

**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/

| | |
|---------------------------|--|
| PRINCIPAL INVESTIGATOR | Karel Tymł |
| DEPARTMENT | Centre for Critical Illness Research, Lawson Health Research Institute |
| ADDRESS | VRL, RM A6-144, 800 Commissioners Rd East, London |
| PHONE NUMBER | 519-685-8300 ext. 55076 |
| EMERGENCY PHONE NUMBER(S) | 519-661-0784 |
| EMAIL | Karel.tymł@lhsc.on.ca |

Location of experimental work to be carried out: Building(s) Victoria Research Laboratories Room(s) A6-118

*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: HSFO
 GRANT TITLE(S): Hypoxia/Reoxygenation, Systemic Inflammation and Vascular Cell Coupling (currently held)

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>Daniel Secor</u> | <u>dsecor@uwo.ca</u> | <u>October 2006</u> |
| <u>Scott Swarbreck</u> | <u>sswarbre@uwo.ca</u> | <u>October 2007</u> |
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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 will harvest and culture primary endothelial cells of mouse skeletal muscle origin. The cells will be grown in a standard incubator and passaged up to fifteen times. The cells will be grown into monolayers and treated with various noninfectious pharmacological agents. At the end of the experiment, cells, culture medium, glass and plasticware will be disposed of in the biohazardous waste container and sent to Stericycle for decontamination.

Please include a one page research summary or teaching protocol.

2008-2011 HSFO grant application summary
(note : a part of the work has been done)

Project title : Hypoxia/reoxygenation, systemic inflammation, and vascular cell coupling

The proposal deals with local microvascular blood flow control, focusing on intercellular coupling within the vascular wall. Although this coupling is a key component of vascular function, the effect of pathophysiology in general on this coupling is not known. We have shown that hypoxia (0.1% O₂, 1 h) followed by reoxygenation (H/R) rapidly reduces electrical coupling between microvascular endothelial cells (EC). However, the mechanism of this reduction has not been clarified. Further, our preliminary experiments suggest that low levels of inflammatory agents (lipopolysaccharide or TNF alpha) applied concurrently with H/R synergistically enhance this reduction in coupling, indicating that H/R+cytokines could greatly aggravate vascular function impairment during systemic inflammation. The proposal addresses the overall hypothesis that endothelial cell electrical coupling and vascular connexins play important role in the microvascular response to hypoxia/reoxygenation plus inflammatory cytokines.

Based on our models of mouse microvascular EC monolayer in vitro and of arteriolar conducted response in the mouse cremaster muscle in vivo, and on electrophysiological approach to assess EC coupling, the proposal consists of four Aims. Aims #1 and 2 address the molecular mechanism whereby PKA mediates the H/R-induced reduction in coupling in the monolayer. Here, I will collaborate with Dr. D. Laird, an expert in gap junction molecular biology, as well as with Drs. A Babwah and T. Peng (experts in transfection/infection of primary cells). We hypothesize that the gap junction protein connexin40 (Cx40) is chiefly responsible for this effect, involving one or more of the four PKA binding sites on the cytoplasmic tail of Cx40 (i.e., residues 345, 348, 349 and 353).

Aim #3 addresses the hypothesis that H/R applied concurrently with lipopolysaccharide (LPS) synergistically reduces the electrical coupling in EC monolayer in vitro, and that ischemia and reperfusion (I/R) plus LPS synergistically reduces the arteriolar conducted response in vivo. We have previously reported that LPS alone reduces coupling PKA- and Cx40-dependently. In the EC monolayer model in vitro, we will examine whether the synergistic effect is (i) inhibited by PKA activation, (ii) associated with Cx40 serine dephosphorylation, and (iii) inhibited by mutation at one or more of the four Cx40 residues. In the arteriolar model in vivo, we will determine whether the possible synergistic effect on the conducted response is PKA- and Cx40-dependent.

Aim #4 examines the generality of the H/R+cytokines synergistic effect on microvascular function. We will test the hypothesis that TNFalpha alone, and H/R together with TNFalpha, reduce vascular cell coupling. Using the EC monolayer model in vitro, we will determine whether the effect of TNFalpha alone is tyrosine kinase-, MAP kinase-, PKC- and PKA-dependent, and whether one or more EC connexins (i.e., Cx37, Cx40 or Cx43) are targeted by TNFalpha-induced intracellular signalling. We will also determine whether TNFalpha alone reduces the arteriolar conducted response. Finally, we will examine if concurrent H/R+TNFalpha, and I/R+TNFalpha, have synergistic effects on vascular cell coupling in vitro and in vivo, respectively.

Systemic inflammation (i.e., whole body inflammatory response to local insult) precipitates cardiovascular dysfunction. One of the prominent features of this dysfunction at the tissue level is impaired capillary blood flow, including micro-regional H/R (i.e., intermittent capillary blood flow). The present proposal will determine whether H/R+cytokines may lead to synergistic impairment of vascular function, and whether therapeutic strategies aimed at restoring capillary blood flow should be sought to minimize the life-threatening effects of systemic inflammation.

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 checked="" type="radio"/> No | | Not applicable |
| Rodent | <input checked="" type="radio"/> Yes <input type="radio"/> No | Mouse Endothelial Cells From Skeletal Muscle | 2010-284 |
| Non-human primate | <input type="radio"/> Yes <input checked="" type="radio"/> No | | |
| Other (specify) | <input type="radio"/> Yes <input checked="" 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 checked="" type="radio"/> No | | | |
| Rodent | <input type="radio"/> Yes <input checked="" type="radio"/> No | | | |
| Non-human primate | <input type="radio"/> Yes <input checked="" type="radio"/> No | | | |
| Other (specify) | <input type="radio"/> Yes <input checked="" 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 type(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 _____

6.3 AUS protocol # _____

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

7.3 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 # _____ 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) ___lipopolysaccharide from *Escherichia coli* 055:B5_
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used. (**Attached**)

8.3 What is the LD₅₀ (specify species) of the toxin ___ 3 mg/kg body weight (Norimatsu et al., 1995)

8.4 How much of the toxin is handled at one time*? ___10 microgram/mL, final concentration _____

8.5 How much of the toxin is stored*? ___10 milligrams, maximum _____

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

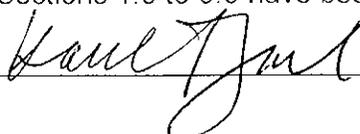
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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: Unknown
 NO, please certify
 NOT REQUIRED for Level 1 containment

*Level 2 certified
MARCH 29, 2011
by GAIL RYAN
Michele*

13.3 Please indicate permit number (not applicable for first time applicants): BIO-LHRI-0069

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.

All work will be conducted in a flow hood, waste will be disposed of in biohazardous waste

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:

First aid and medical treatment will be provided if necessary, workspace will be cleaned

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 *Karl Faus* Date: *May 1, 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: *Maire Ryan*
Date: *MAY 24, 2011*

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

Special Conditions of Approval:

New Info

----- Original Message -----

Subject:Re: Fwd: Fwd: Fwd: Biological Agents Registry Form: Tymł

Date:Mon, 29 Aug 2011 17:45:53 -0400

From:Karel Tymł <Karel.Tymł@LHSC.ON.CA>

To:Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Re why the containment level for the cell line is marked as level 2 in section 2.4, I used the same level 2, as in my previously approved Registry Form from Nov 29, 2007. Then we used the same mouse microvascular endothelial cell as now.

I added info on LPS in section 8 in updated Registry Form today (attached). I also append the MSDS for LPS.

I hope these changes suffice.

Karel

>>> Jennifer Stanley <jstanle2@uwo.ca> 8/29/2011 10:32 AM >>>
Hi Dr. Tymł -

Please make the changes outlined below:

The committee does not understand why the containment level for the cell lines is marked as level 2 in section 2.4. The committee also noted that section the MSDS for the LPS that is to be used in the research is missing. In addition, section 8 should be filled out for the use of LPS.

Regards
Jennifer

SIGMA-ALDRICH

Material Safety Data Sheet

Version 3.3

Revision Date 03/22/2011

Print Date 08/29/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : Lipopolysaccharides, from *Escherichia coli* 055:B5

Product Number : L2880

Brand : Sigma

Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

Telephone : +1 9058299500

Fax : +1 9058299292

Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300

Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

Not WHMIS controlled.

Not WHMIS controlled.

Not a dangerous substance according to GHS.

HMIS Classification

Health hazard: 3

Flammability: 0

Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.

Skin May be harmful if absorbed through skin. May cause skin irritation.

Eyes May cause eye irritation.

Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : lps

| CAS-No. | EC-No. | Index-No. | Concentration |
|--|--------|-----------|---------------|
| Lipopolysaccharide from E. coli serotype 055:B5 | | | |
| no data available | - | - | - |

4. FIRST AID MEASURES

If inhaled

If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact

Wash off with soap and plenty of water.

In case of eye contact

Flush eyes with water as a precaution.

If swallowed

Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIRE-FIGHTING MEASURES**Conditions of flammability**

Not flammable or combustible.

Suitable extinguishing media

Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters

Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

Explosion data - sensitivity to mechanical impact

no data available

Explosion data - sensitivity to static discharge

no data available

6. ACCIDENTAL RELEASE MEASURES**Personal precautions**

Avoid dust formation. Avoid breathing vapors, mist or gas.

Environmental precautions

Do not let product enter drains.

Methods and materials for containment and cleaning up

Sweep up and shovel. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE**Precautions for safe handling**

Provide appropriate exhaust ventilation at places where dust is formed.

Conditions for safe storage

Keep container tightly closed in a dry and well-ventilated place.

Keep in a dry place. Keep in a dry place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment**Respiratory protection**

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection

Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection

Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures

General industrial hygiene practice.

Specific engineering controls

Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES**Appearance**

| | |
|--------|---------------------|
| Form | powder, lyophilized |
| Colour | no data available |

Safety data

| | |
|--|-------------------|
| pH | no data available |
| Melting point/freezing point | no data available |
| Boiling point | no data available |
| Flash point | no data available |
| Ignition temperature | no data available |
| Autoignition temperature | no data available |
| Lower explosion limit | no data available |
| Upper explosion limit | no data available |
| Vapour pressure | no data available |
| Density | no data available |
| Water solubility | no data available |
| Partition coefficient: n-octanol/water | no data available |
| Relative vapour density | no data available |
| Odour | no data available |
| Odour Threshold | no data available |
| Evaporation rate | no data available |

10. STABILITY AND REACTIVITY**Chemical stability**

Stable under recommended storage conditions.

Possibility of hazardous reactions

no data available

Conditions to avoid

no data available

Materials to avoid

Strong oxidizing agents

Hazardous decomposition products

Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.
Other decomposition products - no data available

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50

no data available

Inhalation LC50

no data available

Dermal LD50

no data available

Other information on acute toxicity

no data available

Skin corrosion/irritation

no data available

Serious eye damage/eye irritation

no data available

Respiratory or skin sensitization

no data available

Germ cell mutagenicity

no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)

no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)

no data available

Aspiration hazard

no data available

Potential health effects

Inhalation

May be harmful if inhaled. May cause respiratory tract irritation.

Ingestion

May be harmful if swallowed.

Skin

May be harmful if absorbed through skin. May cause skin irritation.

Eyes

May cause eye irritation.

Signs and Symptoms of Exposure

Fever

Synergistic effects

no data available

Additional Information

RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability

no data available

Bioaccumulative potential

no data available

Mobility in soil

no data available

PBT and vPvB assessment

no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product

Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging

Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)

Not dangerous goods

IMDG

Not dangerous goods

IATA

Not dangerous goods

15. REGULATORY INFORMATION

WHMIS Classification

Not WHMIS controlled.

Not WHMIS controlled.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

Copyright 2011 Sigma-Aldrich Co. License granted to make unlimited paper copies for internal use only. The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.



TOXIN USE RISK ASSESSMENT

| | |
|-----------------------------|-------------------------|
| Name of Toxin: | LPS from E. coli 055:B5 |
| Proposed Use Dose: | 10 µg |
| Proposed Storage Dose: | 10000 µg |
| LD ₅₀ (species): | 3000 µg |

| | | | |
|---|------------|---|--------------|
| Calculation: | | | |
| | 3000 µg/kg | x | 50 kg/person |
| Dose per person based on LD ₅₀ in µg = | | | 150000 |
| LD₅₀ per person with safety factor of 10 based on LD₅₀ in µg = | | | 15000 |

Comments/Recommendations: