GENERAL INFORMATION & APPENDICES:
To assist in the completion of Animal Welfare Approval Forms

GENERAL INFORMATION AND PURPOSE OF THE ANIMAL WELFARE APPROVAL FORM

The Animal Protection Act, Animal Protection Regulation, and the Canadian Council on Animal Care require that complete information be supplied about all manipulations involving animals. The primary mandate of the Animal Welfare Committee is to review protocols from the perspective of the ethical and humane treatment of animals.

The following is the mission statement of the Canadian Council on Animal Care (CCAC):

> *"The Canadian Council on Animal Care works to ensure that animal-based science in Canada takes place only when necessary and that the animals in the studies receive optimal care according to high quality, research-informed standards."*

The Animal Welfare Committee (AWC) at the University of Lethbridge is charged with the responsibility of ensuring that all use of animals proceeds according to the standards established by the CCAC.

The Animal Welfare Approval Forms (research, teaching, field) are intended to help animal users at the University of Lethbridge communicate, in an orderly fashion, relevant information to the AWC in order to facilitate approval of animal use. Animal users will justify the use of animals in research, teaching or testing, demonstrate knowledge regarding the procedures they propose to employ, identify, and assess the potential for these procedures to cause animals to experience pain, discomfort or distress, and indicate measures adopted to eliminate or minimize such pain, discomfort or distress. Details of procedures that will be employed now and, in the future, can be relegated to Standard Operating Procedures (SOPs), thus simplifying current and future Animal Welfare Approval Forms.

Because each use of animals is different and specific, it is difficult to specify the level of detail required in an Animal Welfare Approval Form. Users should be guided by the instructions within the applicable Animal Welfare Approval Form (research, teaching, field). **A reader of this form should be able to easily understand when and how an animal is obtained and maintained, when it enters the protocol, at what point or points it experiences various procedures, and how the animal exits the protocol. Implications for health, pain and distress must be identified and addressed wherever pertinent.** Excessive and extraneous details will distract the reader from this understanding, whereas insufficient details will result in a request for additional information.

If anyone questions the use of animals at the University of Lethbridge, the University should be able to demonstrate that all animal use is carefully evaluated and justified, is regulated and monitored, follows excellent scientific and veterinary standards, and is on par with our established excellence in research and teaching. The protocol assessment and approval process is fundamental to such a demonstration.

For more information visit the Research Ethics Website, or click here for guidelines and forms

Note: The CCAC mandates that in the case of animal-based projects involving two or more institutions, the animal work is reviewed by the researchers' home institution as well as the host institution. All animal research conducted at the University of Lethbridge or by University of Lethbridge researchers at host institutions requires approval from the Animal Welfare Committee (AWC) to ensure that it meets home institution and CCAC standards. It is the responsibility of all researchers involved in the collaborative research to obtain the necessary approvals, permits or certifications before conducting collaborative studies. Please submit a copy of your approved protocol from the collaborating institute (home agency) at least 2 weeks before start-up.

REPONSIBILITIES OF PROJECT PERSONNEL AND PROTOCOL AUTHORS

Protocol authors have responsibility for all aspects of the protocol, including:

1. **ensuring that the AWC receives all the information required to conduct an informed review of the proposed animal use, and that it is approved before any animal use begins;**
2. **considering the Three Rs (replacement, reduction and refinement of animal use) and documenting that the proposed animal use is necessary, that the requested animal numbers are justified and that all appropriate refinements will be made (more information on the implementation of the Three Rs is available from the CCAC's Three Rs microsite located at: http://ccac.ca/en/ThreeRs);**
3. **ensuring that any amendments to the protocol are submitted to and approved by the AWC in a timely manner;**
4. **reporting back to the AWC on the work on at least an annual basis;**
5. **ensuring that all those in their team who will handle animals are appropriately trained and competent to undertake the procedures, and that they understand what is in the approved protocol. NOTE: Individuals performing invasive procedures must be appropriately trained. If the PI is on leave for more than one month, they must inform the AWC and the Director of Animal Care Services of the arrangements made regarding supervision of these individuals.**
6. **ensuring that the work is undertaken in practice, as approved in principle by the AWC, and meets institutional and CCAC standards.**

Scientists, teachers, technicians and students all have the responsibility to:

- **not use animals if a replacement alternative is available and appropriate;**
- **work with the AWC and veterinary and animal care staff in a collegial and respectful manner when animal use is necessary;**
- **treat all animals with respect and dignity; and**
- **respect institutional and CCAC standards.**

For additional questions, contact the Animal Research Ethics Officer: animal.ethics@uleth.ca

LIST OF APPENDICES:
To assist in the completion of Animal Welfare Approval Forms

Please consult the appendices below (as referenced in the Animal Welfare Approval Forms) for guidance.

