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	GF Wagner
DEPARTMENT	Phys-Pharm
ADDRESS	Schulich School of medicine & Dentistry
PHONE NUMBER	83966
EMERGENCY PHONE NUMBER(S)	226 234 4448
EMAIL	Graham.wagner@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) ___HSA____ Room(s)_216/210_____

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES:_____NSERC_____
GRANT TITLE(S): __Hormonal control of calcium and phosphate in fishes and invertebrates_____

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

Name	UWO E-mail Address	Date of Biosafety Training

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 are using mice to explore the regulation of kidney gene expression. These two species are being dehydrated for up to 48 hours (water withdrawl) or injected with vasopressin. The animals are then sacrificed with CO2.

We are not using the dog kidney cell line from ATCC at present. But when we do, we raise the levels of NaCl in the culture media to stimulate gene expression. The cells are then harvested 1-24 hours later for analysis of gene expression.

Please include a one page research summary or teaching protocol.

In this proposal we are seeking to further our understanding of stanniocalcin-1 (STC-1) functions in fish (rainbow trout) and invertebrates (aquatic leeches).

Studies in Fish. One area that remains unexplored in fishes is the distribution and regulation of STC-1 receptors. In mammals, we have developed the tools to quantify and localize STC-1 receptors histologically. On account of the high homology between fish and mammalian STCs, these same tools work with equal efficacy on fish tissues. Thus far we have quantified and localized histologically, high affinity binding sites in fish kidney, gut and gill, as well as pseudobranch, muscle, heart, fat, cartilage and brain.

We would now like to focus on receptor regulation in those tissues principally involved in calcium balance (gill, gut, kidney). This will entail comparing receptor levels in fish held in freshwater, as compared to those in 1/3, 2/3 and full strength seawater. In addition, we also intend to examine the effects of high and low serum STC levels on receptor densities in these same tissues.

We also intend to explore the notion that STC-1 and its receptor are targeted to subcellular organelles such as mitochondria, as is the case in mammals. The likihood of this is high given that other labs (J.H. Youson) have localized STC-1 to kidney mitochondria at the EM level.

Lastly, we intend to purify and characterize the ovarian variant of STC from rainbow trout ovary. Ovarian STC is more heavily glycosylated than corpuscle of Stannius-derived STC (12 kDa vs 5 kDa of sugar) and appears to have a smaller protein core. Our intent is to test the purified hormone for biological effects on oocyte growth.

Studies in leeches. We will continue in our efforts to purify the leech form of STC and have at hand several kilograms of frozen starting material for this project. This project was set back by the withdrawal of Teri Dickinson, who was slated to purify the leech protein. In addition, we want to explore in greater depth the regulation of calcium transport across the leech skin.
Questions to be addressed include:
1) whether leeches undergo cyclical rates of calcium uptake, as occurs in fishes, and if so, what their frequency is.
2) whether calcium uptake is governed in any way by environmental calcium levels.
3) whether the rate of calcium uptake can be modulated by injections of calcium, phosphate, or antibodies to fish STC.

We also want to study the ontogeny of the STC cell in the leech, so as to determine the origin of the cells and their stage of appearance.

Lastly, we have discovered that leech nephridia contain high levels of STC immunoreactivity. Therefore, we intend to explore this finding in more detail, to determine if these cells make STC, are targets of the hormone and what effect if any STC might have on nephridia function.

Studies in Mice. Here we are trying to map out the pathway whereby vasopressin upregulates the stanniocalcin-1 gene in the mouse kidney. This involves injecting mice i.p. with vasopressin and then sacrificing the mice with CO2 for studies on the kidneys by immunocytochemistry (stanniocalcin protein), in situ ligand binding (stanniocalcin receptors) and in situ hybdridization (stanniocalcin RNA).

Dog kidney cell line (MDCK) studies (section 2.3): this line has been stably transfected with the human stanniocalcin cDNA fused to human placental alkaline phosphatase. The cells in culture secrete the engineered protein which is them purified by affinity chromatography.

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:
_____
_____
_____

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
	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? YES xxO 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)	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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	O Yes O No			
Rodent	O Yes O No			
Non-human primate	O Yes O No			
Other (specify) Dog	x Yes O No	Madin-Darby canine Kidney	1	ATCC

*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 x 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES 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) of bacteria and/or cells 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 xNO
(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___C57Bl/6 mice_____

6.3 AUS protocol # ____2008-042-06_____

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

6.5 Will the agent(s) be shed by the animal: O YES O NO, please justify:

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES x No If no, please proceed to section 8.0

7.2 Will live animals be used? x YES O No

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

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) x YES, please specify _____mice_____ O NO

7.4 If no live animals are used, please specify the source of the specimens:

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES x 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 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:

_____

_____

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. x 1 O 2 O 2+ O 3

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

O YES, date of most recent biosafety inspection: _____
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

14.0 Procedures to be Followed

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

n/a

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

the agent we inject, vasopressin, is not toxic to humans at the concentrations employed in the syringe. So, a needle stick from a syringe containing this compound would be treated as if it contained sterile saline. The wound would be swabbed with 70% ethanol, bandaged and observed for any signs of infection.

14.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: Sept 8/2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

Info on Cell Line(s)

Cell Biology

ATCC® Number: **CRL-2935™** Order this Item Price: **$379.00**

Designations:	MDCK.1
Depositors:	Y. Reid, E. Cedrone and E-Eckard-Amar, ATCC
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Canis familiaris*
Morphology:	fibroblast-like 
Source:	**Organ:** kidney; distal tubule **Disease:** normal
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	Isolation date: Jan 2007
Antigen Expression:	E-cadherin (epithelial cell adhesion molecule), expressed Zona Occludens (ZO-1) (tight junction protein), not expressed CD29, expressed CD18, not expressed fibroblast-specific protein (FSP), not expressed cytokeratin (CK1, 4, 5, 6, 8, 10, 13, 18, 19),expressed
Cytogenetic Analysis:	hyperdiploid canine cell line with a modal chromosome number of 76 with low polyploidy rate. Several unidentifiable marker chromosomes were present in most of the cells examined.
Age:	adult
Comments:	Cell line was derived by cloning (limited dilution) the parental cell line MDCK (CCL-34). This cell line is insensitive to epsilon toxin from *C.perfringens* and is a control for MDCK.2 (CRL-2936)
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Subculturing:	**Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 1.0 to 2.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 1 X 10(4) to 3 X 10(4) viable cells/sq. cm is recommended. 6. Incubate cultures at 37C. Subculture when the cell concentration is between 8 X 10(4) and 2 X 10(5) cells/sq. cm. **Subcultivation ratio:** A subcultivation ratio of 1: 3 to 1:8 is recommended. **Medium renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium, 95%; DMSO, 5% liquid nitrogen vapor phase
Doubling Time:	approximately 14 hours
Related Products:	Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-30-2003 Recommended serum: ATCC 30-2020 0.25% (w/v) Trypsin - 0.53mM EDTA in Hank's BSS (w/o Ca++, Mg ++): ATCC 30-2101 Phosphate-buffered saline: ATCC 30-2200 Cell culture tested DMSO: ATCC 4-X Erythrosin B vital stain solution: ATCC 30-2404 parental cell line - CCL-34 derived from same individual - CRL-2936

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