THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 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

PRINCIPAL INVESTIGATOR	Stephen Karlik
DEPARTMENT	Pathology
ADDRESS	Dental Sciences Building
PHONE NUMBER	83376
EMERGENCY PHONE NUMBER(S)	519-284-1836
EMAIL	skarlik@uwo.ca

Location of experimental work to be carried out: Building(s) ____Dental Sciences_____
Room(s)__4035, 4035B, 4035A, 4036

*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:___Elan Pharmaceuticals, Critical Outcome Technologies
GRANT TITLE(S): "Angiogenesis in Chronic Neuroinflammation"__and "Integrin-based therapy for multiple sclerosis"

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

Name	UWO E-mail Address	Date of Biosafety Training
Wendi Roscoe	wendi_roscoe@hotmail.com	
Robin Smith	rsmit28@uwo.ca	
Claire Poppe	cpoppe@imaging.robarts.ca	
Laura Bowie	bowie.laurae@gmail.com	To be done

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.

Pertussis toxin is an essential component of the immunization of mice to produce experimental autoimmune encephalomyelitis. Pertussis toxin is stored in a locked box in our chemical fridge in DSB 4035A. Mice receive an i.p. injection of 200 ng. on days 0 and 2 post immunization. The mice are housed in a Biohazard 2 room in the Heath Sciences Animal Quarters (Rm 5547) for 4 days post immunization. This assures that the in the remote case that there is some excretion, the mice will be in a suitable facility.

Please include a one page research summary or teaching protocol.

We use experimental autoimmune encephalomyelitis in mouse and guinea pig models to model aspects of multiple sclerosis. The specific aims are to investigate potential new methods for treatment of the patients. Currently, we target two main (and related) processes in neuroinflammation: angiogenesis and the binding of immune cells to the vascular endothelium.
For mouse experiments, we use 40 mice which are immunized and disease develops approximately on day 12-14. Treatment with a variety of compounds and approved drugs is then initiated, clinical signs are followed and CNS tissues are dissected at the end of the experiment for histopathological analysis.

For example see:
Roscoe WA, Welsh ME, Carter DE, Karlik SJ. J Neuroimmunol. 2009 Apr 30;209(1-2):6-15. VEGF and angiogenesis in acute and chronic MOG((35-55)) peptide induced EAE.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES X 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 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 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 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
	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? O YES X 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 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:

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 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? X YES O 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/NO**	**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)	The UK Multiple Sclerosis Tissue Bank, Multiple Sclerosis Human Neurospecimen Bank	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 x 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) involving viral vectors be made? O YES, complete table below O 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 _____ 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?

4.7 Will this be expected to increase the containment le

See E-mail

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES O 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? x YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_Guinea pig and mouse_____

6.3 AUS protocol # 2007-080, "Angiogenesis in Chronic Neuroinflammation" and #2007-052, "Integrin-Based Therapy for Multiple Sclerosis"

6.4 Will any of the agents listed in section 4.0 be used in live animals x YES, specify: _pertussis toxin

6.5 Will the agent(s) be shed by the animal: O YES X NO, please justify:
We initiated a country-wide review of the use of this material for EAE and pertussis toxin is rapidly absorbed from the intraperitoneal injection location and it binds permanently to endothelial cells upon absorption.

It is not filtered by the kidneys. Our 96 hours of Biohazard 2 housing is a suitable precaution for the remote chance of any shedding.

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 x No If no, please proceed to section 8.0

7.2 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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)___pertussis toxin_____
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_____18 micrograms/Kg ip_____

8.4 How much of the toxin is handled at one time*? _____8 micrograms_____

8.5 How much of the toxin is stored*? _____50 micrograms _____

8.6 Will any biological toxins be used in live animals? X YES, Please provide details:____as above O NO

*For information on biosecurity requirements, please see
http://www.uwo.ca/humanresources/docandform/docs/he

See E-mail

df

9.0 Insects

9.1 Do you use insects? O YES X 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? O YES X 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
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___ X 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

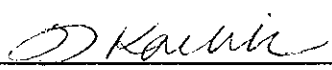
11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

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 

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. O 1 X 2 O 2+ O 3

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

X YES, permit # if on-campus___6026_____
O NO, please certify
O 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 _____ Date:___2010-09-20_____

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

Insufficient pertussis toxin would be transferred in a needle stick to warrant excessive concern. As per the MSDS, if skin pricking should occur, we will induce bleeding and flush with copious amounts of water.

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:

LIST BIOLOGICAL LABORATORIES, INC.

540 DIVISION STREET • CAMPBELL • CALIFORNIA 95008-6906 • USA
408-866-6363 • 800-726-3213 • FAX 408-866-6364 • EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Product #180-181

MATERIAL SAFETY DATA SHEET
Pertussis Toxin
Pertusis Toxin (Salt-Free)

Ingredients:

Each vial contains 50.0 µg of lyophilized pertussis toxin (islet-activating protein). Product 180 also contains 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M sodium chloride, when reconstituted with 0.5 ml water.

Health Hazard Data:

The LD_{50} in mice is 18 µg/kg i.p. There is no LD_{50} information for humans.

Emergency Procedures:

Pertussis toxin is degraded by the low pH in the gut and is not absorbed. If swallowing occurs, induce vomiting.

If skin pricking should occur, induce bleeding and flush with copious amounts of water.

If i.v. or i.m. injection should occur, consult a physician. Attempt to obtain hyperimmune globulin to pertussis from the CDC. In an adult immunized versus whooping cough, no long term ill effects are likely to result.

