Avian Wildlife Euthanasia Techniques

Renée Schott, DVM, CWR
LafeberVet Non-Interactive Webinar
AAVSB RACE Program #776-41764

I. Wildlife rehabilitation is the act of providing temporary care to injured, sick, or orphaned wildlife with the goal of releasing them back into the wild.[1]

II. Legality
   a. If the animal is wild, its "owner" is the State, Federal, or Tribal authority
   b. Some endangered or migratory species may require approval by the appropriate federal agency prior to euthanasia.[2]
      i. Migratory birds = USFW
      ii. Marine mammals = NOAA
      iii. Remainder usually = State

III. Definition
   a. Derived from the Greek terms [3]
      i. "*eu*" = well or good
      ii. "*thanatos*" = death
   b. Ending the life of an animal in a way that eliminates or minimizes pain and distress.[4]
   c. The technique employed should result in rapid loss of consciousness followed by cardiac or respiratory arrest and, ultimately, a loss of brain function.
   d. Actually determining the pain or distress of a method of euthanasia can be very difficult because as humans we will never fully know/understand the subjective experience of the animal[3].
      i. We use our best judgement; paddling, vocalizations, convulsions before apparent loss of consciousness is obviously suffering.
      ii. We also use our knowledge of physiology and assume suffering in the absence of behaviors if a physiological process theoretically leads to suffering.
   e. General rule: A gentle death that takes longer is preferable to a rapid but more distressing death and conversely, if all other methods are equally humane, the quickest method should be chosen.[5]
   f. Taking a life is abhorrent to many (McMillan)[6] we often forget this and need to keep it in mind
   g. What's the difference between euthanasia and humane killing? [4,6,7]
      i. Most veterinary/animal fields define euthanasia simply as "good death", however, the human field does not.
         1. Humane slaughter
         2. Humane depopulation
      ii. The decision of whether or not to end a life must be based on the animal's welfare, that is, to anticipate what is in the animal's best interest.

IV. Evaluating euthanasia methods [4]
   a. Animal factors
      i. Ability to induce loss of consciousness and death with a minimum of pain and distress; compatibility with species, age, and health status
      ii. Time required to induce loss of consciousness (CCAC)[3]

iii. Reliability/irreversibility

b. Human factors
   i. Safety of personnel
   ii. Documented emotional effect on observers or operators
      - Emotional impact
      - Drug availability and human abuse potential
   iii. Ability to maintain equipment in proper working order
   iv. Legal requirements

c. Remains (carcass) factors
   i. Compatibility with intended animal use and purpose
   ii. Compatibility with subsequent evaluation, examination, or use of tissue
   iii. Environmental impacts

d. Unacceptable methods

V. We must view the above within a practical systems view/process flow:

a. Handling/restraint
b. Euthanasia methods/agents: two phases [8]
   - Loss of consciousness (unaware of surroundings, cannot feel pain, fear, distress)
      - Sedation= animal may be aroused to a conscious state with sufficient stimulation [4]
      - Anesthesia= unconsciousness and cannot be aroused [4]
   - Causing death
c. Confirmation of death
d. Disposal of remains, necropsy needed (and why)

VI. Techniques

a. Inhalant agents
   i. Gas displacement rate is critical for the humane application of inhalant euthanasia when using a flowmeter [4]
   ii. Isoflurane [4]
   iii. Carbon dioxide
      1. Rodents: recommendations changed = 30-70% of the chamber/cage volume/min (fill it faster?)
      2. May cause distress in animals by three mechanisms [4]: (CO_2→respiratory acidosis)
         a. Pain due to formation of carbonic acid on respiratory and ocular membranes (pain starts at about 40% CO_2 in people and apparently rats/cats
         b. Feeling of breathlessness→ in humans, this starts at 8% and intensifies with higher concentrations
         c. Direct stimulation of ion channels with the amygdala associated with the fear response

b. Sodium pentobarbital
   i. How sodium pentobarbital works in mammals [9]
   ii. Routes of administration
   iii. Sodium pentobarbital solutions are caustic and it can hurt if given in an inappropriate spot

c. Physical methods:
    i. "*When properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane and practical than other forms of euthanasia*." [4]

VII. Case examples

a. SONGBIRD: @ vet clinic describe case, medical issues, state why we are euthanizing (i.e. spinal trauma with no deep pain)
b. Ask what euthanasia method would others use for this? Carbon dioxide and isoflurane (with or without sodium pentobarbital injection) will be suggested.
c. Avian respiratory physiology:
    i. Much for efficient at gas exchange vs mammals because of:
        1. Unidirectional air flow through lungs (no mixing of inspired and expired air)
        2. Countercurrent gas exchange (which is much more efficient and increases surface area over which gas can be exchanged and more/smaller air capillaries
        3. → more sensitive to inspired toxicants

d. Inhalant agents:
    i. Gas displacement rate is key: critical to the humane application of inhalant euthanasia when using a flowmeter [4]
        1. Time constant (τ) = volume/flow rate
        2. 1 τ required for concentration of inflowing gas to reach 63.2%
        3. 2 τ required for concentration of inflowing gas to reach 86.5%
        4. 3 τ required for concentration of inflowing gas to reach 95%
    ii. Isoflurane[2]:
        1. Smells noxious, irritates respiratory epithelium → animals may breath hold → may cause distress
        2. Expose only to vapors, not the liquid (irritating to skin)
        3. Maximum 33% isoflurane vapor can be produced at 20°C
        4. Feeding euthanized animals to patients? whole body still retained between 4% and 13% of the absorbed dose. Differences between sevoflurane and desflurane were obvious only during the final stages of elimination; large amounts of anesthetics are absorbed during anesthesia and significant amounts remain in the body for days after apparent recovery.[10]
    iii. Best for small animals, less than 7 kg
    iv. Best when no aversion behaviors are noted → exposure to rapid high concentrations preferred in some species
    v. Expose the animal to a high gas concentration using an anesthetic vaporizer or soaked gauze in a closed container [11]; gauze or cotton soaked in container [12]
        1. Vapors are inhaled until respiration ceases and death ensues [11]
        2. Vapor exposure should be maintained for at least 3 minutes after apparent clinical death [11]

e. Sodium pentobarbital

i. Sodium salts (phenobarbital, phenytoin, methotrexate) are considered weak acids and must be formulated at a high pH to ensure solubility.[13] Phenytoin sodium = pH 12

ii. Sodium pentobarbital alone; ~390 mg/ml, dose ~1 ml/4.5 kg (10 lbs)

1. Fatal-Plus: DEA schedule II
    a. Thin (not viscous)
    b. pH=9.6-11 (caustic! Hurts if given near nerves!)
2. Pentasol: pentobarbial sodium powder, reconstitute in 250 ml=392 mg/ml Virbac
3. Socumb
4. Sleepaway Zoetis

iii. Sodium Pentobarbital (390 mg/ml) plus phenytoin sodium (50 mg/ml) →hastens cardiac arrest, so you can't get high…you just die…humans are less likely to abuse

- Euthasol
    - pH=12-13 (caustic!)
    - Thick, viscous
- Beuthanasia-D: Merck Animal Health
- Euthanasia III

iv. How sodium pentobarbital works in mammals [9]

Stage 1: voluntary excitement

- Bloodstream → heart → cerebral cortex
- Begins to lose consciousness/coordination, hyperesthesia, loses voluntary motor, loss of superficial pain → disorientation, possible movement

Stage 2: involuntary excitement

- Bloodstream → heart → cerebral cortex → cerebrum
- Loss of consciousness → possible uncontrolled motor activity (paddling, vocalizations)

Stage 3: surgical anesthesia

- Bloodstream → heart → cerebral cortex → cerebrum → cerebellum (~5 seconds)
- Cannot feel pain, doesn't respond to visual/auditory, reflexes begin to disappear

Stage 4: medullary paralysis

- Bloodstream → heart → cerebral cortex → cerebrum → cerebellum
- Depresses breathing, heartbeat, blood pressure→anoxia→brain cell death (~40 seconds) → possible heart fibrillation, agonal breaths, muscle spasms (unconscious, reflex)

v. ROUTES of sodium pentobarbital administration: discuss the various routes it can be given, the pros and cons of each

1. Sodium pentobarbital solutions are caustic and it can hurt if given in an inappropriate spot
    a. pH humane blood 7.35 [13]
    b. pH between 4.5-8 doesn't hurt?
    c. pH 5-9 safe per some nursing place
    d. *In vitro* experiments have demonstrated that solution pH values of 2.3 and 11 kill venous endothelium cells on contact. [13]

2. IV likely only after anesthesia because wild animals

3. IP likely only after anesthesia because wild animals [9], consider air sacs in avian and generally avoid [12]
4. IC only after anesthesia because painful
5. Intraosseous and intramuscular, only after anesthesia because painful [12]
6. Intrahepatic only after anesthesia because requires careful placement and still animal[12]
7. PO, tastes bitter, but works well in gaping baby birds, prohibited in certain states [9]

f. WHAT IF DRUGS NOT AVAILBLE?

Carbon dioxide:

i. Rodents: recommendations changed = 30-70% of the chamber/cage volume/min (fill it faster?)
ii. Neonatal mice (that is, pups younger than 6 d) must be exposed to carbon dioxide (CO_2) for as long as 50 min to achieve euthanasia.[14]
    1. Neonatal mice (n = 76; age, 1 or 2 d) were exposed to isoflurane in a sealed, quart-size (0.95-L) plastic bag at room temperature. Righting and withdrawal reflexes were absent in less than 2 min. After 30 min of exposure to isoflurane, pups were removed and monitored for recovery. All pups were cyanotic and showed no detectable signs of life when they were removed from the bag. However, after 30 to 120 min after removal from the bag, 24% of isoflurane-overexposed pups began gasping and then resumed normal respiration and regained a normal pink coloration. These results demonstrate that isoflurane overexposure at saturated vapor pressure for 30 min is insufficient to euthanize neonatal mice and that isoflurane overexposure must be followed by a secondary means of euthanasia.[14]
    2. → require extended exposure times [4]
    3. Also true for newly hatched chickens (>5 min CO_2 exposure) (rabbits and mink have prolonged survival times in CO_2 as well)
iii. May cause distress in animals by 3 mechanisms (Leary) [2]: (CO_2 → respiratory acidosis)
    1. Pain due to formation of carbonic acid on respiratory and ocular membranes (pain starts at about 40% CO_2 in people and apparently rats/cats
    2. Feeling of breathlessness → in humans, this starts at 8% and intensifies with higher concentrations
    3. Direct stimulation of ion channels with the amygdala associated with the fear response
iv. Exposure to CO_2 does cause unconscious wing flapping in chickens (emotional observers)
v. Slow rise in CO_2 concentration creates less aversion
vi. Maintain CO_2 flow for at least 1 min after respiratory arrest
vii. Do NOT use combustion (i.e. from a vehicle) or dry ice
viii. Preferably administered from compressed gas cylinders/tanks [15]
ix. The use of volatile agent as induction to anesthesia before introducing CO_2 to euthanize has been described.[16,17]

x. PROS for CO_2
    1. Rapid depressant, causes anesthesia at high concentrations in the blood
    2. Readily available in compressed gas cylinders, non-explosive, not controlled,
    3. Safe for people

4. Remains can be fed to animals

xi. CONS for CO_2
- So many studies have conflicting results about aversion/breathlessness, within/among species, strains, breeds, etc. → no rule of thumb
- Can/does cause aversiveness/distress
- Heavier than air
- Immature, aquatic, burrowing may have high tolerances for CO_2
- May take longer?
- Need regulators/flow meters because if CO_2 flows too quickly it will freeze

Physical methods:
- "*When properly used by skilled personnel with well-maintained equipment, physical methods of euthanasia may result in less fear and anxiety and be more rapid, painless, humane and practical than other forms of euthanasia*".[4]
- Exsanguination, stunning and pithing should NOT be done w/o prior anesthesia
- Aesthetics and humane can be in conflict, i.e. maceration for DOC → death is almost instantaneous
  - https://vimeo.com/174954377: video of day old chick maceration (will not show in presentation)—it appears horrible and visceral yet the amount of suffering is just a fraction of a second—probably the most humane euthanasia method for day old chicks

