

Southeast Coast Network

National Park Service
Inventory & Monitoring Program



Standard Operating Procedure (SOP) 1.4.18

Amphibian Euthanasia Procedures

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Summary

This SOP outlines the SECN technique to euthanize an amphibian if necessary. The methods for monitoring amphibian communities have been selected and designed to minimize the amount of animal handling necessary to meet the protocol's objectives. Prior to conducting field work, it is expected that all members of the field crew read and understand the safe handling procedures, the euthanasia procedures outlined in this SOP, and the circumstances when it is proper to consider humane euthanasia of distressed amphibians. **To date, the use of safe handling procedures by SECN staff while conducting routine amphibian monitoring has *not* warranted euthanasia of any distressed animals**, and it is the intent of the SECN to continue operations in such a manner that use of these procedures remains unnecessary. However, because the potential exists for accidental and inadvertent harm to handled individuals, the following procedures are set forth should they be necessary.

Revision Log

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

Overview

This SOP outlines the SECN technique to euthanize an amphibian if necessary. The methods for monitoring amphibian communities have been selected and designed to minimize the amount of animal handling necessary to meet the protocol's objectives. Occasional handling is only necessary to ensure accurate identification in individuals with atypical patterns or coloration, or to differentiate species that have only subtle differences that warrant closer inspections (e.g., the number of costal grooves, tadpole mouthparts). During the handling process, all measures possible are employed by field personnel to avoid distress or harm in accordance with the Beaupre et al. (2004) guidelines for safe handling of live reptiles and amphibians.

Prior to conducting field work, it is expected that all members of the field crew read and understand the safe handling procedures, the euthanasia procedures outlined in this SOP, and the circumstances when it is proper to consider humane euthanasia of distressed amphibians. **To date, the use of safe handling procedures by SECN staff while conducting routine amphibian monitoring has *not* warranted euthanasia of any distressed animals**, and it is the intent of the SECN to continue operations in such a manner that use of these procedures remains unnecessary. However, because the potential exists for accidental and inadvertent harm to handled individuals, the following procedures are set forth should they be necessary.

If specimens are not destroyed during the euthanasia process or in postmortem analysis, they will be preserved and maintained in accordance with NPS specimen-management guidelines (NPS 2006, Russel 2006, Wright et al. 2008).

Circumstances Warranting Euthanasia

The determination to euthanize an amphibian is made by the Wildlife Biologist and although it should be made quickly to avoid prolonged suffering, the decision should be based upon the situation that caused the injury, other facts at hand, knowledge of the species, extent of injury, experience, and any other pertinent information before the final determination that the animal is moribund and in need of euthanasia.

It is important to note that the euthanasia techniques presented here are appropriate **ONLY** for amphibians and should **NOT** be used on any other injured animals. These procedures should also be used only in the event that an amphibian is mortally injured during the implementation of SECN activities.

It is the Wildlife Biologist's decision on what to do if, during the course of SECN activities, other injured animals are encountered (either non-amphibians or amphibians injured by causes unrelated to SECN activities). In such cases it may be appropriate to notify park resource staff, implement an appropriate technique the biologist is trained to conduct, or other options that are deemed appropriate in the best professional judgment of the Wildlife Biologist.

Materials

- One 500-mL wide-mouth plastic bottle containing 500 mL of DI water.
- One 500-mL wide-mouth plastic bottle containing 320 mL of DI water.
- Two 250-mL wide-mouth plastic bottles containing 160 mL of DI water.
- Two 125-mL wide-mouth plastic bottles containing 80 mL of DI water.
- Two 60-mL wide-mouth plastic bottles containing 40 mL of DI water.
- Two labeled pre-weighed / pre-packaged sealed bags of MS222 for 320 mL of DI water.
- Two labeled pre-weighed / pre-packaged sealed bags of MS222 for 160 mL of DI water.
- Two labeled pre-weighed / pre-packaged sealed bags of MS222 for 80 mL of DI water.
- Two labeled pre-weighed / pre-packaged sealed bags of MS222 for 40 mL of DI water.
- Vinyl gloves, several sizes.
- Pithing probes / straight disposable dissecting (disinfect between uses or dispose).
- Plastic graduated cylinder.
- Hemostats or tweezers.
- Bottle labels.
- Dust mask.
- Safety goggles.
- Permanent marker.
- MSDS for MS222.
- Printed version of this SOP.
- Carrying case for all euthanasia supplies.
- Disinfecting materials identified in SOP3a, organized in a separate container.

Preparation

Read instructions and the Material Data and Safety Sheet (MSDS) for MS222 in their entirety before proceeding. Preparation of MS222 should take place in a ventilation hood while wearing all necessary personal protective equipment (PPE). All euthanasia materials are prepared before the field season and stored together in a dark and dry storage field container. Several different size bottles are used to accommodate different sized amphibians. To avoid under-dosing, the bottles and corresponding MS222 dosage packets are labeled identically. The labels are: A = 500mL bottles, B = 250mL bottles, C = 125mL, D = 60mL. Each bottle and packet is labeled with the appropriate corresponding label. The preparation steps are as follows:

- 1) Obtain and organize all materials.
- 2) Print labels for MS222 packets. Figure 1 depicts the packet labels.