APPENDIX I: CCAC CATEGORIES OF INVASIVENESS IN ANIMAL EXPERIMENTS

APPENDIX II: CCAC PURPOSE OF ANIMAL USE (PAU)

APPENDIX III: TEACHING PROTOCOLS
- Complete the Animal Welfare Approval form- Teaching

APPENDIX IVa: CONSULTATION SHEET ON THE SELECTION AND DOSAGE OF DIFFERENT DRUGS (RATS)
APPENDIX IVb: CONSULTATION SHEET ON THE SELECTION AND DOSAGE OF DIFFERENT DRUGS (MICE)
APPENDIX IVc: CONSULTATION SHEET ON THE SELECTION AND DOSAGE OF DIFFERENT DRUGS (FISH)

APPENDIX Va: ANESTHESIA AND SURGICAL PROCEDURES (RODENTS)
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APPENDIX VIa: MOST LIKELY CLINICAL CONDITIONS (RODENTS)
APPENDIX VIb: WELFARE INDICATORS/POSSIBLE CLINICAL CONDITIONS (FISH)

APPENDIX VIIa: MONITORING SCORE SHEET (RODENTS)
APPENDIX VIIb: FISH HEALTH OBSERVATION CHECKLIST
APPENDIX VIIc: WATER QUALITY AND UNIT MAINTENANCE LOG (AQUATIC SPECIES)

APPENDIX VIII: FIELD STUDIES
***- If your field study has a category of invasiveness of D or E, complete this appendix and attach in to your Animal Welfare Approval Form-Research. For field studies with a category of invasiveness of B or C, complete the Animal Welfare Approval Form-Field*.**

APPENDIX IX: TRANSGENIC INFORMATION SHEET
- This appendix must be completed and attached to your Animal Welfare Approval Form, if the work involves transgenic animals

APPENDIX Xa: RECOMMENDED BLOOD COLLECTION VOLUME AND FREQUENCY (RODENTS)
APPENDIX Xb: RECOMMENDED BLOOD COLLECTION VOLUME AND FREQUENCY (FISH)

APPENDIX XI: ANIMAL WELFARE ASSESSMENTS

APPENDIX I: CCAC CATEGORIES OF INVASIVENESS IN ANIMAL EXPERIMENTS

Investigators and teachers who consider it essential to use vertebrates or invertebrates in their research, teaching or testing in the laboratory or in the field, must adhere to humane principles, and take cognizance of the CCAC Ethics of Animal Investigation and other CCAC documentation in assigning a category. Protocols must be submitted to an appropriate review committee for all studies and courses, which involve the use of vertebrates and some invertebrates in Categories B through E. Cephalopods and some other higher invertebrates have nervous systems as well developed as in some vertebrates, and may therefore warrant inclusion in Category B, C, D, or E.

The following list of categories provides possible examples of experimental procedures, which are considered representative of each category.

A. **Experiments on most invertebrates or on live isolates**

Possible examples: the use of tissue culture and tissues obtained at necropsy or from the slaughterhouse; the use of eggs, protozoa or other single-celled organisms; experiments involving containment, incision or other invasive procedures on metazoa.

B. **Experiments which cause little or no discomfort or stress**

Possible examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes; intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category C); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose, or decapitation preceded by sedation or light anesthesia; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

C. **Experiments which cause minor stress or pain of short duration**

Possible examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies, laparoscopy; short periods of restraint beyond that for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed period of abstinence in nature; behavioural experiments on conscious animals that involve short-term, stressful restraint; exposure to non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.

NOTE: During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalization, aggressive-defensive behaviour or demonstrate social withdrawal or self-isolation.

D. **Experiments which cause moderate to severe distress or discomfort**

Possible examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioural stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of Freund's complete adjuvant (see CCAC Guidelines on Acceptable Immunological Procedures).

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress; the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems.

NOTE: Procedures used in Category D studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioural pattern or attitudes, the absence of grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

E. **Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals**

This Category of Invasiveness is not necessarily confined to surgical procedures, but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress; completely new biomedical experiments which have a high degree of invasiveness; behavioural studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; an euthanasia method not approved by the CCAC; any procedures (e.g. the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g. when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

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APPENDIX II: CCAC PURPOSE OF ANIMAL USE (PAU)

PAU 0 Breeding Colony/Stock

Animals held in breeding colonies (e.g., fish, rodents) that have not been assigned to particular research, teaching or testing protocol.

PAU 1 Studies of a **fundamental nature** in sciences relating to essential structure or function (e.g., biology, psychology, biochemistry, pharmacology, physiology, etc.).

Possible examples: studies designed to understand the cellular and/or molecular basis of inflammatory reactions or other basic physiological or biochemical reactions; studies designed to understand one or some of the various facets of the role played by a hormone or other compound produced by mammals; studies designed to better understand the behavior of various species; studies designed to better understand the population dynamics of various species.

PAU 2 Studies for **medical purposes**, including veterinary medicine, that relate to human or animal diseases or disorders.

These are studies carried out to better understand a specific disease or disorder and to help find therapies for it.

Possible examples: development of a mouse model for a specific type of cancer or other disease; studies to determine which antibodies are the most likely to contribute positively to the therapy of a specific type of cancer; studies to determine which molecule within a particular class of compounds is the most likely to contribute to maintaining stable blood glucose levels in an animal model of diabetes.

PAU 3 Studies for **regulatory testing** of products for the protection of humans, animals, or the environment.

Possible examples: safety testing, regulatory toxicology, vaccine efficacy trials and testing of new therapeutic compounds (if it is to generate data that is going to be used in a submission for an Investigational New Drug Application (IND) or for a New Drug Submission (NDS)); shellfish toxin.

PAU 4 Studies for the **development of products** or appliances for human or veterinary medicine.

These are the studies carried out to investigate potential therapies (as determined following studies of PAU 2) for humans or animals, before regulatory testing (PAU 3) is carried out on the most promising therapies.

Possible examples: studies undertaken in animals to investigate the role and effects of a specific drug or immunotherapy candidate for cancer; studies undertaken to develop physical devices to assist heart function; studies undertaken to develop artificial organs.

PAU 5 Education and training of individuals in post-secondary institutions or facilities.

These are teaching or training programs where animals are used to introduce students to scientific work and teach manual skills and techniques.

APPENDIX III: TEACHING PROTOCOLS

Complete the “Animal Welfare Approval form- Teaching” for PAU 5.

The use of animals for educational purposes is markedly different in its objectives than the use of animals in research or testing. Animals used for educational purposes are not being used to discover, prove or develop new ideas or techniques, but rather to demonstrate principles which are already well-known or to learn manual skills and techniques. The repetitive use of animals in this manner should be based on sound ethical justification and proven educational objectives.

There should be justification provided for the use of animals over the use of alternatives such as models, videos, computer simulations and emulations, etc. The level and type of training of the students (graduate/postgraduate, specialized/non-specialized) are important considerations in ascertaining the need to use animals.

Teaching protocols are subject to relevant review considerations, as well as to the factors of student inexperience and "group" projects. Thus, the description should include the number of students per animal, and the student/teacher ratio. The level of experience and competence of the instructors and/or teaching assistants must be adequate to assure successful preparations and procedures. The disposition of the animals at the end of the teaching session must be clearly described.

Painful experiments or multiple invasive procedures on an individual animal, conducted solely for the instruction of students in the classroom, or for the demonstration of established scientific knowledge, cannot be justified.

APPENDIX IVa: CONSULATION SHEET ON THE SELECTION AND DOSAGE OF DIFFERENT DRUGS (RATS)

Injectable anesthetic agents for RATS

Anesthetic drugs	dose (mg/kg)	route	duration
Ketamine/Xylazine	40-90/5-10 (mg/kg)	IM, IP	20-30 minutes
Ketamine/Xylazine/Acepromazine	50/5/1 (mg/kg)	IM, IP	20-40 minutes

Inhalation anesthetic agents for RATS

Phase of anesthesia	Oxygen (L/min)	Isoflurane
Induction	2.0-4.0 L/min	4-5%
Maintenance	0.5-1.0 L/min	1-2%
Recovery	0.5 then to 00	0

Local anesthetic agents for RATS

Local anesthetic drug	dose (mg/kg)	route	frequency
Lidocaine with epinephrine	2-4 mg/kg	SC	once

Analgesic agents for RATS

Analgesic drugs	dose (mg/kg)	route	frequency
Metacam	1 mg/kg	SC	every 24 hours
Buprenorphine (diluted as per Veterinarian or Manufacturer instructions)	0.03-0.05 mg/kg	SC	every 8-12 hours

*The buprenorphine should be injected pre-operatively and repeated only 8-12 hours later if required. Metacam is used post-operatively as an anti-inflammatory/analgesic in conjunction with the pre-operative dose of buprenorphine as per protocol. Metacam can be administered pre-operatively in the event that buprenorphine cannot be used (consult veterinarian for more information).

Anti-convulsing agents for RATS

Anti-convulsing drugs	dose (mg/kg)	route	frequency
Diazepam	2.5-10.0 mg/kg	IP	can be repeated as needed
Phenobarbital	12.5-100 mg/kg	IP	every 12 hours

Antibiotic agents for RATS

Antibiotic agent	dose (mg/kg)	route	frequency
Baytril (diluted as per Veterinarian or Manufacturer instructions)	10 mg/kg	SC	every 24 hours
Tribrissen	30 mg/kg	SC	every 12 hours

APPENDIX IVb: CONSULATION SHEET ON THE SELECTION AND DOSAGE OF DIFFERENT DRUGS (MICE)

Injectable anesthetic agents for MICE

Anesthetic drugs	dose (mg/kg)	route	duration
Ketamine/Xylazine	75-150/16-20 (mg/kg)	IP	20-30 minutes
Ketamine/Xylazine/Acepromazine	75-100/8-10/3 (mg/kg)	IP	20-40 minutes
Ketamine/Medatomidine	75-150/0.5-1 (mg/kg)	IP	20-30 minutes

Inhalation anesthetic agents for MICE

Phase of anesthesia	Oxygen (L/min)	Isoflurane
Induction	1.5-2.0 L/min	4-5%
Maintenance	0.5-1.0 L/min	1-2%
Recovery	0.5 then to 00	0

Local anesthetic agents for MICE

Local anesthetic drug	dose (mg/kg)	route	frequency
Lidocaine with epinephrine	2-4 mg/kg	SC	once

Analgesic agents for MICE

Analgesic drugs	dose (mg/kg)	route	frequency
Metacam	5-10 mg/kg	SC	every 24 hours
Buprenorphine (diluted as per Veterinarian or Manufacturer instructions)	0.05 – 0.1 mg/kg	SC	every 8-12 hours

*The buprenorphine should be injected pre-operatively and repeated only 8-12 hours later if required. Metacam is used post-operatively as an anti-inflammatory/analgesic in conjunction with the pre-operative dose of buprenorphine as per protocol. Metacam can be administered pre-operatively in the event that buprenorphine cannot be used (consult veterinarian for more information).

Anti-convulsing agents for MICE

Anti-convulsing drugs	dose (mg/kg)	route	frequency
Diazepam	5 mg/kg	IP	can be repeated as needed
Phenobarbital	30 mg/kg	IP	every 12 hours

Antibiotic agents for MICE

Antibiotic agent	dose (mg/kg)	route	frequency
Baytril (diluted as per Veterinarian or Manufacturer instructions)	10 mg/kg	SC	every 24 hours
Tribrissen	30 mg/kg	SC	every 12 hours

APPENDIX IVc: CONSULATION SHEET ON THE SELECTION AND DOSAGE OF DIFFERENT DRUGS (FISH)

Immersion anesthetic agents for fish

Anesthetic drugs	dose (mg/L)[1]	induction time	recovery time
Tricaine methaneslfonate (TMS or MS-222)[2]	60-185 mg/L (fathead minnows)[3]	< 8 minutes	< 10 minutes
	80-300 mg/L (goldfish)	< 5 minutes	< 10 minutes
	80-300 mg/L (rainbow trout)	< 5 minutes	< 10 minutes
Metomidate hydrochloride (Aquacalm)	5 mg/L (rainbow trout)	< 5 minutes	< 20 minutes

[1]Dose at low end of range should be first tested on a small sample of fish as the effect of an anesthetic can vary with local water conditions, as well as the species, life stage, and size of the fish.
[2]TMS is acidic and must be buffered to pH 7.4 +/- 0.2 in holding tank water with sodium bicarbonate before use. The amount of sodium bicarbonate should be double the amount of MS-222 added.
[3]A dose of 60 mg/L TMS will anesthetize a fathead minnow in roughly 5-8 minutes. Doses in the range of 120-185 mg/L TMS will result in deep anesthesia in a matter of seconds and have a higher risk of accidental euthanasia. It is recommended that procedures that require TMS for anesthesia are first tested with a dose in the range of 60-100 mg/L.

APPENDIX Va: ANESTHESIA AND SURGICAL PROCEDURES (RODENTS)

ANESTHESIA

Animals under anesthesia require constant monitoring until they are recovered. Provide details of the anesthetic protocol used.

Include:

1. **Pre-surgical medication, if given (i.e. drug name, dose and route).**
2. **Induction (i.e. drug name, dose and route). If gas anesthesia is used, state the drug name and whether the animal is masked down or placed in an induction chamber.**
3. **Maintenance. State the oxygen flow rate and % isoflurane used (or other gas, if applicable) If using injectable drugs for general anesthesia, record any additional drugs given during the procedure. Provide an oxygen source in case of an emergency.**
4. **Recovery. Indicate how often the animal is observed, and when the animal will be expected to return to its home cage.**

SURGICAL PROCEDURES

Is the surgical procedure survival (chronic) or non-survival (acute)? If survival, provide a description of the preparative regimen. If non-survival, omit points 2, 6 and 8.

Include the:

1. **patient preparation procedures**
2. **detail regarding pain/distress management. NOTE: Analgesics should be given to animals prior to recovery from anesthesia and for a minimum of 24 hours following surgery. More invasive surgeries require longer analgesic therapy.**
3. **type of monitoring during and following surgery**
4. **method to alleviate hypothermia**
5. **method of fluid therapy, if needed**
6. **antibiotic to be administered, if needed (i.e. dose and route)**
7. **brief technical description of the surgical procedure(s). Indicate SOP# in lieu of a description. Include the expected time course for the surgery.**
8. **post operative care. Include any expected conditions that will require further treatment (i.e. paralysis, or seizures). Describe the management plan.**

APPENDIX Vb: ANESTHESIA AND SURGICAL PROCEDURES (FISH)

ANESTHESIA

Animals under anesthesia require constant monitoring until they are recovered. Provide details of the anesthetic protocol used.

Include:

1. **Pre-surgical medication, if given (i.e. drug name, dose and route).**
2. **Induction (i.e. drug name, dose and route).**
3. **Recovery. Indicate how often the animal is observed, and when the animal will be expected to return to its home cage.**

SURGICAL PROCEDURES

Survival surgery involving fish requires attention to operating table set-up, surgical techniques, and selection of materials. Careful attention must be paid to very specific housing and maintenance requirements before, during and after surgery to ensure the survival and the return to normal physiological function of the fish. Please indicate if the surgical procedures are for survival or non-survival surgery. For non-survival surgery, omit point 5.

Include the:

1. **animal preparation procedures (e.g. fasting period prior to handling, procedures to minimize damage to mucus-skin barrier, procedures to minimize exposure to light and air)**
2. **details regarding pain/distress management (e.g. incision placement, surgical preparation and skin disinfection, suture materials and techniques)**
3. **type of monitoring during and following surgery**
4. **brief technical description of the surgical procedure(s). Indicate SOP# in lieu of a description. Include the expected time course for the surgery.**
5. **details regarding post operative care (e.g. monitoring of water quality, return to normal feeding and other behaviours, consultation with the University Veterinarian regarding antibiotic use if necessary)**

APPENDIX VIa: MOST LIKELY CLINICAL CONDITIONS (RODENTS)

Body Condition
Blue and cold extremities (ears, paws)
Decreased body condition
Decreased body weight compared to control
Hunched posture
Lack of grooming (porphyrin staining)
Lethargic
No peer interaction (isolated)
Not responsive
Ocular discharge
Ruffled hair coat
Skin lesions (alopecia, redness, ulceration)

Oral / GI / Liver
Constipation
Decreased food/water intake, anorexia
Diarrhea
Feces altered in volume, color, consistency (black with blood, pale)
Fluid accumulation in body cavities, subcutaneous tissues, etc.
Jaundice
Mucus gel-like stool
Prolapsed rectum
Salivation
Vomiting

Tumours/Neoplasia
Multiple tumours
Palpable internal mass
Ulceration/infection of tumour site
Tumour location interferes with normal bodily function
Tumour size

CNS, PNS, Muscle, Skeletal
Convulsions
Head tilt
Muscular flaccidity, rigidity or weakness
Lameness
Paralysis
Paresis
Twitching

Heart/Respiration
Difficulty breathing (dyspnea)
Heart rate changes (increased/decreased)
Nasal discharge
Noisy breathing from congestion in lungs (rales)
Respiratory rate changes (increased/decreased)

Demeanor (pain)
Aggressive
Restless (circling)
Vocalizing

Dehydration
Skin tenting
Sunken eyes

APPENDIX VIb: WELFARE INDICATORS / POSSIBLE CLINICAL CONDITIONS (FISH)

In any study where there is expected morbidity and mortality, the criteria for early euthanasia should be defined. Frequency of monitoring should allow for the timely removal of fish, before severe morbidity occurs.

Physical Appearance
Abnormal
Eye condition
Fin and skin condition
Mucus production
Colour change (usually a darkening associated with disease or bilateral blindness, however some parasites and bacterial infections can cause a paleness or loss of colour in fish)

Measurable Clinical Signs
Feed consumption
Respiratory rate
Position in water column, i.e. the individual's position in the water (upright, upside down, tilted, etc.)

Unprovoked Behaviour
Position in the water column (e.g. crowding near the inlet or outlet pipe, shoaling, etc.)
Gasping at the surface
Irregular swimming behaviour
Social interactions – direct attack, domination of choice tank locations, schooling, social isolation (i.e. fish either socially isolated or choosing to isolate themselves from the group), not responsive to external stimulation
Hyperactivity / hypoactivity – movement (abnormal movements such as flashing or scraping the body), unexpected jumping or escape behaviour

Provoked Behaviour
Feeding activity
Threat response
Avoidance reaction to mechanical prod
Avoidance reaction to light beam

APPENDIX VIIa: MONITORING SCORE SHEET (RODENTS)

Animal ID #:	Score	Date/Time	Date/Time	Date/Time	Date/Time	Date/Time	Date/Time
Appearance:							
Normal (smooth coat, clear eyes)	0						
General lack of grooming (mildly rough coat)	1						
Moderately rough coat, ocular, nasal discharge or rough skin	2						
Severe piloerection, wounds, cysts, malclusion	3						
Body weight:							
Normal (0% decrease)	0						
Possible body weight loss (0-5% decrease)	1						
Clear decreased body weight (6-25%)	2						
Significant body weight loss (>25% decrease)	3						
Clinical signs:							
Normal body temperature, cardiac, respiratory rate and hydration	0						
Slight changes (increase or decrease)	1						
Moderate changes T+/-1C, C/R rates up/down 30%, measurable dehydration	2						
Significant changes T+/-2C, C/R rates changed 50%, severe dehydration	3						
Behaviour:							
Normal – Alert/calm (food/water consumption, cage exploration)	0						
Minor changes (e.g. awkward grooming, but mobile and alert)	1						
Less mobile AND less alert	2						
Minor movement, hunched, no grooming, frequent vocalization, no food and water intake	3						
TOTAL							
Scorer's Initials:							

Humane Endpoints and Actions:

0-4 Normal
5-7 Monitor carefully and more regularly
7-8 Monitor cautiously, likely suffering (most likely requires analgesics)
>9 Likely experiencing pain or distress. **Obtain a second opinion to terminate animals**

APPENDIX VIIb: FISH HEALTH OBSERVATION CHECKLIST

Daily Fish Health Observation Checklist

Page #: ____

Laboratory: _____ **ARF File #: _____**

Unit ID: _____ **Protocol #: _____**

Date *dd/mm/yy*	Time	Feeding			Lying at surface / not swimming off	Crowding around water inlet / Gulping at surface	Excitable / darting / tilted / erratic swimming / twirling	Bloated / eyes bulging out	Normal movement of gills / operculum	Number of fish with deformed body / lesions	Lost or damaged scales/ fins / opercula / tail	Change of any environmental enrichment	Comments	Initial
		Consumed within a few minutes	Reduced	Stopped										
	am:													
	pm:													
	am:													
	pm:													
	am:													
	pm:													
	am:													
	pm:													
	am:													
	pm:													

Humane Endpoints and Actions:

Conditions indicated in shaded columns should be marked as present (+) or absent (-). By convention negative signs indicate normality. If fish with any of these symptoms are found, a report should be made with the ARF technicians.

Identification of 'sick fish':

APPENDIX VIIc: WATER QUALITY AND UNIT MAINTENANCE LOG (AQUATIC SPECIES)

Recognition of the initial symptoms of a 'sick fish' before outbreak of disease is very complicated. It is important to monitor and ensure that fish are apparently healthy in the tank. Fish health is evaluated by carefully observing the physical appearance and behavior of fish in every tank. If a tank having mortalities >10% over a 3-day period should be suspected of having disease or environmental (water quality) problems, immediately contact the principal investigator and/or ARF technicians. Common symptoms of 'sick' and 'weak' fish include but are not limited to:

i. Body shape emaciated ("skinny") or bent; visible spots or red streaks, lesions, lumps, or white patches on the fish's body or fins.
ii. Fish tail or fins are jagged or frayed at the edges or are breaking off or disappeared.
iii. Scales bloating with raised scales, resulting in a fuzzy appearance.
iv. Skin discoloration or change coloration.
v. Fins held close or folded rather than stretched out and spread wide open. Clamped fins can be a very vague finding but a good indication for an unhealthy fish.
vi. Torn or abnormally truncated fins.
vii. Fish gasping at the surface of the water.
viii. Gills are puffy or swollen or flared and gill tissues are bright red or even a grayish color.
ix. Damaged or missing operculum (gill covering).
x. Eye bulging or protruding.
xi. Fish crashes or prolonged resting on tank bottom or floating at the surface indicates that the fish no longer has the energy to swim and doesn't have much longer to live.
xii. Fish refuses or reduces the amount of regular food ration for more than 2 days indicates that the fish is in stress due to parameters such as water quality, environmental, overcrowding etc.
xiii. Erratic swimming (head-up, twirling, tilting etc.) indicates loss of balance to stay in water column.

If fish with any of these symptoms are found, a report should be made with the ARF technicians.

Water Quality and Unit Maintenance Log

Biofilter activation ☐ **Quarantine** ☐ **Experimental** ☐

Page #: ____

Laboratory: WE00____ Seas ☐ **Ecol** ☐ **Phys** ☐ **Tox** ☐ **ARF File #:** ________________

Unit / Tank ID: ________________________ **Protocol #:** ________________

Water Quality								Feeding		Waste removed	Comments	Initial
Date (dd/mm/yy) and Time	**Water Temp.** (Monitron)	**pH** (calibrate daily)	**Dissolved oxygen** (Monitron)		**TAN[1] (N-NH_3)** *Program 64*	**Nitrite[2] (N-NO_2)** *Program 60*	**Nitrate[3] (N-NO_3)** *Program 51*	**Time**	**grams of feed and/or % of total body mass**			
	(°C)		%	(mg/L)	(mg/L)				g / %	(✓)		**Print**
	T1:							***am***	g ☐ ☐%			
	T2:				*UA =*			***pm***	g ☐ ☐%			
	T1:							***am***	g ☐ ☐%			
	T2:				*UA =*			***pm***	g ☐ ☐%			
	T1:							***am***	g ☐ ☐%			
	T2:				*UA =*			***pm***	g ☐ ☐%			
	T1:							***am***	g ☐ ☐%			
	T2:				*UA =*			***pm***	g ☐ ☐%			
	T1:							***am***	g ☐ ☐%			
	T2:				*UA =*			***pm***	g ☐ ☐%			

