Addendum 6 -Laurentian University
Animal Care Facility Policies

Laurentian University maintains a policy supporting the responsible use of animals in research for the purpose of obtaining knowledge essential to preventing and curing human and animal disease, eliminating pain and suffering, and in teaching for the purpose of scientific and technical education. Laurentian University co-operates and complies with all agencies regulating the use of laboratory animals.

The reader should also familiarize themselves with the University's Animal Care Committee Terms of References, Animal Use Protocol (AUP) and Standard Operating Procedures (SOP's) as it pertains to their research protocol. If your methodology differs from the prescribed Animal Care Committee (ACC) accepted SOP you will need to substantiate this in your AUP.

PRINCIPLES OF ANIMAL CARE

The provision of humane care of animals in research and teaching will be assured by adherence to the following principles:

1. All projects involving the use of animals must be approved by the Animal Care Committee in accordance with the regulations of the Animals for Research Act of the Province of Ontario and the guidelines and policies of the Canadian Council on Animal Care.
2. Animals will only be used when alternative procedures are not feasible.
3. The species will be carefully selected to ensure the most effective use of animals.
4. The least invasive techniques possible will be employed.
5. The number of animals used will be the minimum required to achieve the objectives of the research/teaching programme.
6. Alleviation/reduction of pain and distress will be of prime concern during and following all procedures.
7. All animals will be cared for according to current veterinary standards.

Access to the Facility: investigators and students requiring access will first have to show that they have completed an adequate Training program see "Training Guidelines". The University Administration will have final decision re. admission and key disbursements and the privilege of having a key is contingent on satisfactorily following the university guidelines as per the Terms of Reference and University Policies. These rights can be withdrawn if the ACC should decide that the researcher is contravening University policies. This decision will be given in writing to the person involved. The appeal process will follow the process under the Terms Of Reference.

Access to Space: Space may become an issue in the facility and this may result in a proposal not being approved or contingent on space requirements. The University Administration will have the final say on availability. A per diem rate will be applied for animal use and the rate schedule can be obtained from the Vice-President, Research. All drugs must be approved by the veterinarian.

Animal procurement: Must be approved prior to purchase. Newly acquired animals can introduce disease into established colonies. In addition, production colonies maintained by suppliers occasionally experience outbreaks of disease. The Animal Care Facility (ACF) under the auspices of the University Veterinarian monitors animal health quality from different suppliers and maintains quality control data provided by vendors. This information can be provided to investigators to assist in choosing appropriate sources of animals. These sources have to be approved by the Animal Care Committee prior to ordering. Animals to be ordered or bred have to have an approved protocol. If it is found that an investigator has either ordered or is breeding animals without an approved protocol, the ACC can withdraw access to the facility and the ACC may order the humane euthanasia of the animals. See SOP's LU Trangenic Animals", "LU Breeding Colonies".

Quarantine and stabilization: With some species of laboratory animals, quarantine is necessary to minimize the introduction of disease into established colonies. The extent of the quarantine period is determined by the species and by knowledge of the animal's source and previous history as well as by regulatory requirements. Arriving animals, regardless of source, should be allowed a stabilization period before use. Such a period allows the animal to recover from shipping stress, adapt to its new surroundings and become physiologically stable. A minimum acclimation period of 72 hours is suggested for all species. A longer time period may be necessary if abnormally high shipping stress is detected eg. very cold or hot weather.

Separation of species: Physical separation of animals by species is generally required to reduce the possibility of transmission of latent diseases and to prevent possible inter-species aggression or distress. This separation is usually accomplished by housing different species in separate rooms. Even when animals of the same species are obtained from multiple sources, their microbiological status may differ, in which case separate housing as provided by barrier caging or separate rooms may be advisable. It should be noted that transgenic animals may be considered a distinct species.

Transgenic Animal Use : Because of the many complications utilizing these animals, it is imperative for the researcher to be fully aware of the issues involved. **See SOP's "LU Transgenic Animals", "Policy on Endpoints for Transgenic Animals".** These animals are regarded as a possible societal issues and measures for confinement, phenotypic display and plans for capture upon escape as well as close monitoring of animals is stringently required by the Principal Investigator. Breeding protocols are also imperative. **See SOP "Transgenic Breeding".**

Peer Review and Scientific Merit : All animal users need to be fully cognizant of the University's Terms of Reference and Animal Use Protocols which spell out the required procedures. **See. SOP "Peer Review".**

Endpoints: It is extremely important for researchers to be fully aware of their animals' distress level and to develop observational skills to assess this so that animals do not reach a point where death frequently occurs. Investigators need to be able to write down objective criteria so that they can make decisions when that animal needs to be euthanized. The endpoint should never be the death of the animal unless the researcher can convince the ACC of the necessity of this. **See SOP ""endpoints LU", "Endpoints Checklist"**. The researcher should be very aware of the CCAC Guidelines on Endpoints.

Surveillance, Diagnosis, Treatment and Control of Disease

A comprehensive veterinary medical program is in effect for all animals maintained at Laurentian University. ACF personnel check animals in ACF maintained rooms daily, including weekends and holidays, for signs of illness, injury, or abnormal behavior. In cases where such observation will interfere with experimental objectives, prior arrangements must be made with the ACF to ensure adequate monitoring of animals and environmental systems as well as maintaining proper standards of cleanliness. If these standards are not met, the Facility staff will clean these areas after notifying the principal investigator. The investigator may be charged for this service.

To ensure the health status of animals maintained on campus, general medical surveillance procedures have been developed for each species. For example, in-house health monitoring is done for rodents as part of a sentinel monitoring program and is in effect in all ACF managed facilities. Serology, bacteriology, and parasitology samples are periodically taken from each sentinel cage and submitted to a diagnostic laboratory for mouse or rat comprehensive testing. If evidence of murine viruses, parasites, or bacterial pathogens are discovered and confirmed in the sentinel animals, investigators are notified. **See SOP's "Sick Animal Report", "Mouse Sentinel Program Design", "Incident/Sickness Report" and "LUQ (Laurentian Quality Assurance program".**

Health surveillance information, including information provided to us by our vendors, is maintained on file and available to investigative staff upon request. For more information on the surveillance, diagnosis, treatment, and control of animal diseases, including the possible affects of disease on experimental animal models, contact the University Veterinarian.

Emergency Care

Veterinary care is provided 24 hours a day, 7 days a week. Any health problem noted by any animal user at any time, including evenings, weekends and holidays, should be reported immediately to the Facility technicians, Animal Care Committee (ACC)chair or the university veterinarian. If the veterinary technician is not immediately available, a detailed message including the animal's identification, room number where it is located, the species, nature of the clinical problem, and the telephone number of the person making the report, should be left on

the university veterinarian phone line or answering service.

Anesthesia and Analgesia

Animal procedures are reviewed by the ACC to ensure that proposed anesthetics and/or analgesics are appropriate for the species and research objectives. The ACF veterinary staff is available upon written request to provide assistance with, or training in the proper administration and use of anesthetics.

Written documentation of all surgical procedures, including the types, amounts, and time of administration of anesthetic, analgesic or tranquilizing drugs used and the physiologic parameters (i.e., heart rate, respiratory rate, body temperature, etc.) monitored during the procedure, is required **see SOP for " Recovery Surgery " and SOP for "Post Op Monitoring**". The CCAC Guide for the Care and Use of Laboratory Animals requires that any proposal to conduct painful procedures without anesthesia or analgesia must be scientifically justified by the investigator and approved by the institutional animal care and use committee. Such procedures must be directly supervised by the responsible investigator. Suggested dosage for pre-anesthetics, tranquilizers, anesthetics, and analgesics are provided in the appropriate **SOP' for "Rodent Anesthesia" and "Rodent Analgesia" and "Mice and Rat Anesthesia**".

Surgery and Postoperative Care

Survival surgery: Survival surgery is defined as any surgery from which the animal recovers consciousness. Major surgery is defined as any surgical intervention that penetrates a body cavity or has the potential for producing a permanent handicap in an animal that is expected to recover. Minor surgery is any operative procedure in which only skin or mucous membrane is incised (e.g., vascular cutdown for catheter placement or implanting pumps in subcutaneous tissue). Because they are minimally invasive, gonadectomies on rodents and lower vertebrates are usually considered minor surgical procedures. Multiple major survival surgery is defined as two or more major survival surgical procedures performed at separate times on a single animal. It is permitted only under special circumstances, such as when the surgeries are essential and related components of a single scientific study. Cost alone is not an adequate reason for performing multiple major survival surgeries on an animal.

