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## 1. PURPOSE

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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.

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## 2. RESPONSIBILITY

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Principal investigator (PI) and their research staff.

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## 3. MATERIALS

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- 3.1. Injectable agents (sodium pentobarbital or T61)
  - 3.2. Needles, syringes
  - 3.3. Inhalant agents (CO<sub>2</sub>, isoflurane) and clear chamber
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## 4. PROCEDURES

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- 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. CO<sub>2</sub>: (RECOMMENDED)

- 4.5.2.1.1. Use compressed CO<sub>2</sub> from cylinders.
- 4.5.2.1.2. Use the CO<sub>2</sub> 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 CO<sub>2</sub>. Prolonged exposure to high concentrations of CO<sub>2</sub> will be required to produce death (e.g., up to 5 minutes in 60–70% CO<sub>2</sub> 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.

**SOP 403 AVIAN EUTHANASIA**

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*Approved by the BGU Animal Policy and Welfare Oversight Committee*