[1] Un-ionized ammonia **(UA)** in mg/L = TAN (mg/L x fraction of un-ionized ammonia (from Table 1);
Un-ionized ammonia (NH_3) tolerances: coldwater (5^{0}C – 17^{0}C) = 0.0125 mg/L; warm water (18^{0}C – 30^{0}C) = 0.02 mg/L
[2] Nitrite (N-NO_2) tolerances: coldwater (5^{0}C – 17^{0}C) = <0.2 mg/L; warm water (18^{0}C – 30^{0}C) = <0.1 mg/L
[3] Nitrate (N-NO_3) tolerances: 13 mg/L for all life stages of finfish other than egg stage (3 mg/L)

Unit maintenance:	**Date**	**Initials**
a) Floor disinfected:	______________	______
b) D.O. membrane cleaned (weekly):	______________	______
c) D.O. probe calibrated (monthly):	______________	______
d) Filter/activated carbon screen cleaned (for holding tank, 2x weekly):	______ / ________	___ / ___
e) Cartridge filter cleaned:	______________	______
f) Aragonite/carbon filter cleaned:	______________	______
g) Virkon Aquatic solution test strip:	______________	______
h) Virkon Aquatic replaced:	______________	______

APPENDIX VIII: FIELD STUDIES

The Animal Protection Act, Animal Protection Regulation, and the CCAC require that complete information be supplied about all manipulations involving animals. The primary mandate of the Animal Welfare Committee (AWC) is to review protocols from the perspective of the ethical and humane treatment of animals.

Experimental procedures involving the capture, handling and release of wild animals are of special concern as a lack of conditioning results in a high degree of stress in captured wild animals. The necessity for capture, handling and/or administration of drugs or other compounds must be clearly established. Detailed descriptions of all pursuit, capture, handling and chemical restraint procedures, and explanations of their appropriateness, are essential. Criteria used to assess suitability for release must be clearly stated. Provision for recovery, treatment, or euthanasia of injured animals and disposal of carcasses must be specified.

If traps are to be used, the type of trap, its injury potential, and the monitoring frequency of the traps are important considerations. The collection of samples and affixing of devices to the animal(s) must be described (weight, method of attachment, duration) and be clearly related to the objective(s) of the study. Protocols for field studies involving population sampling by killing of animals (e.g. using methods such as shooting), must include justification for the method used. The use of such methods must be by individuals with sufficient experience and expertise to ensure that the animals are humanely killed.

Wildlife research may involve the use of specialized holding areas and transportation of animals. The potential for injury to personnel and the animals during these procedures should be minimized. The holding of wild animals in highly confined enclosures for extended periods should be avoided.

Ecological disruption may result from the performance of some types of field studies. The type and degree of disruption expected (or its potential) must be indicated (e.g., the adverse consequences to survival and reproduction experienced by the herd, colony, or individual animal due to the study procedures).

Provide the following information:

1. **Method of capture/restraint, duration of captivity, and monitoring frequency**
2. **Transportation and/or housing of animals in the field**
3. **Release of captured animals (i.e. will they be released at or near the capture site, or will they be relocated to other locations and/or populations?)**
4. **Capture of non-target species**
5. **Potential injury/mortality**
6. **Special handling**
7. **Ecological impacts**
8. **Other pertinent information (i.e. address additional risk factors associated with returning animals to the wild successfully, such as preventing the transmission of disease)**

It is the Principal Investigator's responsibility to obtain the necessary wildlife permits. Permit numbers must be sent to the Animal Welfare Coordinator when they have been obtained.

APPENDIX IX: TRANSGENIC INFORMATION SHEET - ***This appendix must be completed and attached to your Animal Welfare Approval Form, if the work involves transgenic animals.*** *Revised September 24, 2018*

Consult the Canadian Council on Animal Care's (CCAC) Guidelines on Transgenic Animals (http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/TRANSGEN/TRANSGE1.HTM) and provide the following information:

Principal Investigator	
Department/Faculty	
Telephone Number	
Email Address	
Project Title	
Animal Species	
Strain (full nomenclature)	
Heterozygotes and/or Homozygotes and/or Hemizygotes	
Indicate the % of offspring with the desired genotype when this TG strain is generated	
Housed in Room	

1. **What is the health profile of the source colony? Provide the most recent serology report.**
2. **What known traits will affect breeding and lifespan?**
3. **What abnormalities are known to exist (or do you expect) in these animals?**
4. **If you expect these abnormalities will cause pain or distress, how will you minimize or alleviate it?**
5. **Describe your monitoring and recording procedures for detecting physical and behavioral abnormalities which are indicative of pain and distress.**
6. **What objective criteria will be used to determine if an animal will be removed from the study prematurely?**
7. **If biological containment is required, state reasons and the level required. Describe your containment and security procedures. How will you deal with breach of containment? Will there be any risks to human health, wild populations or environment generally if containment fails?**
8. **If you are generating a novel transgenic strain, provide a timetable for this process and indicate when you expect to report back to the Animal Welfare Committee on the phenotype obtained.**

APPENDIX Xa: RECOMMENDED BLOOD COLLECTION VOLUME AND FREQUENCY (RODENTS)

Procedures

1. **Do not collect blood from a site presenting inflammation or a hematoma.**
2. **Limit the number of venipunctures to four punctures per day with no more than two punctures per site.**
3. **The following table shows the maximum volume of blood which can be collected at once or over a 24-hour period, and the corresponding recovery time during which the animal should not be subjected to blood collection again (based on the percentage of total blood volume collected). Example: for a mouse, the sum of blood volumes collected over 24 hours cannot exceed 0.4 mL and the animal cannot be collected again before 4 weeks.**

Percent of blood volume collected at once (Single Sampling)	Recovery period in weeks	Percent blood volume collected over a 24 hour period	Recovery period in weeks
7.5%	1	7.5%	1
10%	2	10-15%	2
15%	4	20%	4

Species	Total Blood Volume (mL)	7.5% (mL)	10% (mL)	15% (mL)	20% (mL)
Mouse (26 g)	1.8	0.1	0.2	0.3	0.4
Rat (250 g)	16	1.2	1.6	2.4	3.2
Rabbit (4 kg)	224	17	22	34	45
Dog (10 kg)	850	64	85	127	170
Cat (3 kg)	168	12.8	17	25.5	34
Pig (30 kg)	1950	146	196	292	390
Ferret (1 kg)	70	5.3	7	10.5	14
Guinea Pig (200 g)	14.6	1.2	1.5	2.3	3.0
Hamster (100 g)	7.8	0.6	0.8	1.2	1.6

APPENDIX Xb: RECOMMENDED BLOOD COLLECTION VOLUME AND FREQUENCY (FISH)

Compared to mammals, the volume of blood per unit of body weight is considerably less in bony fish. In general, it is recommended that no more than 0.1% of the fish's body weight (i.e. 1 mL/kg) be collected during a single survival blood collection from a non-compromised, healthy fish (*Blood Sampling of Finfish*, Canada Department of Fisheries, 2004). Species-appropriate sedation or anaesthesia should be used to restrain fish for blood collection purposes, and sufficient time must be allowed for a fish to recover its hematocrit prior to subsequent blood collection. Hematocrit recovery times are temperature-dependent and highly variable between species.

Proposed methods for blood collection must be detailed in the protocol or appended as a standard operating procedure. Blood collection should only be undertaken by trained personnel using sterile equipment.

APPENDIX XI: RECOMMENDED ANIMAL WELFARE ASSESSMENT

The CCAC published guidelines, in 2021, on completing animal welfare assessments:

> *"This specific document was developed based on the recognition that animals used for scientific purposes should have good welfare, and that this requires more than ensuring they are healthy. Good welfare is characterized by maximizing animals' positive experiences while minimizing their negative ones. This approach to ensuring good welfare is already at the core of many existing practices, such as health monitoring, humane intervention point implementation, post-approval monitoring, and the assignment of categories of invasiveness. Formal welfare assessments are another tool to ensure that animals have the best possible welfare."*

The frequency of animal welfare assessments, and the behaviours or conditions that requiring scoring, will vary from project to project. As invasiveness increases, so should the frequency of assessment. A project looking at tumor growth may include assessment variables that would not be included in a project that looks at behaviour. Amended projects, where the experimental endpoint has been extended, may require more frequent assessments. Animals that are expected to be sick or develop higher levels of stress, based on the project they are involved in, will have different humane endpoints than animals that are not expected to become sick or significantly stressed – and this should all be reflected in your assessment plan.

Please use the following checklists as the starting point for developing your welfare assessment template and provide an outline in your protocol or renewal describing the frequency of assessment, in addition to any behaviours or signs of illness that may be specific to your research.

At a minimum, each animal should be assessed once per week. If you have any questions, please reach out to the Animal Research Ethics Officer or the University Veterinarian for clarification.

SEE SOP#614 (ANIMAL WELFARE ASSESSMENT) FOR ADDITIONAL GUIDANCE AND TOOLS.

ANIMAL WELFARE ASSESSMENT – FISH

Date *dd/mm/yy*	Feeding			Lying at surface / not swimming off	Crowding around water inlet / gulping at surface	Excitable / darting / tilted / erratic swimming / twirling	Bloated / eyes bulging out	Normal movement of gills / operculum	Number of fish with deformed body / lesions	Lost or damaged scales/ fins / opercula / tail	Change of any environmental enrichment	Comments	Initial
	Consumed within minutes	Reduced	Stopped										