

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

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Location of experimental work to be carried out: Building(s) __BGS, Duckhouse_ Room(s)3010, 0104, 1C

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: __NSERC__

GRANT TITLE(S): __Mitochondrial metabolism in hibernation, torpor and stress-resistance__

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Jason Brown</u>	<u>jbrow63@uwo.ca</u>	
<u>Dillon Chung</u>	<u>dchungch@uwo.ca</u>	
<u>Katie Belgrave</u>	<u>kbelgrav@uwo.ca</u>	
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We study live animals that have no zoonotic diseases. Hamsters and mice are obtained from commercial or certified research breeding programs. Ground squirrels are wild-caught, and undergo a 6-week quarantine under ACVS supervision. Animals are housed in Duckhouse 1C or BGS 0104. After euthanasia within these rooms tissues are harvested and carcasses are sent for incineration by RPR.

Previously such work did not require specific biosafety training, so if the rules have changed and we require such training, please inform us. All of the people listed have the following training: Laboratory and Environmental/Waste Management Safety, WHMIS, Employee Health and Safety Orientation, as well as specific ACVS animal training.

Previously we had transported some ground squirrels to Robarts for imaging, but that work has been completed and will not be repeated.

Formerly we worked with E. coli XL-1 Blue, Schizosaccharomyces pombe and the approved GMO pGEMT-Easy, pREP1, but we know longer use them and they have been disposed of by approved methods. We do not plan to use them again so they can be "removed" from our permit.

Hibernators, such as ground squirrels, are capable of drastically reducing their metabolism and body temperature for several days at a time. This conserves energy which helps them to survive the energetic stress of cold climates, which would otherwise require high metabolism to maintain body temperature at a time when food is scarce. Daily torpor (seen in dwarf Siberian hamsters, and mice) is a similar phenomenon, but body temperature and metabolic rate are reduced only modestly, and for a few hours. Understanding how metabolism is suppressed in these animals will be very valuable in our basic understanding of metabolism, and may also have medical applications for extending the viability of transplanted organs, for example.

Every spring we trap wild 13-lined ground squirrels (*Ictidomys tridecemlineatus*) in southern Manitoba and return them to UWO. The animals are held in the Duckhouse (room 1C) during an ACVS-supervised 6 week quarantine (SOP available on request). They go through minor surgery to implant temperature-sensitive radio transmitters within the abdominal cavity, allowing us to monitor body temperature remotely. In late autumn, after recovery from surgery, animals are transferred to our controlled environment chambers in BGS 0104, and ambient temperature is reduced from 25C to 5C by 3C a day, and photoperiod is reduced to 2L:22D. After a week in these conditions, virtually all of the animals hibernate. Body temperature, monitored remotely, is used to track hibernation status. Animals will not eat during hibernation, so food is removed from cages to prevent mould formation. During hibernation metabolic rate is measured in some animals by placing them in air-tight plexiglas metabolic chambers with bedding and nesting material. Air is pumped through the chambers and the oxygen content of the exiting air is used to calculate metabolic rate. Flow rate is adjusted to ensure that oxygen in the chamber never falls below 20% (ambient air is 20.9% oxygen).

At various stages throughout the hibernation season animals are euthanised and their tissues (liver, muscle, brain) are removed for mitochondrial isolation.

While in hibernation euthanasia is performed by cervical dislocation. This is necessary because: 1) ventilation rate is much reduced (2-5 breaths/minute) in hibernation, so CO₂ euthanasia is not effective, 2) blood flow and body temperature are much reduced, so euthanasia by barbituate overdose would take a very long time, 3) handling an animal for either of these standard euthanasia methods would induce arousal, negating the scientifically essential hibernating sample group. Control, non-hibernating summer animals will be euthanised by overdose of euthanyl.

Both hamsters and mice will be implanted with temperature transmitters (see above). Daily torpor in hamsters can be induced by acclimating animals to long daylight (16L:8) and warm (18C) temperatures followed by short days (8L:16D) and cool temperatures (15C). Again this will be done in our environment chambers (BGS 0104). Mice will be initially acclimated to thermoneutral temperatures (27C) for 3 weeks. This will be followed by a reduction of ambient temperature to 22C and the removal of food. Animals will be monitored daily by weighing, and by remote measures of body temperature. Our preliminary experiments indicate that this treatment typically results in torpor within 24 hours in a strain-specific manner. About 85% and 75% of Balb/C and CD1 mice, respectively enter torpor, while C15 mice do not enter torpor (providing an interesting comparison group). Any animal that loses 20% of its initial weight will be immediately refed and removed from the experiment. Torpid animals will be euthanised by cervical dislocation, while control non-torpid animals will be euthanised by euthanyl overdose. Liver will be collected for mitochondrial isolation.

After tissues have been harvested carcasses are sent for incineration through UWO Occupational Health and Safety.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO
 If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____
 Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES NO
 If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="radio"/> Yes <input type="radio"/> No			
Rodent	<input type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No			

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required 1 2 2+ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO

If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? YES, complete table below NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.

** Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify _____ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? YES NO

4.7 Will this be expected to increase the containment level required? YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
 (including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
 If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
 Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? YES NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

6.1 Will live animals be used? YES NO If no, please proceed to section 7.0

6.2 Name of animal species to be used __Ground squirrels, dwarf Siberian hamsters, mice

6.3 AUS protocol # __2008-055_____

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Will live animals be used? YES No

7.2 → Yes

7.3 If yes, please specify the animal(s) used:

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # ground squirrel, Manitoba NO
- ◆ Birds YES, please specify species _____ NO
- ◆ Others (wild or domestic) YES, please specify _____ NO

7.4 If no live animals are used, please specify the source of the specimens:

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin _____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? Initiate and maintain colony, give location: _____
 "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

9.7 Do you use insects that require a permit from the CFIA permit? YES NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO

If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

10.8 Is the CFIA permit attached? YES NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____



13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, date of most recent biosafety inspection: _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
- thick leather gloves worn to protect against animal bites, as well as standard personal protective equipment

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
- no such risk.

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE  Date: 27 Jan, 2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval: