





OFFICE OF LABORATORY ANIMAL CARE

Working Instructions

WIN Number:	207	Euthanasia of Aquatic Frogs via Tricaine Methanesulfonate (MS-222)	Revision #:	0
Date Effective:	8/18/23		Supersedes:	0

Author(s):	Jennifer Frohlich	Sign:		Date:	8/18/23
Director:	Dr. Gregory Lawson	Sign:		Date:	8-21-23

PROCEDURE

1. All work instructions must be demonstrated by the trainer and performed by the trainee.
2. Required PPE:
 - a. N-95 respirator
 - b. Safety glasses or goggles
 - c. Gloves
3. Solution preparation:
 - a. Don gloves and safety glasses/goggles.
 - b. Fill a clean container with DI water (to fully submerge the number of frogs to be euthanized).
 - c. Wear the N-95 respirator mask if powder MS-222 is being handled outside of a fume hood. If working within a fume hood or once the powder is completely dissolved into water, the N-95 mask can be removed
 - d. Measure/weigh 2g MS-222 powder per 1L of water using a gram scale and disposable weigh boat.
 - e. Measure/weigh 4g sodium bicarbonate per 1 L of water using a gram scale and disposable weigh boat.
 - f. Mix powdered MS-222 and sodium bicarbonate into DI water until completely dissolved
 - g. Measure pH using pH strip (7.0-7.4) by dipping it into the MS-222 solution and matching the color changes to the key on the bottle.
 - h. If pH is still too acidic (below 7.0), add more sodium bicarbonate little by little, then retest pH until the solution is within this range.

4. Euthanasia:

- a. Place frogs into the buffered MS-222 solution.
- b. Cover the container and wait until frogs lose their righting reflex, usually about 15-30 minutes.
- c. Firmly pinch the toes once the animal has lost righting reflex to ensure no response to pain. If a withdrawal reflex is present (animal pulls leg away when toe is pinched), place back into the MS-222 solution and retest in 5-10 minutes.
- d. Once the absence of withdrawal reflex is confirmed, perform secondary (physical) method of euthanasia, such as:
 - Removal of the heart via dissection
 - Decapitation
 - Double pithing
- e. Place the carcass in plastic bag and store in the Weill Hall Basement necropsy designated freezer (room 36)

REFERENCE DOCUMENTS

REVISION HISTORY			
REVISION NUMBER	AUTHOR(S)	EFFECTIVE DATE	REVISION(S)