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AMPHIBIAN RESEARCH & MONITORING INITIATIVE
NATIONAL WILDLIFE HEALTH CENTER
6006 SCHROEDER ROAD
MADISON WI 53711-6223
Phone: (608) 270-2400 Fax: (608) 270-2415

ANESTHESIA OF AMPHIBIANS IN THE FIELD

STANDARD OPERATING PROCEDURE

ARMI SOP No. 104

I. PURPOSE. Outline the use of benzocaine and MS222 for reversible anesthesia of adult amphibians in the field, so that blood samples and toe/skin biopsies may be collected, and passive integrated transponders (PIT) tags or dyes may be implanted.

II. SCOPE. The described procedures and anesthetic solutions are intended for use on adult (post metamorphic) frogs, toads and salamanders in field situations and generally will require 10 to 90 minutes to induce anesthesia, collect samples, and recovery. While these techniques may be used on tadpoles, caudate larvae and neotenes, the procedures and solutions have not been tested on the latter two groups of amphibians.

III. EQUIPMENT & SUPPLIES.

A. Jar or plastic container with lid (250, 500 or 1,000 ml).
B. Anesthetics: Benzocaine or MS222 (tricaine methanesulfonate).
C. Buffer (sodium bicarbonate powder or 1-normal sodium hydroxide solution)
D. Water, clean & chemical-free (i.e., free of iodine, chlorine, bleach, etc).

IV. BACKGROUND.

A. Definitions.

1. Induction time: The amount of time it takes for an animal to become anesthetized
2. Duration: The amount of time an animal remains anesthetized for painful procedures
3. Recovery time: The amount of time necessary for an animal to fully awaken or recover from an anesthetic. Recovery begins as soon as the animal is removed from an anesthetic solution.

B. Chemicals and Procedures.

Benzocaine and MS222 are closely related chemicals; MS222 is ten times as expensive as benzocaine. There is some preliminary evidence that MS222 may cause mild damage to red blood cells and the mesonephroi (kidneys).

Few chemicals are absorbed through the skin of vertebrates, but the skin of amphibians is highly permeable to many chemicals, hence, soaking the amphibian in a solution is an effective method to induce anesthesia.

Anesthesia is not a simple procedure. It is well to remember that all drug treatments are controlled poisoning of the body; the emphasis is on "controlled." Aspirin, penicillin, and benzocaine are all drugs that are used in controlled doses to cause changes in the body. Two important aspects of inducing anesthesia are understanding the **delirium phase** and that **induction of anesthesia is not instantaneous**. As animals go through the various levels of anesthesia, one alarming phase to inexperienced persons is the agitation or delirium phase. Early during induction of anesthesia, the animal may appear to be agitated and may thrash about as if panicked or irritated; this is normal. Within a few seconds or minutes, the animal will fall into a

deeper level of anesthesia in which thrashing ceases. Anesthesia is not instantaneous, hence, an animal will continue to fall into deeper levels of anesthesia after it is removed from the anesthetic solution, because it takes several moments for chemicals in the skin and blood to reach equilibrium and then be carried to the brain. Hence, the concentration of the anesthetic solution should be sufficient that the depth of induced anesthesia can be controlled. A higher concentration of anesthetic solution could put the amphibian into an unwanted, very deep anesthesia that would require 2-4 hours, or more, for recovery or could even kill the animal.

V. PREPARATION.

A. Anesthetic Solutions. Making the anesthetic solution in the field requires mixing the powder anesthetic in clean, chemical-free water and adjusting the pH. If sites are accessible by vehicle, then the anesthetic solutions may be mixed in the laboratory and transported pre-mixed to the site, but for sites that are remote and require backpacking of supplies, it probably will be desirable to carry the anesthetic in a powder form and mix the solution in the field. Benzocaine is poorly soluble in water, while MS222 is much more soluble in water. Hence, if benzocaine is to be used, it must first be dissolved in alcohol, and then the tincture may be added to water.

B. Anesthetic powders. It is recommended that the anesthetic powders be measured in the laboratory into 1 gram lots, and carried in small vials or blood tubes into the field.

C. Benzocaine preparation. Dissolve 1 gram of benzocaine powder in 3-4 ml of 75 to 95% ethanol. When all or most of the white powder has dissolved, the tincture may be mixed into 500 to 1000 ml of fresh clean water. It is likely that some white powder may remain undissolved in the 500-1000 ml of water. The solution, even with the undissolved white powder, may then be used to anesthetize toads.

D. MS222 preparation. A solution of 1:2000 is recommended for anesthesia of tadpoles, larvae, and frogs. Dissolve 0.5 gram of MS222 into one liter of fresh clean water. With pH paper, check the pH of the solution. If the solution is less than 6, then the pH should be adjusted with 1 to 5 drops of 1 N sodium hydroxide or 1-3 grams of sodium bicarbonate powder until the solution is a pH of 6 to 8.

VI. PROCEDURE.

A. Benzocaine. Benzocaine is recommended for true toads, spadefoots and large salamanders. A 0.2% solution recommended for anesthesia of toads and large salamanders. In each field kit, one gram aliquots of benzocaine dissolved in ethanol are provided in one dram screw-cap vials.

1. Pour contents of one prepared aliquot of tincture of benzocaine in 500 ml of chemical-free water (drinking water that is free of iodine & bleach, or stream water).

2. After the tincture is thoroughly mixed into the water, check the pH of the solution. The pH of the solution should be between 6 and 8. Benzocaine is mildly acidic and may decrease the pH of the water by 1 to 2 pH points. If the water was at pH 7 or less to start with, then it may be necessary to adjust the pH of the solution. This is best done with 1-3 grams of sodium bicarbonate crystals or powder or 1 to 3 drops of 1 normal sodium hydroxide solution (NaOH).

3. Temperature of the anesthetic solution should be equivalent to ambient atmosphere, pond or stream water at the site. Warm water (>25° C) or solutions that have been left in direct sunlight or warm tents should be chilled to <25° C before being used to anesthetized amphibians.

4. Induction of anesthesia. The amphibian is placed into a container of the solution that is 1-2 cm deep. The anesthetic solution only needs to cover the ventral abdomen and thighs. For salamanders, a greater depth and quantity of solution will be needed to completely immerse the gills and body.

5. Levels of anesthesia. Usually, the amphibian does not respond to immersion in the anesthetic for several minutes. After several minutes the amphibian may appear to thrash or become hyperactive; this is normal and is to be expected. In a few more minutes, the amphibian will become quiet and inactive; at this point the animal must be carefully monitored every 1-2 minutes. When the amphibian fails to respond to light touches of the eyelids (by blinking and retracting the eye into the socket) or to a gentle pinch to a toe-tip, then

the animal is suitably anesthetized to collect blood, biopsies or implant dyes or PIT tags. Immediately remove the amphibian from the anesthetic solution and thoroughly rinse its skin with fresh water. This rinse will prevent the animal from falling into deeper levels of anesthesia by removing unabsorbed chemical from the skin surface.

B. MS222 (tricaine methanesulfonate). MS222 is recommended for tadpoles, small larvae, and post metamorphic frogs. MS222 will anesthetize toads, but it may take 30-90 minutes.

1. Mix 0.5 gram of MS222 powder in one liter of fresh, clean water and stir until dissolved.

2. Check the pH of the solution with pH paper. If the solution is less than pH 6, then the pH should be raised to 7 by the addition of 1-3 grams of sodium bicarbonate powder or 1-3 drops of 1 normal sodium hydroxide (NaOH).

3. Temperature of the anesthetic solution should be equivalent to ambient atmosphere, pond or stream water at the site. Warm water (>25° C) or solutions that have been left in direct sunlight or warm tents should be chilled to <25° C before being used to anesthetized amphibians.

4. Inducing anesthesia. The amphibian is placed into a container of the solution that is 1-2 cm deep. The anesthetic solution only needs to cover the ventral abdomen and thighs of post metamorphic frogs, but should be deep enough to completely cover tadpoles, larvae and neotenes.

5. Levels of anesthesia. Usually, the amphibian does not respond to immersion in the anesthetic for several minutes. After several minutes the amphibian may appear to thrash or become hyperactive; this is normal and is to be expected. In a few more minutes, the amphibian will become quiet and inactive; at this point the animal must be carefully monitored every 1-2 minutes. When the amphibian fails to respond to light touches to the eyelids (by blinking or retracting the eye into the socket) or to a gentle pinch to a toe-tip, then the animal is suitably anesthetized to collect blood or perform other minor surgical procedures (e.g., implantation of a PIT tag, toe-clipping or skin biopsy). Immediately remove the amphibian from the anesthetic solution and thoroughly rinse its skin with fresh water. This rinse will prevent the animal from falling into deeper levels of anesthesia by removing unabsorbed chemical from the skin surface.

C. Duration of Anesthesia. Once the amphibian is anesthetized and thoroughly rinsed in fresh water, it should remain at a level of anesthesia sufficient for collection of blood and biopsies for 10-20 minutes.

D. Recovery from Anesthesia. The length of time required for an amphibian to recover from anesthesia depends on the life stage, anesthetic, temperature, species, and depth of anesthesia. Usually, recovery is to be expected in 30 to 90 minutes after the animal is rinsed in fresh water. The amphibian should be placed in fresh water while recovering, and kept away from direct sunlight and temperatures >25° C. When the amphibian appears to be able to swim or walk normally, it may be released. If possible, the amphibian should be released in a hidden, cool, dark place in order to minimize the urgency for the amphibian to immediately flee and to minimize the chances of being detected by predators.

E. Reuse of Anesthetic Solutions. Sufficient anesthetic should remain in the solution after one amphibian is anesthetized so that 2-3 more adult amphibians or 3-5 tadpoles or larvae could be anesthetized. Each successive amphibian that is placed into the solution will require more time than the previous animal(s) for induction of anesthesia. Anesthetic solutions should be reused only on normal-appearing amphibians that are present at the same site or pond.

Discard used anesthetic whenever:

1. Moving from one pond or site to another
2. A sick or abnormally behaving amphibian has been anesthetized
3. The anesthetic solution becomes fouled with feces or chunks of molted skin
4. The solution becomes cloudy or discolored

VII. MISHAPS.

A. Anesthetic deaths. The key to avoiding deaths of amphibians due to anesthesia is to not let the animal become too deeply anesthetized. Careful monitoring of each animal is needed so that the amphibian is not

allowed to remain in the anesthetic solution after becoming unresponsive to the eyelid-touch and toe-pinch tests. Brief but thorough rinsing of the anesthetized amphibian in fresh water should be done immediately after there is no response to the eyelid-touch and toe-pinch tests. Some amphibians with serious infections, cancers, poisonings, or weight loss (not all of which can be detected by external examination of the recently captured animal) may die during anesthesia. In such cases, the carcass should be saved for thorough diagnostic examinations. On average, death rates of 0.25 to 2.0% have occurred in amphibians in California that were anesthetized with benzocaine. Even in hospitals, death rates of 0.1 to 1% of anesthetized humans may occur. Note: Overdosing with anesthetics is a common humane method to euthanize amphibians.

B. Prolonged Anesthesia. Occasionally, amphibians may take longer than 30-90 minutes to recover from anesthesia. In such cases, it is prudent to hold the animal all day or overnight to assure full recovery before release.

VIII. DISPOSAL OF ANESTHETIC SOLUTIONS.

A. Field sites. At remote sites, used anesthetic solutions should be poured into a hole in the ground and the hole covered with soil. Do not discard anesthetic solutions into surface water. If possible, the anesthetic solutions should be discarded in a laboratory.

B. Laboratories. If anesthetic solutions are used in the laboratory situation, then most may be discarded in the sink along with other sewage. However, some regions of the country have strict regulations on what chemicals may be placed into the sewage, so inquiries as to proper disposal methods in your community should be made.

IX. SURGICAL MANAGEMENT with or without anesthesia.

A. Antiseptic sprays. If anesthesia was done to perform an Ainvasive procedure@ on the amphibian, such as collection of blood from the heart, toe-clipping, or implantation of a PIT tag, it is recommended that Bactine7 be sprayed on the injection, biopsy or amputation site. At present, the only commercially available antiseptic spray that is recommended for use on amphibian skin is Bactine7. This is because, at the time this SOP was prepared, the only available antiseptic spray for use on humans and animals that does NOT contain alcohol, is Bactine. All other antiseptic and antibiotic sprays contain alcohol. Alcohol is not recommended for use on amphibians because it may be absorbed through the skin, and alcohol may dissolve vital secretions on the amphibian skin that protect the individual from dehydration and infections.

B. Ointments. Likewise, creams and ointments should not be used on amphibian skin because of the potential to disrupt protective secretions and interfere with respiration and water balance.

X. REFERENCES

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D. Earl Green, DVM, DACVP
U.S.G.S., National Wildlife Health Center
6006 Schroeder Road
Madison WI 53711-6223
Tel: 608-270-2482