2.0 Anaesthesia of Finfish

2.1 Introduction:

This template is intended for use by instructors to train the Department of Fisheries and Oceans (DFO) staff and students in the anaesthesia of research fish. Templates are used to provide the minimum requirements necessary in a training exercise, but the instructor may add additional material.

An experienced instructor must demonstrate the methods outlined in this template, and trainees must be deemed qualified in carrying out the procedures, before they can be permitted to perform these methods on fish without an instructor present. Hands-on training of staff is a requirement for facility approval by the Canadian Council on Animal Care, of which DFO is a member. This template is part of a comprehensive DFO Science Branch series on training for users of aquatic research animals.

The template covers immersion anaesthesia using compounds approved by Health Canada. The drug dosages described in this template are for salmonids; instructors may have to modify the dosages and techniques described when different species are anaesthetised.

This template contains an introductory and an advanced exercise; the former demonstrates the effects of different dosages of a single chemical and the latter demonstrates the effects of sedation prior to anaesthesia.

2.2 Rationale:

Removal from water and handling by humans are very stressful experiences for fish, even if no additional pain is experienced. When experimental animals must undergo procedures that cause pain or distress, there is a moral obligation of the researcher to minimize or eliminate the adverse effects on those animals.

Anaesthetics should be used in experiments where there is expected to be painful or noxious stimuli, and in experiments entailing extensive handling or manipulation when there is a reasonable expectation of trauma and physiological insult to the fish.

2.3 Authority:

The staff or consultant Veterinarian or Animal Care Committee is responsible for providing information about the anaesthesia methods performed on the fish species used for scientific study in their respective regions. Animal Care Committees may delegate training of these procedures to an instructor who has demonstrated knowledge of and experience in the areas of anatomy and anaesthesia and has demonstrated excellent survival of fish that have been anaesthetized using the techniques outlined in this training template. Staff must be

trained in the proper anaesthesia methodology for the fish species being studied prior to performing the procedure(s).

Health Canada regulates the use of drugs and chemicals on animals; the Workers Compensation Board requires safety measures to be taken to protect human health. In general only compounds that are approved by Health Canada for use on fish should be used as anaesthetics. (Note: All experimental studies certificates for clove oil were revoked by Health Canada in 2002).

2.4 Goals of this training exercise:

2.4.1 Introductory exercise:

1. Understand and be able to perform drug dosage calculations.
2. Understand safe handling of anaesthetic compounds.
3. Learn how to monitor fish during anaesthesia.
4. Understand the importance of water quality monitoring during anaesthesia.
5. Learn techniques for gentle restraint and handling of finfish.
6. Become familiar with the type of containers and equipment required to prepare anaesthetic baths.

2.4.2 Advanced Exercise:

1. Learn to mitigate handling stress using sedation prior to anaesthesia.
2. Understand the principle of balanced anaesthesia.
3. Increase understanding of the stages of anaesthesia.

2.5 Theoretical training – to be completed before hands on session:

1. 'The Experimental Fish'
2. CCAC guidelines: Section H: Experimental Procedures 3.3 Anaesthesia
3. WHMIS training.
4. Summary theory material provided with this training template (Appendix C).

2.6 Details of the Introductory Exercise:

Fish are exposed to four different concentrations of TMS™ while being monitored for behavioural and physiological changes.

2.6.1 Time estimate:

Introductory exercise

Set up: 1 hour

Instruction and training: 3 hours

2.6.2. Equipment Required (for a single group of trainees):

- 2.5 grams of TMS™
- 5 grams Sodium bicarbonate (only if performed on freshwater fish)
- 100 mls of distilled water
- Calculator
- Weigh boats and scupula

- Safety glasses and mask or perform drug measurements in a fume hood.
- Digital scale
- 2 Amber coloured Nalgene bottle or other light proof container for holding stock solutions
- 5, 10 and 20 cc syringe for measuring stock solution into anaesthetic containers
- 2 glass or plastic aquaria for anaesthesia (or other available containers) 22.7 litre (5 gallon) aquarium is a good size, the container should be able to hold 10 litres of water
- 2 glass or plastic aquaria for recovery (or other available containers)
- 2 Airstones, airline tubing and access to compressed air
- Knotless dip net
- Thermometer
- Dissolved oxygen meter
- pH paper or pH meter (freshwater only)
- Stopwatch
- 4 healthy fish – try to use smaller fish due to ease of handling, (50-100 gram salmonids are ideal).

2.6.3 Procedure

The instructor should demonstrate the procedures prior to trainees performing the exercise. Fish anaesthetized during this session can be used for training in other training templates (e.g. length weight sampling, blood sampling or tagging).

2.6.3.1 Before the procedure:

- Print Appendix A (Introductory exercise) and Appendix C (Review Theory) and provide the material to the trainees prior to the exercise.

- Ensure trainees have read the required materials, performed the practice calculations and reviewed the training procedure prior to commencing the exercise.

- Take fish off feed for 18 – 72 hours prior to the exercise. It is advisable to have trainees place a card onto the tank holding the fish that clearly indicates that fish are not to be fed; card may simply read NPO (nil per os) until a certain date or time as determined by the instructor.

- Supervise the trainees as they weigh out the drug and ensure that appropriate safety gear is used and inventory records of drug use are kept.

- Have trainees make 50 mls of a 50 mg/ml stock solution of TMS™. 2.5 grams of TMS™ are mixed with distilled water to make a total volume of 50 mls.

- Prepare a 100 mg/ml stock solution of sodium bicarbonate if this exercise is being preformed in fresh water. (5 grams of NaHCO3 are mixed with distilled water to make a total volume of 50 mls).

- Transfer the stock solution of TMS™ to an amber Nalgene bottle.

2.6.3.2 Anaesthetic procedure:

- In this procedure fish will be exposed to four different concentrations of TMS™, 25 ppm, 50 ppm, 75 ppm and 100 ppm (instructors may need to change these concentrations depending on the species and age of fish being anaesthetized).

- Fill each aquarium (or other container) with 10 litres of water.

- Add airstones to maintain water quality, set airstones to the smallest bubble size possible to maximize surface area for gas transfer.

- Add anaesthetic to the first tank (25ppm). Use a syringe to measure and add 5 mls of TMS™ stock solution to this tank. If this demonstration is in freshwater buffer with 5 mls of $NaHCO_3$ stock solution.

- Measure starting temperature and dissolved oxygen; also measure pH if freshwater is being used.

- Add fish to the tank using a knotless dip net and start stopwatch.

- Measure respiratory rate by counting the number of opercular movements in a 15 second interval. This is done three times while fish is in the anaesthetic bath; at the start of the procedure, at loss of equilibrium and at the time when there is lack of response to stimulus.

- Note time to loss of equilibrium.

- Note time it takes for fish to stop responding to stimulus.

- Transfer fish to aerated recovery bath and note time on stopwatch.

- Measure respiratory rate when fish starts to regain equilibrium and when fish appears to be exhibiting normal behaviour.

- Repeat this procedure for each concentration of anaesthetic to teach dosage effects of drugs. Amounts of stock solution to add to each tank are outlined in Chart 1.

- Measure temperature, dissolved oxygen and pH (in freshwater) at end of the exercise.

Chart 1: Volume of stock solution to add to each 10 litre aquarium to achieve the desired final concentration.

	25 ppm	**50 ppm**	**75 ppm**	**100 ppm**
Volume of MS222 stock required (50 mg/ml)	5 mls	10 mls	15 mls	20 mls
	50 ppm	**100 ppm**	**150 ppm**	**200 ppm**
Volume of NaHCO3 buffer required (100 mg/ml)	5 mls	10 mls	15 mls	20 mls

2.6.3.3 Instructors should emphasize the following during the procedure:

- Gentle restraint and handling.

- Correct choice of equipment, (this is a good opportunity to demonstrate the types of dip nets and buckets to be used).

- Monitoring water quality.

- Choice of airstone and importance of small bubbles for maximum surface area for gas exchange.

- Identification of the different stages of anaesthesia.

2.6.4. After the training exercise:

- Trainees should have clear instructions for carcass disposal if fish were euthanized.
- Increase monitoring for 2 – 3 weeks after the handling event if fish are recovered from anaesthesia.
- Anaesthesia baths must be disposed of in accordance with local waste management regulations.
- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).
- Trainees must wash hands with disinfectant soap.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

2.7 ACC Notes

- Locally significant differences required in training (e.g. species).
- Authorization required to teach/list of possible instructors for your region.
- Any other requirements for your region.

2.8. Details of the Advanced Exercise:

Combining sedation and anaesthesia using two chemicals: fish are sedated with Aquacalm™ prior to anaesthesia with TMS™.

2.8.1 Time estimate:

Set up: 1 hour
Teaching: 2 hours

2.8.2 Equipment Required (for a single group of trainees):

- TMS™
- Sodium bicarbonate (if performed in freshwater).
- Aquacalm™
- Calculator
- Weigh boats and scupula
- Safety glasses and mask or drug measured in fume hood.
- Digital scale
- Glass or plastic aquaria for anaesthesia (or other available containers)
- Glass or plastic aquaria for recovery
- Airstones, airline tubing and access to oxygen.
- Knotless dip net
- Thermometer
- Dissolved oxygen meter
- pH paper or pH meter if this procedure is performed in freshwater
- Stopwatch
- 6 - 12 healthy fish – try to use smaller fish due to ease of handling, 50 to 100 gram salmonids are ideal.

2.8.3 Procedure:

Fish anaesthetized during this session can be used for training in other training templates (e.g. length weight sampling, blood sampling or tagging).

2.8.3.1 Before the Procedure:

- Print Appendix B (Advanced exercise) and Appendix C (Review Theory) for trainees to review prior to the exercise.

- Take fish off feed for 18 – 72 hours prior to the exercise. It is advisable to have trainees place a card onto the tank holding the fish that clearly indicates that fish are not to be fed; card may simply read NPO (nil per os) until a certain date or time as determined by the instructor.

- Prepare a stock solution of TMS™ and Aquacalm™ or weigh out the required amount of each chemical. This will vary depending on the volume of anaesthesia baths that you choose to use.

- Prepare a stock solution of $NaHCO_3$ if this exercise is being performed in freshwater or weigh out the desired amount of the chemical.

- Sedate the fish in their home tank prior to being transported to the anaesthesia bath. The goal of sedation prior to handling is to mitigate the stress response.

- Prepare tank for sedation (0.5 ppm metomidate), anaesthesia bath (70 ppm MS222) and recovery bath. Note: These are appropriate dosages for most salmonids; the instructor may have to vary these depending on the species and age of fish being used.

2.8.3.2 Sedation with Aquacalm™

- Add air stones with supplementary oxygen to the home tank.

- Stop water flow into and out of the home tank.

- Dissolve Aquacalm™ in a volume of water and distribute it evenly around the tank. A dosage of 0.5 ppm should achieve the desired level of sedation for juvenile salmonids. Start the stopwatch.

- Monitor fish behaviour and respiration rate. Ask the trainees fill out the chart for respiration rate and behaviour.

- Monitor dissolved oxygen and temperature throughout the procedure.

- Inform the trainees that the goal of the sedation is to have fish sedate but still able to maintain equilibrium prior to introduction to the anaesthetic bath. You should be able to net the fish without having to pursue them around the tank; fish should not struggle in the dip net.

- Emphasize to students the importance of sedation prior to any handling: this will allow the sedative to interfere with the production of cortisol.

- The fish can remain in the sedative for up to 8 hours.

2.8.3.3 Anaesthesia with TMS™:

- Once fish are sedate, transfer a single fish to the anaesthetic bath (for juvenile salmonids 70 ppm works well). Ask the trainees to monitor behaviour and respiratory rate throughout anaesthesia.

- Determine when the fish has completely lost equilibrium and muscle tone and spinal reflexes are lost, but opercular movements are still regular. This is the stage appropriate for handling, injecting or biopsying.

- Add in any of the handling or live sampling templates if desired by the instructor. Blood sampling, tagging or marking techniques can be performed during this stage of anaesthesia.

- Move fish to the recovery bath and have trainees monitor time to recovery and respiratory rate.

- Allow one or more fish to remain in the anaesthetic bath to the stage of medullary collapse. Complete the euthanasia template and use the individual for sampling demonstrations or anatomy training.

2.8.4 After the training session:

- Trainees should have clear instructions for carcass disposal if fish were euthanized.

- Increase monitoring for 2 – 3 weeks after the handling event if fish are recovered from anaesthesia.

- Anaesthesia baths must be disposed of in accordance with local waste management regulations.

- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).

- Trainees must wash hands with disinfectant soap.

- Update inventory records to reflect the number of fish euthanized for this session (if any).

- Update drug use records to include anaesthetic use.

2.9 ACC Notes

- Locally significant differences required in training (e.g. species).

- Authorization required to teach/list of possible instructors for your region.

- Any other requirements for your region.

APPENDIX A: Introductory Exercise

In this exercise fish will be exposed to four different concentrations of TMS™, 25 ppm, 50 ppm, 75 ppm and 100 ppm. They will be monitored for behavioural and physiological changes. Water quality will be monitored throughout the procedures.

Complete Practice Calculations

Information you must have to correctly calculate an anaesthetic dosage:

- Volume of container in litres
- Conversion of gallons (UK) to litres if required. 1 gallon (UK) = 4.545 litres
- Dosage of drug required (refer to prescription for dose). Remember ppm = mg/l
- Conversion of milligrams to grams. 1 gram = 1000 mg
- Concentration of stock solutions if required.

