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) __NCB_____ Room(s)_417_____

*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): _Photosynthetic acclimation and adaptation to extreme environments: photostasis, excitation pressure and psychrophilly_____

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

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Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

We study photoacclimation of the green algae, Chlamydomonas raudensis, Chlamydomonas reinhardtii and Chlorella vulgaris. None of these organisms is biohazardous. These studies involve liquid cultures.
We routinely dispose of the liquid cultures by autoclaving the liquid cultures and then disposing of the dead, autoclaved cultures in the sink. The media used in all cases are basic minimal salt media.

Please include a one page research summary or teaching protocol.

(I) Chloroplasts as Global Energy Sensors. We define photostasis as the maintenance of cellular energy balance in photoautrophs. In contrast, excitation pressure (EP) is a measure of the imbalance in cellular energy budget. It is measured as the relative reduction state of PSII reaction centres ($Q_{Ared}/Q_{Aox} + Q_{Ared}$) ie. the extent to which PSII reaction centres are 'closed' and unable to perform photochemistry. PSII closure is sensed by the redox state of the , intersystem PQ pool of the photosynthetic electron transport chain (PETC). The redox state of the PQ pool, in turn, acts as a global energy sensor that regulates chloroplastic as well as nuclear gene expression which, in turn, govern remodelling of the photosynthetic apparatus. Although plants, green algae and cyanobacteria sense ΔEP, the molecular response, and therefore, the phenotypic response to ΔEP is species dependent due to inherent differences in electron sink capacity to consume photosynthetically-generated reductants. An emerging consensus is that the PETC not only transforms light energy but also acts as a primary sensor of energy imbalance.

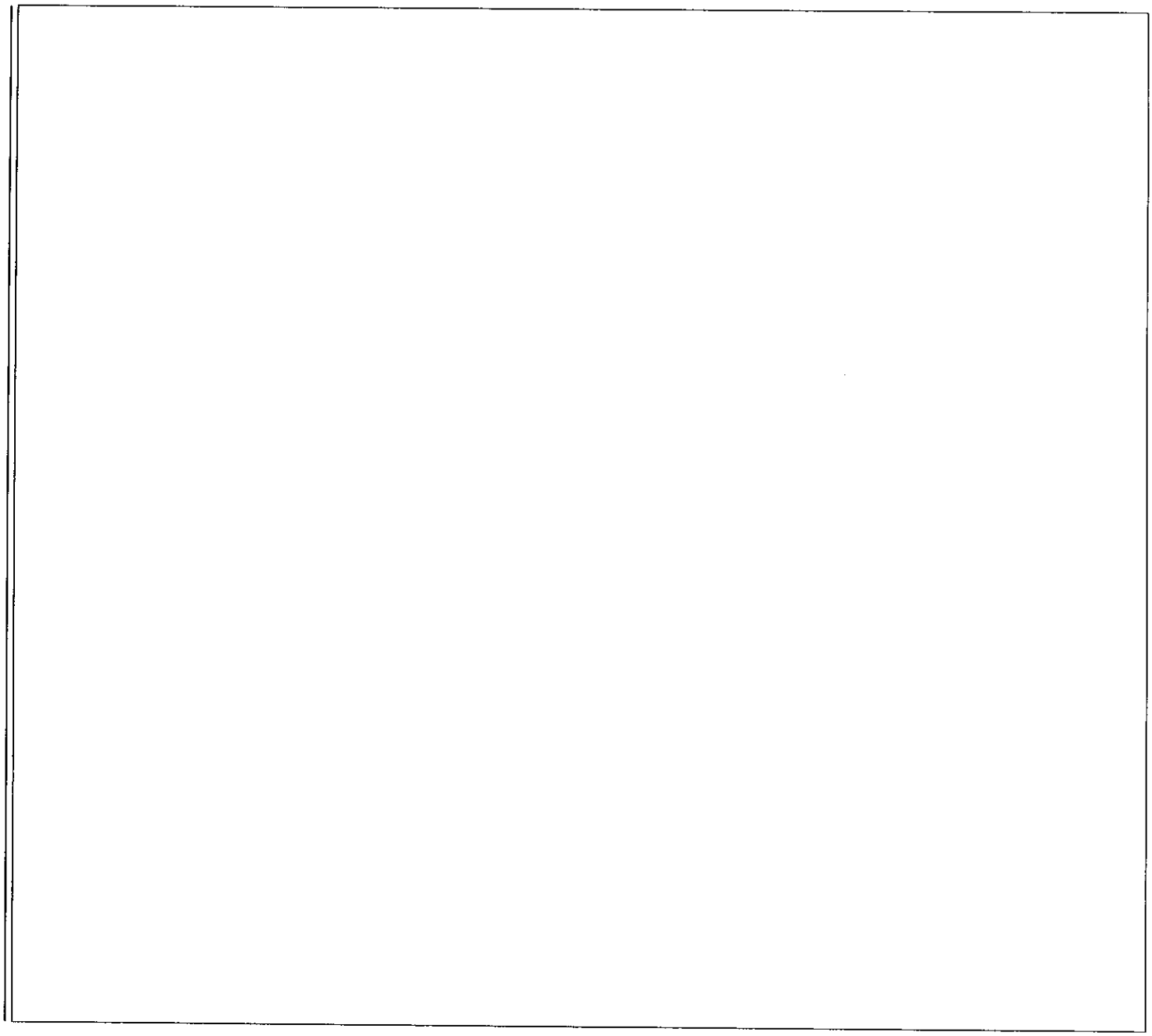
(II) EP, PTOX & Variegation. Because of its capacity to oxidize thylakoid plastoquinol (PQH_2), it was proposed that IMMUTANS(IM) is a plastid terminal oxidase (PTOX) that acts as a safety-valve to protect PSII from EP. By comparing the knockout mutant (*im*) and two Arabidopsis *IM* overexpressing lines with the WT, we reported that PTOX in fully developed Arabidopsis leaves does not act a safety valve because of its inability to compete with $P700^+$ for PSII generated electrons and to protect either PSII or PSI against photoinhibition[1]. Furthermore, the absence of *IM* is necessary but not sufficient to account for the variegated phenotype of *im*! High EP (HEP) controls the extent and sectoring patterns of variegation not only in *im* but also *spotty*, *var1* and *var2*. IM does act as a safety valve and minimizes EP during the very early stages thylakoid biogenesis and assembly and protects the developing photosynthetic apparatus from photo-oxidation prior to the attainment of full photosynthetic competence.

