Center for Biomedical Research / IACUC
Standard Operating Procedure - Guidelines

Euthanasia of Animals

Euthanasia is required at the end of studies, to harvest tissues, for culling animals due to welfare reasons or when animals exceed requirements. The PHS Policy and the AWRs require that an IACUC review and approve methods of euthanasia. The CBR will humanely euthanize research animals upon written request. The researcher, prior to animals being euthanized, **MUST** fill out an Animal Euthanasia Request Form. These cards are located in each animal room. Euthanasia is performed according to AVMA guidelines to ensure a rapid and painless death and to prevent or eliminate undue suffering.

CBR Recommended methods:

- CO_2: The CBR has the equipment necessary for CO_2 euthanasia and utilizes this method for sacrificing animals.
- Barbituric Acid Derivatives: Sodium pentobarbital (Nembutal) is the most common barbiturate agent for euthanasia. CBR requires sedation of animals prior to the use of this agent. Justification as sole agent use must be approved through IACUC review.
- Both methods above must be followed by cervical dislocation or decapitation.

Unacceptable

- Chloral hydrate, chloroform and cyanide
- Decompression
- Neuromuscular blockers
- Various pharmacological and physical methods
- Dry ice-generated CO_2

Guidelines for Euthanasia of Rodent Fetuses and Neonates

For mouse or rat fetuses E15 days to birth: A physical method of euthanasia (decapitation or cervical dislocation) is required in addition to euthanasia of mother or removal of fetus.

For mice or rat neonates up to and including 10 days of age: Decapitation, cervical dislocation, or injection with a chemical anesthetic (e.g., pentobarbital 800 mg/kg IP) are acceptable means of euthanasia. Neonates 10 days of age or less are resistant to hypoxia; if CO_2 is used, prolonged exposure time is needed to cause loss of consciousness or death. A secondary physical means of euthanasia (decapitation or cervical dislocation) is required when CO_2 is used.

Perfusion as Euthanasia vs. Non-survival Surgery

Perfusion as Euthanasia:

Perfusion is considered the means of euthanasia when an animal is anesthetized for immediate perfusion that results in death**.** When this is the case, the following steps must be taken:

- The lab must record that the animal was adequately anesthetized prior to opening the body cavity for perfusion or prior to initiating percutaneous perfusion.
- Standard anesthetic monitoring records are required for perfusion procedures in which death is not rapid (>5 min). For the majority of non-rodent perfusions, anesthetic monitoring records will likely be necessary.

Perfusion as Non-survival Surgery:

Perfusion is considered a non-survival surgery when an animal is anesthetized for surgical or invasive procedures and is euthanized prior to anesthetic recovery. This includes perfusion preceded by collection of cells, tissues or organs.

Guidelines for Euthanasia of Amphibians, Reptiles, and Fish

Chemical methods: (follow safety recommendations when using chemicals)

- Immersion: The intentional overdose via immersion of fish in anesthetic solutions
    - Recommended using a minimum of 10 times the anesthetic dose when used for euthanasia. **Fish should remain in the anesthetic solution for a minimum of 30 minutes after cessation of operator movement.**
    - Adjunctive methods can be used to ensure death.
- There are currently no drugs approved by the FDA for euthanasia of fish in the U.S.

    - Pentobarbital injection: given at a dose of 60-100 mg/kg IV or intracoelomic (ICo).
      Appropriate for: fish, amphibians, reptiles
      As results can be variable, verify that the animal is dead before carcass disposal.
      Check for respiratory movements, and lack of response to a painful stimulus. If still in doubt, follow with a physical method to ensure death.

    - Tricane methane sulfonate (MS-222): animal is placed in an MS-222 solution with a minimum concentration of 250 mg/L until death occurs.
      Appropriate for: amphibians, fish
      Time to death is variable. After removal from the solution, monitor the animal for a few minutes to ensure respiratory movements have ceased. MS-222 is

acidic and at concentrations greater than 500 mg/L, it should be buffered with sodium bicarbonate to achieve a pH of 7.0 – 7.5 to prevent discomfort to the animal. Alternatively, MS-222 can also be injected in the lymph or pleuroperitoneal spaces.

- Benzocaine hydrochloride: animal is placed in a benzocaine hydrochloride bath with at least a 250 mg/L concentration
  Appropriate for: amphibians, fish

- 2-phenoxyethanol: animal is placed in a bath of 2-phenoxyethanol solution at a concentration of 0.3 – 0.4 mg/L
  Appropriate for: amphibians, fish

- Inhalants (Isoflurane, Sevoflurane, CO_2): require long exposure times to achieve death. They should be followed with physical methods after loss of consciousness. CO_2 can be bubbled into a closed container for at least 30 seconds to displace O_2. Isoflurane is administered by extended induction.
  Appropriate for: fish and some fully aquatic forms of amphibians. Not recommended for land-dwelling amphibians or reptiles due to their ability to breath-hold for long periods of time and survives long periods of anoxia.

Physical Methods: (preceded by anesthesia/sedation unless justified and approved by IACUC) Results in rapid loss of neuronal transmission

- Pithing followed by decapitation (2-step): use a very sharp instrument of appropriate size to ensure a rapid separation of head from body. Immediately follow with double pithing. See below.
  Appropriate for: reptiles, amphibians and fish

    - Cervical Transection followed by pithing (2-step)
    - Blunt force trauma (cranial concussion) followed by pithing to cerebrum and brainstem
    - Maceration

- Pithing: insertion of a metal rod into the foramen magnum of the brain and proximal end of the spinal cord causing mechanical destruction. Appropriate for: amphibians, fish and reptiles. Not suitable for Xenopus, due to anatomical features that can lead to inconsistent unconsciousness and death.

- Thermal shock (freezing): controversial method since ice crystals may form in larger animals prior to death, causing pain. Only permissible with small fish. A container holding tank water and the small fish to be euthanized can be put in a freezer to cause a slowing of metabolism prior to freezing. The fish can be wrapped or put in a small container which is then placed on the surface of ice

slush can be used for more rapid cold water exposure.
Appropriate for: very small fish only (e.g., zebrafish)

- Perfusion: with a fixative may be used when justified. Animal must be sedated prior.
  Appropriate for: reptiles, amphibians, and fish

    - Exsanguination, Stunning, or Pithing are NOT recommended as a sole means of euthanasia, but may be considered as adjuncts to other agents or methods.

    - Adjunctive Methods

        - Decapitation, Pithing, freezing and other physical or chemical methods for destroying brain function may be used as the second step of a 2-step procedure when fish have been rendered unconscious prior to their application by a first method

(This sign is posted in the Procedure Rooms)

GUIDELINES- $C0_2$ EUTHANIZATION

Place provided cage top with hole onto cage with mice or rats to be euthanized. Insert green hose into hole on cage top. Turn gold knob located on top of $C0_2$ tank to the left. Next, move silver flow regulator knob located to the left until small ball rises to "4" for a mouse cage and "8" for a rat cage. Allow the $C0_2$ to release into the cage. Monitor the mice/rats for breathing. Once breathing has stopped, turn off $C0_2$ gas by turning gold knob at top of tank to the right. (Once gas is turned off, it will take a little while for the small ball inside the flow regulator to lower.)
Immediately following euthanasia with CO_2, a secondary confirmation of death must be performed including either cervical dislocation or decapitation. Once confirmed dead, place animals into carcass bag and seal, then place bag into appropriately labeled freezer.

If $C0_2$ tank is empty or you have any questions please contact CBR staff. **Do not change out $C0_2$ tank.**