University of Windsor

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Animal Care Handbook

Produced by the
University of Windsor Animal Care Committee

September 2006 and revised January 2011

Preamble

The University of Windsor ensures that researchers among the faculty, students and staff at the University have facilities to hold and care for vertebrate animals for use in teaching and research in accordance with the guidelines of the Canadian Council on Animal Care (CCAC) and the Animals for Research Act of the Province of Ontario.

The use of animals in research, teaching, and testing is acceptable only if it promises to contribute to understanding of fundamental biological principles, or to the development of knowledge that can reasonably be expected to benefit humans or animals. Animals should be used only if the researcher's best efforts to find an alternative have failed.

All research and teaching at the University of Windsor involving the use of animals (including fish and invertebrates), whether laboratory or field-based, must receive approval from the Animal Care Committee (ACC) before such research or teaching begins. Submission of an Animal Utilization Project Proposal (AUPP) to the ACC will initiate the process.

The University has three sites on campus for holding and care of vertebrate animals. The sites are in the Biology building (1965), Chrysler Hall South (1967) and the Great Lakes Institute for Environmental Research (GLIER, 2003). The first two sites noted above are interconnected and share several common facilities such as cage washer and animal surgery rooms. During 2009-2010 the Biology and Chrysler Hall sites underwent major renovations in the heating, cooling and humidity control systems, and reconfiguration of rooms to accommodate research involving immune-comprised and transgenic mice, to ensure compliance with Provincial and CCAC guidelines for the use of vertebrate animals in teaching and research. The renovations will allow some expansion of research space for faculty in the newly opened Schulich School of Medicine and Dentistry as the need arises.

Part 1: Legislation and Regulations Pertaining to the Use of Animals in Research and Teaching

All persons who wish to use animals for research or teaching at the University of Windsor must be aware of their legal and regulatory obligations. There are three regulatory bodies which are responsible for setting out guidelines pertaining to the use of animals in research and teaching:

- The Canadian Council on Animal Care (CCAC),
- The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and
- The University of Windsor Animal Care Committee (ACC).

Also, there are moral, legal, and ethical issues regarding animals in research which are the concerns of not only the Canadian Council on Animal Care, but also other organizations. It is important to recognize that there are various aspects and views regarding the use of animals in research and teaching.

Canadian Council on Animal Care (CCAC)

The CCAC was established in 1968 and is the national peer review agency responsible for setting and maintaining standards for the care and use of animals in teaching, research and testing throughout Canada. Programs on guideline development, assessment, and education help the CCAC accomplish its goals. Essentially, the Council encourages adherence to the Russell-Burch "3R" tenet of "Replacement, Reduction, and Refinement" wherever possible. Those using animals should employ the most humane methods on the smallest number of appropriate animals required to obtain valid information.

CCAC Guidelines

The CCAC program is based on guidelines that give clear direction to institutional animal care and use programs. The *CCAC Guide to the Care and Use of Experimental Animals*, Vol. 1, second edition, 1993, lays down the principles for good animal care and use. In addition, volume 2 of the Guide (1984) provides information on the husbandry of experimental animals on a species by species basis. Additional guidelines on issues of current and emerging concerns are developed in conjunction with the needs of the CCAC Assessment Program. These guides and others can be found at the CCAC website: http://www.ccac.ca/en/CCAC_Main.htm

CCAC Assessment Program

Each institution is subject to regular peer review by the CCAC, which involves an assessment of the effectiveness of an institution's ACC and the appropriateness of its animal care facilities, practices, and procedures. Assessments are based on CCAC guidelines and policies and are carried out by CCAC assessment panels every three years. Each assessment panel is composed of at least one scientist and a veterinarian, selected for their experience in animal experimentation and care relevant to the institution to be visited. Each panel also includes a Canadian Federation of Human Societies appointee, usually drawn from the geographical area of the institution. A CCAC Assessment Director is present at every assessment visit as an ex-officio member of the assessment panel.

CCAC Education Program

The Education Program of the CCAC interacts with the assessment and guideline development programs to provide information and training necessary for CCAC constituents. Education occurs at a number of levels. CCAC assessments of institutions are based on a system of peer review. This facilitates the ready exchange of information on best practices and encourages the continual striving for the highest standards of animal care and use across Canada. CCAC sponsored workshops are held on a regular basis to provide information and training, in particular for members of animal care committees. An orientation package including a video has been developed by the CCAC to assist animal users in fulfilling their responsibilities. Lastly, the CCAC has developed a National Institutional Animal User Training (NIAUT) program to ensure all personnel involved with the use of animals in research, teaching, and testing are adequately trained in the principles of laboratory animal science and the ethical issues involved in animal use. The NIAUT program can be found at:

http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/NIAUT/NIAUTCOV.HTM#top

Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA)

The Ontario Ministry of Agriculture, Food and Rural Affairs registers all research facilities in Ontario. Facilities must meet the minimum requirements of the regulations. The Director of the Animal Industry Branch may refuse to renew or suspend a registration of a facility that has not met requirements of the *Animals for Research Act*, R.S.O., 1990, c.A22 and its Regulations.

Animals for Research Act, 1990

The Ontario *Animals for Research Act* continues to be one of the most comprehensive pieces of legislation protecting research animals in North America. Since May 1971, the *Ac*t has provided a focus for humane care and use of all animals in research through minimum standards. The *Act* requires all researchers to be accountable to animal care committees for their use of animals.

Originally, the *Animals for Research Act* was a response to the needs of medical research for a regulated source of dogs that did not rely on animal dealers. The *Act* eliminates the chance of dog-napping for research. In addition, the *Animals for Research Act* protects all vertebrates including fish, rodents, reptiles, birds, primates, and livestock. The *Act* also covers animal use in educational exhibits, classroom, and laboratory teaching. Community colleges, public, separate and private schools, science centres, and teaching displays are all governed by the *Act*.

Objectives of the Act

- To maintain a minimum standard of care and well-being for all animals used in research,
- To protect research animals from unnecessary pain, and
- To ensure that dogs used for research are obtained legally and are not wanted as pets.

Key Elements of the Act

Minimum Standards for Housing and Care. The regulations set the minimum standards for housing and care of all research animals. These include standards for cage and pen size, construction, maintenance, and cleanliness of animal rooms. Proper food, water and bedding are also required. Ventilation, temperature, and humidity of the animal rooms must be suitable for the comfort of the animals. Secure transportation containers, safe vehicles, and competent animal attendants are also assured.

Animal Care Committee. The *Animals for Research Act* and the CCAC guidelines require that each research facility have an animal care committee responsible for the ethical use of animals in their institution. Membership includes scientists from departments using animals, a veterinarian, non-animal users, and a community member. Animal care committees must approve each protocol involving animals as being acceptable before animals are used. The Committee's purpose is to assure the well-being of the research animals and the avoidance of

unnecessary suffering. The members are present in the facility, monitor research activities, and meet regularly to assure the minimum standards of animal husbandry, management, training of staff, disease control, housing, and environmental conditions. The committee is responsible for the proper use of anaesthetics and analgesics in preventing unnecessary pain.

Research Proposals. The *Act* requires that every researcher submit a research proposal to the Animal Care Committee (ACC) before starting any research. The proposal or "protocol" outlines all the animal procedures and the number and types of animals needed. All protocols must be filed with and approved by the ACC before the initiation of any research. The Committee can order changes to any procedure. To avoid any unnecessary pain, the *Animals for Research Act* requires adequate use of anaesthetics and analgesics on research animals. The research protocol must outline the types of anaesthetics to be used. All anaesthetic use is subject to approval of the ACC. The ACC may not approve a research protocol if it believes that he animals would be subject to unnecessary pain. The *Animals for Research Act* gives the Committee the authority to issue orders to stop any research.

Veterinary Inspectors. Ontario Ministry of Agriculture, Food and Rural Affairs inspectors inspect registered research facilities on a regular, unannounced basis. The inspectors have the power to enter any premise in which they believe animal research is taking place. The responsibilities of the inspectors include:

- Inspection of animal housing,
- Inspection of procedure and surgery rooms,
- Examination of animals,
- Research protocol review, and
- Monitoring performance of the Animal Care Committee

Inspectors can request hearings to refuse to renew, or revoke registrations of research facilities. Facilities not following the *Animals for Research Act* and its regulations can face court charges.

University of Windsor Animal Care Committee

The University of Windsor Animal Care Committee (ACC) reports directly to the Vice-President, Research on the procurement, maintenance, and use of experimental animals in research and teaching in the university community. It is the responsibility of the Committee to ensure that all these activities are carried out within the regulations established by the Ontario *Animals for Research Act*, the guidelines of the Canadian Council on Animal Care, and any regulations established by the ACC itself.

The ACC recognizes the importance and necessity of using animals in research and teaching. Such research contributes to both human welfare and the understanding of the biosphere. At the same time, animal care and experimental procedures must be monitored to ensure that all animals are well cared for and do not undergo unnecessary suffering or discomfort. These criteria have a high priority in the evaluation of research and teaching protocols. Additional aspects used in the review of protocols are that a minimum but suitable number of animals are

employed, that the species used is justified, and that the study itself is of scientific merit. The ACC works on the principle that the use of animals must have suitable justification.

The mandate of the ACC is to ensure that all research with animals is conducted in accordance with the highest ethical and humane standards and that the animals, the public, the researchers, and the University are all protected from harm. All ACC policies and procedures are designed to comply with those of the Canadian Council on Animal Care.

Authority of the ACC

The ACC is authorized and required to:

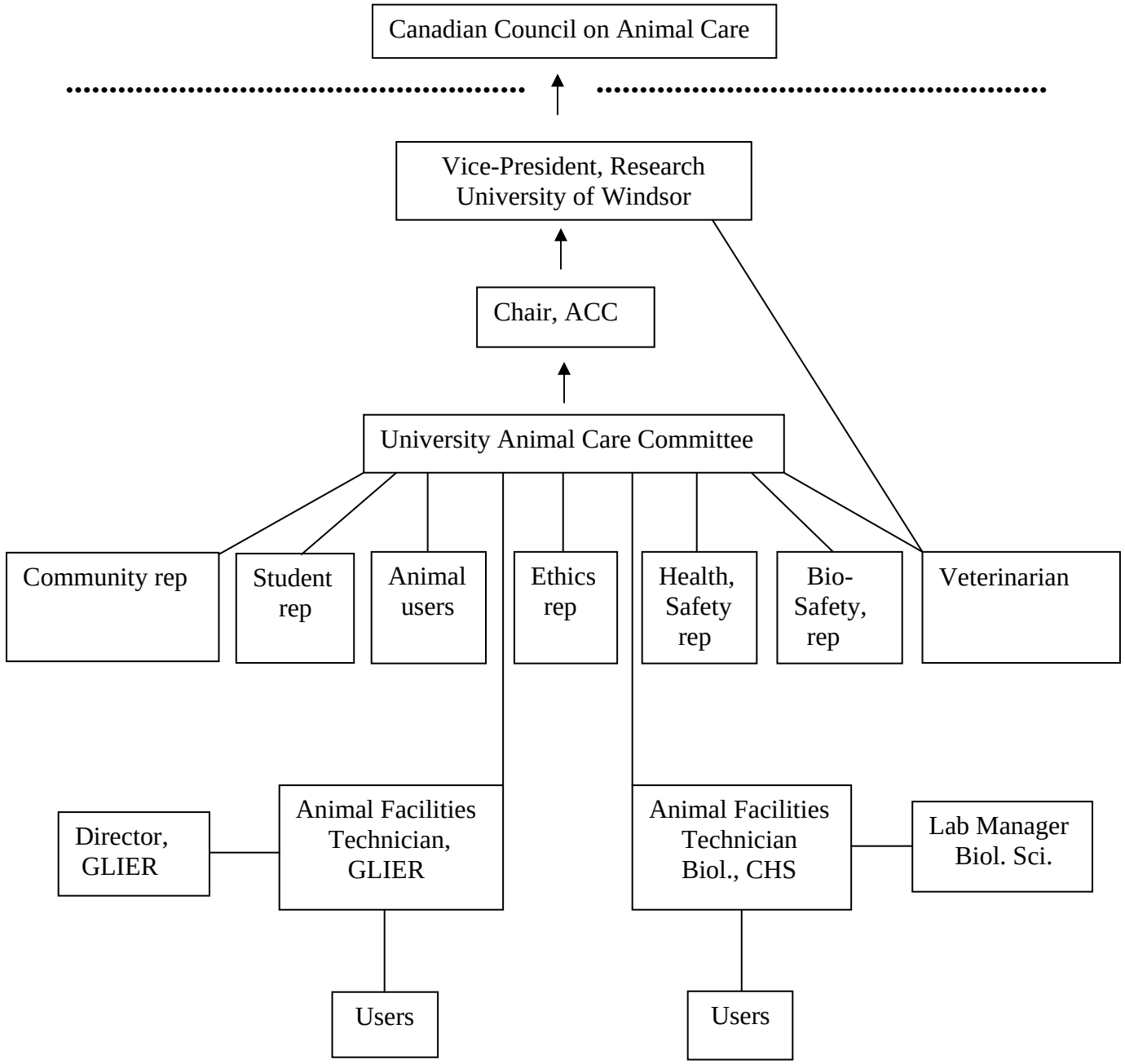
- Stop any procedures it considers inappropriate,
- Stop any procedures that are causing unnecessary pain or discomfort to animals, and
- Humanely euthanize an animal if pain or distress caused to the animal cannot be alleviated.

Responsibility of the ACC

The responsibility of the ACC is to:

- Ensure that no research or teaching program involving animals (including field studies) is begun without prior ACC approval of a completed Animal Utilization Project Proposal (AUPP). The appropriate approval protocol form must be used which can be found on the ACC web site (http://www.uwindsor.ca/acc). Information on the objectives and procedures must be presented so that it can be readily understood by all members of the ACC,
- Keep abreast of any substantial changes in the protocols and to inform the researchers that any major changes require the submission of a new AUPP,
- Review all protocols annually since protocols are approved for a twelve-month period,
- Inform all animal users of the CCAC Guidelines and in any changes in Provincial Statutes governing the use of animals in teaching and research,
- Ensure that veterinary care is available on a consultative basis,
- Establish mechanisms to ensure that unnecessary pain or distress is avoided,
- Ensure that any anaesthetics or analgesia are used effectively,
- Ensure that appropriate post-operative care is provided, consistent with accepted veterinary practices,
- Develop and foster the implementation of policies regarding the training and qualifications of animal users and animal care personnel,
- Meet at least three times a year to consider protocols, inspect facilities, and discuss general policy.
- Ensure that each member of the Committee visits the animal holding facilities at least once a year,
- Foster and sponsor attendance at seminars or workshops on the use of animals in research and teaching and the ethics of such use, and
- Maintain liaison with the CCAC secretariat, provincial authorities, and bona fide animal welfare organizations.
- Conduct post-approval monitoring of active research protocols

Governance of Animal Research at the University of Windsor (as of Sept 21, 2010)



Submitting an Animal Utilization Project Proposal (AUPP)

Researchers considering the use of vertebrate animals in their research, or for use in teaching laboratories, should visit he ACC website and contact the ACC to ensure that their requirements can be met.

Research projects must be peer reviewed for scientific merit by either a funding agency or two independent reviewers if the project or teaching function is unfunded (see Appendix for access to the most recent forms and guidelines). All proposals must describe the **endpoint** in the experimentation in accordance with CCAC guidelines. The proposal must include information regarding the **replacement** alternatives, **reduction** in the number of animals with the appropriate experimental design, and **refinements** in procedures employed to protect and enhance animal health and welfare.

The most current AUPP form is available on the ACC website: **www.uwindsor.ca/acc.** This form must be completed in full and returned to the Research Ethics Coordinator for review by the ACC. Approval must be granted before any research or teaching using animals can begin. The animal user (usually the principle investigator or laboratory teacher) will receive a copy of the front page with the assigned protocol number indicating approval. The protocol number is to be used when ordering animals.

AUPPs are valid for one year from the date of approval, but they can be renewed for **up to three consecutive years** using the Progress Report Form available on the ACC web site. In the event that the project is to be continued beyond four years, a new AUPP must be submitted and approved before research involving animals can be continued, funded or unfunded, beyond he initial four years.

Protocol Changes

Investigators are required to notify the ACC of any changes in experimental procedure, animal species or numbers, granting agencies, and/or in the use of anaesthetics by submitting a **Request to Revise Form** which is available on the ACC web site.

Protocols Involving Biohazards or Radioisotopes

The injection of any infectious agent or radioactive substance into an animal must be clearly described in the AUPP, and have prior approval by the appropriate committee (ie., Radiation Safety or Biosafety Committee). All radioactive substances must be obtained through the Chemical Control Centre at the University. Animals injected with a biohazard must be maintained in approved, isolated rooms in the animal holding facilities.

Procedure for Approving Protocols

Any individual wishing to use animals in teaching or research must submit an Animal Utilization Project Protocol (AUPP) to the ACC at least two weeks before a scheduled meeting of the ACC. In an emergency a subcommittee of the ACC composed of the Chair, the veterinarian, the Community Representative, and one other member of the ACC could provide interim approval provided that:

- the general experimental design and the experimental procedure have already been peer-reviewed and approved, or
- the subcommittee agrees that there will be a low level of discomfort caused to the animals involved.

Final approval of the proposal will rest with the entire ACC at the next general meeting. If the approval of the AUPP is denied, either by the subcommittee or the full ACC, the researcher has the right to address the full ACC. If the ACC refuses to approve the AUPP, the researcher may appeal the decision of the ACC to the Vice-President, Research. Note that appeals will not be considered by the CCAC.

Moral, Legal, and Ethical Issues Regarding Animals in Research

Animal Welfare Movement

Animal welfare has been defined as "an animal's state as regards to its attempts to cope with its environment" and "all aspects of animal well-being, including proper housing, management, nutrition, disease prevention and treatment, responsible care, humane handling and, when necessary, humane euthanasia." (*CCAC: Guide to the care and use of experimental animals*, Vol. 1, p. 51).

Public concern regarding the humane treatment of animals has lead to the animal welfare movement. Humane treatment is an issue as more and more pets are perceived as members of the family. This human-animal bond is acknowledged widely in the veterinary community. The public may be fearful that stray animals or lost and kidnapped pets are being taken for laboratory use. However, the Canadian Veterinary Medical Association (CVMA) position statement states that it "accepts the use of pound animals for research, provided that every reasonable effort has been made to find homes for them and the research institution complies with the guidelines of the CCAC." Please note that pound animals are not currently being used for research at the University of Windsor.

Concern with pain caused by the experimental protocol (i.e. applying chemical irritants in the eyes) is another reason for the public questioning the use of animals for experiments.

An increased awareness of the environment has also led to people being concerned about studies using wildlife. Removing animals from their natural environment may lead to consequences such as a disruption of that habitat, a decrease in genetic diversity, or behavioural abnormalities in the captive animal.

Humane endpoints are a list of criteria of adverse effects to the animal beyond which research may not be permitted. If the animal is affected according to those criteria, it should be euthanized humanely. These endpoints are helpful in refining the experiment so as to reduce animal suffering. The CCAC has been developing guidelines on how to establish these criteria.

One definition of **animal rights** is "that animals, like humans, have interests that cannot be sacrificed or traded away just because it might benefit others. However, the rights position does not hold that rights are absolute; an animal's rights, just like those of humans, must be limited, and rights can certainly conflict" (www.peta.org). There are numerous organizations and businesses dedicated to this issue. Some examples of these, internationally and locally, include:

- People for the Ethical Treatment of Animals (PETA);
- OPRIG Windsor's Animal Action Group;
- New England Anti-Vivisection Society (NEAVS);
- The Body Shop.

Humane societies – The Canadian Federation of Humane Societies (CFHS: www.cfhs.ca) "is opposed to procedures and experiments which inflict pain, suffering or injury upon animals used in research, testing, and teaching." However, they are members and supporters of CCAC programs. Their participation helps direct us to better care and use of animals in the laboratory.

Foundations for Ethical Considerations Concerning Animal Use

Why should people use animals to conduct research?

This can be considered a privilege or an abuse, depending upon the point of view. One of the reasons for using animals in medical and scientific research is that they provide models of human systems in which we can study diseases. However, the aim of such scientific research is to replace, reduce, and refine animal use.

Researchers and the responsibility for animal care

In a laboratory setting, it is our responsibility to provide food, water, housing, and environmental enrichment. The animals do not have the capacity to change any of these factors and so are dependent on us. However, providing these basic requirements is not enough. It is important to avoid "doing it because it is the routine." One should assess regularly whether the animals' needs are being satisfied. Daily observation of the colony is ideal and can be done by anyone in the lab.

The principal researcher should try to examine the animal(s) at least once weekly. Each person working with the animals should be asking themselves several questions: Is the animal/colony showing any signs of stress? Are all the animals eating enough? Is there any weight loss or gain? Is the cleaning schedule being adhered to? Do I need to change it? In other words, is the animal "happy"? This is perhaps an anthropomorphic statement but it can make us more aware of their environmental needs. Sick animals do not make ideal research subjects.

Canadian Veterinary Medical Association

The position of the Canadian Veterinary Medical Association is:

> "The Canadian Veterinary Medical Association (CVMA) recognizes the need to use animals for research, teaching, and testing purposes to improve the overall well-being of humans and animals, when there are no scientifically acceptable replacement alternatives, and when the studies have been shown to be scientifically and ethically justified.
>
> "The CVMA recognizes and upholds the Canadian Council on Animal Care (CCAC) program. Animal-based studies should be carried out in accordance with CCAC guidelines and policies by institutions holding a valid CCAC Certificate of Good Animal Practice." (www.canadianveterinarians.net)

Contentious Issues – Recognition of Various Aspects and Views

Examples of Evaluation of Competing Ethical Demands

Which life is more important, human or animal? Do humans have the right to use animals for research purposes? There is no one correct answer to this very complex question. There have been many cases whereby research using animals has helped treat human illness. Studies on human multiple sclerosis have been done using a guinea pig model (not being done at the University of Windsor). Medical therapy for human diabetes was developed using dogs.

Non-animal methods in biomedical research are important to review before deciding to do a research study using live animals. www.neavs.org/articles/alternatives/html is a web site that offers ideas on alternative experimental protocols that do not involve or limit the use of live animals. Some of the methods that should be considered when developing a research project include the following:

- Epidemiological studies;
- Clinical studies;
- Autopsies and post-mortem studies;
- Non-invasive imaging techniques (CAT/MRI/PET/SPECT);
- Tissue cell culture;
- Microbiological studies;

- Computer and mathematical models;
- Chromatography and spectroscopy.

Another helpful resource may be the **Canadian Centre for Alternatives to Animals in Research (CCAAR)**. This is a group based at the University of Ottawa with the mission of developing a database of techniques for refinement of experimental protocols to minimize animal suffering.

The ethical problem of resource allocation refers to the limited amount of funding available for animal care and welfare. Provision of enrichment is an important issue in animal housing that may not be addressed due to limitations in the availability of personnel, cost of equipment, the size of facility, and difficulty in cleaning. Attempts to address environmental enrichment, at this and other institutions, have included the following: toys for rats/mice, radios/music for auditory stimulation, provision of paper towels and rolls for bedding/teething, and group housing.

Level of Invasiveness vs. Potential Benefit

Level of invasiveness refers to anything from blood collection and injections to behavioural observation to invasive surgical procedures. The question to be asked is, “Will the information derived from this procedure be worthwhile of the method in which it was obtained?”

Submission of research protocols for review by the University of Windsor Animal Care Committee and granting agencies, such as NSERC, are methods used to assess **scientific integrity and scholarly activity**. Peer review also helps to determine the **responsible use of animals** by assessing the proposed numbers of animals used, the length of the project, level of invasiveness, etc.

The **Canadian Council on Animal Care** (CCAC; www.ccac.ca) is the federal group responsible for the oversight of animals used in research, teaching, and testing. Please refer to information discussed previously in these notes.

Part 2: Rodents

Overview of the Biology and Disease in Mice and Rats

Possible Diseases/Problems in Mice

- Digestive - diarrhea (mouse hepatitis virus, parasites)
- Skin - barbering, trauma, mites, abscess, tumour, bacterial dermatitis
- Respiratory - sneeze, sniffle, chatter and laboured breathing (Sendai virus, *Mhycoplasma pulmonis*), pulmonary abscess
- Reproductive - neoplasia
- Urinary - bacterial infection

Table 1. Biodata of Mice

Adult body weight - male	20 - 40 g
Adult body weight - female	25 - 40 g
Birth weight	0.75 - 2.0 g
Rectal temperature	36.5 - 38^{o}C
Karyotype – diploid number	40
Life span	1 - 3 yr
Food consumption	12 - 18 g/day
Water consumption	15 ml/day
GI transit time	8 - 14 h
Breeding onset: male	50 days
Breeding onset: female	50 - 60 days
Cycle length	4 - 5 days
Gestation period	19 - 21 days
Postpartum estrus	Fertile
Litter size	10 - 12
Weaning age	21 - 28 days
Breeding duration	210 - 279 days
Commercial	6 – 10 litters
Young production	8/month
Milk composition	12.1% fat, 9.0% protein, 3.2% lactose

Possible Problems/Diseases in Rats

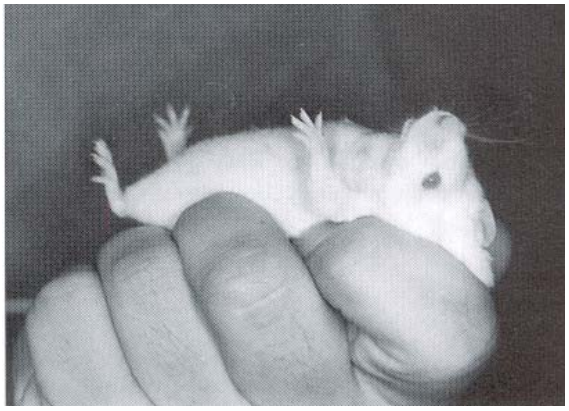
- Digestive – infected salivary gland (sialodacryoadenitis virus), overgrowth of incisors/broken teeth
- Skin – bacterial dermatitis, neoplasia, ringtail (= avascular necrosis of the tail)
- Respiratory – pneumonia (bacterial and viral)
- Reproductive – tumour
- Urinary – chronic nephrosis
- Ocular – red tears (acute onset stress)

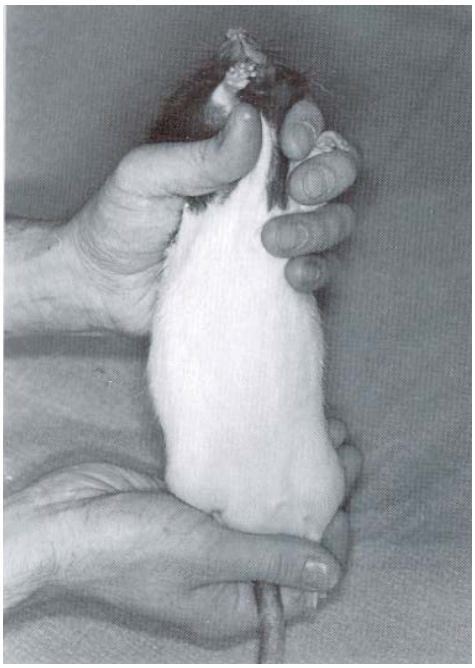
Table 1. Biodata of Rats

Adult body weight - male	450 - 520 g
Adult body weight - female	250 - 300 g
Birth weight	5 - 6 g
Rectal temperature	35.9 – 37.5^{o}C
Karyotype – diploid number	42
Life span	2.5 – 3.5 yr
Food consumption	5 - 6 g/100 g/day
Water consumption	10 – 12 ml/100 g/day or more
GI transit time	12 - 24 h
Breeding onset: male	65 - 110 days
Breeding onset: female	65 - 110 days
Cycle length	4 - 5 days
Gestation period	21-23 days
Postpartum estrus	Fertile
Litter size	6 - 12
Weaning age	21 days
Breeding duration	350 - 440 days
Commercial	7 – 10 litters
Young production	4 - 5/month
Milk composition	12.1% fat, 9.0% protein, 3.2% lactose

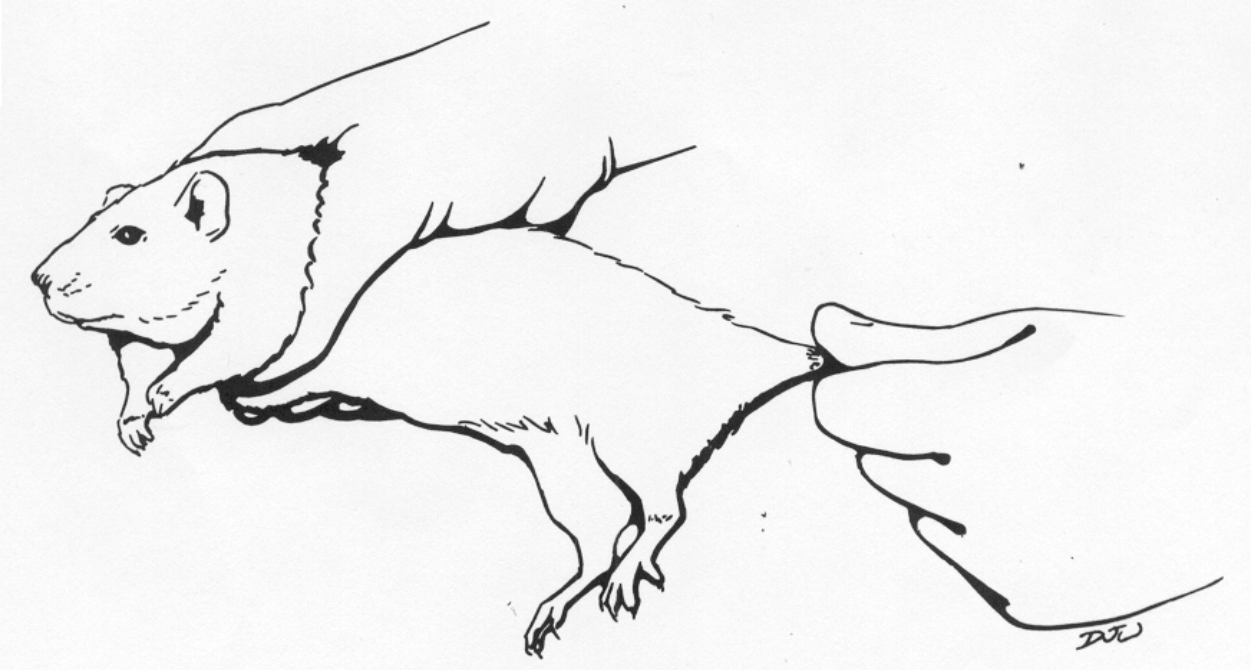
Handling and Restraint (Diagrams)

Mice (see film from the Norwegian Reference Centre for Laboratory Animal Science and Alternatives at http://film.oslovet.veths.no/)



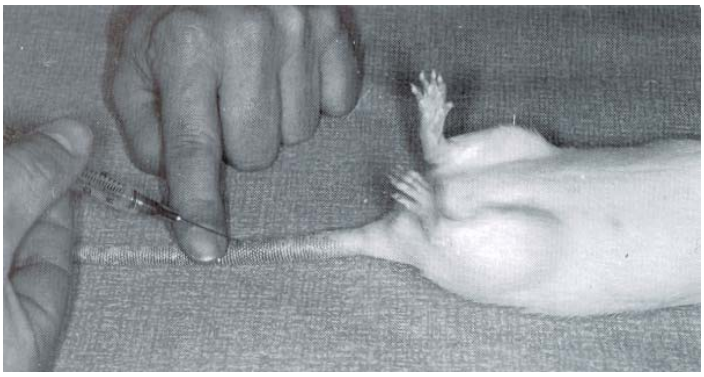


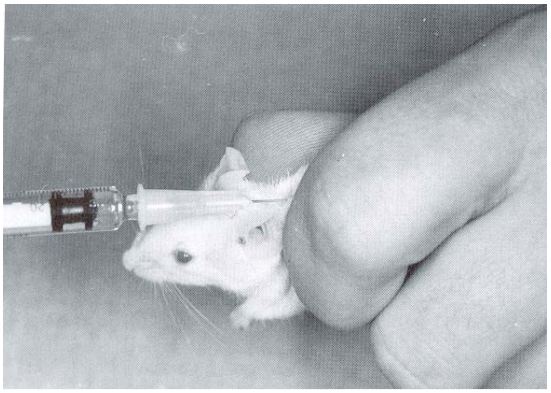
Rats

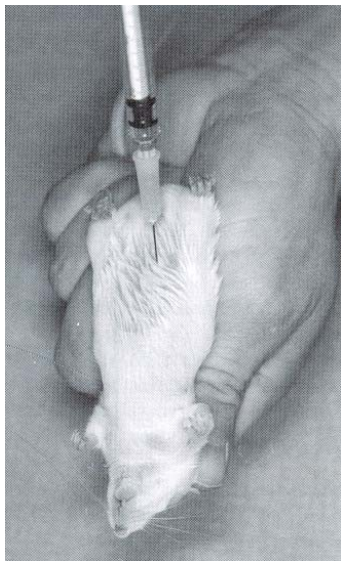


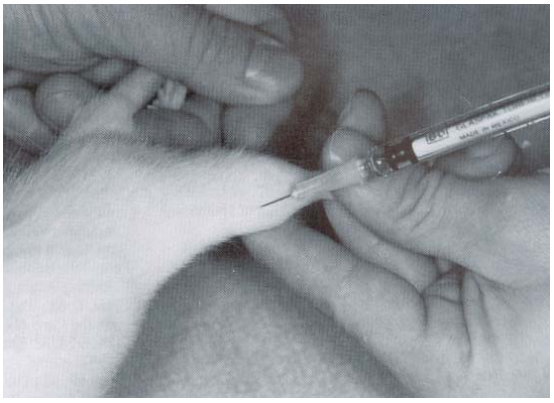
Blood Sampling and Injection Techniques

Mice and Rats (Diagrams) (see http://www.procedureswithcare.org.uk/category/rat/mouse/)









Necropsy Technique

Necropsy, or post-mortem, examination of a dead animal can provide helpful clues in diagnosing illness in that animal and/or its colony. Good necropsy techniques can also aid in better dissection of organs that need to be analyzed as part of the experimental protocol.

Recognition of Pain and/or Distress

Vocalization, anorexia, and lethargy are common clinical signs that can indicate illness in rodents.

Sedation and Anaesthesia

Injectable drugs can be used for sedating animals to permit easier handling for blood collection, reducing the amount of anaesthetic agent required during surgery.

Inhalant anaesthesia uses volatile gases delivered through a breathing system. The advantage of such anaesthesia methods is rapid recovery afterwards since the gas is excreted from the body by the lungs during exhalation. Disadvantages include the use of expensive anaesthetic machines, a scavenging system to remove volatile fumes that can be hazardous to personnel working.

Surgical Principles

Aseptic technique is the method whereby surgery is performed in a sterile environment. This is especially important in invasive, recovery procedures. The introduction of infectious organisms into the animal will result in serious disease, death of the animal, and invalidation or termination of the experiment.

Instrument sterilization can be done via autoclaving (i.e. dry high heat). It is important to use monitoring devices to ensure that the correct sterilization temperature has been achieved. The date of sterilization of each pack must be noted on the outside covering. For non-invasive procedures, a cold sterile solution can be used to bathe the instruments.

Multiple survival surgeries on a single animal are not recommended at the University of Windsor, as there are limited facilities, equipment, and staff to monitor animals for pain and post-operative recovery.

Surgical procedures on a series of animals may be a part of the research protocol. This involves organization to make the most efficient use of time (i.e. all procedures done on same day or on consecutive days).

Pain management is a high priority in all techniques/surgeries performed. **Post-operative monitoring** is covered under this category but, since recovery surgeries are not recommended, this topic is beyond the scope of this discussion.

Records documenting the following topics are recommended: the date/time/duration of the procedure to be performed; the animal's condition before/during/after the procedure; type and volumes of any sedatives/anaesthetics used; researcher's impression of procedure (i.e. any improvements).

Specialized Techniques

Food deprivation and motivation techniques are being used in the Department of Psychology research protocols. Food deprivation must not be for too long a period (only until research has been done for your species of interest) or done at the wrong time of day. There is no sense in food deprivation at night for an animal that sleeps all night long! Motivation also requires that the animal enjoy/want the food treat it is being offered. This may involve some experimentation to find the "right" treat. The treat should also not be harmful to the animal. Food deprivation should not last longer than 24 hours.

Diet manipulations may involve a change in the formulation of the food. For example, previous research at the University of Windsor involved feeding hamsters a high lipid pellet diet. There was some difficulty in keeping this high lipid diet in a solid form at warmer room temperatures. This illustrates the importance of practicality when trying to institute a diet change. Diet manipulations may also require gradual introduction. Changes in texture, taste, format, etc., may require an adjustment period, during which time it is crucial to ensure the animal continues to eat and not lose weight. Animals should be monitored and weighed daily during this adjustment.

Adjuvant use may be required to produce monoclonal antibodies. See *CCAC guidelines on: monoclonal antibody production*.

Transgenic animal refers to "an animal in which there has been a deliberate modification of the genome – the material responsible for inherited characteristics – in contrast to spontaneous mutation" See *CCAC guidelines on: transgenic animals*, 1997.

Euthanasia and Disposal of Animal Remains

Carbon dioxide (CO_2) is the preferred method of humane euthanasia in University of Windsor's animal lab facilities. The animal is placed in a chamber that is slowly filled with CO_2. CO_2 competes with O_2 for binding sites on haemoglobin. When these sites cannot be occupied by O_2 the animal dies by asphyxiation. If performed properly, this is an acceptable method of human euthanasia in small rodents.

Pentobarbital is an injectable intravenous agent used for humane euthanasia. It is a controlled substance requiring a permit to purchase or prescription from the veterinarian. This method does require technical expertise in locating the appropriate peripheral vein or through intra-cardiac puncture. Pentobarbital can also be injected into the peritoneal cavity, whereby the solution is absorbed by intra-abdominal vessels. However, this method does require time for absorption and thereby prolongs the time before euthanasia is complete. Due to concerns with misuse of this solution, this drug is not regularly available at University of Windsor animal lab facilities.

Animal remains are kept frozen until group incineration is available. Any animals treated with biohazardous and radioactive materials are disposed of in accordance with the regulations at the University of Windsor.

Cervical dislocation is not recommended as the sole means for humane euthanasia, unless you have been properly trained. When done properly, this procedure can result in rapid death. If you are not familiar with this technique, it is preferable to sedate the animal first. Cervical dislocation is performed by rapid traction on the spinal cord/vertebrae while restricting movement of the head/skull. The spinal cord is severed at the neck. This method should not be attempted without appropriate training, either by your supervisor or at a recognized animal training course. According to the CCAC, cervical dislocation is suitable for poultry, mice, or immature rats, or other similar small species.

Personal Health and Safety

Allergies to lab animals (ALA) can develop in people after handling them for any period of time. The most common allergens are due to urinary protein, fur, and dander. Rats, guinea pigs, and mice are the species most commonly involved. Using a mask to limit inhalation of allergens and washing hands after animal contact will help to limit the severity of symptoms. Should you have pre-existing allergies to animals, asthma, or other respiratory illnesses, please contact your family physician or Student Health Services as to the safety of you working in this environment. Always wear a mask when dumping soiled bedding out of cages.

Bites are better prevented than treated. Therefore, handling of all animals should be done with respect. If you have any doubt as to how to approach, restrain, or handle any of the animals that you work with, you should not hesitate in asking your supervisor for their assistance. Should an animal bite occur:

- use the first aid kit to be found near Room 15 in the Biology building,
- have the wound checked by Student Health Services, and,
- report and document the incident to your supervisor.,
- Ensure that you have an updated tetanus vaccination

Zoonotic diseases can be passed between lab animals and humans. They can be transmitted via bites, aerosol, or contact. The following are some of the possible infectious diseases to be aware of:

- Rabies by bite wounds,
- Ringworm (any species) by skin contact,
- Hantaan virus (rats) causing hemorrhagic fever,
- Leptospira (bacterial kidney disease from rats),
- Salmonella (various species) causing gastrointestinal disease,
- Various other bacterial infections.

Should there be any concern about an illness that occurs during or after a period of animal handling, it is important to mention to your family physician that you had been exposed to lab animals.

Record Keeping and Identification

Animals should be identified by group or individually (ear tagging, tattoo, microchip, banding, etc.). Additions to the colony, in the form of births or new purchases, as well as euthanasias or deaths, should be recorded in the binder kept in each animal holding room.

Handling Guidelines

The laboratory rat is generally very docile. In approaching a rat, your movements should be quiet and slow. The use of gloves is rarely justifiable because it impairs your sense of touch and will be resented by the animal. Rats will be gentled by the warmth of a bare hand, cease to struggle and become progressively easier to handle.

You are required to wear either a lab coat or a long sleeve cotton shirt over your street clothes. The material should be of a tight weave to prevent the rat's nails from being caught and possibly injured.

When handling your rat for the first few times, follow this procedure: Remove the water bottle from the cage and set it on the cage rack. Slowly and quietly remove the cage, with the rat inside, from the cage rack and place it on a table top with a sheet of paper towel placed on the tabletop and under the cage floor. Occasionally, when a rat is being handled for the first time it may, because of fear, defecate or urinate. This response will cease almost immediately with a gentle, quiet handling technique.

With finger rings removed and secure, let the rat know that you are there, by gently stroking its back. This procedure prevents startling the rat. Do not be afraid, as nervousness is contagious. Now place one hand over its back and ribs so that your thumb and fingers are behind its front legs. Do not squeeze too tightly, as this will impede breathing and the rat will struggle. With a steady lifting motion, place the rat on your arm and sit down in a chair. Release the rat on your arm and usually it will stick its head into the crook of your arm and next to your chest. Gently stroke its back. While still seated and if you have your lap covered, try letting the rat walk around on top of your legs being careful that the rat doesn't jump or fall to the floor. A rat doesn't like to be held and restrained in a hand for more than a few seconds.

Practice picking the rat up from your arm and placing it in its home cage, then again sitting down with it on your arm or lap. Never pick your rat up by the tip of its tail because the covering skin and tissue will break off. Do not stick your finger in front of its mouth; it just might investigate whether your finger is soft or hard – remember those chisel-shaped incisors! Usually the only time a person is bitten by a laboratory rat is when the rat is startled or injured.

It cannot be overemphasized that the more frequently you handle your rat, the more quickly both of you will become used to each other.

Feeding Instructions

Since your rat receives very small food pellets as a reward for its correct responses in the operant-test chamber, it is necessary that the rat be motivated by hunger before it is placed in the test chamber. For this reason the rats are maintained at 90% of their Ad Libitum weight (what they would weigh if they had food to eat at all times).

Using the balance in the lab, weigh and record your rat's weight on the weight chart. Rats generally receive 20g of food per day. Talk to your supervisor or technician before altering this amount. Weigh out the required ration and then place it quietly inside the cage. Rat weights should be measured and recorded weekly.

Do not use food that has fallen on the floor. Also please remember to check that your rat's cage is in the correct order and completely closed on the cage rack, and that the water bottle has been replaced on the cage bottle holder. If you become ill and are not available to weigh and feed your rat, you must notify your partner if you have one, or your teaching assistant or technician (University of Windsor phone number – (519) 253-3000, ext. 2707).

Hygiene and Housekeeping

Please remember to wear a lab coat or a long sleeve cotton shirt over your street clothes and that eating, drinking, and smoking are not permitted in the lab. After you have handled your rat and equipment, and are about to leave the lab, remember to thoroughly wash your hands.

Please keep the room clean at all times. Sweep up any spilled food and place clean paper towels in the operant-test chamber tray. If you break a water bottle, carefully sweep up with broom and dust pan and place glass in container specifically marked for broken glass. Rinse off the stopper and sipper tube and place on counter top. Clean bottles and stopper/sipper tubes will be found in the cage wash room (Room 01 Biology).

Health Concerns

The Director of Medical and Health Services at the University of Windsor highly recommends that all students be protected against tetanus. If you have had a tetanus booster shot within the last ten years you will probably have adequate protection. If you need advice on this matter, please consult your personal physician or visit the Medical and Health Services facility on the second floor of the CAW Centre. While the risk of contracting tetanus is probably quite low, it is better to err on the side of caution.

It should be noted also that animal dander, serum, urine, and other animal tissue products can be allergenic. It is advisable that students with a history of susceptibility to allergens be skin tested for contact with rodents prior to commencing laboratory contact. A surgical type mask or respirator may be required. Please seek the advice of your physician in this matter.

If you are bitten by your rat, which is unlikely if you follow the proper procedures, please immediately notify your technician and NOTE that the First Aid Kit is mounted on the wall near Room 15 Biology. Then wash your hands and wound thoroughly with soap, letting the wound bleed freely for a few seconds. Dry your hands and, if necessary, apply direct pressure over the wound, using a 2-inch sterile compress to stop the bleeding.

If the bite wound is not severe and is in the fleshy part of your finger or hand, standard first aid treatment should suffice. However, if the bite is on the back of the hand near tendons or in or near knuckle joints, the chance of infection should be considered more seriously. Please see your personal physician or a physician at the Medical and Health Services facility on the second floor of the CAW Student Centre as soon as possible.

