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	Dr. Morris Karmazyn
DEPARTMENT	Physiology & Pharmacology
ADDRESS	Dept of Physiology & Pharmacology
PHONE NUMBER	519-661-3872
EMERGENCY PHONE NUMBER(S)	519-471-4330
EMAIL	Morris.Karmazyn@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) Medical Sciences Bldg.__ Room(s)_242,247,259

*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: CIHR and HSFO_____
GRANT TITLE(S):__; Role of Leptin in Cardiac Hypertrophy and Heart Failure CIHR)_____
Role of Adenosine in Myocardial Hypertrophy and Heart Failure (HSFO)__
Nitric Oxide as an Endogenous Antihypertrophic Factor (HSFO)__

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

Name	UWO E-mail Address	Date of Biosafety Training
Venkatesh Rajapurohitam	Venkatesh.rajaprohitam@schulich.uwo.ca	June 2009
Tracey Gan	tgan@uwo.ca	June 2009
Jim Haist	James.Haist@schulich.iwo.ca	June 2009
Melissa Moey	mmoey@uw.ca	Oct 2009
Eduoardo Martinez	Eduardo.martinez@schulich.uwo.ca	May 2009
Cathy Huang	Cathy Xiauling.Huang@schulich.uwo.ca	June 2009
Nazo Said	nsaidfa@uwo.ca	July 2008
Seiichi Taniai	New employee – does not yet have email	Not yet completed

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.

The laboratory produces and uses purified heart cells from 1 to 5 day-old Sprague Dawley rats. Other cell types isolated during the process are killed by autoclaving soon after the initial production. The cells are kept in an incubator or a laminar flow hood for 4 to 6 days. One endpoint of cell use is to kill the cells and scrape out of the dishes as much as possible for measurement of cellular DNA or protein. The other endpoints are photography of the dishes or measurement of light or fluorescence in dishes. After wards all dishes are autoclaved. Pipets, pipet tips, filters, and other equipment used in the preparation and use of the cells are also autoclaved.

Please include a one page research summary or teaching protocol.

The majority of experiments will be done on either in cultured neonatal rat ventricular myocytes particularly to examine hypertrophic responses and in rats subjected to coronary artery ligation. Our laboratory has experience with most of the methodology proposed; otherwise key collaborations have been established to assure successful completion of the proposed studies. It is anticipated that this study will provide a comprehensive picture of the role of leptin in heart failure and provide a detailed and comprehensive evaluation of underlying mechanisms. The following studies will be performed to study leptin: 1. We will determine the effect of leptin inhibition on extracellular remodelling in the postinfarcted rat myocardium and determine the role of gender in these responses. 2. We will determine the role of mitochondria and the regulation of mitochondrial function in the postinfarcted myocardium and the influence of leptin inhibition. 3. As cell to cell communication represents a key defect in heart failure, we will determine the relationship between inhibition of both hypertrophy, remodelling/heart failure, and myocardial connexin expression. 4. We will study the mechanisms underlying the salutary effect early brief administration of a leptin receptor antagonist against the development of myocardial remodelling and heart failure. To determine the role of NO as an endogenous antihypertrophic factor and to study the underlying mechanisms involved, the experiments proposed here will be carried out using animals subjected to coronary artery ligation in which the heart failure process can be monitored. In addition, to obtain a better understanding of mechanisms underlying hypertrophic responses we will use cultured adult rat ventricular myocytes which will be studied after in vivo treatment or exposed to hypertrophic stimuli in culture and in which specific mechanisms can be closely studied. Among these mechanisms include the role of the RhoA/ROCK system as a target for the effects of NO To study the effect of obesity, we will produce obesity in rats by feeding them a high fat diet for either a 12 or 24 week period. We will also carry out experiments in which mice with specific genetic nitric oxide synthase (NOS)isoform deletions will be subjected to coronary artery ligation and resultant heart failure to determine the contributions of specific NOS isoforms to the remodelling process. Thus, overall, we will utilize pharmacological, physiological, cellular and molecular approaches to determine the role of the NO system in the hypertrophic and heart failure process. This study, we believe, will provide a comprehensive assessment into the role of NO in the hypertrophic program and delineate the mechanistic bases for these complex effects. The following represent the major general research goals aimed at determining the role of adenosine in cardiac hypertrophy and heart failure: 1. To determine the potential modulatory role of adenosine receptor agonists on other (non PE) G-protein linked hypertrophic factors 2. To assess the cellular mechanisms underlying the antihypertrophic effect of adenosine receptor activation in cultured myocytes with particular emphasis on the role of the RhoA/ROCK system. 3. To determine whether adenosine receptor expression is altered in hypertrophied cultured myocytes and postinfarcted remodelled myocardium. 4. To determine the response of adenosine receptor activation to hypertrophy in the postinfarcted myocardium 5. To determine the antihypertrophic effects of adenosine receptor modulation in the aging myocardium and to determine whether aging influences adenosine receptor expression profiles and the response to insult. 6. To determine whether treatment delay with adenosine receptor modulators can reverse the maladaptive response and affect the remodelling and heart failure process.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES xOx 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 X 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? xOx 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 O No		Not applicable
Rodent	xOx Yes O No	Rat heart	
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