(III) Psychrophily and Energy Partitioning. We have established a very powerful biological system to address the molecular basis of psychrophily through comparisons of the psychrophile, *Chlamydomonas raudensis Ettl* UWO241, with its mesophilic strain SAG 49.72. My approach to elucidating the molecular basis of psychrophily is novel since it focuses on the molecular, biochemical and physiological basis for the sensitivity of UWO241 to high non-permissive growth temperatures. Why is UWO241 restricted to growth at temperatures below 20°C? Recently, we reported that shifting UWO241 from a permissive 8C to a nonpermissive 24C induces a classic, time-pendent heat shock response coupled with an increase in EP with no loss of pigmentation! Furthermore, exposure of UWO 241 to increased growth temperature decreases PSI/PSII stoichiometry coupled with a shift in energy partitioning from antenna to reaction centre quenching. This is correlated with Thr phosphorylation of a specific 17kD subunit associated with a purified PSI supercomplex rather than the expected phosphorylation of LHCII in the PSII supercomplex as observed in SAG 49.72. Using mass spectroscopic sequencing, the 17kD subunit, we have identified this 17kD PSI-associated subunit to a PsbP- like (PsbPL) protein. In contrast to UWO241, no phosphorylated subunits were detected in the PSI-supercomplexes of either SAG 49.72 or *C. reinhardtii*.

(IV) Photoperiod-Temperature Interactions and EP. Contrary to expectations, we reported that increasing autumn temperatures from 7°C to 22°C combined with a short photoperiod inhibits CO_2 assimilation in *Pinus banksiana* as a result of enhanced EP due to an inhibition of electron transport between the Cyt b6/f complex and P700. Thus, the autumnal photoperiod signal to induce dormancy in *Pinus banksiana* over-rides any potential benefit in C-gain associated with warm autumn temperatures. Although photoperiod is unaffected by climate change, it is the photoperiod signal that actually limits C-gain of this conifer in a warmer world, not the fluctuating temperature!