Please fill out an incident report (located near the sink in Room 15 Biology), detailing the nature, location, and treatment of the bite wound, time and state of injury, and identification number of the rat involved. This report is to be given to the technician as soon as possible.

Other cuts and abrasions received in the lab should be treated with concern, using standard first aid procedures. Inform the technician of any such injury. Any cuts or abrasions inflicted outside the lab should be kept covered and asepticzed.

Your rat's health is also important. Please be vigilant and notify your technician if you observe your rat sneezing or if it appears congested. Occasionally a rat may break off one of its incisors (teeth) and this can lead to eating difficulties. If your rat is injured in any way (cuts, lifted nails), please inform the technician. If you notice a steady decline in a rat's weight, please notify the technician as soon as possible. This is usually the first sign of any illness.

If a rat dies unexpectedly, remove the body from the cage, wrap it in a sheet of bench paper or a garbage bag and place it in the fridge in Room 17 Biology (outside the double doors). Put a label on the bag with the rat's cage number and notify the technician.

References

Literature

CCAC Guide to the Care and Use of Experimental Animals, Volume 1, Second Edition

CCAC guidelines on: animal use protocol review, 1997

CCAC guidelines on: transgenic animals, 1997

CCAC guidelines on: monoclonal antibody production

CVMA Animal Welfare Position Statements

Harkness, J. E. and J. E. Wagner. 1995 *The Biology and Medicine of Rabbits and Rodents*

Hillyer, E. V. and K. E. Quesenberry. 1997. *Ferrets, Rabbits and Rodents: Clinical Medicine and Surgery*. W.B. Saunders. Philadelphia.

Wolfensohn, S. & M. Lloyd. 1998. *Handbook of Laboratory Animal Management and Welfare*. Blackwell Science. Oxford.

Websites

www.ccac.com = Canadian Council on Animal Care

www.cfhs.ca = Canadian Federation of Humane Societies

www.peta.org = People for the Ethical Treatment of Animals

www.neavs.org = New England Anti-vivisection Society

Other Sources of Information

Canadian Centre for Alternatives to Animals in Research (CCAAR)

Gilly Griffin, Ph.D.
University of Ottawa
Faculty of Health Sciences, Office of the Dean
451 Smyth Road, Room 3028
Ottawa, Ontario K1H 8M5
Phone: (613) 562-5800, ext. 8051
Fax: (613) 562-5627

Part 3: Aquatic Animals

General Introduction

Aquatic animals are particularly fragile and need special care. There is considerable variation in the type of care required depending on the type of animal one is handling. You should make every effort to find out more about your particular species than is contained in this manual.

For amphibians, a very detailed manual is available at the following web site: http://www.nap.edu/readingroom/books/amphibian/index.html. If you cannot download this manual, an electronic or print version can be supplied to you. Since this manual is fairly complete, the rest of this chapter will just provide general details for amphibians that can be supplemented by referring to this manual.

For fish, there are several sources available, depending upon the species you are using.

Husbandry

General

Aquatic animals are quite permeable to substances in the water around them. In the case of fish, this is because they absorb oxygen through gills and for amphibians, it is because their skin is very permeable. This means that they need clean water provided by filtration. The filtration may be mechanical (such as a Power filter) on a contained system or a flow-through system which changes the water continuously. Advice on setting up these systems is available from the Great Lakes Institute for Environmental Research (GLIER) aquatic facility manager. Because the food for these animals is either added directly to, or ends up in, the water, regular cleaning to prevent bacterial and other pathogen build-up is a must.

Amphibians

Handling

When handling the frogs, latex lab gloves should always be worn. Frogs will only move forward, not backwards so take this into account when trying to pick them up. Frogs should be held around their waist region and care taken to ensure they are not dropped as injury occurs relatively easily. Efforts should be made to make any handling as quick and as stress free as possible to reduce damage caused by panicking.

Individual Identification and Monitoring (leopard frogs, bullfrogs, and green frogs)

All frogs are photographed on arrival and the spot pattern on their back used as a method of identification. Other distinguishing characteristics will include colour (whether brown or green), sex, length, and weight. A log will be kept to monitor weight changes, growth, and experimental history (e.g. when they were bled and what exposures they have received).

Parasite control (leopard frogs, bullfrogs, and green frogs only)

On arrival all frogs can be injected intraperitoneally with a commercial de-wormer to clear parasite infestations which occur naturally. If a parasite load is not a problem to the experimental protocol, after the assays have been completed, representatives can be euthanised by immersing them in a solution of MS222. Post mortems will then be performed to determine the parasite load. Parasites can be identified by using a standard parasite key.

Feeding (leopard frogs, bullfrogs, and green frogs only)

Frogs are generally fed until satiated. Frogs will eat Red Wiggler (or Nightcrawler) earthworms or crickets. The worms or crickets are placed on purpose-built worm trays that are made from acrylic or on rocks placed in the tank. There should be a one-inch lip around the edge to make it harder for them to escape. If the worms escape into the water the frogs will not eat them, but they will eat any crickets that land in the water.

Cricket Care:

Crickets should be fed prior to feeding the frogs, as there is little nutritional value in the exoskeleton alone. This can be achieved using commercial products such as “Gutload”. Crickets can be kept at room temperature in an aquarium with a lid.

Earthworm Care:

Worm Bin. Worms are kept in a large plastic container. Since earthworms prefer darkness, a box that isn’t clear or translucent is preferred. The container should be thoroughly washed and rinsed before the worms and bedding are added. The bin size depends on the number of worms you plan to keep, approximately one square foot of surface area for 2,000 Red Wigglers or 1,000 Nightcrawlers. The reason for this is that the worms require oxygen which is introduced from the surface. Small holes should be drilled (approximately 1/16 inch) around the sides, just below the top edge for aeration.

Bedding. Worms will be bedded on peat moss which has been thoroughly soaked. It is preferable to let the peat soak at least overnight before moving to the next step.

The ideal moisture content for worm bedding is approximately 60%. To achieve this, take hands full of your soaked peat and "wring" it out by squeezing it. When only a couple drops of water are coming out, it is ready to add to your bin. Crumble it into the bin so that you don't end up with large "clumps". Continue until you have the bedding a depth of six to seven inches.

Food and Feeding. The most convenient food is chicken mash, either chick starter or laying mash. Generally, the laying mash has a higher protein content. Lightly sprinkle the mash on the top of the bedding. It is far better to put too little food than too much. Do not add additional food until what is there is completely gone. After spreading the feed, lightly spray the food which will allow the worms to easily consume it.

If the worms are to be harvested, do so before they are feed. Never mix food into the bedding. It will cause a condition known as "protein poisoning," which is a build-up of acid within the bedding caused by the decomposition of the food. If the worms start dying, it will be a result of this condition.

General Maintenance. The worm bin should be kept out of direct sunlight. Although the worms can stand heat, this will cause excessive heat. Every week or two, the bedding should be lightly tossed, allowing the bedding at the bottom of the bin to be on the top. Since worms absorb oxygen through their bodies, this process allows sufficient oxygen to be throughout the bedding. If the worm bedding appears to be getting dry, lightly spray it.

Xenopus – African Clawed Frogs

Feeding

The xenopus are fed twice weekly with floating trout chow pellets ordered through the Essex Feed Warehouse. The water flow to the frog tanks must be turned off. The tanks must be drained well past the outflow valve on the tanks or the food will clog the drains, causing the tanks to overflow. Once the tanks have drained to a proper level, close the drains and add food. Spread approximately 2-3 cups of food between the tanks. Let the tanks sit with the food for about an hour. After the frogs have fed, use the large nets hanging in the room to scoop excess food from the tanks. Rinse the nets with hot water between tanks. When the tanks are clean, turn the water flow to the tanks back on. Check the gauge on the wall to make sure that the water is at the correct temperature (19^{o}C). If temperatures are above or below the desired setting, please let your supervisor or the animal quarters technician know.

Housing and Cleaning

The xenopus are kept in a flow-through housing system. There is a slow but constant flow of water going in and out of the tanks. This system keeps the tank water relatively clean so they are very low maintenance. Xenopus are completely aquatic and do not require any basking areas.

They do require dechlorinated water as they are very sensitive to chemicals. The water flow into the tanks is tested weekly for chlorine leaching. The frogs do occasionally manage to escape from their tanks. If you happen to find an escaped frog, use the net to scoop it up and place it into the tank labelled ‘escapees.’

The sides of the tanks and tank lids occasionally need to be scrubbed to remove algae build-up. Use a scrub pad with no additional cleaning agents to clean them.

Distinguishing Between the Sexes

It is quite easy to sex adult xenopus. The females are generally much larger than the males and have a visible cloaca. The males are smaller and develop dark pads on the undersides of their hands and forearms.

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Short Term Care of Laboratory Frogs

Maintaining Adult Frogs

The most convenient method of ‘holding’ adult frogs (Rana Pipiens) until needed is to render them inactive by reducing their metabolic rate. This method makes feeding unnecessary, reduces waste build-up, and reduces the occurrence of various frog diseases.

