

Title	Euthanasia of Avians SOP
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I. PURPOSE

To define a set of approved procedures for performing euthanasia on avians. This standard operating procedure provides euthanasia methods and procedural details in accordance with the IACUC Euthanasia Policy.

The PHS Policy requires that euthanasia be conducted according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020 Edition). The AVMA Guidelines set criteria for euthanasia and specify appropriate euthanasia methods and agents based upon the best literature and empirical evidence that minimizes pain and distress to animals during euthanasia.

II. POLICY

Per the IACUC Euthanasia Policy, “Methods not covered in approved IACUC standard operating procedures need to be approved by the IACUC before utilization. The Principal Investigator should confer with the Attending Veterinarian prior to submitting the protocol to the IACUC.”

This SOP discusses the following approved euthanasia methods for avians:

- CO2 Euthanasia
- Injectable Barbiturate Euthanasia
- Physical Methods for Field Studies

III. DEFINITIONS

Euthanasia – In the context of this SOP, euthanasia is the act of inducing humane death in an animal with rapid unconsciousness and death with a minimum of pain, discomfort, or distress.

IV. PROCEDURES

A. General:

1. All individuals responsible for euthanasia of research animals must be qualified and trained to perform euthanasia humanely, per the IACUC policy on Training for Research and Animal Care Personnel (IACUC-003).
 - a. The Principal Investigator is responsible for ensuring that their personnel have been sufficiently trained, and for maintaining appropriate documentation.
 - b. Documentation of personnel training may be reviewed by the IACUC.

2. These procedures must be posted near the routine area of use – e.g., procedure or laboratory spaces, necropsy rooms. An abbreviated version may be posted at carbon dioxide euthanasia stations. Refer to Appendix A for an example template.
3. Euthanasia should occur in a procedure room or laboratory, away from other animal housing. Satellite facilities may not euthanize animals in close proximity to the housing area.
4. Euthanasia procedures should be documented. See Appendix B for an example.
5. Death must be confirmed by a second physical form of euthanasia for narcotic or inhalant euthanasia processes. Examples:
 - a. Bilateral thoracotomy
 - b. Collections of vital tissues to assure the animal will not recover
 - c. Exsanguination
 - d. Decapitation
 - e. Cervical dislocation
 - f. Thoracic depression
6. Animals must not be left alone at any point during the euthanasia processes.
7. Pentobarbital and pharmaceutical formulations containing pentobarbital are controlled substances and are regulated by the Drug Enforcement Agency and the State of Michigan. Refer to university policy on Oversight of Controlled Substances in Research (3-44) for more information.

B. Hazards and Safety Precautions:

1. Wear appropriate PPE as required per facility standards (e.g., lab coat, gloves)
2. Ensure appropriate ventilation when working with CO₂ (e.g., HVAC is operational, fume hood, down draft table). Build-up of CO₂ gases in the area can be a safety hazard negatively impacting research and facility staff.

C. Methods

1. Carbon Dioxide Euthanasia:

- a. Carbon dioxide (CO₂) is a frequently used euthanasia agent for small laboratory animals due to its rapid onset of action, safety, and availability.
- b. Mechanism of Action
Inhalation of high concentrations of CO₂ result in a rapid decrease of intracellular pH, rapidly resulting in decreased function in the CNS and death.
- c. Administering CO₂
 - i. A gradual fill rate of 30-70% chamber volume per minute displacement is required. Refer to Appendix A for information using the NIH ARAC Guidelines for calculating euthanasia chamber size and flow rate instructions.
 - ii. Compressed gas is the only acceptable source of CO₂. The following are not permitted: dry ice, fire extinguishers, and other sources of CO₂.
 - iii. Euthanasia chambers should be constructed of a clear material (e.g., Plexiglas®) to facilitate observation of the animals during the euthanasia procedure.
 - iv. CO₂ is denser than room air and will remain at the bottom of the chamber, thus the chamber will need to be emptied between groups of cages. The chamber must be cleaned between uses.
 - v. Prefilled chambers are unacceptable.
 - vi. Maintain CO₂ flow for a minimum of 5 minutes.
 - vii. All avians must be kept within the CO₂ chamber until death, usually within 10

- minutes (an additional 5 minutes after the CO₂ has been turned off).
- viii. Observe each animal to ascertain for lack of respiration, heartbeat and noting fixed, dilated pupils, and faded eye color. If all these signs are observed, then remove the animal from the chamber otherwise repeat the procedure.
 - ix. If unconsciousness does not occur, check the chamber fill rate. Examine the system for a defective flow meter, regulator, absence of CO₂ gas supply, and leaks.
 - x. Confirmation of euthanasia must be performed by an approved secondary method. Please see Section IV.A5.

2. **Injectable Barbiturate Euthanasia**

- a. The advantage of using barbiturates for euthanasia far outweighs the disadvantages, and barbiturate overdose is a common euthanasia method for mammals. Intraperitoneal injection of a barbiturate, such as pentobarbital, is an acceptable method of euthanasia for avians.
- b. The recommended intraperitoneal dosage of sodium pentobarbital varies by species. Example: the minimum recommended IV or IP dosage for white carneau pigeons is 75 mg/kg or 3x the anesthetic dose, however, increasing the dosage to 4 or 5x the anesthetic dose may be appropriate.
- c. Observe the animal to ascertain death (lack of respiration, heartbeat and noting fixed, dilated pupils, and faded eye color).
- d. Confirmation of euthanasia must be performed by an approved secondary method. Please see Section IV.A.5 for approved methods

3. **Physical Methods for Field Studies**

- a. Euthanasia procedures described above may not be practical for field settings, particularly in emergent situations.
- b. In those situations, physical methods may be warranted, as recommended by the AVMA Guidelines for Euthanasia of Animals.
- c. These methods include cervical dislocation and decapitation.

D. Disposal

- 1. Death must be confirmed prior to bagging carcasses for disposal.
- 2. Animal carcasses must be disposed of properly via biohazardous waste disposal.

V. REFERENCES

American Veterinary Medical Association, Panel on Euthanasia. (2020). *AVMA guidelines for the euthanasia of animals: 2020 edition*. American Veterinary Medical Association.
<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>

Appendix A: Example Template - Procedures for CO₂ Euthanasia

1. Determine the necessary flow rate, see the two options for how to determine the minimum and maximum flow rates below.

a. Double click the table below to use the Flow Rate Calculator:

Flow Rate Calculator		
Instructions:		
- If chamber volume is already known, simply enter the volume in the first row, below. - Otherwise, enter length, width and height/depth measurements into the table to obtain chamber volume and required flow rate.		
Volume (in L), if known		
	Inches	Centimeters
Length		
Width		
Height/Depth		
Volume (L):	0.00	
Required Flow Rate*:	From 0 to 0 L/min	
*Current OLAW and AVMA Guidance requires displacement of air with CO₂ at a rate of 30% -70% of chamber volume per minute.		

b. Perform the calculations manually:

- i. Determine chamber volume – If known, use the volume of euthanasia chamber. Otherwise calculate the interior volume of the euthanasia chamber as Length X Width X Depth (all dimensions are inside measurements, in centimeters).

Example: A euthanasia chamber measuring 20 cm X 25 cm X 20 cm has a volume of 10,000 cm³, or 10 L.

- ii. Calculate minimum flow rate as 0.3/minute X chamber volume (in L, from preceding). Example:

For a 10 L chamber, the minimum flow rate should be 0.3/minute X 10 L = 3 L/minute.

- iii. Calculate maximum flow rate as 0.7/min X chamber volume (in L, from preceding).

Example: For a 10 L chamber, the maximum flow rate would be 0.7/minute X 10 L = 7 L/minute.

2. The chamber must be cleaned to remove debris and pheromones between animals. Line the chamber with a paper towel. Place the animal(s) in the chamber.
3. Fasten the chamber lid and securely attach the hose to the top port of the chamber.
4. Fill the chamber completely with CO₂ using a fill rate of 30-70% of the chamber volume per minute.
5. CO₂ is denser than room air and will remain at the bottom of the chamber, thus the chamber will need to be emptied between groups of cages.
6. Unconsciousness typically occurs within two to three minutes. Observe each animal for lack of respiration. Maintain CO₂ flow for one minute after respiration ceases. If all signs of death (lack of respiration, fixed, dilated pupils and faded eye color) are observed at this point, then remove the animals from the chamber. Otherwise, continue exposing them to CO₂. If unconsciousness does not occur, check the chamber fill rate. The system should also be checked for a defective flow meter, an empty CO₂ cylinder and/or system leaks.
7. It is important to verify death after CO₂ exposure. This process must be followed by a secondary method of euthanasia, such as bilateral pneumothorax, decapitation, or cervical dislocation.

Euthanasia Record

[illegible]

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