Surgical procedures on mammals require ACC approved surgical facilities intended for that purpose and using aseptic techniques. **See SOP for "survival Surgery**"

Minor surgical procedures may be performed in a suitably located and equipped laboratory area, subject to approval by the ACC. Appropriate aseptic technique for these procedures includes a clean uncluttered work area, preparation of the surgical site including clipping of the hair, disinfection of the skin and draping of the surgical site with sterile drapes; the use of sterile supplies and instruments; and the use of sterile gloves and a surgical mask by the surgeon and any assistants working in the surgical field.

Pre- and Postoperative care: Animals (other than some rodents) should generally be fasted prior to anesthesia and surgery to prevent vomiting, aspiration, and problems associated with a distended intestinal tract. Animals should be evaluated by performing a brief physical examination and while recording baseline physiologic measurements of such parameters as body temperature, heart rate, and respiratory rate prior to the administration of an anesthetic agent are of benefit, these criteria may be waived in some cases. Animals should be weighed and dosages of agents administered calculated individually according to body weight measurements.

Postsurgical care includes clinical observation of the animal to ensure uneventful recovery from anesthesia and surgery. Rodents must be fully recovered prior to returning to their usual cage and room. Once the animal has been returned to its normal housing area, subsequent care may be necessary. This may include supportive fluids, analgesics, and other drugs as required; monitoring of the animal to include daily body temperatures, clinical observations for signs of pain, abnormal behavior, appetite and excretory functions, and providing adequate care of surgical incisions. The investigator is responsible for supportive care unless arrangements have been made to contract ACF veterinary staff for these services. Written post-operative records including date, time, person making the observations, condition of animals, and any treatments/procedures performed should be maintained at the animal room where the animals are held for inspection by the veterinarian, technicians or governmental agencies incl. OMAF and the CCAC. For examples of surgical record forms and postoperative regimens, see the veterinarian or facility technicians **see "post op monitoring**".

Non-survival surgery: Non-survival surgery is defined as any surgery in which the animal will not regain consciousness after being anesthetized. Such procedures may be performed in a suitably located and equipped laboratory, subject to ACC evaluation and approval.

Euthanasia

Euthanasia is generally performed at the end of a project or, if possible, during a procedure in which animals experience severe or chronic pain or distress that cannot be relieved. Since there may be a need to euthanize animals for unanticipated reasons even on protocols that do not include euthanasia as part of the planned project, at least one method must be documented for each species used in a protocol. The euthanasia method chosen must be appropriate for the species and research use described by the protocol, and must be consistent with the recommendations of the American Veterinary Medical Association Panel on Euthanasia (2012), or succeeding revised editions) and CCAC standards eg. CCAC Guidelines on euthanasia of animals in science. If the method deviates from AVMA recommendations, the deviation must be justified scientifically and approved by the ACC. Euthanasia should be performed quickly and efficiently in a nonpublic area but generally not in rooms in which animals are housed. **See "Rodent Euthanasia SOP**".

This document describes the methods of animal euthanasia that are recommended. Additional methodologies may be approved under certain circumstances. ACF staff are available to demonstrate and/or discuss these techniques. Part 1 lists methods by species, while Part 2 includes a general discussion of each method. Conditionally acceptable methods must be

scientifically justified by the Principal Investigator and approved by the ACC.

Part 1

Recommended Agents and Methods of Euthanasia Listed by Species

FISH

- Tricaine methane sulfonate (MS222)
- Benzocaine
- Barbiturates
- Inhalant anesthetics
- CO_2
- 2-phenoxyethanol

- Conditionally acceptable: stunning followed by decapitation/pithing; decapitation and pithing

RABBITS

- Inhalant anesthetics
- Barbiturates
- Exanguination (under general anesthesia)

- Conditionally acceptable: cervical dislocation (< 1 kg); decapitation ; CO2

RATS, MICE AND OTHER SMALL MAMMALS

- Inhalant anesthetics (halothane, isoflurane) or followed by CO2
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- Barbiturates

- Conditionally acceptable: methoxyflurane; cervical dislocation (< 200 g); decapitation & CO2

Part 2

Comments on Recommended Agents and Methods of Euthanasia

INHALANT ANESTHETICS - Because in the liquid state most inhalant anesthetics act as topical irritants, animals should be exposed to the vapors of the anesthetic only. Air or oxygen must be provided during the induction period. All agents are given "to effect" until respiratory and cardiac arrest occurs.

