1. PURPOSE

This Standard Operating Procedure (SOP) describes the acceptable methods for euthanasia of avian species. All methods described are under the AVMA 2021 Guidelines for Euthanasia.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. MATERIALS

3.1. Injectable agents (sodium pentobarbital or T61)
3.2. Needles, syringes
3.3. Inhalant agents (CO₂, isoflurane) and clear chamber

4. PROCEDURES

4.1. Ensure that all individuals responsible for euthanasia:
   4.1.1. Receive appropriate training to perform the procedure.
   4.1.2. Adhere to Facility Animal Care Committee (FACC)-approved protocols and institutional policies.
   4.1.3. Select the method of euthanasia based on the species and the protocol's objectives.
4.2. Minimize distress to the animal and the operator by handling the bird gently and carefully.
4.3. Avoid the euthanasia of birds in the presence of other birds and animals.
4.4. Verify death prior to disposal of the body. Confirm by:
   4.4.1. Observing for the absence of movement.
   4.4.2. Observing for the absence of respiratory and heartbeat activity for at least 3 minutes.
   4.4.3. Check the eye and toe pinch reflex.
4.5. Non-Physical Methods:
   4.5.1. Barbiturate overdose (RECOMMENDED)
      4.5.1.1. Inject sodium pentobarbital intra-celom (IC) or intravenously at a dose of 150 mg/kg
      4.5.1.2. When the IC route of administration is used, place the bird in a small cage in a quiet area to minimize excitement and trauma, as birds may slowly become sedated.
   4.5.2. Inhalant overdose:
4.5.2.1. **CO₂ (RECOMMENDED)**

4.5.2.1.1. Use compressed CO₂ from cylinders.

4.5.2.1.2. Use the CO₂ in a transparent chamber so the bird can be observed.

4.5.2.1.3. Do not overcrowd the chamber or mix species within the chamber.

4.5.2.1.4. Set a flow rate that displaces 20% of the chamber volume per minute.

4.5.2.1.5. Sanitize the chamber after each session.

4.5.2.1.6. Chicks, hatchlings, and diving birds tolerate high concentrations of CO₂. Prolonged exposure to high concentrations of CO₂ will be required to produce death (e.g., up to 5 minutes in 60–70% CO₂ for 1-day-old chicks).

4.5.2.2. **Inhalant anesthetics (Isoflurane):**

4.5.2.2.1. Should be followed by other methods (i.e., cervical dislocation) to ensure death.

4.6. **Physical methods:**

4.6.1. Anesthesia or sedation must be given before using any of the following physical techniques.

4.6.2. Use cervical dislocation (dislocation of the neck) for birds less than 200 g.

4.6.3. Perform decapitation with clean, sharp equipment that will keep the head from the body rapidly and completely.

4.6.4. Verify death as in Section 4.4.

4.7. **Embryos, Eggs:**

4.7.1. For embryos, eggs >50% gestation, use methods appropriate for hatched birds (e.g., decapitation, an overdose of anesthetic)

4.7.2. For embryos, eggs <50% gestation (under field conditions) destroy the viability of eggs by one of the following methods: shaking, puncturing, freezing, or coating eggs with oil.