

SOP-160 Euthanasia

Methods of Euthanasia

Methods of euthanasia utilized at the Bowling Green State University Lab Animal Facilities will conform with the 2007 AVMA Guidelines on Euthanasia (<http://www.avma.org/resources/euthanasia.pdf>).

Determining Death

Death can be verified in the following ways:

- Cessation of heartbeat
- Cessation of breathing
- Onset of rigor mortis

Secondary means of determining death can be by either opening the thoracic cavity or by leaving the animal exposed to ambient air for 20 to 30 minutes after CO₂ asphyxiation prior to freezing.

Carbon Dioxide Asphyxiation

This is the most common method for euthanizing mice and rats. It can also be used for pigeons, rabbits under 3 kilos and other small mammals. Unless a drug overdose is necessary as part of a procedure, the primary method is CO₂ inhalation to effect.

Procedure: Pre-fill euthanasia chamber with CO₂ gas. Place animal(s) in chamber and add more gas to compensate for displacement. Shut off gas. Wait for all movement to stop. Remove animals and appropriately label for placement in the carcass freezer.

Euthanasia Solutions

Commercially available euthanasia solutions are available for purchase. Label directions will be followed. Sodium pentobarbital solutions are dosed at 100 mg/kg IP or IV.

Other drugs may be used to induce euthanasia. These would require a case by case review by the Attending Veterinarian and approval by the IACUC.

Decapitation – IACUC approval only

Pre-anesthetized

Rats and mice that are anesthetized may be decapitated by a trained individual.

No anesthetic

Explicit scientific justification of this method on unanesthetized rats and mice must be provided. A trained individual may perform this procedure with explicit IACUC approval.

Cooling and/or Freezing

Liquid nitrogen

Total immersion of prenatal rats and mice or larval forms of fish in liquid nitrogen.

Immobilization of reptiles by cooling is considered inappropriate and inhumane even if combined with other physical or chemical methods of euthanasia. Snakes and turtles, immobilized by cooling, have been killed by subsequent freezing.

This method is not recommended. Formation of ice crystals on the skin and in tissues of an animal may cause pain or distress. **Quick freezing of deeply anesthetized animals is acceptable.**

Tricaine methane sulfonate (MS 222, TMS)

MS 222 is commercially available as tricaine methane sulfonate (TMS), which can be used for the euthanasia of amphibians and fish. Tricaine is a benzoic acid derivative and, in water of low alkalinity (< 50 mg/L as CaCO₃); the solution should be buffered with sodium bicarbonate. A 10 g/L stock solution can be made, and sodium bicarbonate added to saturation, resulting in a pH between 7.0 and 7.5 for the solution. The stock solution should be stored in a dark brown bottle, and refrigerated or frozen if possible. The solution should be replaced monthly and any time a brown color is observed. For euthanasia, a concentration \approx 250 mg/L is recommended and fish should be left in this solution for at least 10 minutes following cessation of opercular movement.

Benzocaine hydrochloride

Benzocaine hydrochloride, a compound similar to TMS, may be used as a bath or in a recirculation system for euthanasia of fish or amphibians. Benzocaine is not water soluble and therefore is prepared as a stock solution (100 g/L), using acetone or ethanol, which may be irritating to fish tissues. In contrast, benzocaine hydrochloride is water soluble and can be used directly for anesthesia or euthanasia. A concentration \approx 250 mg/L can be used for euthanasia. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement.