Standard Operating Procedure (SOP)

Euthanasia of Reptiles Incidentally Injured During Research, Inventory or Salvage Projects Performed by Qualified Environmental Professionals

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Purpose

- To provide current Standard Operating Procedures (SOP) for the humane euthanasia of reptiles that are incidentally injured during research, inventory or salvage projects performed by Qualified Environmental Professionals (QEP).

Application

- This SOP applies to QEP and those under their supervision who are involved in the euthanasia of reptiles in British Columbia (BC) to ensure that all animals are euthanized in a humane and safe manner.
- The QEP must directly oversee and ensure compliance with this SOP and that all animals are confirmed to be dead prior to disposal.
- The QEP must find a veterinarian willing to provide veterinary care to injured reptiles that do not require euthanasia and for over the phone consultation. The veterinarian's contact information must be provided in the permit application.

Background

- British Columbia currently has 9 species of snakes, 3 species of lizards and 3 species of turtles that are protected under the BC *Wildlife Act*. It is illegal to kill, collect, possess or harass them without a permit.
- Field conditions present limitations to methods than can be safe and practical to use, but do not reduce the need for safe and humane euthanasia techniques to be applied when required.
- Acceptable and unacceptable methods are outlined based on current scientific evidence and field safety. As new information, equipment and pharmaceuticals become available, revisions to this protocol will occur.
- Acceptable chemical euthanasia methods have been extrapolated from the limited research currently available on non-controlled reptile euthanasia agents. Species differences in response to chemical euthanasia methods are likely to exist within BC reptile species and may require using higher concentrations or alternative methods (physical euthanasia techniques).
    - Any instances where chemical euthanasia appears ineffective or has reduced efficacy must be reported after the reptile(s) has been humanely euthanized with physical methods.

Overview of Methods

- Euthanasia techniques must prioritise methods that minimise or prevent pain and stress and result in rapid loss of consciousness, followed by death.
    - Techniques that do not meet this standard and/or are not currently approved methods in the scientific literature are not considered acceptable.

- Techniques must be reliable and repeatable.
    - Where operator fatigue may cause reduced animal welfare, alternative options or additionally trained personnel must be available.
- Proper handling and restraint of reptiles must occur to minimize the amount of pain, and/or distress experienced by them while ensuring human safety.
    - Continuous analysis and refinement of handling methods must occur to optimize the welfare of animals prior to and during euthanasia.
- All animals euthanized must be confirmed dead.
    - This can be challenging to determine in reptiles due to their ability to cope with hypoxia and hypotension. The heart can beat after death (brain electrical activity has ceased) and therefore, secondary methods of euthanasia should be used to ensure death (AVMA 2020).
- Reptiles must be kept within their preferred optimal temperature and humidity zone (POTHZ) prior to euthanasia or the conditions that they were found in.
- If transport is required, reptiles must be transported in secure containers that provide adequate ventilation and maintain the animals in their POTHZ.
- When direct handling is required, animals should be handled with powder free gloves to reduce the risk of contracting zoonotic diseases (e.g. Salmonella).

Equipment

- All equipment must be inspected regularly to ensure good working condition.
- All equipment must be properly cleaned and disinfected between different locations and/or populations of animals.
    - Refer to the amphibian and reptile decontamination protocol authored by the Canadian Herpetofauna Health Working Group (CHHWG 2017) for specific details.
        - https://www.canadianherpetology.ca/conservation/doc/HHWG%20Decontamination%20Protocol%202017-05-30.pdf
        - NOTE: If other decontamination protocols are in place for the region where work occurs, follow the more stringent protocols.
- Containers used to hold reptiles should not result in trauma to the animals. Pillowcases or other small, cloth bags can be used for holding snakes and lizards and will prevent trauma when placed inside a larger plastic container.

Chemical Euthanasia Methods

MS-222 (Tricaine methanesulfonate)

Product Information

- MS-222 is an isomer of benzocaine and a Health Canada approved drug.

- It is an AVMA approved method for the humane euthanasia of reptiles (AVMA, 2020) and is commonly used in field conditions to effectively euthanize a variety of reptile species including snakes, lizards and turtles (Conroy et al., 2009; Doss and Sladky, 2021; Wirth et al., 2020).
- MS-222 deteriorates in sunlight and breaks down into harmful by-products when in contact with metal containers. Only use this solution in plastic containers and away from sunlight.
- The product is commonly available in Canada under the tradename Syncaine® and in 100g and 1kg amounts.
- A veterinary prescription is required.
    - Contact a veterinarian with amphibian, fish or reptile experience and establish a valid Veterinary Client Patient Relationship (VCPR) to obtain a prescription.

Safety and Disposal

- Wear gloves and eye protection and always work outside with MS-222 whenever possible.
- If working inside with powered MS-222 a N95 mask should be worn.
- Animals euthanized with MS-222 should be handled with gloves and are not fit for consumption by people or other animals.
- Contact local authorities for specific disposal requirements in your jurisdiction. Generally, disposal of animals can occur at a landfill and disposal of the MS-222 solution down a sanitary sewer.
    - Do not dispose of animals euthanized with MS-222 or solution in the field.

Anesthetic Technique

Injectable Method

Summary of Equipment Needed:

- MS-222
- Sodium bicarbonate
- Measuring containers
- pH test strips or reader.
- Clean, de-chlorinated water.
- Gloves, eye protection, +/- mask.
- Plastic, sealable bag.
- Tweezers or pliers.
- Towel.

- Syringes (1ml-12ml)
- Needles (3/4 inch, 20-25 gauge)

Technique:

- MS-222 must be buffered prior to use as it is acidic and causes aversive behaviours in conscious animals when not buffered. Buffering also improves the efficacy of the drug, with lower concentrations needed to anesthetize animals (Gentz, 2007).
    - Use sodium bicarbonate (baking soda) to buffer the solution to a final pH of 7.0 (Conroy et al., 2009).
    - Generally, this requires 1:1 to 2:1 ratio of sodium bicarbonate to MS-222.
    - Add the sodium bicarbonate to the MS-222/water mixture. Do not pre-mix the powders together.
- Follow the procedure steps outline in Conroy et al., 2009 to make the 1% and 50% solutions at various volumes depending on the size and/or number of reptiles to be euthanized.
    - Briefly, make the 50% solution by adding equal parts MS-222 to water. For example, add 1 teaspoon (5 grams) of MS-222 powder to 5ml of water. Stir until dissolved and the solution is clear.
    - To make a small amount of the 1% (10mg/ml) solution, add 0.1-0.2ml of the above 50% solution for every teaspoon (5ml) of water.
        - Add sodium bicarbonate to the 1% solution until it reaches a pH of 7. At this point the solution will be slightly cloudy (Conroy et al., 2009).
- Euthanasia of reptiles with MS-222 requires 3 steps.

Step 1:

- Intracoelomic injection of a buffered 1% stock solution of MS-222 at a dosage of 250-500mg/kg (Conroy et al., 2009).
- To euthanize a 100 gram snake, 5mls of the above 1% solution is required for a 500mg/kg dosage.
- Rapid loss of consciousness within 30 seconds to 4 minutes should occur (Conroy et al., 2009).
- Intracoelomic injections in reptiles should be performed with the animal on its side (lizards and turtles) or on their backs (snakes) so that dependent organs move away from the injection site. No air,

blood or fluids should be present when drawing back on the plunger prior to injection. If these are found, retract the needle and redirect.

- The injection must be performed near the rear of the animal to avoid injection into the lungs.
- In lizards, insert the needle just in front of a rear leg and with the needle directed towards the opposite shoulder.
- In turtles, insert the needle in front of the hindlimb (prefemoral fossa) into the ventral portion of the coelom.
- For snakes, restrain the animal so the ventral (bottom) of the snake is facing upwards.
    - The injection is performed just forward of the vent. From the vent count 4-6 ventral scales towards the head to ensure the needle enters the caudal coelomic cavity.
    - The intracoelomic injection can be performed with the snake angled down at about a 45-degree angle so the internal organs move away from the injection site. Insert the needle at the junction between the ventral and lateral scutes.

Step 2:

- Once confirmed unconscious (lack of righting reflex) a second injection of unbuffered, 50% MS-222 is injected intracoelomically at 0.1-1.0ml depending on the size of the reptile (Conroy et al., 2009).
- After injection, respirations and heart beat should cease within a few seconds to minutes (Conroy et al., 2009).
- However, because confirming death in reptiles can be challenging, a third step is required to ensure death.

Step 3:

- A physical method(s) is used to ensure a humane death.
- Confirm deep anesthesia by forcefully pinching a toe (lizard and turtle) or tip of the tail (snakes) with tweezers or pliers.
- Firm pressure is required, otherwise a false result could occur.
- The reptile will not respond to a toe/tail pinch when deeply anesthetized.
- If reflexes are present, re-administered another full dose of the 50% MS-222 solution, wait 5 minutes and reassess.
- When confirmed to be deeply anesthetized, pith the brain by flexing the head down, inserting the needle into the depression at the back of the skull and directed towards the mouth.

- After the needle enters the brain, move it from side to side multiple times (4-6) to ensure brain destruction.
- Alternatively, decapitation followed immediately by pithing can be performed to ensure death when locating the pithing site is difficult.

Physical Euthanasia Methods

Application

- These methods are acceptable when applied by properly trained and skilled operators and result in rapid loss of consciousness.
- Provides a practical method of euthanasia if pharmaceutical agents are not available or there are concerns about contamination of the carcass with chemicals (CCAC, 2023).
- When emergency euthanasia is required to alleviate immediate suffering of an animal due to a severe injury in the field (AVMA, 2020; CCAC, 2010; CCAC, 2023) and other methods are not feasible or timely.

Overview

- Techniques that are conditionally acceptable when it results in rapid loss of consciousness and is immediately followed by a secondary physical method to ensure death (AVMA, 2020; CCAC, 2010; CCAC, 2023).
- Must only be performed by experienced and skilled operators.
- Must be conducted in a separate area from other animals.

Captive Bolt

- Captive bolts are an approved tool for euthanizing reptiles (AVMA, 2020; Doss and Sladky, 2021; Nevarez et al., 2014).
- Non-penetrating captive bolts (NPCB) used for poultry or small mammals under 10kg (e.g. CASH® Small Animal Tool; Frontmatec Accles & Shelvoke, 2024) can be used as a 2-step euthanasia process for small snakes, lizards and turtles if the turtle is unable to retract their head into the carapace due to a severe injury or illness.

Step 1:

- The NPCB must be placed directly on the skull, just behind the eyes to be effective. See Figure 1 for landmarks and NPCB placement.
    - Draw an imaginary “X” between the eyes and back of the jaw.
    - Where the lines intersect at midline is the target area (circle in Figure 1).
    - Apply this technique perpendicular to the top of the skull to ensure maximum impact.

- Use the flat faced concussion head and the brown charge if using the CASH® Small Animal Tool.
- Hearing protection should be worn when using a captive bolt.
- Proper maintenance and cleaning after each use is required to ensure the device remains functional. Refer to the NPCB manual for details.

Step 2:

- Pithing the brain must occur after applying the NPCB. Pithing may occur through the skull fracture created by the NPCB or at the base of the skull.

Manually Applied Blunt Force Trauma

- Used in remote field conditions where immediate euthanasia is required to end the suffering of an animal and other methods are not feasible or timely.
- Can result in immediate unconsciousness and death if sufficient force is used, but a secondary physical technique (e.g. pithing) is required to ensure death.

Technique:

- The reptile is held with the head against a hard, flat surface that will not move or compress when the technique is applied (e.g., against a flat rock or a piece of hardwood). Soft ground is not acceptable as it will compress on impact.
- While holding a flat metal punch (0.5-1cm diameter) against the skull, a metal hammer, or similar hard object is used to make forceful impact with the punch.
- The appropriate skull location is illustrated in Figure 1 and summarized as:
    - Draw an imaginary "X" between the eyes and back of the jaw.
    - Where the lines intersect at midline is the target area.
    - Apply this technique perpendicular to the top of the skull to ensure maximum impact.

Secondary Euthanasia Methods

Application

- These methods are unacceptable as the primary means of euthanizing reptiles and must only be used when the animal is already unconscious and/or has no deep pain sensation.
- They must only be performed by experienced and skilled team members.

Pithing

- Pithing with a metal probe/rod (e.g., 20-22 gauge, 1-1.5 inch needle), is performed by holding the reptile facing away from you, flexing the nose down and locating the soft depression (foramen magnum) at the end/base of the skull.

- Insert the probe into the skull 1-2 cm and move it from side to side to destroy the brain.
- Double pithing is performed by redirecting the probe towards the body to also sever the spinal cord.

Decapitation Then Pithing

- Performed after deep anesthesia or blunt force trauma to the brain results in unconsciousness.
- Decapitation will require a sharp metal knife or large pruning shears.
- Remove the head just behind the back of the jaw where the neck meets the skull.
- Because the central nervous system (CNS) of reptiles can tolerate low oxygen and blood pressure, decapitation must always be followed by pithing to ensure immediate death (AVMA, 2020).
- Decapitation is used in situations when locating the site for pithing is challenging.
    - Once decapitated, the brain and spinal cord are exposed, making the pithing location obvious.

Figure 1: Snake and lizard brain locations illustrated by drawing an imaginary 'X' from the eyes to the back of the jaw. Where the lines intersect is the location of the brain and where the captive bolt or blunt force trauma should be applied (circle). Photo credit: author.

Unacceptable Methods

1. Cooling then Freezing or Freezing Alone
    - Cooling then freezing or freezing alone is not an acceptable euthanasia method (CCAC, 2010; CCAC, 2023).

2. Pithing and Decapitation on Conscious Animals
    - Animals must be unconscious prior to using these methods.
    - Only in emergency situations where the animal is already severely injured and in pain can decapitation followed immediately by pithing be used to alleviate suffering in conscious animals and only if other methods are not available.

3. Cervical Dislocation
    - Due to the CNS of reptiles being able to tolerate hypoxia and hypotension, cervical dislocation will not result in rapid loss of consciousness or death and therefore, is unacceptable in conscious animals. If performed in unconscious reptiles it must be immediately followed by pithing.

4. Alcohol Immersion
    - This method does not result in rapid loss of consciousness prior to death.
    - Animals become distressed when placed in concentrated alcohol solutions and therefore, this method is unacceptable.

5. Gunshot
    - Gunshot is an acceptable method of euthanizing larger animals when applied appropriately. However, the risk of ricochet of projectiles at point blank range while small reptiles are restrained is a safety concern that does not warrant the risks of this method for euthanasia of small reptiles.

6. Other
    - Any other method not described above as an acceptable technique and/or not previously approved by the BC government.

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