Formulas:

Calculating the amount of powder to add:

1. Calculate the total amount of drug needed in the final volume of water:

   (Volume of container)(Dosage in mg/l) = mg of drug required

2. Convert mg to grams for ease of weighing
   Milligrams of drug ÷ 1000 = grams required

or

Calculating the amount of a stock solution to add:

1. Calculate the total amount of drug needed in the final volume of water:
   (Volume of container)(Dosage in mg/l) = mg of drug required

2. Determine volume of stock solution to add
   (Milligrams of drug) ÷ (Stock solution mg/ml) = mls of stock solution to add.

Example calculations:

Example #1: **Adding powder directly to the anaesthetic bath**

You wish to anaesthetize a 100g salmon smolt in a 20 litre container of water with TMS™ at a drug dosage of 70 ppm.

Container volume = 20 l
Drug dosage = 70 ppm = 70 mg/l

- (20 litres) x (70 mg/l) = 1400 mg of drug
- (1400 mg) ÷ (1000 mg/g) = 1.4 g

Add 1.4 grams of TMS™ to the 20 litre anaesthetic bath.

Example #2: **Adding stock solution to the anaesthetic bath**

You wish to anaesthetize a 100 g salmon smolt in a 20 litre water bath with TMS™ at a drug dosage of 70 ppm. Your stock solution contains 50 mg/ml.

Drug dosage = 70 ppm = 70 mg/l

- (20 litres) x (70 mg/l) = 1400 mg of drug
- (1400 mg) ÷ (50 mg/ml) = 28 mls of stock solution to be added to the 20 l bath.

Practice calculations:

1. How many litres does a 5 gallon (UK) bucket contain?

2. You wish to anaesthetize a group of adult sablefish to assess their spawning condition. Your container volume is 150 litres and the required dosage is 180 ppm. Calculate the amount of TMS™ to add.

3. You need to anaesthetize a group of brook trout in order to biopsy the fish. The anaesthesia induction bath volume is to be 25 litres and the TMS™ dosage is 80 ppm. The water will be buffered with 160 ppm of $NaHCO_3$.
   Calculate the amount of TMS™ and $NaHCO_3$ required.

4. A group of cod are to be sedated for transport to another facility. They will be transported in a transport tanker that can hold 1 cubic meter of water (1000 l). Calculate the amount of Aquacalm™ required to achieve a dosage of 0.25 ppm in the transport tank.

5. You wish to sedate a group of salmon prior to anaesthesia to minimize the stress of being captured. The fish are in a 500 litre holding tank. The water flow has been stopped and supplementary oxygen provided.

   Calculate the amount of Aquacalm™ required to achieve a dosage of 0.4 ppm the 500 litre container?

   The fish are to be induced in a 20 litre water bath with TMS™ at a concentration of 50 ppm. Calculate the amount of TMS™ required for the induction bath?

6. You need to anaesthetize some salmon fry in order to remove their adipose fin for marking purposes. The anaesthetic bath contains 10 litres of water and the TMS™ dosage is 50 ppm; 100 ppm of sodium bicarbonate will be used to buffer the anaesthetic bath. Calculate the amount of TMS™ and $NaHCO_3$ required.

7. Some researchers prefer to use stock solutions of anaesthetics and sedatives. How would you prepare 500 mls of a stock solution of TMS™ at a concentration of 50 mg/ml?

8. You wish to anaesthetize a 250 gram Chinook in TMS™ at a dosage of 70 ppm. Your stock solution has a concentration of 100 mg/ml. Calculate the amount of stock solution required to add to a 20 litre anaesthetic bath to obtain a dosage of 70 ppm.

Answers:

1. 1 gallon (UK) = 4.545 litres
   5 gallons x 4.545 l/gallon = 22.73 litres in a 5 gallon bucket

2. (150 litres)(180 mg/l TMS™) = 27000 mg
   27000 mg ÷ 1000 mg/g = 27 g of TMS™ to add to the 150 litres of seawater

3. (25 litres)(80 mg/l TMS™) = 2000 mg
   2000 mg ÷ 1000mg/g= 2 grams of TMS™ to add to the 25 litre induction bath

   (25 litres)(160 mg/l $NaHCO_3$) = 4000 mg
   4000 mg ÷1000mg/g = 4 grams of $NaHCO_3$ to add to the 25 litre bath.

4. (1000 litres)(0.25 mg/l) = 250 mg
   250 mg ÷ 1000 mg/l = 0.25 g

5. Aquacalm™ sedation
   (500 litres)(0.4 mg/l) = 200 mg
   200 mg ÷ 1000 mg/l = 0.20 g

   TMS™ Induction
   (20 litres)(50 mg/l) = 1000 mg
   1000 mg ÷ 1000 mg/l = 1.0 grams

6. TMS™
   (10 litres)(50 mg/l) = 500 mg
   500 mg ÷ 1000 mg/g = 0.5

   $NaHCO_3$
   (10 litres)(100 mg/l) = 1000 mg
   1000 mg ÷ 1000 mg/g = 1 gram

7. Desired concentration is 50 mg/ml, desired final volume is 500 mls
   (500 mls)(50 mg/ml) = 25000 mg
   (25000 mg) ÷ 1000 mg/g = 25 g
   Add 25 grams of TMS™ to the 500 mls to get a 50 mg/ml stock solution.

8. (20 litres)(70 mg/l) = 1400 mg required
   Stock solution contains 100 mg/ml

   1400 mg ÷ 100 mg/ml = 14 mls of stock solution to be added to the 20 litre container.

Calculations for introductory exercise:

Prepare stock solution:

50 mls of a stock solution of TMS™ at a concentration of 50 mg/ml:

(Volume of solution required in mls)(Concentration desired in mg/ml) = mg to add

(50 mls) (50 mg/ml) = _____ mg

Convert mg to g for ease of weighing:

_____ mg ÷ 1000 mg/g = _____ g

Add _____ g of TMS™ to distilled water to make up to 50 mls.

Preparation of buffer stock solution (freshwater only):
If fish are anaesthetized in fresh water with TMS™ the water must be buffered. The amount of bicarbonate buffer used is double the concentration of the TMS™.

50 mls of a stock solution of $NaHCO_3$ at a concentration of 100 mg/ml:
(Volume of solution required in mls)(Concentration desired in mg/ml) = mg to add

(50 mls)(100mg/ml) = _____mg
Convert to grams for ease of weighing

_____mg ÷ 1000 mg/g = _____g

Add _____ g of $NaHCO_3$ to distilled water to make up to 50 mls.

Calculate the volume of stock solution to add to each 10 litre aquarium to achieve the desired final concentration and fill out in the chart below:

	25 ppm	50 ppm	75 ppm	100 ppm
Volume of TMS™ stock required (50 mg/ml)				
	50 ppm	**100 ppm**	**150 ppm**	**200 ppm**
Volume of $NaHCO_3$ buffer required (100 mg/ml)				

The Anaesthetic Procedure

- In this procedure fish will be exposed to four different concentrations of TMS™, 25 ppm, 50 ppm, 75 ppm and 100 ppm.
- Fill each aquarium (or other container) with 10 litres of water. Ensure that both anaesthetic bath and recovery bath is ready to receive the fish.
- Add air stones to anaesthetic and recovery baths, set air stones to the smallest bubble size possible to maximize surface area for gas transfer.
- Maintain water quality parameters.
- Add anaesthetic to the first tank (25ppm). Use syringe to measure and add TMS™ stock solution to this tank. If anaesthetising in freshwater buffer with a $NaHCO_3$ stock solution. Do not mix the stock solutions together or they will precipitate out. Add the TMS™ to the water bath first followed by the buffer.
- Measure starting temperature and dissolved oxygen, also measure pH if freshwater is used. Record starting water quality parameters.
- Lift the fish with care from the source tank using a knotless dip net, place fish into the anaesthetic bath and start the stopwatch. Fish should not be out of the water for more than a few seconds.
- Measure the respiratory rate by counting the number of opercular movements in a 15 second interval. This is done three times while the fish is in the anaesthetic bath; at the start of the procedure, at loss of

equilibrium and at the time when there is lack of response to stimulus. Record number of opercular movements per 15 second time interval.

- Record the time to loss of equilibrium.
- Record the time it takes for the fish to stop responding to stimulus. The animal should still be respiring slowly and regularly.
- Transfer the fish to an aerated recovery bath and note the time on the stopwatch.
- Measure and record the respiratory rate when fish starts to regain equilibrium and when fish appears to be exhibiting normal behaviour.
- Repeat this procedure for each concentration of anaesthetic to understand the dosage effects of drugs.
- Measure and record temperature, dissolved oxygen and pH (in freshwater) at the end of the procedure.
- Calculate time intervals for induction and recovery, calculate respiratory rate by multiplying opercular beats measured in each 15 second interval by 4 to get respirations per minutes.

After the training exercise:

- Trainees should have clear instructions for carcass disposal if fish were euthanized.
- Increase monitoring for 2 – 3 weeks after the handling event if fish are recovered from anaesthesia.
- Anaesthesia baths must be disposed of in accordance with local waste management regulations.
- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).
- Trainees must wash hands with disinfectant soap.
- Update inventory records to reflect the number of fish euthanized for this session (if any).
- Update drug use records to include anaesthetic use.

Chart to be completed during anaesthesia exercise

25 ppm	Stopwatch time	Calculate min and sec between events	Opercular beats per 15 seconds	Calculated resps per minute
Loss of equilibrium				
Loss of response to stimuli (fish is induced)				
Transfer to recovery				
Regain response to stimuli				
Regain equilibrium				
Normal behaviour (fish is recovered)				

Water Quality in 25 ppm anaesthetic bath	Start of Procedure	End of Procedure	
Dissolved Oxygen (mg/l and %sat)			
Temperature (° C)			
pH			

50 ppm	Stopwatch time	Calculate min and sec between events	Opercular beats per 15 seconds	Calculated resps per minute
Loss of equilibrium				
Loss of response to stimuli (fish is induced)				
Transfer to recovery				
Regain response to stimuli				
Regain equilibrium				
Normal behaviour (fish is recovered)				

Water Quality in 50 ppm anaesthetic bath	Start of Procedure	End of Procedure	
Dissolved Oxygen (mg/l and %sat)			
Temperature (° C)			
pH			

75 ppm	Stopwatch time	Calculate min and sec between events	Opercular beats per 15 seconds	Calculated resps per minute
Loss of equilibrium				
Loss of response to stimuli and decreased respiratory rate (fish is induced)				
Transfer to recovery				
Regain response to stimuli				
Regain equilibrium				
Normal behaviour (fish is recovered)				

Water Quality in 75 ppm anaesthetic bath	Start of Procedure	End of Procedure	
Dissolved Oxygen (mg/l and %sat)			
Temperature (° C)			
pH			

100 ppm	Stopwatch time	Calculate min and sec between events	Opercular beats per 15 seconds	Calculated resps per minute
Loss of equilibrium				
Loss of response to stimuli (fish is induced)				
Transfer to recovery				
Regain response to stimuli				
Regain equilibrium				
Normal behaviour (fish is recovered)				

Water Quality in 25 ppm anaesthetic bath	Start of Procedure	End of Procedure	
Dissolved Oxygen (mg/l and %sat)			
Temperature (° C)			
pH			

APPENDIX B: Advanced exercise in fish anaesthesia

Combining sedation and anaesthesia using two chemicals: Fish are sedated with Aquacalm™ prior to anaesthesia with TMS™.

Theoretical training required:

1. Completed 'The Experimental Fish'
2. CCAC guidelines: Section H Experimental Procedures: 3.3 Anaesthesia.
3. Completed training module in introductory anaesthesia or has experience or training in this area.
4. WHMIS training.
5. Review theory provided with this training exercise (Appendix C).

Before the exercise:

- Take fish off feed for 18 – 72 hours prior to the exercise. Place a card onto the tank holding the fish that clearly indicates that fish are not to be fed; card may simply read NPO (nil per os) until a certain date or time as determined by the instructor.
- Consult with instructor to determine container volumes and drug dosages to be used for this training exercise.
- Prepare a stock solution of TMS™ and Aquacalm™ or weigh out the required amount of each chemical.
- Prepare a stock solution of $NaHCO_3$ if this exercise is to be conducted in freshwater or weigh out the desired amount of the chemical.

Details of the Procedure:

- Fish will be sedated in their home tank prior to being transported to the anaesthesia bath. The goal of sedation prior to handling is to mitigate the stress response.
- Prepare tanks for sedation (0.5 ppm Aquacalm™), anaesthesia (70 ppm TMS™) and recovery.

Sedation in home tank:

- Add air stones with supplementary oxygen to the home tank.
- Stop water flow into and out of home tank.
- Add Aquacalm™ to the tank and start the stopwatch.
- Monitor fish behaviour and respiration rate; fill out chart provided to follow depth of anaesthesia throughout the procedure.
- Emphasize that the goal is for the fish to be sedate but still able to maintain equilibrium prior to addition to anaesthetic bath. The response to being picked up in a dip net is minimal.

Anaesthesia:

- Transfer a single sedated fish to the anaesthetic bath. Monitor behaviour and respiratory rate throughout anaesthesia and record observations on the charts provided with this exercise.
- Determine when the fish has completely lost equilibrium and muscle tone and spinal reflexes are lost, but opercular movements are still regular. This is the stage appropriate for handling, injecting, blood sampling, tagging or marking, biopsying, or other minor invasive procedures.

Recovery:

- Move fish to the recovery bath, monitor time to recovery and respiratory rate and record findings on the chart provided.

or

- Allow one or more fish to remain in the anaesthetic bath to the stage of medullary collapse. Complete the euthanasia template. The fish may be used for sampling demonstrations or anatomy training.

After the training exercise:

- Trainees must wash hands with disinfectant soap.
- Disinfect the area where fish were handled (provide trainees with site biosecurity SOP).
- Increase monitoring for 2 – 3 weeks after the handling event.
- Update inventory records to reflect the number of fish euthanized for this session (if any).

- Update drug use records to include anaesthetic use.

Charts to be completed during Advanced Exercise in fish anaesthesia:

Time (m:sec)	Respiratory Rate	Behaviour	Response to stimulus	Comments
0.00				**Add metomidate to home tank.**

Time (m:sec)	Respiratory Rate	Behaviour	Response to stimulus	Comments
				Add fish to anaesthetic tank.

Time (m:sec)	Respiratory Rate	Behaviour	Response to stimulus	Comments
				Move one or more fish to recovery tank

Time (m:sec)	Respiratory Rate	Behaviour	Response to stimulus	Comments
				Allow one or more fish to progress beyond surgical plane to euthanize.

APPENDIX C: Review Theory

References:

Alpharma Technical Bulletin 5/2001. MS222 (Tricaine methane sulphonate).

Bowser, P.R. 2001. Anaesthetic options for fish in Recent Advances in Veterinary Anaesthesia and Analgesia: Companion animals, R.D. Gleed and J.W. Ludders (Eds.) International Veterinary Information Service (www.ivis.org) Ithaca, New York, USA.

Brown, L. 1993. Anaesthesia and Restraint. In: Textbook of fish medicine. M. Stoskopf. W.B. Saunders Company. Philadelphia 882 pages.

Burka, J.F., Hammell, K.L., Horsberg, T.E., Johnson, G.R. Rainnie, D.J., Speare, D.J. 1997 Drugs in salmonid aquaculture – A Review. J. Vet. Pharmacol. Therap. 20: 333-349.

Introductory Theory

Definitions:

Acidosis: A pathological condition resulting from accumulation of acid or depletion of the alkaline reserve (bicarbonate content) in the blood and body tissues, and characterized by a decrease in pH.

Anaesthesia: Generally defined as a state caused by an applied external agent resulting in a loss of sensation through depression of the nervous system.

Analgesia: The relief from or the absence of pain.

Aquacalm™: Canadian trade name for metomidate.

Asphyxia: A condition due to lack of oxygen resulting in actual or impending cessation of life.

Hypnosis: Artificially induced sleep or a trance resembling sleep from which the patient can be aroused by stimuli.

Hypoxia: Diminished availability of oxygen to the body tissues.

Induction: The production of anaesthesia or unconsciousness by the use of appropriate agents.

Narcosis: Drug induced stupor or sedation in which the patient is oblivious to pain, with or without hypnosis.

Sedation: A state of lowered awareness or anxiety. It is often used to make handling and transportation of fish less stressful. It is important to remember that sedation does not ensure analgesia.

Synapse: The junction between the processes of two neurons or between a neuron and an effector organ.

TMS™: Canadian Trade Name for Tricaine Methane Sulphonate.

ppm: parts per million, an equivalent measurement to milligrams per litre

- Fish must be taken off feed for 18 – 72 hours prior to being anaesthetized; this prevents fecal contamination of the water during the procedure and prevents vomiting. Gill damage, skin infections and disease transfer can occur when water is contaminated with feces or vomit.

- Anaesthetics approved by Health Canada for use on fish are obtained by veterinary prescription only (drug schedule 1); approved products available are limited to Tricaine methane sulphonate (TMS™) and metomidate (Aquacalm™).

- Benzocaine is a non-prescription drug (schedule 2 or 3) and has been approved for use on terrestrial species thus its use for fish is considered an 'extra label'[1] use of the drug.

- Compounds such as clove oil and 2-phenoxyethanol are not approved by Health Canada for use on fish. Researchers must seriously consider the legal and physiological consequences of the use of these compounds on research animals in the absence of thorough testing by the Health Canada approval process.

- Known concentrations of anaesthetic baths are to be used. Too low a dosage results in the stress of prolonged induction times and too high a dosage can result in prolonged recoveries, hypoxia, acidosis and death.

- Factors including but not limited to age, size, species, water temperature and pH, presence of disease and reproductive status will affect response to anaesthesia. Thus a few fish should always be tested

[1] Extra-label drug use sometimes referred to as "off label use" is defined as the use of a drug product in a manner that is not consistent with what is indicated on the label, package insert of any drug product approved by Health Canada.

in a fresh anaesthetic bath to gauge induction time and behavioural response prior to anaesthetizing a large number of fish.

- Be familiar with the stages of anaesthesia as outlined in Table 1 at the end of this summary.

- If anaesthetizing a species with which you are not familiar, always start with the label dosage of the drug and then increase gradually as needed. Induction times should not exceed 2 – 3 minutes.

- Anaesthetic and recovery baths are aerated to ensure adequate water quality. Ensure that the smallest bubbles possible are generated through air stones; large bubbles have too small a surface area to provide efficient gas transfer

- Fish are monitored continually throughout the anaesthesia and recovery process.

- Water quality must be monitored during anaesthetic procedures. Temperature and dissolved oxygen are the most important parameters to monitor. For most cold-water bony fish a temperature change of more than 2°C or a dissolved oxygen level less than 5 mg/l requires that the anaesthetic bath be renewed. The appearance of waste products from the fish in the water bath is also an indication for bath renewal.

- Water quality affects response to anaesthetic. Water temperature and pH affect the animal's metabolic rate and drug uptake across the gills.

- Anaesthesia with Tricaine Methane Sulphonate (TMS™) in freshwater requires buffering to maintain a neutral pH as TMS™ is relatively acidic. As fish respire they release CO_2 into the water contributing to a declining pH. Seawater is already well buffered so it does not require the use of an additional buffer.

- Stock solutions of TMS™ and $NaHCO_3$ will precipitate out if mixed together. Add the TMS™ to the anaesthetic bath first followed by the buffer; the fish must be placed in the bath last.

- Fish and anaesthetic baths should not be exposed to direct sunlight. Exposure of solutions of TMS™ to strong sunlight will make them toxic; anaesthetize fish in shaded areas or indoors.

- Only inert containers (glass or plastic) should be used for fish anaesthesia. TMS™ becomes toxic upon exposure to metal containers (e.g. zinc or copper).

- All equipment, hands/gloves used to handle fish must have smooth surfaces and be wetted down prior to touching fish. Damage to the fish cuticle from handling can result in increased susceptibility to disease outbreaks after handling.

- Mucus protectants such as Vidalife™ or Stresscoat™ can be used to protect the fish cuticle during handling.

- Anaesthesia and handling are stressful events that can predispose fish to disease. After a stressful event monitoring of morbidity, mortality and mortality rate should be increased. This period of increased monitoring should occur for the incubation period of diseases of concern (usually 2 – 3 weeks post handling).

- Anaesthetic solutions must be disposed of in accordance with local waste management regulations.

Table 1. Stages of Anaesthesia (Bowser, 1991)

Stage	Descriptor	Behavioural Response of Fish
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
1	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
2	Deep sedation	Total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate; equilibrium normal
3	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
6	Medullary collapse (stage of asphyxia)	Opercular movements cease; cardiac arrest usually follows quickly

Advanced Theory

- Balanced anaesthesia: In terrestrial vertebrate species it is common to use multiple drugs to achieve narcosis, muscle relaxation and analgesia. Generally, the use of several compounds together decreases the total amount of each drug necessary.

- There are limited drug options available for use on fish so true balanced anaesthesia is currently not possible. The combination of Aquacalm™ as a hypnotic and TMS™ as a general anaesthetic (and a probable analgesic) is the only recommended combination; this combination provides excellent control of stress and is expected to function as a local anaesthetic and control pain.

- Mechanism of action of metomidate (Aquacalm™)
  - Aquacalm™ is an imidazole-based non-barbiturate hypnotic agent.
  - The mechanism of action for central nervous system depression is not clear. Imidazoles are thought to function by enhancing GABA inhibitory pathways in the central nervous system.
  - The use of hypnotics alone does not provide analgesia.
  - Aquacalm™ suppresses cortisol synthesis through the suppression of 11-B-hydroxy-lation of cholesterol. (Brown 1993). Blocking cortisol production can prevent the negative consequences of the stress response in fish.
  - A withdrawal time has not been established for Aquacalm™.
- Mechanism of action of tricaine methane sulphonate (TMS™)
  - TMS™ is a benzocaine derivative and has a similar function.
  - The compound blocks inward sodium currents on nerve cell membranes blocking generation and conduction of nerve impulses. It acts directly on the central nervous system, cardiovascular system, neuromuscular junctions and ganglion synapses. (Alpharma, 2001)

- The ability of this compound to act at the neuromuscular junction results in muscle relaxation and the loss of spinal reflexes.

- The liver is the primary site of metabolism of TMS™ though some metabolism also occurs in the kidney, blood and muscle tissue. There is production of a polar and non-polar metabolite. The polar metabolite is excreted via the kidney; the non-polar metabolite is excreted via the gills. (Alpharma, 2001)

- The withdrawal time of TMS™ is 5 days at water temperatures greater than 10°C and 21 days at water temperatures less than 10°C.

- Further discussion of other chemicals and methods is beyond the scope of this template. Researchers must give serious consideration to the physiological and legal consequences of the use of any chemicals on research animals.