Title: Euthanasia of Avians, IACUC Standard Operating Procedure	
Approval Date: September 16, 2021	
Authorized by: Vice President for Research and Innovation and the Institutional Animal Care and Use Committee (IACUC)	
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I. PURPOSE

To define a set of approved procedures for performing euthanasia on avians using carbon dioxide (CO_2). CO_2 is an inhaled agent that may be used to euthanize avians. This standard operating procedure provides the euthanasia method and procedural details in accordance with the IACUC Policy on Euthanasia.

The PHS Policy requires that euthanasia be conducted according to the American Veterinarian Medical Association (AVMA) Guidelines for 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 IACUC Policy on Euthanasia, "Methods not covered in approved IACUC standard operating procedures need to be approved by the IACUC. The Principal Investigator should confer with the attending or clinical veterinarian prior to submitting the protocol to the IACUC. The IACUC committee must grant an exemption to any deviation from approved policies and procedures."

III. DEFINITIONS

Euthanasia – In the context of this policy, 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 training policy (P-003-02).
    a. The Principal Investigator is responsible for ensuring 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. laboratory, procedure room, necropsy room. An abbreviated version may be helpful to post for carbon dioxide 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. There should be a log used for tracking euthanasia procedures, see Appendix B for an example.
5. Confirmation of death must be ensured 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 CO_2 (e.g. adequate air exchanges, fume hood, down draft table). Build-up of CO_2 gases in the area can be a safety hazard negatively impacting research and facility staff.

C. Method:

1. Carbon Dioxide Euthanasia:

a. Carbon dioxide (CO_2) is a frequently used euthanasia agent due to its rapid onset of action, safety, and availability.
b. Mechanism of Action: Inhalation of high concentrations of CO_2 result in a rapid decrease of intracellular pH, rapidly resulting in decreased function in the CNS and death.
c. A gradual fill rate of 30-70% chamber volume per minute displacement is expected at all avian euthanasia locations at CMU.
   Note: Refer to Appendix A for calculating euthanasia chamber size and flow rate instructions.
d. Compressed gas is the only acceptable source of CO_2. Dry ice, fire extinguishers, and other sources of CO_2 may not be used.
e. Euthanasia chambers should be constructed of a clear material (e.g. Plexiglas®) to facilitate observation of the animals continuously during the euthanasia procedure.
f. CO_2 is denser than room air and will remain at the bottom of the chamber, thus the chamber will need to be emptied and cleaned between animals (prefilled chambers are unacceptable).
g. CO_2 flow should be maintained for a minimum of 5 minutes.
h. All avians must be kept within the CO_2 chamber until death, usually within 10 minutes (an additional 5 minutes after the CO_2 has been turned off).
i. Observe the animal to ascertain death (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 continue exposing them to CO_2.
j. If unconsciousness/death does not occur, check the chamber fill rate. Examine the system for a defective flow meter, regulator, absence of CO_2 gas supply, and leaks.
k. It is important to verify death after CO_2 exposure. This method must be followed by a secondary method of euthanasia, such as bilateral pneumothorax, decapitation, or cervical dislocation.

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. Intravenous (IV) or intraperitoneal (IP) injection of a barbiturate, such as pentobarbital, is an acceptable method of euthanasia for avians.
b. The recommended 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). This method must be followed by a secondary method of euthanasia, such as bilateral pneumothorax, decapitation, or cervical dislocation.

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.

4. **Disposal:**
   a. Death must be confirmed prior to bagging carcasses for disposal.
   b. 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

Fedde, M. R. (1978). Drugs used for avian anesthesia: A review. *Poultry Science, 57*(5), 1376-1399. https://doi.org/10.3382/ps.0571376

Appendix A: Example Template - Procedures for CO_2 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 CO_2 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 cm^3, 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. Place the animal(s) in the chamber. If not leaving animals in their original cage, line the chamber with paper towel. If animals are not euthanized in their home cage, the chamber must be cleaned to remove debris and pheromones between animals.
3. Fasten the chamber lid and securely attach the hose to the top port of the chamber.
4. Fill the chamber completely with CO_2 using a fill rate of 30-70% of the chamber volume per minute.
5. CO_2 is denser than room air and will remain at the bottom of the chamber, thus the chamber will need to be emptied between cages.
6. Unconsciousness typically occurs within two to three minutes. Observe animal for lack of respiration. Maintain CO_2 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 animal from the chamber. Otherwise, continue exposing them to CO_2. If unconsciousness does not occur, check the chamber fill rate. The system should also be checked for a defective flow meter, an empty CO_2 cylinder and/or system leaks.
7. It is important to verify death after CO_2 exposure. This process must be followed by a secondary method of euthanasia, such as bilateral pneumothorax, decapitation, or cervical dislocation.

Appendix B: Example - Euthanasia Log

Euthanasia Record

Date	Subject ID*	Species	Time Start	Time End	Gauge Level (if CO_2) or Method	Name

*In the event of euthanizing cages of animals without identification, indicate the protocol number and number of animals.