  
**UNIVERSITY OF WINDSOR ANIMAL CARE COMMITTEE**

##### ANIMAL UTILIZATION PROJECT PROPOSAL

All research and/or teaching projects conducted at the University of Windsor with live non-human vertebrate animals must be covered by a corresponding Animal Utilization Project Proposal (AUPP) which is approved by the Animal Care Committee (ACC) **prior to** 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 (CCAC) and the *Ontario Animals for Research Act*.

**Notes to the Applicant:**

1. Send **one electronic copy with all electronic signatures and dates** to the Animal Care Coordinator; acc@uwindsor.ca.

2. Any changes in experimental procedures must be reported to the ACC by submitting a Request to Revise.

3. AUPPs are valid for **one year** from the date of approval. Ongoing research projects or teaching projects **must be reviewed** **annually** but they may be **renewed for three consecutive years** by using the Progress Report form.

**Today’s Date:** Click here to enter text. **AUPP #** Click here to enter text.

**Title of Research Project/Course:** Click here to enter text.

**Research Project/Course Status: New Project/Course Renewal of AUPP #:** Enter text.

**Anticipated Start Date:**Click here to enter text. **Anticipated Completion Date:**Click here to enter text.

**Emergency Contact Person:** Click here to enter text. **After Hours Phone Number:** Enter text.

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|  | **Name** | **Dept.** | **Emergency Phone** | **E-mail** |
| **Principal Investigator/ Instructor** | Enter text. | Enter text. | Enter text. | Enter text. |
| **Co-Investigator** | Enter text. | Enter text. | Enter text. | Enter text. |
| **Co-Investigator** | Enter text. | Enter text. | Enter text. | Enter text. |
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**A. ANIMAL HANDLING EXPERIENCE AND RELATED TRAINING**

**A.1.** Describe briefly the qualifications and experience of personnel with respect to the procedures they will perform on animals. If personnel will not have direct contact with the animals, please describe their role in this protocol.

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**A.2.** **What is the chain of command for reporting observations to supervisors?**

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**B. RESEARCH PROJECTS/COURSE INFORMATION**

**B.1.** **Purpose of Project**:  Research Pilot Study  Other  Teaching **Course Number:** Enter text.

**B.2. Level of Research Project/Course:** Faculty  Post-doctoral  Graduate  Undergraduate

**B.3. Does this work involve field work and wild animals?**  **Yes** **No**

If **yes**, you will need to complete all applicable sections of this form and the [*Field Work Involving Wild Animals* form*.*](http://www1.uwindsor.ca/acc/system/files/Field%20Work(1).pdf)

**C. SUMMARY OF EXPERIMENTAL PROCEDURES**

Describe **concisely** (in 250 words or less), as if you are explaining your work to a group of Grade 9 students or for a media release, the experimental procedures of the project, including *the rationale and/or the objectives, the anticipated potential benefits to scientific knowledge, or to human and/or animal health and the procedures*.

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**Key Words:** Provide 6 to 8 key words to identify this project.

*(Refer to Appendix A, below, after the Signing Page, for Key Words)***.**

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**D. DESCRIPTION OF ENDPOINT MONITORING PROCEDURES**

*The CCAC guidelines recommend the use of endpoints. Endpoint is the point at which an experimental animal’s pain and/or distress is terminated, minimized or reduced, by taking actions such as killing the animal humanely, terminating a painful procedure, or giving treatment to relieve pain and/or distress. Death of an animal is not an acceptable endpoint. Since the end-point for each animal may vary depending on the treatments given the animal, all animal care personnel should consult the appropriate ACC-approved SOP endpoint document when working with animals. (Please refer to* ***ACC SOP*** [***AH25A***](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah25a_endpoint_for_aquatics_revised_june_2018.pdf)***,*** [***AH25B***](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah25b_endpoint_for_frogs_june_2018.pdf)***,*** [***AH26A***](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah26a_endpoint_for_mouse_revised_july_2018.pdf)***,*** [***AH 26B***](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah26b_endpoint_for_rat_june_2018.pdf) ***or*** [***AH34***](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah34_endpoint_for_avian_revised_april_2017_0.pdf)*)*

**D.1.** **Indicate who is responsible for monitoring the condition of the animals, the frequency of the monitoring, and how this frequency was determined. *(Note: All non-surgical animals must be observed at least once per day; monitoring must be initially more frequent in post-surgical animals).***

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**D.2.** **What are the “indicators” of pain and/or distress that are evident as a result of the experimental manipulation(s) (e.g. clinical conditions, behavioural changes, and abnormalities)? How were these indicators chosen? Could a pilot study help you to determine this?**

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**D.3. What procedures will be used to treat any adverse effects and/or complications?**

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**D.4. Specify what health condition(s) or other criteria would trigger a decision to humanely euthanize an animal. If this is necessary, when might this occur during the experiment?**

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**D.5.** **Animals that die unexpectedly or are euthanized may be submitted for post-mortem examination by the ACC veterinarian. Are there any special instructions for sample collection?**

**Yes** **No**

If **yes**, explain.

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**D.6.** **Specify what health condition(s) or other criteria would trigger the decision to terminate the experiment.**

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**E. EXPERIMENTAL DESIGN**

Describe in sequence all experimental procedures to be done on the animals, including surgical procedures, behavioural manipulations, physiological assessments, restraint procedures (type and duration), etc. Procedures that could or will potentially impact the well-being of the animal(s) must be clearly outlined. Use of a flow chart is encouraged. Please attach additional pages.

**REPLACEMENT, REDUCTION *AND* REFINEMENT (3 R’S)**

Consideration of the **3 R’s** should be undertaken before a study using animals is proposed. The CCAC publishes an excellent [online resource](http://3rs.ccac.ca/en/) dedicated to the 3 R’s, which has many helpful links to alternatives that can remove or reduce the need to use animals. For assistance in performing a 3Rs search, see the CCAC’s 3R’s Search Guide (http://3rs.ccac.ca/en/searches-and-animal-index/guide/).

To assist in deciding if your Three Rs search is complete, it may be useful to review the following questions (<http://3rs.ccac.ca/en/searches-and-animal-index/questions.html>):

* Is the proposed experiment or test duplicative?  **Yes**  **No**
* Are there any in vitro techniques that could replace use of animals?  **Yes**  **No**
* Have any computer simulations been developed that relate to the study?  **Yes**  **No**
* Are there any alternative animal models of lower sentience?  **Yes**  **No**
* Is there useful and current information about the proposed animal model?  **Yes**  **No**
* Has a particular strain of animal been shown to be more sensitive to the   
  effects that will be studied?  **Yes**  **No**
* Is there information on the proposed model that might allow the use of   
  fewer animals or might reduce any pain experienced by the animals?  **Yes**  **No**
* Could in vitro methods be incorporated into the protocol in any way to   
  reduce the number of animals used (e.g., for early screening)?  **Yes**  **No**
* Have any statistical models been developed for use in this type of study,  
   and would these affect the design of the experiment?  **Yes**  **No**
* Is there a way to decrease the level of invasiveness of the protocol   
  without compromising the scientific objective?  **Yes**  **No**
* Could the proposed anaesthetics, analgesics or other drugs pose a  
  confounding influence on the experimental outcome?  **Yes**  **No**
* Is there information about assessing welfare and the level of pain  
   of the animals?  **Yes**  **No**

**F.1. PROCEDURES**

List all procedures where pain, distress, discomfort and/or suffering could occur in the animals. Indicate what measures will be taken to alleviate or minimize these effects. Include post-operative care; specify analgesics and anaesthetics with dosages and routes of administration and special procedures used.

**\***Distress levels may be nil, low, medium or high.

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**F.2.** **Explain the scientific hypothesis(es) to be investigated in this project.**

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**F.3.** **Explain what new information is expected from the conduct of this project and its anticipated value.**

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**F.4.** **Explain why it is necessary to use live animals in this project and what alternatives have been considered (e.g. mathematical models, computer simulations, or in vitro systems).**

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**F.5.** **Explain the rationale for the choice of species.**

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**G. FUNDING INFORMATION FOR RESEARCH PROJECTS**

**G.1.** **Is this project currently funded?**  **Yes**  **No**

**Title of the funded project:** Click here to enter text.

**Agency Name**: Click here to enter text.

**Business Unit**: Click here to enter text.

Period of funding from: Click here to enter text.

**G.2.** **Is funding being sought?**  **Yes**  **No**

**Title of The Funded Project:** Click here to enter text.

**Agency Name:** Click here to enter text.

**Business Unit**: Click here to enter text.

**Period of Funding From**:Click here to enter text. **To** Click here to enter text.

**G.3. Does this proposal involve a collaborative project with other institutions?**  **Yes**  **No**

**If yes, please attach documentation indicating that this proposal has been reviewed by the other institutions ACC.**

**H. PEER REVIEW FOR SCIENTIFIC MERIT FOR RESEARCH PROJECTS**

**H.1.** Has this project already been peer-reviewed for scientific merit?   **Yes**  **No**

**If yes**, please provide details.

**Name of Granting Agency:** Click here to enter text.

**Other:** Click here to enter text.

**H.2. If no**, the project must be peer-reviewed prior to commencement of the research.

All non-funded and non-externally peer-reviewed research projects involving animals must undergo prior review for scientific merit as per [*SOP AD-04* *Assessment of Research Protocols in the Absence of Peer Reviews*](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ad04_assessment_of_research_protocols_in_absence_of_peer_reviews_revised_june_2017_1.pdf). This can be found at [www.uwindsor.ca/acc](http://www.uwindsor.ca/acc). This includes pilot research and contract or grant research.

The ACC itself does not conduct formal scientific or educational merit review for non-funded, non-externally reviewed projects. This is done by the VP Research at the request of the principle investigator or Chair of the ACC. Once a research proposal has received positive external reviews, the applicant will invited by the VP Research to submit an AUPP to the ACC.

1. **PEDAGOGICAL MERIT REVIEW FOR TEACHING PROTOCOLS**

All non-funded and non-externally peer reviewed teaching protocols involving animals **must** undergo prior review for pedagogical merit. Please refer to [SOP AD05 Assessment of Teaching Protocols](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ad05_review_and_assessment_of_teaching_protocols_march_2018.pdf) to aid you in completing this section of the AUPP. Once you’ve submitted your teaching AUPP to the ACC, the ACC coordinator will forward your AUPP to an independent referee within the University of Windsor community in order to evaluate pedagogical merit.

**I.1.** Has this teaching protocol already been reviewed for pedagogical merit?  **Yes**  **No**

**If yes,** please provide a completed form [**(SOP AD05)**](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ad05_review_and_assessment_of_teaching_protocols_march_2018.pdf)

**I.2. If no, please provide at least 1 referee**; Please note that Deans, Chairs and Directors are suitable referees.

**Referee:**

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**J. FOR COURSES WITH LABORATORIES INVOLVING USE OF ANIMALS**

**(\*\*Please indicate if not applicable)**

**J.1.** **What on-site supervision will be provided for the students in this course?** **N/A**

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**J.2.** **What training is provided to Teaching Assistants to ensure they are adequately prepared for their role in this course?**  **N/A**

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**J.3.** **Explain why the use of live animals is required in this project and identify what alternatives to live animals you have considered (e.g. demonstration, film, videotape, etc.)?** **N/A**

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**J.4.** **What are students expected to learn from this project that justifies using live animals?** **N/A**

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**J.5.** **What is the student to animal ratio in this project?** **N/A**

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**J.6.** **What alternative will be provided to any student that does not wish to participate in animal based teaching either with the AUPP as a whole or with individual procedures within the AUPP.** **N/A**

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**K. Pedagogical Merit.**

In the space below please describe the intended learning outcomes in lay terms for the use of animals in the AUPP (250 words or less)

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**Please note the reviewer will judge the suitability of the AUPP in terms of pedagogical merit based on the following questions. Please consider these when preparing your AUPP**.

1. Are the composition, learning level and needs of the student group(s) compatible with the goals and objectives of the animal-based teaching/training?
2. Is the timing of the inclusion of animals in the teaching/training suitable for the projected timing of the expected outcome(s)?
3. Does the course incorporate any feedback from student assessments and course or session evaluations regarding the benefit of the animal-based teaching/training?
4. Is the review of the obstacles and opportunities for implementing Three Rs by the animal based teaching/training instructors’ is sufficiently thorough
5. Is the criteria proposed for assessing the completed animal-based teaching/training suitable and will it contribute to the optimization of the use of animals for the benefit of future students?
6. Do the learning objectives specify the proportion of the objective that must be achieved?

# L. USE OF ANIMALS AND SUPPLIERS

**L.1.** Indicate the number of animals in each species required for a **one year period** and the source/suppliers. Please provide an explanation when Transgenic/mutant/knockout strains are used. If you require more space, please include on separate sheet.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Commercial Supplier & Address** | **Number Purchased** | **Number Used in Procedures** |
| Enter text. | Enter text. | Enter text. | Enter text. |
| Enter text. | Enter text. | Enter text. | Enter text. |

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Own breeding stock** | **Number Bred on site** | **Number Used in Procedures** |
| Enter text. | Enter text. | Enter text. | Enter text. |
| Enter text. | Enter text. | Enter text. | Enter text. |

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Source of donation** | **Number Donated** | **Number Used in Procedures** |
| Enter text. | Enter text. | Enter text. | Enter text. |
| Enter text. | Enter text. | Enter text. | Enter text. |

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| **Total Number of Animals Required** | |
| **Species Used** | **Total Numbers to be used in Procedures of each Species** |
| Enter text. | Enter text. |
| Enter text. | Enter text. |
| Enter text. | Enter text. |

**L.2.** **Outline how the number of animals to be used was determined (e.g. number of groups, replicates etc.).**

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| **Species** | **Commercial Supplier & Address** | **Number Purchased** | **Number Used in Procedures** |
| Enter text. | Enter text. | Enter text. | Enter text. |
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| **Species** | **Own breeding stock** | **Number Bred on site** | **Number Used in Procedures** |
| Enter text. | Enter text. | Enter text. | Enter text. |
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| **Species** | **Source of donation** | **Number Donated** | **Number Used in Procedures** |
| Enter text. | Enter text. | Enter text. | Enter text. |
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**L.3.** **If this is a continuation from a previous AUPP, what was the actual number of animals used during the previous year?**

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| **Total Number of Animals Required** | |
| **Species Used** | **Total Numbers to be used in Procedures of each Species** |
| Enter text. | Enter text. |
| Enter text. | Enter text. |
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**L.4.** Explain any change in numbers between those requested in I.3 and the number approved for use under the last year of the previous AUPP. (Details of any animals bred from purchased animals should also be included here.)

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# M. HOUSING INFORMATION

**M.1. Experiments.** **Are experiments going to be performed?**  **Yes**  **No**

If **yes**, indicate the areas which will be used. CACF GLIER LaSalle **Room No.:** Enter text.

**M.2. Surgery.** **Are surgical procedures involved in this project?** **Yes**  **No**

If **yes**, indicate the areas which will be used. CACF GLIER LaSalle **Room No.:** Enter text.

