Curtin University Standard Operating Procedure

ANAESTHESIA OF FISH

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Aims / Objectives: To provide guidelines on the anaesthesia of fish for any researchers using fish and requiring anaesthesia of the fish as part of any research projects at Curtin Aquatic Research Laboratories (CARL) and / or fish research projects under the control of the Curtin Animal Ethics Committee (AEC).

Definitions:
Anaesthesia: Involves the loss the consciousness, loss of sensation (analgesia) and muscle relaxation.

AQUI- S: A sedative and anaesthetic agent, easily dispersed in water with a wide safety margin. It is the main anaesthetic used in fish to be used for animal consumption.

Benzocaine Hydrochloride: A form of local anaesthetic commonly used in fish as a sedative, anaesthetic agent, and euthanasia agent. It is relatively cheap, easy to use, and relatively safe.

MS 222 – or Tricaine methanesulfonate: A white powder used for anaesthesia, sedation, or euthanasia of fishes. It is a muscle relaxant that operates by preventing action potentials.

Procedures:
Anaesthesia of fish requires knowledge of the different drugs and their method of action, along with the ability to monitor the appropriate depth of anaesthesia for the procedure required.

- It is recommended that the fish are fasted for 12-24 hours prior to the anaesthetic to minimise the risk of regurgitation under the anaesthetic and the subsequent complications if that was to occur. It also minimises the risk of faecal contamination in the water during and after the procedure.

- Anaesthetics are generally administered by dispersal of the drugs in a water bath and the fish are placed into the water bath. The temperature should be maintained at the optimum temperature for that particular species of fish.

- Ideally using water from the holding tank for the procedure is recommended to minimise any extreme changes in pH level, ammonia etc.

- Oxygenation of the fish is recommended to ensure normal physiological homeostasis, through the use of portable oxygen tanks.

- The depth of anaesthesia is monitored through the opercular rate, reaction to deep pain, and assessment of muscle tone (see below)

Level	Description	Signs displayed
0	Normal behaviour	Reactive to stimuli. Good muscle tone, normal equilibrium and operculum rate.
1	Sedation	Equilibrium maintained. At lighter levels there is some reaction to external stimuli and normal opercular rates. Deeper levels show no reactivity to mild external stimuli and reduced opercular rates.
2	Light anaesthesia	Opercular rate increases initially, then decreases as anaesthesia deepens. Progressive loss of equilibrium. Reacts to only deep pressure stimuli. Colour changes may be seen.
3	Surgical anaesthesia	No reaction to any stimuli. Slow opercular rate, with operculum spread. No muscle tone, no equilibrium control.
4	Medullary collapse	Cessation of operculum movements, followed some time later by cardiac arrest.

Taken from Barker et al (2009)

- The anaesthetic chosen should allow for a rapid and safe return to normal physiological and behavioural status

Recommended Drugs at CARL and for any research fish under the guidance of the Curtin AEC are:

a. AQUI-s – this is a very effective liquid anaesthetic. It is generally made up to a stock solution and this can be added directly to a water bath.
b. Immersion of fish in Benzocaine. Information on the best way to prepare and administer benzocaine can be found in Barker et al. (2015)
c. Immersion of fish in MS 222 – this is very water soluble and used regularly; however, it is quite expensive. Also there is a very thin margin of error- in that there is a very fine line between anaesthesia and toxic levels.

Recommended dose rates of fish anaesthetics

Anaesthetic	Handling	Concentration (mg/L) Anaesthesia	Euthanasia
AQUI-s	25	60	175mg/L (20 mins)
Benzocaine	20-35	50-75	100
MS-222	25-35*	50-100*	>250-500

* MS-222 has a variable effect depending on the type of fish and the size of fish so the dose must be calculated after investigations into the specific fish type.
Prior to any project occurring in CARL, the researcher must complete a risk assessment for the whole project, and if any chemicals are to be used, an individual chemical risk assessment sheet must be completed. This must then be assessed and approved for use by the Health and Safety Manager for Curtin University, prior to AEC approval being granted.
The Safety Data Sheets for individual chemicals can be found on the Curtin University Website at http://healthandsafety.curtin.edu.au/hazardous_substances/chemicals.cfm

References:

Barker, D., Allan, G.L., Rowland, S.J., Kennedy, J.D. and Pickles, J.M. (2015): A Guide to Acceptable Procedures and Practices for Aquaculture and Fisheries Research (4th Edition). Primary Industries (Fisheries) Animal Care and Ethics Committee, NSW Government.

NHMRC (2008) Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes. The Assessment and Alleviation of Pain and Distress in Research Animals.
Wingerter, K. (2010) Aquarium Fish – Use of MS-222 to Induce Sedation and Anaesthesia in Oriental Fish. Advanced Aquarist, Volume IX Nov 2010.