The metabolic rate is lowered by refrigerating the frogs. If the frogs are to be held for 1-3 days, they may be placed in a mesh covered pan secured tightly with clips with 2.5-5cm of water (tap water may be used). If not covered properly, frogs can and do escape from their holding tanks. Place the container in a refrigerator. For longer holding periods (1-3 weeks) slight modifications are necessary. Cool the warm frogs for several hours in the refrigerator before unpacking. Have a container of water 15-30 centimetres deep (tap water may be used) cooling at the same time. This container must be well aerated at all times through the use of an air stone and air pump. Frogs may be placed in the container as soon as the above temperatures are reached. Change the water with fresh water which has been pre-cooled to holding temperature.

Adult bullfrogs may also be held in a similar fashion. Cool the warm frogs for several hours in the refrigerator. Prepare cages (use rodent shoe box cages) with a thick layer of soaked paper towels on the bottom. Place two frogs in the cage and cover them with a layer of soaked paper towels. Place the cage into the refrigerator. The paper towels must remain wet at all times. Add extra water to the cages as necessary. Completely change the paper towels twice weekly. Frogs should not be held for more than 3 weeks in this fashion.

Fish

Handling

When handling fish, latex lab gloves should always be worn. Fish should be removed from tanks using dip nets specific for that task. Collection efforts should be made as quickly as possible to reduce stress and shock. Keep the time out of water down to the bare minimum and allow a couple of minutes for the animals to recover before handling them again. If extended periods out of water are required, lay the animal on wet paper towels and cover any areas you don't need access to on top of the animal with wet paper towels.

Individual identification

If possible, individual fish within a tank should be identified by their natural features. If some other method of identification is required, non-invasive methods, such as the injection of a small amount of fluorescent latex paint under the skin of the operculum, should be tried before more invasive methods such as fin clipping.

Feeding

Be sure to buy the size of pellet appropriate for the size of your fish. Other types of fish may eat other types of food (e.g. goldfish can be fed pet fish food). Food is simply thrown into the water for the fish to consume. Do not leave uneaten food in the water for extended periods of time, as this can be a source of harmful bacteria and/or pathogens. A good rule of thumb for many species is to only feed enough food for them to consume in five minutes. If fish are not use to artificial food, it may take them a few days to become conditioned to the new food type. Always monitor new fish to make certain they are eating. It may be necessary to augment the artificial food with a more natural food source for a couple days until they make the switch.

Facilities

Tanks in the Biology Animal Quarters and in the GLIER aquatic facility are available to faculty, post-docs and graduate students. For tanks in the Biology Animal Quarters, contact the Animal Quarters Technician, Biology, extension 2707. For tanks in the GLIER aquatic facility, contact the facility manager, extension 4846.

Amphibians

Housing (leopard frogs, bullfrogs, green frogs)

Frogs should be kept outdoors, if possible, so that they can receive as much natural UV exposure as possible. If this is not possible, use indoor tanks that have a dechlorinated water source, a drain, and an outlet to run UV lights. Lights will be placed above each frog tank and low dose exposure (approximately two hours a week), should be applied using a timer.

Frog populations should be kept in large tubs suspended in a metal frame or a wooden frame that is either built of cedar or has been varnished to seal it. These tubs should have drains in the base for easy cleaning and have capacity for twenty green frogs or forty leopard frogs. The tubs should be equipped with habitats tailored to suit the species. For the leopard frogs, half of the habitat should be water and the other half should be meadow. The meadow area should be a purpose-built worm tray with about an inch of soil and grass. Worms should be fed directly onto the grass. For the bullfrogs and green frogs, large rocks should be used to allow the frogs to get out of the water and sunbathe. Live plants (such as water hyacinths) should also be added to the tubs so that the frogs can hide underneath them and hold onto them in the water.

Smaller numbers of frogs can be housed in aquariums with a similar habitat set up as previously described. The aquariums should have purpose-built lids which fit tightly so that escapes are as difficult as possible.

Cleaning (leopard frogs, bullfrogs, green frogs)

The tubs should be cleaned every second day by removing the plug and allowing the water to drain out. The frogs should be left in the tank to reduce stress. If possible, plants should be left in if possible so that there is something to hold on to. If water from the hose is used, then dechlorinating solution should be added and thoroughly mixed.

The aquariums should be cleaned using a siphon method. Waste and dirt are siphoned out of the bottom of the aquariums; the aquariums are then refilled with dechlorinated water.

Fish

Tanks

Fish need clean dechlorinated water and tank size should be appropriate for the number and size of fish used. Many species need a current to swim against and this is best arranged by having a flow-through system with a stand-pipe drain in the centre of the tank regulating the water level. Where possible, rocks, plastic pipes, or other items that will provide cover and hiding places should be provided for more benthic species. If this is not possible, covering half of the tank with a cloth or tarp will suffice. For fish in re-circulating water, pH should be checked at least weekly as a guide to water quality.

Cleaning

Tanks need to be cleaned regularly in order to remove old food and prevent the build up of algae. This need will vary with the size of the tank. Flow-through systems will alleviate some of the problems here, requiring less frequent cleaning, but once a week the stand-pipe should be removed or the bottom siphoned in order to clean dirt from the bottom of the tank.

Lab Procedures

Amphibians

Euthanasia

Frogs should be euthanised by overdosing them with MS222. Place them in a solution of 200mg/litre MS222 for five minutes. Ensure the animal is dead before continuing to other procedures.

Fish

Blood Collection Techniques

Fish should be removed from the tank, a one at a time, and individually immersed in a 0.01% solution of tricane methanosulfate (MS222), buffered to pH 7, for approximately five minutes to partially sedate them. The sedation is appropriate when the fish starts to float upside down, but do not leave them in the solution after this point as the MS222 can be lethal. Make sure that the fish's gills are still moving during the whole sedation process. Move the fish to a pile of wet paper towels on a nearby bench and cover the anterior half with another wet paper towel. Blood is taken using an appropriate syringe (for a 450gm fish, a 5 ml syringe is appropriate) with a 28G x 1/2 needle or smaller. Coating the syringe with heparin will reduce clotting and make it easier to remove blood. Insert the needle into the caudal vein by inserting it in to the caudal surface of the animal, half way between the anal and caudal fin. Push the needle in gently until it is stopped by the spine, then pull it back gently about 3 mm. Pull gently on the plunger of the syringe until blood starts flowing into the barrel. If necessary, blood removal can also be done by cardiac puncture but survival is less likely. The amount of blood removed should not exceed 3ml for a 450gm fish. After blood is removed, the fish is placed in a separate container to recover and to avoid confusion with fish which have not been bled yet. The recovery chamber must be well aerated and, for larger fish, they may have to be manually moved around the tank to increase water flow over their gills. The site of blood removal should be dabbed with a Q-tip soaked in MelaFix to aid in healing. Once the fish has been bled, do not bleed it again for at least three days.

Euthanasia

Fish should be euthanised by overdosing them with MS222. Place fish in a solution of 0.1% MS222 for five minutes. Ensure that the gills have stopped moving before removing the animal from the solution. For some species of fish stronger doses of MS222 may be required. Clove Oil is also an acceptable substitute for MS222.

Part 4: Wildlife Collection

General Introduction

Just because a researcher is not on the University of Windsor campus, it does not mean that University rules do not apply to them. When collecting in the field, you are a representative of this University and will be required to adhere strictly to the University policies.

Animal collection should be performed in a humane manner that causes a minimum of discomfort to the animals. If the animals are not to be brought back to campus alive, they should be euthanised according to the procedures outlined in the previous chapter.

Regulations

In order to collect wild animals in the province of Ontario, one must have a collector's permit from the Ontario Ministry of Natural Resources from the appropriate region. Once you have a permit, you can go to another regional office for an extension. There are fines for collecting animals without this permit.

University of Windsor researchers collecting animals in the field must submit an Animal Utilization Project Protocol, along with a Field Work Involving Wild Animals form, to the University Animal Care Committee for approval before proceeding with the collection.

Collecting Animals in the Field

Amphibians

Species Collection

Frogs should be collected from the field at the relevant time of year for each species. Leopard frogs can be collected during the late afternoon to early evening, as they are active and accessible at this time. Green frogs and bullfrogs can be collected after dark by using a flashlight beam to temporarily stun them. The frogs can be found in the following areas and times of the year:

Northern Leopard Frog (Rana pipiens)

The Leopard Frog is found in lakes, grassy ponds, marshes, and wet meadows. During the warmer parts of the year they are found in meadows and grasslands, especially those near

streams, rivers, and ponds. They are often found in cultivated hay fields or meadows grazed by cows or horses. They are especially active at night and are very difficult to catch once they have reached the water so efforts should be made to get them before they jump.

Preferred calling sites have rafts of floating vegetation for the males to cling to as they float on the surface of the water. Females arrive at the breeding ponds approximately one week after the males start calling. Eggs are laid a week later and are attached to submerged vegetation or are occasionally laid on the bottom of the pond. After hatching, the tadpoles take between 10 to 13 weeks to transform into froglets. By September, the froglets have doubled in weight and are about five cm long. They will hibernate in deep pools for the winter, and sometimes be seen on the surface mud under the water.