**M.3. Housing.** **Does this project require housing of animals?**  **Yes No**

**If** **yes**, indicate the areas which will be used. CACF GLIER LaSalle **Room No.:** Enter text.

**M.4.** **Environmental enrichment is an important aspect of stress reduction for most animals, therefore all animals housed in the University facility must be provided with environmental enrichment elements such as group housing, nesting materials, hiding places, etc. Please describe the type of environmental enrichment that you will provide your animals. Please refer to** [**SOP AH31- Laboratory Animal Environmental Enrichment**](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah31_laboratory_animal_environmental_enrichment_revised_september_2018.pdf)**.**

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**N. INVASIVENESS CATEGORY**

What is the Invasiveness Category of this project? *(Refer to Appendix B, below, for examples of Categories of Invasiveness.)*

***Category A (Low):***Experiments on most invertebrates or on live isolates.

***Category B (Nil-Low):***Experiments which cause little or no discomfort or stress.

***Category C (Low-Moderate):*** Experiments which cause minor stress or pain of short duration.

***Category D (Moderate-High):*** Experiments which cause moderate to severe distress or discomfort.

***Category E (High Pain or Severe):***Procedures which cause severe pain near, at or above the pain

tolerance threshold of anaesthetized conscious animals.

# O. CLASSIFICATION CATEGORY

***Acute:*** Any study involving euthanasia of an animal upon receipt or shortly after a brief period of housing;

No manipulations or experiments to be performed on conscious animals (e.g. animals euthanized for tissues, or anaesthetized and not allowed to recover from anaesthesia).

***Chronic:*** Any study that involves recovery of an animal from anaesthesia or maintenance of animals in

University facilities for more than 2 days.

***Other:***Include explanation:

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# P. DISPOSAL OF ANIMALS

**NOTE:** *Researchers are advised to consult the most recent CCAC Guidelines, which can be found* [*HERE*](http://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia_implementation.pdf)*.*

**\* In the case of physical methods of euthanasia, attach a sheet explaining the reason for selecting this method and detailing the relevant training of personnel undertaking this procedure.**

**\*\*Please note that the ACC requires justification if the method of euthanasia proposed is not listed within the CCAC guidelines.**

What will be the disposal of the animals upon completion of the study? (Check all that apply)

Kept for future use (specify project): Click here to enter text.

Humanely euthanized by (specify method):

Anaesthetic overdose (specify agent and dose): Click here to enter text.

C02 (***CO2 euthanasia should be followed by another form of physical euthanasia.) (Please consult ACC*** [***SOP AH23***](https://www.uwindsor.ca/animal-care-committee/sites/uwindsor.ca.animal-care-committee/files/ah23_carbon_dioxide_euthanasia_revised_october_2017_0.pdf)***, subsection 15)***

Cervical dislocation\*

Decapitation**\***

Exsanguinations

Other (Specify and Explain):

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# Q. HAZARDS TO HANDLERS OR TO ANIMALS

**Q.1.** Will any of the following agents be used? Note: Approval from the appropriate oversight committee must be obtained before project commences.

**Radioactive** Yes No **Specify isotope and Dose**: Click here to enter text.

**Biological** Yes No **Specify Agent and Dose:** Click here to enter text.

**Infectious** Yes No **Specify Agent and Dose:** Click here to enter text.

**Chemical/Noxious\*** Yes No **Specify Agent and Dose:** Click here to enter text.

**I acknowledge by checking this box that I am responsible for the chemical(s) utilized during the process of this research.**

**NOTE:** A copy of each SDS/drug insert is required for application review. Please attach.

**Q.2.** Describe the safeguards that will be used to protect other animals.

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**Q.3.** Describe the safeguards that will be used to protect handlers including protective equipment.

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**Q.4.** Please provide a copy of the appropriate documentation from the Research Safety Committee if your protocol required biosafety or radiation safety approval. Attach to the end of the AUPP form.

Biosafety Radiation Safety

Chemical Control Approval  Chemical/Noxious Use Approval

**CERTIFICATE #**: Click here to enter text.

**R. PURPOSE OF ANIMAL USE**

This information is required by the Canadian Council on Animal Care. Choose the item below that best describes the purpose of animal use:

0. Breeding colony/stock that have not been assigned to a particular research, teaching, or testing protocol.

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

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

3. Studies for regulatory testing of products for the protections of humans, animals, or the environment.

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

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

***These appendices are attached below (following the signing page) for your information. You do not need to include them with this AUPP.***

Appendix A CCAC List of Key Words

Appendix B CCAC Categories of Invasiveness in Animal Experimentation

**RESEARCHER’S/COURSE INSTRUCTOR’S DECLARATION**

1. I believe that the proposed animal use conforms to my stated objectives, will advance knowledge and will employ the best methods on the smallest number of animals to obtain valid information.

2. I believe that, wherever possible, all procedures having the potential to cause pain or stress have been refined and/or reduced to minimize animal discomfort.

3. I confirm that the experimental method accurately describes ALL the proposed animal use. I accept responsibility for procedures performed on animals in this project. All procedures will be carried out by, or under the guidance of, trained and competent personnel using recognized techniques.

4. All animals in this project will be used in compliance with the regulations of Ontario’s *Animals for Research Act*, the guidelines of the Canadian Council on Animal Care, and the policies and procedures of the University of Windsor.

5. I am aware that the data provided in this protocol will be entered into the Animal Research Protocol Management System and submitted to the Canadian Council on Animal Care.

6. I will ensure that any individual who will perform any procedure(s), as describe in this protocol, **will be** **familiar with the contents of this document**.



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**FOR OFFICE OF RESEARCH SERVICES USE ONLY**

**INTERIM APPROVAL**

**(to be reviewed at the next formal ACC meeting)**

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ACC Chair Approval Date

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**FINAL APPROVAL**

This AUPP has received ACC approval and is valid for a period of twelve months from the approval date. It is the responsibility of the Principal Investigator to ensure that all procedures are conducted in the manner described and approved in this application.

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ACC Chair Approval Date

**APPENDICES**

**APPENDIX A – KEYWORDS**

The CCAC highly recommends the use of the following keywords:

***General***

• Research, teaching, testing;

• Regulation (are the experiments performed directly in relation to testing regulations in force in Canada and/or the US (FDA, EPA, etc.) and/or elsewhere, type of testing (e.g., cosmetic testing);

• Field work, behavior observation, environmental protection study, fauna conservation;

• Development of techniques, study of the effectiveness of a product (drugs, others) or a method (spectroscopy, others);

• Breeding, breeding colony, sentinel program;

• Antibody production (monoclonal, polyclonal);

• Palatability test, digestibility test, reinforcement/motivation, staged behavioral encounters;

• Primary cell culture, tissue/organ collection, graft, transplants;

• Species, transgenic animal;

• Validation of non‑animal model (*in vitro* test, computational methods...).

***Procedures***

• Trapping/netting, marking/tagging;

• Injection (intravenous, subcutaneous, intramuscular, intraperitoneal);

• Blood sampling/testing (small volume), blood removal (large volume);

• Gavaging, physical restraint, infection induction, whole‑body radiation, physical euthanasia;

• Food deprivation, water deprivation, special diet;

• Altered environmental exposure, physical restraint (duration).

***Agents***

• Radioisotope administration, chemical exposure, infectious agents;

• Immunogenic or inflammatory agents, Freund’s complete adjuvant.

***Surgery***

• Major surgery, minor surgery, stereotaxic surgery, survival surgery, multiple surgeries, cannulation.

**APPENDIX B – CATEGORIES OF INVASIVENESS**

Cephalopods and some other higher invertebrates have nervous systems as well developed as in some vertebrates, and may therefore warrant inclusion in Category B, C, D, or E.

The following list of categories provides possible examples of experimental procedures which are considered to be representative of each category:

***Category A (Low)*** *- Experiments on most invertebrates or on live isolates*.

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

***Category B (Nil-Low)* -** *Experiments which cause little or no discomfort or stress.*

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

***Category C (Low-Moderate)* -** *Experiments which cause minor stress or pain of short duration.*

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

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

***Category D (Moderate - High)* -** *Experiments which cause moderate to severe distress or discomfort.*

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

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

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

***Category E (High Pain or Severe)* -** *Procedures which cause severe pain near, at or above the pain tolerance threshold of unanesthetized conscious animals.*

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

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