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UNIT*: Biomedical Engineering DEGREE SOUGHT: Ph.D.

TITLE OF THESIS: Microencapsulated bile salt hydrolyzing probiotic lactic acid bacteria for use in hypercholesterolemia and associated cardiovascular diseases

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> Dept. of Biomedical Engineering
> McGill University
> 3775 University Street, Room 322
> Montreal, QC H3A 2B4
>
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Co-Author Name	Signature	Date
Christopher Martoni	Chris J. Martoni	1 May/09
Dr. Jasmine Bhathena		
Dr. Aleksandra Malgorzata Urbanska		
Arun Kulamarva	K. G. Arun	30 APRIL '09
Dr. Mitchell Lawrence Jones		May 04/09
Dr. Hongmei Chen		
Meenakshi Malhotra	Meenakshi	30/4/09
Dr. Satya Prakash		

Co-Author Name	Signature	Date
Christopher Martoni		
Dr. Jasmine Bhathena		
Dr. Aleksandra Malgorzata Urbanska	Aleksandra Urbanska	1.05.09
Arun Kulamarva		
Dr. Mitchell Lawrence Jones		
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Co-Author Name	Signature	Date
Christopher Martoni		
Dr. Jasmine Bhathena		
Dr. Aleksandra Malgorzata Urbanska		
Arun Kulamarva		
Dr. Mitchell Lawrence Jones		
Dr. Hongmei Chen		April 30, 2009
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Co-Author Name	Signature	Date
Christopher Martoni		
Dr. Jasmine Bhathena	J Bhathena	30 APRIL 2009
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(must match the title of the funding source application)

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1. Investigator Data:

Principal Investigator: Dr. Satya Prakash **Phone #:** 514-398-3676
Unit/Department: Biomedical Engineering **Fax#:** 514-398-7461
Address: 3775 University Street, Montreal, QC, H3A 2B4 **Email:** satya.prakash@mcgill.ca

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CCs		
DB	~	Sept 17/07
APPROVED		

** All projects that have not been peer reviewed for scientific merit by the funding source require 2 Peer Review Forms to be completed e.g. Projects funded from industrial sources. Peer Review Form available at www.mcgill.ca/research/compliance/animal/forms

Proposed Start Date of Animal Use (d/m/y): ____ or ongoing ☒
Expected Date of Completion of Animal Use (d/m/y): ____ or ongoing ☒

Investigator's Statement: The information in this application is exact and complete. I assure that all care and use of animals in this proposal will be in accordance with the guidelines and policies of the Canadian Council on Animal Care and those of McGill University. I shall request the Animal Care Committee's approval prior to any deviations from this protocol as approved. I understand that this approval is valid for one year and must be approved on an annual basis.

Principal Investigator's signature: [signature] **Date:** 02/Aug/07

Approved by:

Chair, Facility Animal Care Committee:	[signature]	**Date:** SEPT 13, 2007
RESEARCH ETHICS OFF.	[signature]	**Date:** Sept 17 '07
Chair, Ethics Subcommittee (as per UACC policy):	—	**Date:** —
Approved Animal Use	**Beginning:** June 1, 2007	**Ending:** MAY 31, 2008

☐ This protocol has been approved with the modifications noted in Section 13.

☒ **Renewal requires submission of full Animal Use Protocol form**

14 SEP. 2007

4. Research Personnel and Qualifications

List the names of the Principal Investigator and of all individuals who will be in contact with animals in this study and their employment classification (investigator, technician, research assistant, undergraduate/ graduate student, fellow). Indicate if Principal Investigator is not handling animals. If an undergraduate student is involved, the role of the student and the supervision received must be described. Training is mandatory for all personnel listed here. Refer to *www.animalcare.mcgill.ca* for details. Each person listed on this section must sign to indicate that s/he has read this protocol. *(Space will expand as needed)*

Name	Classification	Animal Related Training Information		Occupational Health Program *	Signature
		UACC on-line Theory course	Workshops + others		
Dr. S. Prakash*, Investigator, Theory + Rat/Mouse practical				Y	
Mr. Christopher Martoni, Graduate Student, Theory + Rat/Hamster/Mouse practical				Y	
Ms. Jasmine Bhathena, Graduate Student, Theory course + Hamster/Mouse workshop				Y	
Mr. Arun Kulamarva, Graduate Student, Theory course + Hamster/Mouse workshop				Y	
Dr. Mitchell Jones, Graduate Student, Theory + Rat/Hamster/Mouse practical				Y	
Ms. Aleksandra Urbanska, Graduate Student, Theory + Hamster/Mouse practical				Y	

* will not be handling animals

*** Indicate for each person, if participating in the local Occupational Health Program, see *www.mcgill.ca/research/compliance/animal/occupational* for details.**

5. Summary (in language that will be understood by members of the general public)

5 a) AIMS AND BENEFITS: Describe, in a short paragraph, the overall aim of the study and its potential benefit to human/animal health or to the advancement of scientific knowledge.

COMMON FINDINGS IN OBESE POPULATIONS ARE ELEVATED CHOLESTEROL, ELEVATED TRIGYCERIDES, DIABETES AND FATTY LIVER. RECENT MODALITIES FOR LOWERING BLOOD CHOLESTEROL LEVELS INVOLVE DIETARY MANAGEMENT, BEHAVIOR MODIFICATION, WEIGHT LOSS, EXERCISE, AND DRUG THERAPY. ALTHOUGH BENEFICIAL, THESE METHODS POSE SEVERAL LIMITATIONS. FOR EXAMPLE, PHARMACOLOGIC AGENTS SUCH AS STATINS ARE VERY EXPENSIVE, AND ARE KNOWN TO HAVE SEVERAL SIDE EFFECTS INCLUDING AN ASSOCIATION WITH EXTENSIVE MORBIDITY. ALSO, SO FAR, NO ESTABLISHED SINGLE INTERVENTION HAS CONVINCINGLY IMPROVED LIVER HISTOLOGY FOR PATIENTS WITH FATTY LIVER. GIVEN THE LIMITATIONS OF PRESENTLY AVAILABLE THERAPIES, AN OPPORTUNITY EXISTS FOR THE DEVELOPMENT OF A SAFE, EFFECTIVE, AND ECONOMICAL STRATEGY IN THE TREATMENT OF ELEVATED CHOLESTEROL, DIABETES AND ASSOCIATED FATTY LIVER.

A LESS WELL KNOWN APPROACH TO REDUCING BLOOD CHOLESTEROL, TRIGLYCERIDES, GLUCOSE AND FATTY LIVER IS ORAL LIVE BACTERIAL CELL THERAPY. THIS IS BASED ON THE DEMONSTRATION THAT NATURALLY OCCURRING LACTIC ACID BACTERIA (E.G. LACTOBACILLUS PLANTARUM 80 (PCBH1), LACTOBACILLUS FERMENTUM 11976 ETC.) CAN LOWER ELEVATED CHOLESTEROL, TRIGLYCERIDE AND GLUCOSE LEVELS SIGNIFICANTLY. INDEED A NUMBER OF STUDIES HAVE CONFIRMED THIS CAPACITY AND IT HAS BEEN FOUND THAT ORAL DAILY INTAKE OF LIVE LACTOBACILLUS CELLS CAN LEAD TO SIGNIFICANT REDUCTION IN CHOLESTEROL AND TRIGLYCERIDE LEVELS. HOWEVER, THE THERAPEUTIC POTENTIAL OF LIVE BACTERIAL CELLS HAS BEEN HAMPERED BY INHERENT LIMITATIONS IN THEIR USE. FOR EXAMPLE, OF THOSE BACTERIA INGESTED ONLY 1% SURVIVE GASTRIC TRANSIT LIMITING THE OVERALL THERAPEUTIC EFFECT. ALSO, THERE ARE SOME PRACTICAL CONCERNS REGARDING THE PRODUCTION, COST, AND STORAGE OF SUCH A PRODUCT. FURTHERMORE, ORAL ADMINISTRATION OF LIVE BACTERIAL CELLS CAN CAUSE A HOST IMMUNE RESPONSE, AND CAN BE DETRIMENTALLY RETAINED IN THE INTESTINE, REPLACING THE NORMAL INTESTINAL FLORA. THUS, CONCERNS OF SAFETY HAVE PREVENTED REGULAR USE OF THIS PROMISING THERAPY IN CLINICAL PRACTICE.

IN THE PRESENT PROJECT WE PROPOSE TREATMENT MODALITIES, FOR HIGH BLOOD SERUM CHOLESTEROL, TRIGLYCERIDES, GLUCOSE AND ELEVATED LIVER CHOLESTEROL AND TRIGLYCERIDES BASED ON THE USE OF THE MICROENCAPSULATED LACTOBACILLUS PLANTARUM 80 (PCBH1) AND LACTOBACILLUS FERMENTUM 11976 BACTERIAL CELLS. WE BELIEVE THAT THESE METHODS WILL TAKE ADVANTAGE OF THE INHERENT CHOLESTEROL AND TRIGLYCERIDE-LOWERING PROPERTIES OF LIVE NON-PATHOGENIC LACTOBACILLUS PRODUCING BACTERIA AND AT THE SAME TIME CIRCUMVENT, TO A LARGE EXTENT, ASSOCIATED PROBLEMS WITH THEIR USE. STUDIES THUS FAR HAVE SHOWN THAT MICROENCAPSULATED LACTOBACILLUS CAN LOWER TOTAL CHOLESTEROL, LDL CHOLESTEROL, BLOOD GLUCOSE AND REDUCE ATHEROGENIC RISK IN HAMSTERS WITH HIGH CHOLESTEROL. WE ARE NOW IN THE PROCESS OF OPTIMIZING DOSAGE, EXAMINING THE EFFECT IN ASSOCIATED CASES OF FATTY LIVER AND UNDERSTANDING THE MODE OF ACTION IN MALE GOLDEN SYRIAN HAMSTERS. THE RESULTING CONTROL OVER SERUM CHOLESTEROL MAY GREATLY OUT PERFORM THE ESTIMATES OF ORAL ADMINISTRATION OF FREE BACTERIA, AND PROVE TO WARRANT CLINICAL HUMAN AFFIRMATION. LONGER TERM STUDIES ARE WARRANTED TO PROVE THAT MICROENCAPSULATION OF LACTOBACILLUS IS A SAFE AND EFFECTIVE WAY OF CONTROLLING SERUM CHOLESTEROL.

AS SUCH, THIS THERAPY, IN ADDITION TO ITS BENEFICIAL EFFECTS ON FATTY LIVER WILL HAVE AN ADDED ADVANTAGE IN LOWERING ELEVATED SERUM CHOLESTEROL, REDUCING THE RESISTANCE TO INSULIN IN TYPE 2 DIABETES AND DIMINISH ATHEROSCLEROTIC LESIONS. IF SUCCESSFUL, THE POTENTIAL OF THIS PRODUCT IS WIDESPREAD AS THERE IS AN URGENT NEED FOR A LOW-COST, SAFE AND EFFECTIVE THERAPY TO HELP MANAGE SOCIAL DISTRESSES RELATED TO THESE DISEASES.

5 *b*) SPECIFIC OBJECTIVES OF THE STUDY: Summarize in point form the primary objectives of this study.

1. To study the effectiveness and safety of oral microencapsulated Lactobacillus therapy at different dosages for lowering blood serum cholesterol through interruption of the EHC of bile salts.
2. To investigate the overall suitability and pre-clinical efficacy of orally delivered microencapsulated Lactobacillus cells for lowering hepatic triglycerides, cholesterol, serum glucose and inflammation due to the supplementary action of feruloyl esterase in the GI tract.
3. To investigate the dosage and long term safety of the formulation as a viable NAFLD therapy.

5 *c*) Indicate if and how the current goals differ from those in last year's application.

Objectives remain the same as those listed in last year's protocol and those added through amendments.

5 d) List the section / subsection numbers where significant changes have been made

5 e) KEYWORDS: Using keywords only, list the procedures used on animals (e.g. anaesthesia, breeding colony, injection IP, gavage, drug administration, major survival surgery, euthanasia by exsanguination, behavioural studies). For a more complete list of suggested keywords refer to Appendix 1 of the Guidelines (www.mcgill.ca/research/compliance/animal/forms).

Sapenous vein blood collection, Gavage, Special diet, Intramuscular injections, Intraperitoneal injections, drug administration

6. Animals Use data for CCAC :

6 a) Purpose of Animal Use (Check most appropriate one):

1. ☐ **Studies of a fundamental nature/basic research**
2. ☒ **Studies for medical purposes relating to human/animal diseases/disorders**
3. ☐ **Regulatory testing**
4. ☐ **Development of products/appliances for human/veterinary medicine**
5. **If for Teaching, use the Animal Use Protocol form for Teaching (www.mcgill.ca/research/compliance/animal/forms)**

6 b) Will field studies be conducted? NO ☒ YES ☐ If yes, complete "Field Study Form"
Will the project involve genetically altering animals? NO ☒ YES ☐ If yes, complete SOP #5 or #6
Will the project involve breeding animals? NO ☒ YES ☐ If breeding transgenics or knockouts, complete SOP#4

7. Animal Data

7 a) Please justify the need for live animals versus alternate methods (e.g. tissue culture, computer simulation).

Live animals are necessary for this research because the development of suitable methods for lipid lowering requires animal testing. We have completed in-vitro testing and have obtained encouraging results. Use of animals are the only method available where we can test the pre-clinical efficacy and safety of the oral administration of microencapsulated Lactobacillus yoghurt bacterial cells for lipid lowering therapy.

7 b) Describe the characteristics of the animal species selected that justifies its use in the proposed study (consider characteristics such as body size, species, strain, data from previous studies or unique anatomic/physiological features).

Golden Syrian hamsters are well-established for demonstrating cholesterol and bile acid metabolism that accurately mimics the human condition. In relation to animals of similar size, the hamster is unique in that it contains plasma cholesterol ester transfer protein and LDL-receptor mediated activities at levels similar to humans and its closely suited lipoprotein profile is useful for studying the effects of diet and pharmaceutical products on lipoprotein metabolism. Furthermore, the hamster requires only small increases in dietary cholesterol to induce elevations in plasma lipid and lipoprotein cholesterol concentrations. In particular, the Bio F1B strain (BioBreeders USA) male golden Syrian hamster will be employed because of its characteristic phenotype which promotes diet-induced hyperlipidemia and atherosclerotic lesion formation. When administered a diet of elevated cholesterol and saturated fat, the Bio F1B model shows increased serum cholesterol levels more significantly in the VLDL and LDL fraction, as compared to the HDL fraction, making the hyperlipidemic Bio F1B model more useful for mimicking the human situation than other strains.

7 c) Description of animals

Quality Control Assurance: To prevent introduction of infectious diseases into animal facilities, a health status report or veterinary inspection certificate may be required prior to receiving animals from all non-commercial sources or from commercial sources whose animal health status is unknown or questionable. Quarantine and further testing may be required for these animals.

If more than 6 columns are needed, please attach another page

	Sp/strain 1	Sp/strain 2	Sp/strain 3	Sp/strain 4	Sp/strain 5	Sp/strain 6
Species	Golden Syrian Hamster					
Supplier/Source	Biobreeders					

Strain	Bio F1B					
Sex	M					
Age/Wt	7-8 weeks/ 90-100g					
# To be purchased	180					
# Produced by in-house breeding	0					
# Other (e.g.field studies)	0					
#needed at one time	84					
# per cage	3					
TOTAL# /YEAR	**180**					

7d) Explanation of Animal Usage: BASED ON THE EXPERIMENTAL OBJECTIVES OF THE PROJECT, describe the number of animals required for one year. Include information on experimental and control groups, # per group, and failure rates.

For breeding, specify how many adults are used, number of offspring produced, and how many offspring are used in experimental procedures.

The arithmetic explaining how the total of animals for each column in the table above is calculated should be made clear.
(Space will expand as needed)

We will employ a total of 7 groups each consisting of 12 young male Golden Syrian Hamsters for the experiment. The groups will be as follows:

(A) Normal hypercholesterolemic treatment group #1
(B) Normal hypercholesterolemic treatment group #2
(C) Normal hypercholesteroemic control group
(D) Fatty Liver induced normal hypercholesterolemic treatment group
(E) Fatty Liver induced normal hypercholesterolemic control group
(F) Fatty Liver induced diabetic hypercholesterolemic treatment group
(G) Fatty Liver induced diabetic hypercholesterolemic control group

GROUPS A, B AND C WILL BE FED A HIGH CHOLESTEROL DIET THROUGHOUT THE EXPERIMENT AND GIVEN MICROCAPSULE TREATMENT (TWO DIFFERENT FORMULATIONS) OR CONTROL. GROUPS D, E, F AND G WILL ALL BE INDUCED FOR FATTY LIVER AND GROUPS F AND G WILL BE ADDITIONALLY INDUCED FOR DIABETES. THE EFFECT OF MICROCAPSULE TREATMENT VS. CONTROL WILL BE DETERMINED BOTH FOR HYPERCHOLESTEROLEMIC HAMSTERS WITH FATTY LIVER AND FOR HYPERCHOLESTEROLEMIC HAMSTERS WITH FATTY LIVER AND DIABETES. TWELVE ANIMALS PER GROUP ARE SELECTED TO PROVIDE ADEQUATE NUMBERS OF STATISTICAL ANALYSIS OF THE STUDY DATA. ALTHOUGH ANIMAL LOSS IS NOT EXPECTED, AN ADEQUATE NUMBER IS NECESSARY TO PROVIDE SUFFICIENT DATA IN THE FACE OF UNEXPECTED ANIMAL LOSS OR PREMATURE EUTHANIZATION (INFECTION, ADVERSE EFFECT TO INDUCTION OF DIABETES, ETC.). AS A RESULT, THE TOTAL AMOUNT OF ANIMALS NEEDED AT ONE TIME WILL BE 84.

ADDITIONAL ANIMALS WILL BE REQUIRED FOR SUBSEQUENT EXPERIMENTS TO DETERMINE SAFETY AND SURVIVAL AND EVALUATE ALTERNATIVE MEMBRANE FORMULATIONS FOR EFFECTIVENESS. FOR SAFETY AND SURVIVAL STUDIES, 48 HAMSTERS WILL BE EMPLOYED. SPECIFICALLY, 12 ANIMALS PER GROUP WILL BE NEEDED TO TEST FOR LOW, MID AND HIGH DOSAGE IN ADDITION TO CONTROL. IN ADDITION, FOR THE EVALUATION OF ALTERNATIVE MEMBRANE FORMULATIONS, 48 HAMSTERS WILL BE EMPLOYED. SPECIFICALLY, 12 ANIMALS PER GROUP WILL BE NEEDED TO TEST FOR THREE ALTERNATIVE MEMBRANE FORMULATIONS IN ADDITION TO CONTROL. THUS, IT IS ESTIMATED THAT 180 HAMSTERS WILL BE REQUIRED FOR THE UPCOMING YEAR.

8. Animal Husbandry and Care

8 a) If projects involves non-standard cages, diet and/or handling, please specify

We will be feeding 48 of the Golden Syrian Hamsters two distinct diets over the course of the experiment. Once diabetes has been induced, all animals in all four fatty liver induced groups will be fed a methionine deficient-choline devoid, synthetic diet containing 0.05% cholesterol and 6% saturated fats in addition to other essential vitamins, minerals, nutrients, etc. (from Land O' Lakes Purina Feed, LLC, Test Diet Formulation 5D4F) for 10 days-- [MDCD]

After 10 days of feeding the MDCD diet, 1-2 hamsters in each group will be euthanised to determine the development and progression of fatty liver disease in the animals.
For the remainder of the experiment (15 weeks) the animals will be fed a methionine adequate-choline deficient non-purified diet containing 0.05% cholesterol and 6% saturated fats in addition to other essential vitamins, minerals, nutrients, etc. (from Land O' Lakes Purina Feed, LLC, Test Diet Formulation 5D4E). [MACD].

8 b) Is there any component to the proposed procedures which will result in immunosuppression or decreased immune function (e.g. stress, radiation, steroids, chemotherapeutics, genetic modification of the immune system)?

NO ☒ YES ☐ **if yes, specify:**

8 c) Indicate area(s) where animal use procedures will be conducted:

Building: Lyman Duff **Room:** 710

Indicate area(s) all facilities where animals will be housed:

Building: Lyman Duff **Room:** 710

If animal housing and animal use are in different locations, briefly describe procedures for transporting animals:

9. Standard Operating Procedures (SOPs)

Complete this section if you plan to use any of the UACC SOPs listed below. IT IS UACC POLICY THAT THESE SOPS BE USED WHEN APPLICABLE. Any proposed variation of the SOPs must be described and justified. The Standard Operating Procedures can be found at the UACC website at www.mcgill.ca/research/compliance/animal/procedures. The completed and signed SOP form must be attached to the protocol.

Check all SOPs that will be used:

Blood Collection UACC#1	☒	**Collection of Amphibian Oocytes UACC#9**	☐
Anaesthesia in rodents UACC#2	☒	**Rodent Survival Surgery UACC#10**	☐
Analgesia in rodents UACC#3	☐	**Anaesthesia & Analgesia Neonatal Rodents UACC#11**	☐
Breeding transgenics/knockouts UACC#4	☐	**Stereotaxic Survival Surgery in Rodents UACC#12**	☐
Transgenic Generation UACC#5	☐	**Field Studies Form**	☐
Knockout/in Generation UACC#6	☐	**Phenotype Disclosure Form**	☐
Production of Monoclonal Antibodies UACC#7	☐	**Other, specify:**	☐
Production of Polyclonal Antibodies UACC#8	☐		☐

10. Description of Procedures

10 a) IF A PROCEDURE IS COVERED BY AN SOP, WRITE "AS PER SOP", NO FURTHER DETAIL IS REQUIRED.

FOR EACH EXPERIMENTAL GROUP, DESCRIBE ALL PROCEDURES AND TECHNIQUES, WHICH ARE NOT PART OF THE SOPs, IN THE ORDER IN WHICH THEY WILL BE PERFORMED - surgical procedures, immunizations, behavioural tests, immobilization and restraint, food/water deprivation, requirements for post-operative care, sample collection, substance administration, special monitoring, etc. Appendix 2 of the Guidelines (www.mcgill.ca/research/compliance/animal/forms) provides a sample list of points that should be addressed in this section.

BLOOD WILL BE COLLECTED EVERY SECOND WEEK FROM THE HAMSTER SAPHENOUS VEIN INTO SERUM SEPARATOR TUBES. BLOOD COLLECTION - "AS PER UACC#1 SOP (SEE ATTACHED SOP)". HAMSTERS WILL BE FASTED PRIOR TO BLOOD COLLECTION AND WILL NOT BE ANESTHETIZED. HAMSTERS WILL BE HOWEVER,

MILDLY SEDATED USING ACEPROMAZINE. INJECTION IM OF ACEPROMAZINE- "AS PER UACC#2 SOP (SEE ATTACHED SOP)". SERUM ANALYSIS WITH A CLINICAL CHEMISTRY ANALYZER WILL PROVIDE INFORMATION ON THE LEVELS OF TOTAL CHOLESTEROL, HDL, LDL AND TRIGLYCERIDES. WEIGHT GAIN AND FOOD INTAKE WILL BE MONITORED DAILY AND FECAL SAMPLES WILL BE OBTAINED WEEKLY AND ANALYZED ENZYMATICALLY. AT EXPERIMENTAL ENDPOINT, HAMSTERS WILL BE EUTHANIZED BY CO2, BLOOD WILL BE COLLECTED BY CARDIAC PUNCTURE AND HEARTS AND LIVERS WILL BE REMOVED UNDER THE SUPERVISION OF AN ANIMAL HEALTH TECHNICIAN.

1) GAVAGE - DURING THE "TREATMENT PERIOD". THE ANIMALS WILL NOT BE ANESTHETIZED DURING GAVAGE BUT WILL BE PROPERLY RESTRAINED. FREQUENCY OF GAVAGE WILL BE TWICE DAILY DURING THE ANIMAL FEEDING CYCLE.

2) INDUCTION OF DIABETES:

ACCORDING TO OSHA, IARC, NTP AND ACGH, STZ IS HIGHLY HAZARDOUS. IT IS A SUSPECTED CARCINOGEN AND IS POTENTIALLY HARMFUL TO THE FOLLOWING ORGANS: BLOOD, KIDNEYS, NERVOUS SYSTEM, DIGESTIVE SYSTEM, SKIN, EYES, BONE MARROW, MUSCLE TISSUE AND PANCREAS.

GENERAL PRECAUTIONS:
1. THE FOLLOWING PERSONAL PROTECTION MUST BE WORN WHEN HANDLING STZ: 2 PAIRS OF GLOVES, LAB COAT, N95 MASK, SAFETY GLASSES. ANY HANDLING MUST BE DONE IN A CHEMICAL FUME HOOD OR TYPE II B2 BIOLOGICAL SAFETY CABINET
2. ANIMAL BEDDING IS NOT TO BE CHANGED FOR AT LEAST 3 DAYS AFTER THE DATE OF STZ ADMINISTRATION.
3. FOR THE FIRST CAGE CHANGE FOLLOWING STZ ADMINISTRATION, THE BEDDING IS CONSIDERED CONTAMINATED AND TRANSFER TO CLEAN CAGES MUST BE DONE IN A FUME HOOD OR TYPE II B2 BIOSAFETY CABINET.

INJECTION OF STREPTOZOTOCIN (STZ) METHOD: INJECTION USING CITRIC BUFFER PH 4.5

1) WEIGH AND RECORD HAMSTER WEIGHTS, STZ DOSE: 50MG/KG BW.
2) TRANSFER THE DESIRED HAMSTERS TO THE ARC PROCEDURE ROOM, WORK IN TYPE II B2 BIOLOGICAL HOOD ONLY (HOOD #1 IN DUFF ARC PROCEDURE ROOM 712)
3) THE PREFERRED METHOD OF DIABETES IS VIA INTRAPERITONEAL (IP) INJECTIONS AFTER A 2 HOUR FAST.
4) RESTRAIN THE ANIMAL AND INJECT THE APPROPRIATE VOLUME OF STZ IP USING A 1 ML SYRINGE AND A 25G NEEDLE.
5) RELEASE THE ANIMAL BACK INTO THE CAGE AND CONTINUE IP ADMINISTRATION TO THE REMAINDER OF THE HAMSTERS.
9) HAMSTER CAGES TO BE USED WITH EXTRA BEDDING AND EQUIPPED WITH 2 BOTTLES; ONE CONTAINING WATER, THE OTHER CONTAINING 5% GLUCOSE SOLUTION FOR ANIMALS TO RECOVER FROM DRUG INDUCED HYPOGLYCEMIA.

3) MONITORING OF BLOOD GLUCOSE
COMMENCING APPROXIMATELY 48 HOURS POST-STZ INJECTION, MONITOR BLOOD GLUCOSE LEVELS (BGLS) VIA SAPHENOUS VEIN PRICK, USING GLUCOSE TEST STRIPS AND BLOOD GLUCOSE MONITOR. RECORD BGLS AND EXAMINE CAGES FOR ONSET OF POLYURIA (EXCESSIVE URINE PRODUCTION). IN THE EVENT OF POLYURIA, REPLACE CAGE BEDDING AS REQUIRED. MEASURE NON-FASTING AND FASTING BLOOD GLUCOSE LEVELS AT REGULAR INTERVALS (EVERY 3 DAYS) FOR 1-2 WEEKS, TO CONFIRM THE ONSET OF IRREVERSIBLE DIABETES (200-250 MG/DL).
IF AFTER 2 WEEKS, ANIMALS HAVE REVERTED TO REGULAR BLOOD GLUCOSE LEVELS, A SINGLE ADDITIONAL INJECTION OF 50MG/KG BW WILL AGAIN BE ADMINISTERED IP. ANIMALS WILL BE MONITORED FOR ONSET OF DIABETED AS ABOVE.

10 b) Experimental endpoint – for each experimental group indicate survival time

(A) Normal hypercholesterolemic treatment group #1: 17 weeks
(B) Normal hypercholesterolemic treatment group #2: 17 weeks
(C) Normal hypercholesteroemic control group: 17 weeks
(D) Fatty Liver induced normal hypercholesterolemic treatment group: 19 weeks
(E) Fatty Liver induced normal hypercholesterolemic control group: 19 weeks

(F) Fatty Liver induced diabetic hypercholesterolemic treatment group: 19 weeks
(G) Fatty Liver induced diabetic hypercholesterolemic control group: 19 weeks

10.c) Clinical endpoint – describe the conditions, complications, and criteria (e.g. >20% weight loss, maximum tumour size, vocalizing, lack of grooming) that would lead to euthanasia of an animal before the expected completion of the experiment (specify per species and project if multiple projects involved)

>20% WEIGHT LOSS FOR ALL ANIMALS, INCLUDING DIABETICS, DESPITE SUPPORTIVE THERAPY
Unknown sickness causing distress - acute diarrhea, vocalizing, lack of grooming, lethargy

Frequency of monitoring: Monitored daily, weighed weekly; diabetic animals weighted 2-3 times a week

10.d) Specify person(s) who will be responsible for animal monitoring and post-procedural care *(must also be listed in section 4)*

Name:	**Phone #:**
Ms. Jasmine Bhathena, Graduate Student	514-398-2736
Mr. Christopher Martoni, Graduate Student	514-398-2736
Ms. Aleksandra Urbanska, Graduate Student	514-398-2736
Mr. Arun Kulamarva, Graduate Student	514-398-2736
Dr. Mitchell Jones, Graduate Student	514-398-2736

10.e) Pre-Anesthetic/Anaesthetic/Analgesic Agents: List all drugs that will be used to minimize pain, distress or discomfort. *(Table will expand as needed)*

Species	Agent	Dosage (mg/kg)	Total volume(ml) per administration	Route	Frequency/Duration
Golden Syrian Hamster	Acepromazine	5mg/mL	2 uL/100g body wt	Intramuscular	Once/2 weeks

10.f) Administration of ALL other substances: List all non-anaesthetic agents under study in the experimental component of the protocol, including but not limited to drugs, infectious agents, viruses *(Table will expand as needed)*

Species	Agent	Dosage (mg/kg)	Total volume(ml) per administration	Route	Frequency/Duration

10.g) Method of Euthanasia

Specify Species	
	☐ **Anaesthetic overdose, list agent/dose/route:**
	☐ **Exsanguination with anaesthesia, list agent/dose/route:**
	☐ **Decapitation without anaesthesia *** ☐ **Decapitation with anesthesia, list agent/dose/route (including CO_2):**
	☐ **Cervical dislocation without anaesthesia *** ☐ **Cervical dislocation with anaesthesia, list agent/dose/route (including CO_2):**
	☒ **CO_2 chamber only**
	☐ **Other, specify:**
	☐ **Not applicable, explain:**

*** For physical method of euthanasia without anaesthesia, please justify:**

11. Category of Invasiveness: B ☐ C ☒ D ☐ E ☐

Categories of Invasiveness (from the CCAC *Categories of Invasiveness in Animal Experiments*). Please refer to this document for a more detailed description of categories.
Category A: Studies or experiments on most invertebrates or no entire living material.

Category B: Studies or experiments causing little or no discomfort or stress. *These might include holding animals captive, injection, percutaneous blood sampling, accepted euthanasia for tissue harvest, acute non-survival experiments in which the animals are completely anaesthetized.*
Category C: Studies or experiments involving minor stress or pain of short duration. *These might include cannulation or catheterizations of blood vessels or body cavities under anaesthesia, minor surgery under anaesthesia, such as biopsy; short periods of restraint, overnight food and/or water deprivation which exceed periods of abstinence in nature; behavioural experiments on conscious animals that involve short-term stressful restraint.*
Category D: Studies or experiments that involve moderate to severe distress or discomfort. *These might include major surgery under anaesthesia with subsequent recovery, prolonged (several hours or more) periods of physical restraint; induction of behavioural stresses, immunization with complete Freund's adjuvant, application of noxious stimuli, procedures that produce pain, production of transgenics (in accordance with University policy).*
Category E: Procedures that involve inflicting severe pain, near, at or above the pain threshold of unanaesthetized, conscious animals. *Not confined to but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain or extreme distress or physical trauma on unanaesthetized animals.* According to University policy, E level studies are not permitted.

12. Potential Hazards to Personnel and Animals: It is the responsibility of the investigator to obtain the necessary Biohazard and/or Radiation Safety permits before this protocol is submitted for review. A copy of these certificates must be attached, if applicable.

No hazardous materials will be used in this study: ☐

12 a) Indicate which of the following will be used in animals:

☐ Toxic chemicals ☐ Radioisotopes ☒ Carcinogens
☐ Infectious agents (includes vectors) ☐ Transplantable tumours and/or tissues

12 b) Complete the following table for each agent to be used (use additional page as required):

Agent name	Steptozotocin		
Dosage	50mg/Kg BW		
Route of administration	Intraperitoneal		
Frequency of administration	Once a day		
Duration of administration	3 days		
Number of animals involved	24		
Survival time after administration	12 weeks (expected mortality <5%)		

12 c) After administration the animals will be housed in:

☒ **the animal care facility** ☐ **laboratory under supervision of laboratory personnel**

Please note that cages must be appropriately labeled at all times.

12 d) Describe potential health risk(s) to humans or animals:

According to OSHA, IARC, NTP and ACGH, STZ is highly hazardous. It is a suspected carcinogenic, is a possible mutagenic for humans (based on human data) , is a possible teratogenic for humans and is harmful to the following organs: blood, kidneys, nervous system, digestive system, skin, eyes, bone marrow, muscle tissue and pancreas.
STZ is non-volatile and thus only represents a risk in its crystalline and solubilised forms.

12 e) Describe measures that will be used to reduce risk to the environment and all project and animal facility personnel:

Because it is a highly hazardous chemical and that no exposure limit was established, the following precautions are mandatory when handling STZ.

