THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 also be updated at least every 3 years or when there are changes to the biohazards 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 Biohazard 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

PRINCIPAL INVESTIGATOR	Dr. Susanne Schmid
SIGNATURE	
DEPARTMENT	Anatomy & Cell Biology
ADDRESS	Medical Science Building 470
PHONE NUMBER	519-661 2111 ext. 82668
EMERGENCY PHONE NUMBER(S)	
EMAIL	susanne.schmid@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) MSB Room(s) 473

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC, OMHF, UWO
GRANT TITLE(S): Mechanisms underlying habituation of startle
Mechanisms underlying disruption of prepulse inhibition

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Susanne Schmid
Tyler Brown
Farinna Pinnock
Laura Walker
Megan Clark

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? O YES O 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)*	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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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? O 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 in the table below

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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 O 1 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 Ø 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 Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

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 Ø 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

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

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below O NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O 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 using the viral vector in 4.0? O YES Ø NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus 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? Ø YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used rats

6.3 AUS protocol # 2008-010 and 2008-006

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

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES Ø NO
- Pound source cats O YES Ø NO
- Cattle, sheep or goats O YES Ø NO
- Non-human primates O YES, please specify species _____ Ø NO
- Wild caught animals O YES, please specify species & colony # _____ Ø NO
- Birds O YES Ø NO
- Others (wild or domestic) Ø YES, please specify laboratory rats, purchased from Charles River O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø 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*? _____

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES Ø 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 lifestage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O 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? O Grow and maintain a crop O "One-time" use

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

10.9 Please 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_____
If no, please proceed to Section 10.0 ⊗ NO

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

13.0 Containment Levels

Level 1

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

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____
O NO, please certify
⊗ NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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 S. Sol Date: 04/06/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kidder
Date: 1 May 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: Altzinley
Date: May 1/09

Safety Officer for University of Western Ontario (if different from above): SIGNATURE:_____
Date:_____

Approval Number: B10--UWO--0216 Expiry Date (3 years from Approval): April 30, 2012

Special Conditions of Approval:

Description of procedures

We perform bahvioural experiments using adult Sprague Dawley rats. Rats are handled and placed into startle boxes in order to measure startle responses. Some animals will be injected systemically with specific neurotransmitter agonists and antagonists. Most of them are toxic in higher doses. We use and store them in microgram quantities.

Other animals undergo stereotaxic surgery in order to have chronic cannulae implanted into their skull. They also are injected with tiny amounts (nanomol quantities) of specific neurotransmitter agonists and antagonists.

We transcardially perfuse these animals after experimentation and remove brains from the skull. We perform tissue slicing from frozen brains and stain them using classical histological procedures.

We also perform electrophysiological experiments in acute rat brain slices. Animals are anaesthesized and decapitated for this procedure. Brains are removed and sliced, using a standard vibrating microtome. Slices are kept in a beaker for a day and transferred to the microscope for experiments. They might get perfused with neurotransmitter agonists and antagonists during these experiments.

Modification Form for Permit BIO-UWO-0216

Permit Holder: Susanne Schmid

Approved Personnel
(Please stroke out any personnel to be removed)

Farina Pinnock
Megan Clark
Tyler Brown

Additional Personnel
(Please list additional personnel here)

Bridget Valsamis
Duncan Mac Laren
Clara Wende

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells		
Approved Use of Human Source Material		
Approved GMO		
Approved use of Animals	rats	
Approved Toxin(s)		diphteria fusion toxin

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Classification: 1

Date of Last Biohazardous Agents Registry Form: May 1, 2009

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Diphteria Fusion Toxin

Construction of Toxin (Steward Clark, University of California Irvine)
Toxin construction was done by genetically replacing the targeting domain of Dtx with an alanine and glycine ether (similar in sequence to the N-terminal of the carp UII orthologue) linked to the last eight amino acids of the rat UII sequence (Dtx-UII). One form of the fusion toxin was constructed with a disrupted disulphide bond, dismantling the cyclic nature of UII, which is known to be essential for receptor binding (Itoh et al. 1988). This construct was used as an untargeted control. Targeting domain constructs were obtained from Sigma-Aldrich (St Louis, MO, USA) as complementary oligonucleotides with appropriate sticky splice sites available (list below), and these were then phosphorylated and annealed. The resulting double stranded DNA was then ligated into pETJV127 DAB389-IL-2 (gift from Drs J. Van der Spek and J. Murphy, University Hospital, Boston, MA, USA) which had the sequence for IL-2 removed by excision with SphI and HindIII (NOPE: we have never requested, nor have ever had, the cDNA of the natural form of Dtx which includes that naturally occurring targeting domain). Plasmids were grown in DH5ƒÑ bacteria and verification of the sequence of single clones was done by automated DNA sequencing (Laragen, Los Angeles, CA, USA) with the primer: 5'-CGGGTATCGGTAGCGTAATGG- 3', which anneals to the Dtx sequence.

B) Dtx-CUK, SphI-His6-UII-HindIII

A H H H H H H K K A A G G G A E C F W K Y C I .
ca cat cat cat cat cat cac aaa aaa gcg gcg ggc ggc ggc gcg gaa tgc ttc tgg aaa tac tgc att ta
3' gta cgt gta gta gta gta gta gtg gtg ttt ttt cgc cgc ccg ccg ccg cgc ctt acg aag acc ttt atg acg taa att cga 5'

Material Safety
The urotensin II receptor is known to be expressed in the brain, vasculature, liver and kidney. These organs will be particularly vulnerable to toxin exposure. Therefore, any instances of puncture wounds with contaminated materials should be treated seriously. The effected individual should seek medical attention. Splashes on to skin should be treated by through washing of the affected area. Inadvertent splashes into eyes or other mucosal membranes should be flushed, and medical attention should be sought. The isolated toxin is **NOT** a self-replicating / pathogenic agent. Therefore, much like a chemical toxin, exposure to the toxin will have acute effects. Also, it has been shown in cell culture toxicity assays that the toxin is 10 000 times less toxic to cells which do not express the UII receptor than those that do. Therefore, a superficial exposure which is dealt with quickly should not result in any deleterious consequences.

Handling at UWO
We will use the toxin for stereotaxic injections into a specific area of the rat brain in order to lesion a specific cholinergic cell group. We will receive a total of 1mL, that we will reconstitute and aliquot. We will be using a ~3% solution for our injections with injection volumes being in the submicrolitre range. Once the toxin has been stereotaxically injected into the brain of the animal, and the wound sutured closed, there should be no exposure risk to laboratory or animal care staff. The amounts that are injected have been shown not produce detectable toxicity outside a 5mm3 within the brain, so the risk of this toxin being spread outside the cranium is very low. The toxin is proteiacous in nature and so normal proteiase activity within the brain will deactivate the toxin by cleaving the protein.

During surgery and microinjection laboratory personnel Dry contaminated materials will be collected carefully into biohazard bags (red colour), sealed and autoclaved before disposed as trash. Non-autoclaved disposable glassware will be kept in a separate, clear marked container, containing liquid bleach for 24 hours soaking before it is disposed as regular glassware. Liquid waste will be aspirated directly into collecting bottle which contains 10% bleach solution. Decontamination solution will be disposed as directed for liquid as described below. Solid waste will be placed in a plastic bag and disposed as biohazard waste. Liquids: All toxin solution must be diluted 1:1 with the decontamination solution for one-half hour or more. After decontamination aqueous wastes can be disposed of down the drain.