See E-mail

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	xOx Yes O No	cardiomyocytes	Prepared in our lab
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? O YES x 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)		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 xOx 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 X 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? 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 xOx 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? xx YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used____Sprague Dawley rat_____

6.3 AUS protocol # _____2009-020_____

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

6.5 Will the agent(s) be shed by the animal: O YES xOx 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 xOx 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? O YES xOx 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 xOx 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 xOx 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 Ox 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. xOx 1 O 2 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
xOx 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:___November 30, 2010_____

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:

_____
_____

See E-mail

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): _____

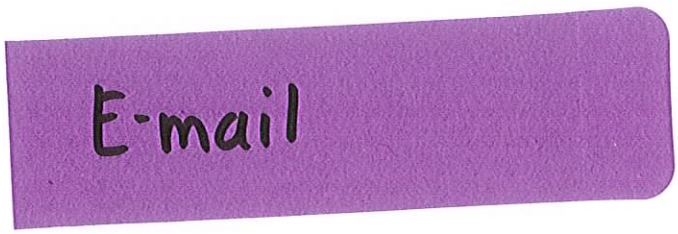
Special Conditions of Approval:

-------- Original Message --------
Subject: Fwd: biohazards 14.3 (BARF for Karmazyn lab)
Date: Thu, 09 Dec 2010 10:38:46 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: James Haist <james.haist@schulich.uwo.ca>, Morris Karmazyn <morris.karmazyn@schulich.uwo.ca>

Thanks Jim

Also, for Section 2, I assume that the rat hearts/cardiomyocytes are obtained from work done under AUS protocol 2009-020?
Question 2.4 would then be Level 1.

Regards,
Jennifer



-------- Original Message --------
Subject: biohazards 14.3
Date: Thu, 02 Dec 2010 10:09:40 -0500
From: James Haist <James.Haist@schulich.uwo.ca>
To: jstanle2@uwo.ca

Hello Jennifer,
re section 14.3 , please fill the form with the following

"If there is a small accidental exposure such as a needlestick injury, a small amount of bleeding is encouraged when there is a cut or scrape, followed by thorough washing with hand soap and warm water, and a bandage (a small first aid kit is kept in the laboratory) is applied if appropriate. If an injury is any more serious, we can proceed to take a person to staff health in the University Community Centre or to emergency at University Hospital both of which are a five minute walk away."

This seems to be the information requested, but if there is anything else, please let me know.
Thanks.

Jim

Jim Haist
Dep't of Physiology & Pharmacology
U.W.O.
phone 661-2111-ext 86699
e-mail James.Haist@schulich.uwo.ca