

AQU_001 Chemical Restraint of Aquatic Animals

I. OBJECTIVE

To describe the procedure for chemically restraining aquatic animals within UQBR facilities via submersion into a light sedation bath in order to both facilitate safe animal handling practices and minimize stress on aquatic animals being handled.

II. DEFINITIONS

Competent - “the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments.” (as per, Australian code for the care and use of animals for scientific purposes, 2013)

III. COMMENTS / RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: rationale for chemical restraint, frequency of use, any intended variation to this procedure.
- As per routine the conditions (see document footer) this procedure must be performed by, or under the direct supervision of, personnel who are competent to perform the procedure.
- This SOP was written with focus on the chemical restraint of aquatic species managed by the BR aquatics division (namely zebrafish, anemone fish spp. and xenopus frogs). Regardless, this SOP should be considered generally applicable to a range of other amphibian and teleost species. Before application to a novel species, ensure you seek veterinary advise to confirm doses and procedures are appropriate for your model.
- Ensure familiarity with UQBR SOP 22 UQBR Veterinary Care Protocol, for management of adverse events.
- Ensure animals are monitored when netting/straining as they may jump out of the tank.

IV. EQUIPMENT

Equipment items

- Complete mating tank with divider or housing tank OR 8L tank with water.
- Net (small -3’)
- Transfer pipette.
- Beaker to measure water volumes for bath.
- Ph test strips.
- Aerator (optional)
- Timer/Clock.

Consumables

- Sedative Agent (Aqui-S or MS-222)
- System Water
- PPE (as required, relative to workplace and procedure being performed)

Administration

- “Agistment Extras” Log

Conditions:

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V. PREPARATION

1. Ensure restraint area is setup for the procedure.
2. Label bath with animal identification and procedure to be performed.
3. Place a timer or clock next to the procedure space (*)
4. Ensure your stock solution have been properly prepared and labelled prior to use (instructions for several products detail below).*

E.g. of minimum labelling requirements:

Product:	Isoeugenol (Aqui-S)
Concentration:	1:10 stock solution with system water
Date:	12/05/21
Name of maker:	J. Smith

**Always check expiry according to manufacture's instructions and recommendations before usage of any anesthetic product.*

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Aqui-S (isoeugenol) Solution

Note: Specific gravity of AQUI-S® is 1.089

Isoeugenol (active ingredient) conc. is 50% of AQUI-S

Aqui-S concentration from bottle = 540g/L isoeugenol = 540mg/mL

Stock solution will be 30mg/mL:

Mix 1mL of Aqui-S into 17mL DI water to a total of 18mL to give 30mg/mL

Working solution will be 300ug/mL (=0.3mg/mL):

Mix 1mL Stock solution into 99mL system water to a total of 100mL to give 300ug/mL

The overdose bath will be 600ug/mL:

Mix 2mL Stock solution into 98mL system water to a total of 100mL to give 600ug/mL

1. Make up stock solution (30mg/ml)
 - a. To make a standard stock solution; Mix 1mL of Aqui-S into 17mL DI water to a total of 18mL to give 30mg/mL. Shake well and ensure that this solution is an even milky-looking mixture.
 - b. Refrigerate stock solution until ready for use.

Note: System Water used to make AQUI-S® stock solutions.

Do not store AQUI-S® stock solutions for more than 48 hours.

Shake vigorously before each use.

2. Make up **sedative bath** (300µg/ml)
 - a. To make a working solution; Add 1ml of standard stock solution per 99 mL of system water to a total of 100mL to give 300ug/mL.
 - b. Stir well, ensuring that no white granules linger at the bottom of the tank.

Note: Stock solution can be stored at 4°C

Sedative bath must be used within one hour.

3. Make up **overdose bath (600µg/ml)**
 - a. If using one overdose bath, place additional 1mL stock solution into sedative bath to create overdose bath, at least 1 minute after the addition of the last aquatic animal.

OR

- b. Make a second bath for overdosing by adding 2mL Stock solution into 98mL system water to a total of 100mL to give 600ug/mL.

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MS-222 (Ethyl 3-Aminobenzoate methanesulfonic acid salt) Solution

1. Make up stock solution at x25 or x50 strength

To make x25 stock solution (3mg/ml)

- a. Add 1.6 g MS-222 powder to 400 mL of DI water.
- b. Add 8.4 mL of 1M tris at pH 9.
- c. Verify pH is neutral (7) using pH test strip. (follow instructions on packaging as different manufacturers may have slightly different operating instructions)

To make x50 stock solution (6mg/ml)

- a. Add 3.2 g MS-222 powder to 400mL of DI water.
- b. Add 16.8 mL of 1M tris at pH 9
- c. Verify pH is neutral (7) using pH test strip.

2. Refrigerate stock solution until use. Extra stock solution may be refrigerated for further use for up to 2 months and must always be shaken well before each use.

3. Light **sedation bath (0.01mg/ml)**

- a. If using x25 stock; Add 0.5 mL of stock solution per 100 mL system water.
- b. If using x50 stock; Add 0.25 mL of stock solution per 100 mL system water.

4. Full strength **sedation bath (0.15mg/ml)**

- a. If using x25 stock; Add 10 mL of stock solution per 100mL system water.
- b. If using x50 stock; Add 5 mL of stock solution per 100mL system water.

Note: Sedation bath must be used within an hour.

5. Make up **overdose bath (0.3mg/ml)**

- a. For x25; add 20mL of stock solution per 100mL fish water.
- b. For x50; add 10mL of stock solution per 100mL fish water.

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VI. PROCEDURE

1. Use a net or strainer to capture the aquatic animal.
2. Add animal to the prepared **sedation bath**.
 - Consider time taken to complete the procedure.
 - If working with large/overly sensitive animals (such as xenopus or anemone fish respectively), limit the number of animals to **one animal / bath at a time**. If working with zebrafish, limit the number of animals to no more than **6 adults or 4 juveniles at a time**. This is to safeguard welfare.
3. Leave the animal(s) undisturbed for at least 1 minute within the bath, to reach stage 3 sedation.

Note: Stage 3 sedation is here defined as:

 - Loss of equilibrium and lack of movement.
 - Lack of response to surrounding stimuli (noises, vibrations to the tank etc.)
 - Lack of response to physical stimuli (no response to tail pinch)
4. Once the animal is sedated (i.e. chemically restrained), they may be safely removed from the bath.
 - Always work quickly with the animal, keeping it out of the water for no longer than **1 minute**.
 - If the animal begins showing signs of reaction to physical stimuli, return to the working bath for a few seconds until stage 3 sedation is again reached.
5. To recover, place the animal into either a static, or aerated recovery bath. Monitor for signs of recovery:
 - Increased opercula movement.
 - Bursts of tail movement.
 - Recovered equilibrium and buoyancy control.

Note: Do not place a recovering animals into a tank with alert tank mates. Fish e.g. Zebrafish display high levels of aggression towards other fish in a weakened state and will try to bite the recovering animal's eyes and gills, potential causing severe injury or death. Once recovered, it is safe to add all fish back in together.

Adverse recovery reactions

1. If the animal does not recover as per step 5 above, try the following:
 - a. Ensure that the recovery bath is being aerated.
 - b. Flush any remaining anesthetic from the gills by using a transfer pipette to gently increase water flow of gills, mimicking the action of buccal pumping.
2. If the gills of the animal begin bleeding, immediately transfer them to the **overdose bath**. Then consider the following:
 - a. The time the animal was in the anaesthetic bath for – this is likely the result of an overdose.
 - b. The amount of time the anaesthetic bath has been in use – remember to make fresh baths every hour if the session length requires.
 - c. Check the date of the stock solution & the concentration (e.g. 25 x or 50x for MS-222).
 - d. Consider switching to another anaesthetic (AQUI-S / MS-222) – this effect can be an allergic reaction to one of the 2 anesthetics used, and it is likely that any related fish from the same cohort will exhibit similar effects.

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Chemical Overdose

Note: In the event of an adverse reaction / poor recovery, euthanise the animal via the **overdose bath**.

Leave animal in the bath for at least 1 hour, before verifying death by checking for:

- a. Lack of opercula/gill movement.
- b. Lack of heartbeat.

Optional final step post 1 hour in bath. Note: May only be required for very old/large aquatic animals

- a. Then either “pith” or decapitate the animal with a sharp instrument following step 5 to ensure death.

VII. SAFETY

- PPE use is essential when completing this task – consult with a biosafety adviser for specifics, as required.
- All accidents, injury or near misses are to be reported immediately to the Facility Manager and recorded on a UQ OHS Incident Report Form.

VIII. REFERENCES:

1. Australian code for the care and use of animals for scientific purposes (8th Edition, NHMRC 2013): <https://www.nhmrc.gov.au/guidelines/publications/ea28>
2. Department of Agriculture and Fisheries (DAF): <http://www.daf.qld.gov.au/>
3. Guidelines to promote the wellbeing of animals used for scientific purposes (NHMRC, 2008): https://www.nhmrc.gov.au/files_nhmrc/publications/attachments/ea18.pdf
4. OGTR PC2 work requirements and regulations: <http://www.ogtr.gov.au>
5. QLD WH&S Act 2011: <https://www.worksafe.qld.gov.au/laws-and-compliance/workplace-health-and-safety-laws/laws-and-legislation/work-health-and-safety-act-2011>
6. UQ Animal Ethics Unit SOPs: <http://www.uq.edu.au/research/integrity-compliance/standard-operating-procedures-sops> UQ OHS Unit: <http://www.uq.edu.au/ohs/>
7. UQ OHS Incident Report Form: <http://www.uq.edu.au/ohs/index.html?page=141331>
8. UQBR SOPs: <V:UQBR/SOPs/Common/UQBR SOPs> and <http://biological-resources.uq.edu.au/secure/uqbr-sops>

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
2	MBS	04/08/2021	04/08/2024

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