Bullfrog (Rana catesbeiana)

Bullfrogs prefer the shorelines of lakes, bays, large beaver ponds, and the mouths of slow-moving rivers. Deep, permanent bodies of water are necessary because the tadpole spends the winter in the water. If one uses binoculars, the bullfrogs can often be seen sitting partially or fully exposed on the bank. Sometimes they are hidden beneath leaves and branches. They are also sometimes in small ledges several feet above the water level. Although active in May, male bullfrogs do not establish their shoreline territories until June or July. Their deep, booming calls may be described by the phrase "Jug O'Rum", "Jug O'Rum", "Jug O'Rum". At dusk they will call from a favourite location, or more often while floating at the surface of the water. Males are very territorial and will defend their breeding territory with a special encounter call. If this call fails to drive away an intruder, the male will jump at the invading male, push, lock arms, kick, and try to flip him over. Once territories are established, the loud "Jug O'Rum" calls are given to attract females to good breeding locations.

It is easiest to find them at night by using a flashlight as they will not move once both their eyes are in the light from the beam. The largest populations exist where both underwater plants and those growing near the margins of the waters are particularly lush.

Green Frog (*Rana clamitans)*

Green frogs tolerate a wide range of habitats and may be found in any permanent supply of water. They typically prefer rich, weedy, warm ponds and lakes, slow-moving rivers, farm ponds, and shallow marshes. They emerge from hibernation in early April, but do not begin to call or breed until June or July. The male's call can be compared to the "twang" of a single banjo string being plucked. Males are most active in the first 30 minutes after sunset. Calling frogs will sit in such a way that their yellow throats are exposed, using a set of four special calls to defend their individual established territories. If competing males ignore the warning calls, a defending male may splash, chase, or wrestle with his competitors.

Tadpoles spend the winter in the water and transform the following summer. The tadpoles may grow to about six to eight centimetres long, but during transformation, the froglets may end up smaller than they were.

Identification

Northern Leopard Frog (Rana pipiens)

Leopard frogs grow to about five inches in length. They are slender, long-legged frogs with two conspicuous dorsolateral ridges. They are marked on the back, sides, and legs with numerous dark, roundish spots that usually have light borders around them. They are usually green, although some may be light brown in colour. The call of leopard frogs is sometimes compared to the sound of wet hands rubbing across a wet balloon.

Bullfrog (Rana catesbeiana)

Bullfrogs grow to be about 10 to 15 cm and are green or greenish brown. There is a fold extending from the rear edge of the eye down over the eardrum, but there is no ridge running down each side of the back. Male bullfrogs have yellow throats, especially in summer. The tympanum is twice as large as the eye on males and the same size as the eye on females. Young bullfrogs have small black spots on their green backs. Eggs are laid in large floating masses. Tadpoles spend the winter in the water, and transform the following summer.

Green Frog (Rana clamitans)

This frog is between 6 and 9 cm long with green or dark brown spots over the back. A bright green area above the upper lip is usually visible, as are black bands across the hind legs. The green frog can be distinguished from the bullfrog by looking for the ridges running down each side of the green frog's back. Male green frogs have yellow throats, particularly during breeding season.

Distinguishing Between the Sexes

Northern Leopard Frog (Rana pipiens)

Both sexes look similar, though males are generally smaller than females, reaching only three and one quarter inches. Males have two vocal sacs that swell out from their armpits when the frogs are calling. In the breeding season, the thumbs on the males' front feet swell in size. Females usually grow to three and a half inches or more in length. During the breeding season they are much fatter than males.

Bullfrog (Rana catesbeiana)

The oldest and largest bullfrogs are invariably females as the males are more exposed to predation. Males have yellow throats and eardrums that are much larger than the diameter of

their eyes. Females, in contrast, have white throats and eardrums that are about the same size or slightly smaller than their eyes.

Green Frog (Rana clamitans)

As is the case with the bullfrog, males have yellow throats and eardrums that are much larger than the diameter of their eyes. Females, in contrast, have white throats and eardrums that are about the same size or slightly smaller than their eyes.

Fish

Fish may be collected by several methods including dip nets, seining, and gill nets. In all cases, these procedures should be performed in as humane a manner as possible. If the animals are not to be brought back immediately, they should be euthanised in a solution of 0.1% MS222 as quickly as possible and prior to their immersion in any fixatives. Live animals should be returned in tanks or buckets that are covered, and provided sufficient oxygen for the trip. Animals should be allowed a week to recover from collection before experiments are initiated.

Appendix: Forms

The Animal Care Committee has prepared several forms to be used by researchers. Please see the ACC web page: www.uwindsor.ca/acc for the most current forms.

Animal Utilization Project Proposal (AUPP)

All research and/or teaching projects conducted at the University of Windsor with live non-human vertebrate animals must be covered with a corresponding Animal Utilization Project Proposal (AUPP). This form is approved by the Animal Care Committee (ACC) prior to the acquisition of any animals for the project. Approval of an AUPP indicates that the ACC is satisfied that humane practices and proper animal care standards will be used, in accord with the requirements of the Canadian Council on Animal Care and the Ontario *Animals for Research Act*.

Field Work Involving Wild Animals

In addition to the AUPP, the Field Work Involving Wild Animals form must be filled out if research involves wild animals in the field.

Progress Report/Annual Renewal

A completed copy of the Progress Report/Annual Renewal must be submitted by the end of January for every AUPP that is still active at the end of the previous calendar year. At its spring meeting, the ACC reviews this document and statistics concerning animals used are compiled and sent to the Canadian Council on Animal Care.

Request to Revise

If there are any changes to the original AUPP, a Request to Revise must be submitted outlining these changes.

Final Report

The Final Report form should be submitted when research outlined in an AUPP has been completed. Like the Annual Renewal, this form provides statistics which must be compiled and submitted to the Canadian Council on Animal Care.

ASSESSMENT OF RESEARCH PROTOCOLS IN ABSENCE OF PEER REVIEWS

Prologue

Research funded by such organizations as NSERC or CIHR is assumed to have been peer reviewed. Research which is not funded by an established funding agency which applies a peer review process must undergo more intensive scrutiny prior to consideration by the Animal Care Committee (ACC).

Teaching Protocols.
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 the relevant aforementioned 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.

Honours thesis projects and all other research undertaken as part of an undergraduate course (including honours thesis courses) do not require external review for scientific merit. Since the primary purpose of such projects is the teaching and/or training of undergraduate students, proposals for work of this nature shall be reviewed by the ACC primarily on the basis of their pedagogical value. However, honours thesis projects which are deemed (by their thesis committee) to require ACC approval should be examined for scientific merit by at least two thesis advisors prior to submission to the ACC.

Research Protocols

Research projects, other than those which constitute part of an undergraduate course, must be reviewed for scientific merit unless they are direct extensions or applications of ongoing peer-reviewed work by the principal investigator. Scientific review is considered adequate if it is performed by a granting agency or by an industry that obtains the opinions of external referees, sets scientific standards, and awards funds on a competitive basis. An investigator who receives funding from a granting agency must have an approved AUPP before funds will be released by the university to that researcher for expenditures. In general, the ACC will review research proposals (other than undergraduate projects) from the viewpoint of the ethical aspects and the acceptability of the proposed procedures.

Research proposals that are not funded by an agency or industry that applies a peer review process, and which are not part of an undergraduate thesis or non-thesis course, must be reviewed for scientific merit. Researchers wishing to undertake research involving vertebrate animals must consult with the Office of the Vice-President, Research and provide the following:

(a) a brief research proposal, including a summary, a brief literature review, clearly stated objectives of the research, proposed methodology, a description of the significance of the work, and a brief time schedule for work lasting up to four years;

(b) evidence of scholarly activities, including a list of publications and conference presentations for the past six years, academic positions held, and evidence of research impact (a current curriculum vitae will suffice);

(c) names, email addresses and phone numbers of at least three experts in the field relevant to the proposed research, none of whom can be the applicant's former supervisor or collaborators within the past three years.

Reprints, submitted manuscripts and other materials demonstrating scientific merit may be submitted as attachments. Within one week of receiving such an application, the VP Research will contact at least two of the experts recommended by the applicant, ensure their participation, and send them copies of the entire proposal. These external referees will be requested to review the proposal within two weeks and to provide a brief, written report as outlined on the Peer Review Form sent to each referee. Within one week of receiving the referee reports, the VP Research will review the reports and indicate whether or not the proposal, either as proposed or with modifications, has scientific merit. The VP Research will notify the applicant in writing of the outcome of the peer reviews, and if positive, invite the applicant to submit a full AUPP to the ACC. Copies of the written reports of the referees will be provided to the applicant after removal of the referee's name upon request.

Animal Care Committee

PEER REVIEW FORM

The Canadian Council on Animal Care, which overseas animal use for research, teaching and testing, requires that all animal-based research projects receive peer review from two independent experts prior to their approval by an Animal Care Committee.

The following set of questions will serve as a guideline for this review (please attach additional pages if necessary), and return the form to the Vice President, Research, University of Windsor, 401 Sunset Ave., Windsor, Ontario, Canada N9B 3P4.

AUPP #

1) Please comment on the objectives and potential contribution(s) of this study to scientific knowledge.

2) Please comment on the following:

   a) Hypotheses of the study and appropriateness of the experimental design involving animals.

   b) Are the number of animals proposed appropriate to the research proposed?

   c) Is the species of animal/model appropriate?

d) Are there alternative experimental procedures which would address the same research questions which would decrease or eliminate the use of experimental animals?

_____

Signature Date

_____

Name and Title (please print)