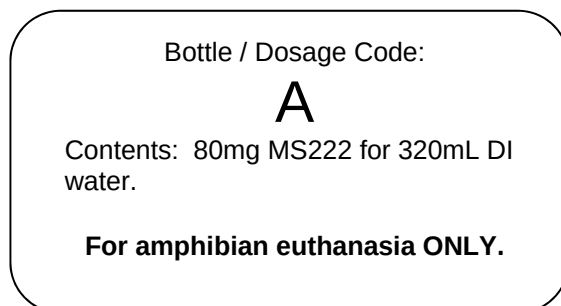


Figure 1. Example label for MS222 prepackaged dosage for field-amphibian euthanasia.

- 3) Affix labels to all empty re-sealable plastic bags.
- 4) Label bottles with a permanent marker, according to Table 1, to indicate their size and necessary dosage. Label the bottle just below the neck, and not on the bottle top.

Table 1. Bottle size, label, deionized water amount, MS222 amount for amphibian euthanasia.

Bottle and Dosage Label	Bottle Size (mL)	Amount of DI Water (mL)	MS222 Dosage (mg)
A	500	320	80
B	250	160	40
C	125	80	20
D	60	40	10

- 5) Fill bottles with the amount of DI water identified in Table 1. This allows for displacement and complete immersion of amphibian.
- 6) Put on PPE (gloves, goggles, long sleeves).
- 7) Place pre-labeled plastic re-sealable bags, measuring scale, and MS222 in ventilated hood.

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- 8) Weigh MS222 according to Table 1 and place prefilled bags, grouped by bottle/packet label (i.e., same dosage and same bottle size) into a larger re-sealable plastic bag.
- 9) When bags are prepared for all different dosage schedules and bagged together, place all four bags in another re-sealable plastic bag.
- 10) Seal MS222 container thoroughly, wipe down scale with a disposable towel, and remove all items from the ventilation hood.
- 11) Place all materials in field euthanasia container, and store remaining unused materials in their proper location.

Procedure

The following should be reviewed quickly prior to euthanization.

The guidelines set forth in American Veterinary Medical Association (2007) identify several standard procedures for humane euthanasia of animals from which a technique recommended for amphibians that was adopted and is detailed here - immersion in Tricaine methanesulfonate (MS222) followed by pithing. The recommended dosage for amphibian euthanasia immersion is 250 mg MS222 per liter of deionized (DI) water for a duration of at least ten minutes; although the duration can vary among species and is checked by evaluating eye-blink response action. MS222 must be mixed on an as-needed basis because premixed MS222 can degrade or convert to toxic substances after standard storage procedures or exposure to sunlight. Solutions greater than 500 mg/L are acidic and not acceptable for euthanasia, therefore the following instructions follow the recommended mixture of 250 mg/L for amphibian euthanasia. A pithing procedure follows the MS222 immersion to ensure the animal is euthanized. The action of pithing in amphibians is when a rigid metal rod is inserted into the cranial vault through the foramen magnum. This severs the spinal cord and destroys the brain. The euthanasia technique chosen was based upon its effectiveness and ability to be administered successfully in the field, while allowing the field team to avoid exposure to sharps or dangerous chemicals.

As with all wildlife handling activities conducted by SECN staff, field personnel are to adhere to strict sanitary measures to prevent the spread of pathogens.

Detailed instructions are as follows:

- 1) Put on PPE (gloves, goggles, long sleeves).
- 2) Determine appropriate bottle size necessary for animal euthanasia. Estimate accurately to allow for fluid displacement from the mass of the animal and to ensure the animal will be fully immersed in the mixture. Choose corresponding packet of MS222.
- 3) Prepare mixture (i.e., mix MS222 packet and DI water) downwind and away from other people.
- 4) MS222 is easily soluble in water, but to ensure the powder dissolves and is suspended relatively equally throughout the solution, shake vigorously for one minute.
- 5) Re-seal the empty plastic bag containing the MS222 and return it to its original bag.
- 6) Place amphibian head-first in bottle with MS222 and DI solution, ensuring the animal is completely submerged.
- 7) Screw the cap on the bottle and place it in cool, dark, place.
- 8) Leave animal in the solution for at least ten minutes; although the duration can vary among species and is checked by evaluating eye-blink response action.
- 9) Remove the animal with hemostats or tweezers for pithing procedure.

- 10) Place the animal flat on its ventral surface facing away from you or hold the animal in your hands facing away from you with your forefinger beneath its chin and thumb on its upper back (Figure 2).

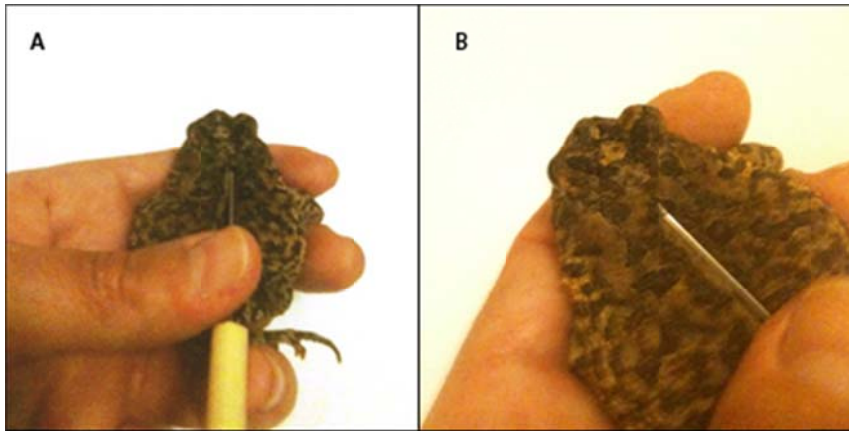
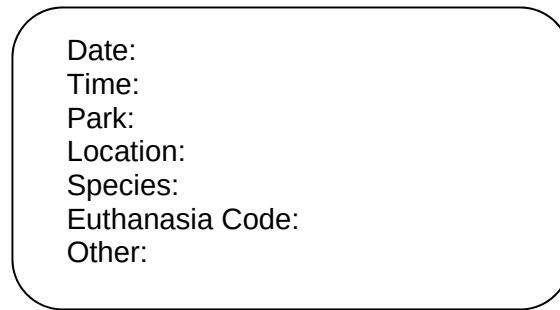


Figure 2. Example of amphibian pithing procedure; a) securely hold anesthetized animal between index/forefinger and thumb, position pithing probe parallel with spine, b) position tip of probe at the base of the skull (foramen magnum) and opening into the cranial chamber. **NOTE:** Animal was not pithed; images are for illustrative purposes only.

- 11) Flex the animal's neck slightly backwards to locate the foramen magnum. This can be identified by a slight midline depression and soft spot at the base of the skull. This is the location where the pithing probe will be inserted (Figure 2).
- 12) Insert the pithing probe quickly, move the probe rapidly in a circular motion several times, then remove the probe. Any movement or noise during the pithing process is most likely a reflex action and should not be of concern. The MS222 immersion alone is very successful at euthanasia, however pithing ensures the animal is euthanized.
- 13) Touch the eye and eyelid with pithing probe to test for a blinking response. If the animal blinks, immediately repeat the pithing process.
- 14) Return animal to plastic bottle.

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- 15) Label bottle with date, time, species, park acronym, location (either sampling location or GPS location), euthanasia code, and any other relevant information (Figure 3). Euthanasia codes: 1 = mortally wounded by field personnel, 2 = found moribund.



Date:
Time:
Park:
Location:
Species:
Euthanasia Code:
Other:

Figure 3. Example label for bottle containing euthanized amphibian in the MS222 and deionized water mixture.

- 16) Place the labeled bottle with the euthanized animal in it in the euthanasia field kit.

Literature Cited

- American Veterinary Medical Association. 2007. AVMA guidelines on euthanasia (formerly Beaver, B.V., W. Reed, S. Leary, B. McKiernan, F. Bain, R. Schultz, B. T. Bennett, P. Pascoe, E. Shull, L. C. Cork, R. Franis-Floyd, K. D. Amass, R. Johnson, R. H. Schmidt, W. Underwood, G. W. Thorton, and B. Kohn. 2001. 2000 Report of the AVMA Panel on Euthanasia. *Journal of the American Veterinary Medical Association* 218: 669-696.) Available online: http://www.avma.org/issues/animal_welfare/euthanasia.pdf
- Beaupre, S. J., E. R. Jacobson, H. B. Lillywhite, and K. Zamudio. 2004. Guidelines for use of live amphibians and reptiles in field and laboratory research, second edition. Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists. 42pp. (Available online at <http://www.asih.org/files/hacc-final.pdf>).
- National Park Service. 2006. Museum Handbook, Part 1. National Park Service Museum Management Program.
- Russell N. 2006. SECN curatorial responsibilities guidance document. Wright, C. J. and K. Dahl-Kearney eds. 18 pages. South Florida Collections Management Center, Everglades National Park.
- Wright, C. J., J. C. DeVivo, K. Funk, S. McCort, B. Blankley, E. Davie, M. W. Byrne, T. Curtis, D. Lagana, and N. Russell, Editors. 2008. Southeast Coast Network Information Management and Archiving Plan Appendices. Natural Resource Report NPS/SECN/NRR—2008/062. National Park Service, Fort Collins, Colorado.