- Blunt force trauma to head:
  - Appropriate for animals with thin craniums
  - Trained/proficient personnel, but personnel can/will become fatigued (physically, emotionally)
  - Strive for other options, this is a good last resort
  - DIFFICULT because of strength needed, resolve, knowledge of anatomy, only have one chance, thick skulls, needs resolve
  - not recommended by OIE (USDA, APHIS)[18]

- Captive bolt: penetrating and non-penetrating
  - Follow with exsanguination to ensure death [3]
  - MUST maintain the tools (clean, etc.)
  - CASH Small Animal Tool: non-penetrating
    - https://www.accles-shelvoke.com/tools/other-cash-tools/cash-small-animal-tool
    - https://www.qcsupply.com/cash-22caliber-small-animal-tool.html
  - Ballista Penetrating Bolt Gun $60, spring loaded
    - https://www.bunnyrancher.com/store/p42/The_Ballista_-_Penetrating_Bolt_Gun_.html
  - PRACTICE, PRACTICE, PRACTICE on dead animals first and dissect skull/brain
    - https://www.inspection.gc.ca/food-safety-for-industry/food-specific-requirements-and-guidance/meat-products-and-food-animals/guidelines-for-stunning-techniques/eng/1538160892409/1538160892704#a110

        - https://www.hsa.org.uk/mechanical-methods-poultry/non-penetrative-captive-bolt

- Gunshot: entire section in AVMA 2020 about gunshot, energy requirements, bullet selection, etc. bullet should go through cranium and into brain…firearm safety![19]

- Cervical dislocation
    - Hindlimbs one hand, head in the other, neck is hyperextended and dorsally twisted to separate the first cervical vertebrae from the skull—pull head and apply a ventrodorsal rotational force to the skull
    - Few studies that affirm the feeling that it is humane…"appears" to be humane
    - Small birds, poultry, waterfowl (birds < 3 kg)
    - Remain conscious ~1+ min after manual cervical dislocation [20]
    - Four sources say poultry likely aren't immediately unconscious [4] (we don't know if consciousness and perception of pain are concurrent, but we do have to assume they are for now)
    - Pliers and other devices seem to prolong reflexes/time to death/etc.[20] and are not recommended (Leary)[4] → these crush the spinal cord/trachea and are not acceptable
    - Practice on dead things first
    - Blunt force trauma may be more humane ☹
    - Broomstick method, in hand method

- Decapitation
    - Birds < 200g
    - Electrical activity in the brain persists for 13-14 seconds after, but other studies this does not necessarily mean pain is perceived
    - However, research has shown that in birds there may be some brain function for up to 30 seconds after decapitation [21], which makes this method unacceptable in welfare terms. Exsanguination without stunning is also unacceptable.
    - "Appears" to be humane but we don't have enough studies

- Exsanguination
    - Only after anesthesia/stunning b/c anxiety associated with extreme hypovolemia
    - Not as a sole method of euthanasia

- Pithing: only have anesthesia

g. BACK TO SONGBIRD CASE:
    i. Which method to use?
    ii. Use chart to compare methods, routes, etc.

h. GOOSE CASE:
    i. Physiologic components to discuss: breath holding in "diving birds", large animal, thrashing

VIII. References

1. Pokras M. Introduction to Wildlife Rehabilitation Purpose and Philosophy. In: Moore AT, Joosten S, eds. *NWRA Principles of Wildlife Rehabilitation*, 2nd ed. St. Cloud, MN: National Wildlife Rehabilitators Association; 2002:I.7.

2. Drew ML. Wildlife issues. In: *American Association of Zoo Veterinarians Guidelines for Euthanasia of Nondomestic Animals*. Yulee FL: American Association of Zoo Veterinarians, 2006: 19-22.

3. Canadian Council on Animal Care. CCAC Overview of Acceptable Euthanasia Methods. In: *CCAC Guidelines on: Euthanasia of Animals Used in Science*; 2010:12-16.

4. Leary S, Underwood W, Anthony R, *et al*. *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*.; 2020.

5. Hawkins P, Prescott MJ, Carbone L, *et al*. A good death? Report of the second newcastle meeting on laboratory animal euthanasia. *Animals* 6(9):1-28, 2016. doi:10.3390/ani6090050 [link to https://www.mdpi.com/2076-2615/6/9/50].

6. McMillan FD. Rethinking euthanasia: Death as an unintentional outcome. *J Am Vet Med Assoc*. 219(9):1204-1206, 2001. doi:10.2460/javma.2001.219.1204 [link to https://avmajournals.avma.org/doi/abs/10.2460/javma.2001.219.1204].

7. Meyer RE. Euthanasia and Humane Killing. In: Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA, eds. *Veterinary Anesthesia and Analgesia The Fifth Edition of Lumb and Jones*. 5th ed. Ames, Iowa: Wiley Blackwell; 2015:130-146.

8. Bexton S, Couper D, Grogan A. Euthanasia. In: Williams K, ed. *Wildlife Rehabilitation: A Comprehensive Approach*, 1st ed. Eugene, OR: International Wildlife Rehabilitation Council; 2016:95-110.

9. Humane Society of the United States. *Euthanasia Reference Manual*, 2nd ed. The Humane Society of the United States; 2013. Available at animalsheltering.org/sites/default/files/content/euthanasia-reference-manual.pdf. Accessed Feb 6, 2020.

10. Lockwood G. Theoretical context-sensitive elimination times for inhalation anaesthetics. *Br J Anaesth*. 104(5):648-655, 2010. doi:10.1093/bja/aeq051 [link to https://academic.oup.com/bja/article/104/5/648/310520].

11. New Zealand Veterinary Association. Euthanasia of avian patients. Sep 2017. New Zealand Veterinary Association web site. Available at nzva.org.nz/page/policyavian. Accessed Feb 6, 2020.

12. Orosz S. Birds. In: *Guidelines for the Euthanasia of Nondomestic Animals*. American Association of Zoo Veterinarians; 2006:46-49.

13. Stranz M, Kastango E. A review of pH and osmolarity. *Int J Pharm Compd.* 6(3):216-220, 2013. PMID: 23979188 [link to https://ijpc.com/Abstracts/Abstract.cfm?ABS=1043].

14. Seymour TL, Nagamine CM. Evaluation of isoflurane overdose for euthanasia of neonatal mice. *J Am Assoc Lab Anim Sci*. 55(3):321-323, 2016. PMCID: PMC4865695 [link to https://www.ingentaconnect.com/content/aalas/jaalas/2016/00000055/00000003/art00012;jsessionid=4c212jul0b8bq.x-ic-live-03].

15. Brown RN. Summary of methods for euthanasia of wild and exotic vertebrates. In: *American Association of Zoo Veterinarians Guidelines for Euthanasia of Nondomestic Animals*. Yulee FL: American Association of Zoo Veterinarians, 2006: 95-102.

16. Edis A. Humane euthanasia of exotic and wildlife species. *Vet Times* 49(43): Oct 28, 2019. Available at vettimes.co.uk/article/humane-euthanasia-of-exotic-and-wildlife-species/. Accessed Feb 19, 2020.

17. Moody CM, Weary DM. Mouse aversion to isoflurane versus carbon dioxide gas. *Appl Anim Behav Sci* 158:95-101, 2014. doi.org/10.1016/j.applanim.2014.04.011 [link to https://doi.org/10.1016/j.applanim.2014.04.011].

18. USDA, APHIS, Health TC for FS& P. Physical methods. In: *NAHEMS Guidelines: Mass Depopulation and Euthanasia*. ; 2011:17-25.

19. American Veterinary Medical Association Panel on Euthanasia. *AVMA guidelines for the euthanasia of animals: 2020 edition*. Available at avma.org/sites/default/files/2020-01/2020_Euthanasia_Final_1-15-20.pdf. Accessed Feb 6, 2020.

20. Woolcott CR, Torrey S, Turner P V., et al. Assessing a method of mechanical cervical dislocation as a humane option for on-farm killing using anesthetized poults and young Turkeys. *Front Vet Sci*. 25(NOV):1-10, 2018. doi:10.3389/fvets.2018.00275 [link to https://www.frontiersin.org/articles/10.3389/fvets.2018.00275/full].

21. Gregory NG, Wotton SB. Effect of slaughter on the spontaneous and evoked activity of the brain. *Br Poult Sci* 27:195-205, 1986. doi: 10.1080/00071668608416872 [link to https://www.tandfonline.com/doi/abs/10.1080/00071668608416872].