Halothane and isoflurane have the most rapid action, and since halothane is better tolerated, it is preferred. Methoxyflurane is less suitable, due to its slow effect and poor market availability. Care should be taken to minimize personnel exposure to vapors. Fume hoods or active scavenging systems will be required.

NON-ANESTHETIC GASES - Most agents in this category require the use of special equipment.

CO_2 - Carbon dioxide is now only conditionally acceptable and needs substantiation and ACC approval since this is not the best humane choice any longer for euthanizing rodents and other small laboratory animals. Use of a sealed chamber filled by a compressed gas cylinder is required. CO_2 generated by other methods, such as from dry ice, is unacceptable because gas flow can't be regulated precisely. Chambers should not be overcrowded. CO_2 concentration of 70% or more should be utilized for euthanasia. Because CO_2 can act as a reversible anesthetic, it is imperative that the animals be kept in the chamber for several minutes after respiratory arrest. Where possible, death should be verified by absence of a palpable heart beat. Due to physiologic characteristics, neonates require prolonged exposure to the gas. **See SOP "Rodent Euthanasia**".

PHARMACOLOGICAL AGENTS - Use of these agents requires adequate restraint and mastery of appropriate injection techniques.

Barbiturates such as pentobarbital are acceptable for mammalian species and birds. These drugs should be administered intravenously (IV) except in rodents where intraperitoneal (IP) administration is an acceptable alternative. Sodium pentobarbital (Nembutal) is the most common barbiturate agent for euthanasia. The dosage is usually at least twice that required for anesthesia, and ranges from 85 mg/kg for larger species to 200 mg/kg for some rodents. A dosage of 120 mg/kg is sufficient for most species, but more should be given if death does not ensue. Commercial barbiturate euthanasia formulations as are also appropriate, and should be used following label directions. Sodium pentobarbital is a narcotic drug which is strictly regulated by Governmental guidelines. Personnel using this agent are required to store it in a locked location and maintain records which include the date and amount of use. **See SOP for "Restricted Drug Use**"

Tricaine methane sulfonate (MS222) can be used either as an injectable agent (200-300 mg/kg of a 1% buffered solution) or as an immersion bath (2 mg/ml in H2O) for amphibians and fish. The immersion time needed to assure death can range from 20 minutes to three hours, so it may be advantageous to use MS222 as an anesthetic followed by a physical method of euthanasia. Benzocaine immersion (100-200 mg/liter H2O) is also acceptable. **Note: Cutaneous exposure to MS222 can cause retinal toxicity. Gloves should be worn at all times when handling fish and amphibians, and in particular when using MS222.**

Neuromuscular blocking drugs are absolutely condemned for use as euthanasia agents.

PHYSICAL METHODS - These methods require that the user have experience and skill in the techniques to be used.

Exsanguination is acceptable for all species if animal is first rendered unconscious by another agent.

Cervical dislocation is acceptable for mice, birds, rats (< 200 gm) and rabbits (< 1 Kg), but proper technique is essential. It is therefore recommended that animals be first sedated with another agent (carbon dioxide, pentobarbital or halothane are suggested). Its use as a sole means of euthanasia requires scientific justification and ACC approval. For more information, see the SOP for "cervical dislocation for euthanasia of rodents".

Decapitation with proper equipment may be performed on small mammals or birds after the animal has been sedated or lightly anesthetized (carbon dioxide, pentobarbital or halothane are suggested). Decapitation of fish, amphibians and reptiles should be followed by pithing. Use as a sole means of euthanasia in any species requires scientific justification and ACC approval. Decapitation should generally be used only when study design requires it due to the potential hazard to personnel. Many species react adversely to the smell of blood, so animals should not be decapitated in the presence of other animals and the person performing decapitation should change gloves and/or wash hands between animals.

Pithing of both the brain and the spinal cord (double pithing) may be used as the sole means of euthanasia in frogs of the genus Rana or other amphibians with anatomic features that facilitate easy access to the central nervous system. In all other amphibian and reptile species pithing should be followed by decapitation.

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