Handling:

Pertussis toxin, in spite of its name, is not considered hazardous. However, as with any biochemical, it should be handled by trained personnel using good laboratory technique. Observe the following practices when working with pertussis toxin: Special care should be taken when working in conjunction with hypodermic needles. Wear protective gloves, avoid contact with cuts or wounds, avoid inhalation, do not mouth pipet, and flush thoroughly any area of the body that comes in contact with this product. Only individuals who were immunized in childhood against whooping cough should work with this product. This product is intended for research purposes only.

Stability:

Stable for months when stored at 4°C. Do not freeze.

Deactivation:

Boil at 100°C for 15 to 30 minutes.

Subject: Re: Biological Agents Registry Form: Karlik
From: Stephen Karlik <skarlik@gmail.com>
Date: Fri, 01 Oct 2010 09:41:55 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

E-mail

Hi Jennifer:
When I was looking for the info, I found a number of values all about the same. The Tocris MDSS gives a value of 17 ug/kg for LD50 for an intraperitoneal injection in the mouse.
Steve

On Thu, Sep 30, 2010 at 2:06 PM, Jennifer Stanley <jstanle2@uwo.ca> wrote:

> Hello Dr. Karlik -
>
> Thank you for your recent submission.
> Please confirm that you have no human therapy gene trials (question 5.1 was not addressed).
>
> Also, for the toxin listed in Section 8.0, what species is the LD50 for (18 ug/Kg)?
>
> Thanks again,
> Jennifer

Western

TOXIN USE RISK ASSESSMENT

TOXIN: Pertussis

PROPOSED USE (DOSE): 8 ug (handled), 50 ug (stored)

LD_{50} (species): 18 ~~m~~ ug / kg (species unknown)

CALCULATION:

18 ug/kg X 70 kg/person = 900 ug per person

Divide by safety factor(s) of 10 (as applicable): 90 ug per person
(one safety factor used)

COMMENTS/RECOMMENDATION:

amount handled + stored < calculated lethal dose

Canadian Centre for Occupational Health and Safety

CCOHS

RTECS Registry of Toxic Effects of Chemical Substances®

Data source: Symyx Software Inc.

Record Contents

Format: All Sections

- Chemical Identification
- Acute Toxicity Data

REFRESH RECORD

CHEMICAL IDENTIFICATION

RTECS Number	XW5883750
Chemical Name	Toxins, pertussis
CAS Registry Number	70323-44-3
Other CAS Registry Nos.	82248-93-9
Last Updated	200808
Data Items Cited	9
Compound Descriptor	Drug Natural Product

Synonyms/Trade Names

IAP
Lymphocytosis-promoting factor
Pertussigen
Histamine-sensitizing factor
Islet activating protein

HEALTH HAZARD DATA

ACUTE TOXICITY DATA

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - rat	114 ug/kg	Sense Organs and Special Senses (Eye) - lacrimation Behavioral - changes in motor	TJEMAO Tohoku Journal of Experimental Medicine. (Maruzen Co. Ltd., Export Dept., P.O. Box 5050, Tokyo Int., 100-31 Tokyo,

				activity (specific assay) Nutritional and Gross Metabolic - weight loss or decreased weight gain	Japan) V.1- 1920- Volume (issue)/page/year: 130,105,1980
LD50 - Lethal dose, 50 percent kill	Intraperitoneal	Rodent - mouse	17160 ng/kg	Details of toxic effects not reported other than lethal dose value	INFIBR Infection and Immunity. (American Soc. for Microbiology, 1913 I St., NW, Washington, DC 20006) V.1- 1970- Volume (issue)/page/year: 31,495,1981
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - mouse	127 ug/kg	Sense Organs and Special Senses (Eye) - lacrimation Behavioral - changes in motor activity (specific assay) Nutritional and Gross Metabolic - weight loss or decreased weight gain	TJEMAO Tohoku Journal of Experimental Medicine. (Maruzen Co. Ltd., Export Dept., P.O. Box 5050, Tokyo Int., 100-31 Tokyo, Japan) V.1- 1920- Volume (issue)/page/year: 130,105,1980
TDLo - Lowest published toxic dose	Intracerebral	Rodent - mouse	200 ng/kg	Biochemical - Metabolism (Intermediary) - other	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1- 1909/10- Volume (issue)/page/year: 299,960,2001
TDLo - Lowest published toxic dose	Intracerebral	Rodent - mouse	10 ug/kg	Behavioral - changes in psychophysiological tests	NEROEW Neuropsychopharmacology. (Elsevier Science, 655 Avenue of the Americas, New York, NY 10010) V.1- 1987- Volume (issue)/page/year: 27,554,2002
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	30 ug/kg	Vascular - other changes	EJPHAZ European Journal of Pharmacology. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1967- Volume(issue)/page/year: 493,139,2004
TDLo - Lowest published toxic dose	Intravenous	Rodent - rat	15 ug/kg	Vascular - BP lowering not characterized in autonomic section	EJPHAZ European Journal of Pharmacology. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1967- Volume(issue)/page/year: 493,139,2004
TDLo - Lowest published toxic	Subcutaneous	Rodent - mouse	333.333 ng/kg	Biochemical - Metabolism (Intermediary) - effect on	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co.,

dose				inflammation or mediation of inflammation	428 E. Preston St., Baltimore, MD 21202) V.1-1909/10- Volume (issue)/page/year: 318,611,2006
TDLo - Lowest published toxic dose	Intraperitoneal	Rodent - rat	15 ug/kg	Vascular - contraction (isolated tissues)	NSAPCC Naunyn-Schmiedeberg's Archives of Pharmacology. (Springer Verlag, Heidelberger, Pl. 3, D-1000 Berlin 33, Fed. Rep. Ger.) V.272- 1972- Volume(issue)/page/year: 369(Suppl 1),R171,2004

END OF RECORD

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