(V) CBFs, EP and Phenotypic Plasticity. Growth and development of cold-tolerant plants at low temperature generally results in a compact, dwarf growth habit with leaves that exhibit increased thickness relative to NA controls. In 2005, we reported that the dwarf growth habit and freezing tolerance in *Brassica napus* appears to be controlled by overexpression of *BNCBF17*. Most recently, we show that overexpression of *BNCBF17* also reduces the low temperature sensitivities (Q_{10}) of CO_2 assimilation and photosynthetic electron transport analogous to that which occurs during cold acclimation of winter rye and winter wheat[6]. This results in an increase in the number photons required to close PSII reaction centres and a decrease in the the need of for energy dissipation through NPQ. Thus, to our surprise, overexpression of a single transcription factor (*BNCBF17*) not only affects phenotype but also affects photosynthetic performance and global energy balance during cold acclimation of *Brassica napus* and by extension, presumably winter cereals.

(VI) *isiA* Expression and Energy Partitioning. The role of the *isiA* gene product in mitigating Fe stress in cyanobacteria has been a source of controversy. Through biochemical localization studies and analyses of composition of the isolated pigment-protein complexes, we showed that the cyanobacterial *isiA* gene product induced by Fe stress is associated with PSI monomers in contrast to PSI trimers as previously reported in Nature. More important, in contrast to these reports, direct, *in vivo* biophysical measurements of PSI absorptive cross sections support our suggestion that the PSI-associated isiA protein acts as a quencherto protect PSI from excess light during Fe stress[2,28] rather than a functional PSI antenna to enhance light harvesting efficiency.



1.0 Microorganisms

1.1 Does your work involve the use of biological agents? —XO- YES O 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? O YES XO 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
C. raudensis	O Yes Θ X No	O Yes XΘ No	O Yes XΘ No	2 L		XΘ 1 O 2 O 2+ O 3
C. vulgaris	O Yes XΘ No	O Yes XΘ No	O Yes Θ No	2 L		XΘ 1 O 2 O 2+ O 3
C. rhinehardtii	O Yes XΘ No	O Yes XΘ No	O Yes XΘ No	0.2 L		XΘ 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 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? XΘ YES O 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	O Yes xΘ No		Not applicable
Rodent	O Yes xΘ No		
Non-human primate	O Yes xΘ No		
Other (specify)	xΘ Yes O No		Arabidopsis plant cell cultrures

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	O Yes xO No			
Rodent	O Yes xO No			
Non-human primate	O Yes xO No			
Other (specify) Arabidopsis plant cell cultures	XO Yes O No	n/a	1	Univ. of Calgary

*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 XO 1 O 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES XO 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		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 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? O YES XO NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O 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?
O YES, complete table below XO 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 O YES, please specify _____ XO NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ XO NO
- SV 40 Large T antigen O YES XO NO
- E1A oncogene O YES XO NO
- Known oncogenes O YES, please specify _____ XO NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ XO NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES XO 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

6.5 Will the agent(s) be shed by the animal: O YES O 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)? O YES XΘ No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

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

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O 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? O YES XΘ 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 LD_{50} (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? O YES, Please provide details:_____ O 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? O YES XΘ 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? O Initiate and maintain colony, give location: _____
O "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? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

10.0 Plants

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

10.2 If YES, please give the name of the species. Secale cereale, Aestivum savitum, Brassica napus, Arabidopsis thaliana_____

10.3 What is the origin of the plant? Ag Canada and TAIR_____

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

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

10.6 Do you do any modifications to the plant? O YES XO 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: n/a We have our own seed stocks. -____

_____

_____

_____

10.8 Is the CFIA permit attached? O YES XO 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? O YES, please give country of origin_____ XO NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 _____ 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. XO 1 O 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
O YES, date of most recent biosafety inspection: _____
O NO, please certify
XO 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.

_____
_____
_____

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:

_The injury/splash will be washed thoroughly and treated if necessary with the first aid kit.
_____
_____

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: Sept 9/2011

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: