must be humanely euthanized unless these conditions are required for scientific objectives that have been documented in a study protocol, justified, and approved by the IACUC.

It is important for researchers to delineate criteria for euthanasia in the study protocol including measurable physiological parameters and observable signs indicative of pain and distress. Euthanasia methods must also be described, including the persons who will be responsible for observations and euthanasia procedures. The veterinarian is available to demonstrate or discuss these techniques.

Distress vocalizations, fearful behavior and release of pheromones by a frightened animal can all cause anxiety and apprehension in other animals. Therefore animals must be euthanized in an area separated from other live animals, especially of their own species. The only exception to this rule is when animals do not share the same environment (as in fish living in separate aquarium tanks) and thus will not be exposed to the effects mentioned above.

Any animal that is moribund (i.e. near death) must be immediately addressed by the Principal Investigator or animal care staff. The Principal Investigator or specified contact person for that study will be contacted immediately and asked to immediately respond. If there is no response in a timely manner, the veterinarian reserves the right to euthanize that animal within an acceptable time period. No animal should be left in a moribund state at the end of the workday if there is no one left to continue to observe the animal and euthanize it if it does not recover.

Regardless of the technique used, <u>all</u> animals must be evaluated following euthanasia to assure that they are dead. This includes observing or palpating the chest for absence of a heartbeat and respiration (animals can have absence of respiration but still have a heart beat and subsequently fully recover.) For lower animals, such as fish and amphibians, a follow up method may be necessary. The veterinarian and IACUC will notify the investigator of this during the protocol review process.

The 2013 Report of the AVMA Panel on Euthanasia provides specific recommendations for the euthanasia of prenatal and neonatal animals and should be followed. Note: The UMASSD IACUC Committee has made the following determination regarding Zebrafish embryos: Because zebrafish embryos at 8 days-post-fertilization are eating external food, the IACUC has determined that zebrafish embryos 8 days post-fertilization are considered animals under IACUC

jurisdiction. Manipulations and euthanasia of embryos less then 8 days-post-fertilization are not under IACUC purview.

The 2013 Report of the AVMA Panel on Euthanasia also gives specific recommendations for the euthanasia of rodents via carbon dioxide which must be followed. Compressed carbon dioxide cylinders must be used and the gas inflow chamber must be regulated so that 10%-30% of the air volume within chamber is displaced per minute. Prefilled chambers are unacceptable. Euthanasia should be conducted within the home cage whenever possible. If euthanasia cannot be conducted in the home cage, chambers should be emptied and cleaned between uses. It is important to verify that an animal is dead after exposure to carbon dioxide. Death may be confirmed by physical examination or ensured by an adjunctive physical method. Addition of oxygen to the carbon dioxide will prolong the time to death and may complicate determination of consciousness and should not be used.

Investigators requiring use of controlled substances for euthanasia should contact Institutional Compliance. Procurment, use, and storage of controlled substances require compliance with federal and state laws.

Persons responsible for euthanizing animals must be properly trained and should be aware of where the equipment and drugs are located.

Acceptable Methods:

1. Carbon Dioxide (CO2) Asphyxiation using Compressed Gas a) Dry ice is not an acceptable source of CO2; b) Recommended for rodents; c) Must confirm death; d) AVMA Guidelines must be followed regarding the flow rate of carbon dioxide into the euthanasia chamber.

2. Barbiturates/Sodium pentobarbital, including euthanasia solutions a) DEA Schedule II or III Controlled Substance must be stored in a secured safe within a secured laboratory; b) Dosage should be 2-3X that acceptable for anesthesia (i.e., 100-200 mg/kg; follow directions on label for euthanasia solutions); c) Route of administration should ensure rapid circulation.

3. Gas Inhalants (e.g. Isoflurane) a) Recommended for rodents; b) Adequate ventilation and scavenging must be provided for safety reasons; c) Animal must not come in contact with the anesthetic liquid; d) Must confirm death.

4. Cervical Dislocation a) Is acceptable as primary means of euthanasia for rodents weighing less than 200 grams.

5. Decapitation with Guillotine or Scissors a) Acceptable for rodents; b) Equipment must be kept sharp and in good working order; c) When followed by pithing, may be acceptable for fish and amphibians if anesthetic can not be used (must be scientifically justified and approved by IACUC).

6. Potassium Chloride (KCL) a) Overdose only permissible in anesthetized animals; b) Animal must demonstrate Stage III anesthesia before administration; c) Leads to cardiac arrest; d) Not a preferred method.

7. Exsanguination and Vital Organ Removal a) Only applicable with anesthetized animals; b) Exsanguination is only appropriate when large volumes of blood are to be collected (i.e., >50% of total blood volume); c) Must confirm death.

8. MS-222 (tricaine methanesulfonate) a) Preferred method for amphibians and fish; b) Doses are generally greater than 250 mg/L (up to 1-3 grams/L in some amphibians); c) Solution must be buffered such that pH of euthanasia water matches home tank.

9. Hypothermia a) Acceptable with conditions for neonatal rodents; b) Should be used only on rodents less than 7 days old; c) Animal must not come in contact with ice or cooled surface.

10. Rapid Chillling a) Acceptable **only** for zebrafish and other similarly-sized tropical fish; b) Must thoroughly mix water with ice; c) Once water is thoroughly chilled, then fish are placed into container that allows rapid immersion into ice-chilled water without coming into contact with ice (such as a colander).

Fetuses and Neonates (mice and rats): See AVMA Guidelines section S2.2.4.

Euthanized animal storage: Euthanized animals will be double-bagged, labeled with the PIs name, protocol number and date. Euthanized or deceased animals may only be stored in a dedicated freezer (or refrigerator for necropsy). The freezer must not be used to store anything else such as food or chemicals. If the investigator would like the veterinarian to perform a necropsy, the carcass should be stored in a refrigerator (not freezer) and the veterinarian must be called as soon as possible within the same day.

Euthanized animal disposal: Contaminated animal carcasses (see EHS Procedure for Disposal of Animal Carcasses) must be double-bagged in leak proof, biohazard-labeled red bags within disposable Bio-box. Contact EHS to obtain Bio-boxes and bags. Uncontaminated animal carcasses must be packaged in double-bagged, air-tight, opaque plastic bags and appropriately labeled. Uncontaminated animal carcasses must be discarded in normal trash.

References:

American Veterinary Medical Association (AVMA). 2013. https://www.avma.org/KB/Policies/Documents/euthanasia.pdf

Jolaine M Wilson, Ralph M Bunte, and Anthony J Carty, Evaluation of Rapid Cooling and Tricaine Methanesulfonate (MS222) as Methods of Euthanasia in Zebrafish (Danio rerio). J Am Assoc Lab Anim Sci. 2009 Nov; 48(6): 785–789.