GENERAL PRECAUTIONS:
1. Pregnant or breast-feeding women cannot work with STZ
2. The following personal protection must be worn when handling STZ: 2 pairs of gloves, lab coat, N95 mask, safety glasses.
3. Any handling (weighing powder, preparing stock injection, injection to rodents) must be done in a chemical fume hood or type II B2 biological safety cabinet
4. Prepare work area in hood by laying down absorbent work surface (blue pad) with the absorbent material facing up.

STORAGE PRECAUTIONS

1. Keep locked, away from heat and sources of ignition
2. Empty containers pose a fire risk, evaporate residue under a fume hood
3. Ground all equipment containing material

WASTE DISPOSAL

1. All items contaminated or potentially contaminated with STZ (needles, gloves, etc) must be discarded in a "cytotoxic" plastic container located inside the fume hood or biosafety cabinet
2. Contact waste management for disposal of cytotoxic containers

CAGE CHANGE

1. Animal bedding is not to be changed for at least 3 days after the date of STZ administration
2. Cages must be labelled "Streptozotocin" along with date of administration
3. For the first cage change following STZ administration, the bedding is considered contaminated and requires the following handling:

- ☐ Transfer to clean cages must be done in a fume hood or type II B2 biosafety cabinet
- ☐ Replace the covers on the soiled and place in a heavy duty plastic bag
- ☐ Twist the ends of full bags, goose-neck and seal with elastic band
- ☐ Label "Streptozotocin" on bag
- ☐ Transport bags of soiled cages to a filtered dumping station that draws air away from the handler

4. After first cage change there is no need for further special precautions to be taken regarding the animals or the cages as long as the animals do not receive any more STZ

12 f) If using cell lines, have they been tested?

☐ **Yes If yes, What human and/or animal pathogens have been tested?**

☐ **No If no, justify:**

13. Reviewer's Comments and Modifications (to be completed by ACC only): The Animal Care Committee has made the following modification(s) to this animal use procedure protocol during the review process. Please make these changes to your copy and comply with the recommended changes as a condition of approval:

MCGILL UNIVERSITY
UNIVERSITY ANIMAL CARE COMMITTEE

UACC Standard Operating Procedure # 1 October 2005 version

BLOOD COLLECTION - RODENTS (Rats, mice and guinea pigs)

1. INTRODUCTION

Standard Operating Procedures (SOPs) provide a detailed description of commonly used procedures. SOPs offer investigators an alternative to writing detailed procedures on their protocol forms. Any deviation from the approved procedures must be clearly described and justified in the Animal Use Protocol form (AUP). Approval of the protocol indicates approval of the deviation from the SOP for that project only. A signed SOP cover page must be attached to the Animal Use Protocol form. The relevant SOP number must be referred to in the Procedures section.

2. INFORMATION REQUIRED

2.1 Species: Golden Syrian Hamster Bio F1B
Age: 7-8 weeks
Approximate weight: 91-100 g

2.2 Route: Put an **X** next to the one to be employed:

X	Route
Survival procedures:	
☒	1. Lateral saphenous vein - *preferred*
☐	2. Tail artery
☐	3. Jugular vein (percutaneous)
☐	4. Tail tip

Terminal procedures:	
☒	7. Cardiac puncture – *preferred*
☐	8. Trunk blood
☐	9. Retro-orbital sinus

☐	10. Other, specify: _____

2.3 Volume per collection: 225 ul

2.4 Frequency of collection: Every 2nd week for 14 weeks
if repeated how frequent (interval)? _____
Replacement volume: No ☒ Yes ☐ **specify:** _____

2.5 Are there changes to this SOP indicated in the AUP form? ☐YES ☒NO
If yes, specify changes: _____

2.6 PI signature: _____ **Date:** 28/05/07

PLEASE ATTACH ONLY THIS SIGNED COVER SHEET TO THE BACK OF EACH RELEVANT AUP FOR ANIMAL CARE COMMITTEE APPROVAL.

MCGILL UNIVERSITY
UNIVERSITY ANIMAL CARE COMMITTEE

UACC Standard Operating Procedure # 2 October 2005 version

GENERAL ANAESTHESIA IN ADULT EXPERIMENTAL ANIMALS

1. INTRODUCTION

Standard Operating Procedures (SOPs) provide a detailed description of commonly used procedures. SOPs offer investigators an alternative to writing detailed procedures on their protocol forms. Any deviation from the approved procedures must be clearly described and justified in the Animal Use Protocol form (AUP). Approval of the protocol indicates approval of the deviation from the SOP for that project only. A signed SOP cover page must be attached to the Animal Use Protocol form. The relevant SOP number must be referred to in the Procedures section.

2. USE THIS SOP IF NOT USING *'RODENT SURGERY SOP#10'* OR *'STEREOTAXIC SURVIVAL SURGERY SOP#12'*

3. INFORMATION REQUIRED

3.1 Species/strain(s): ***(must refer to the Sp/strain column # of the table in "Description of animals" section in main protocol)*** Golden Syrian Hamster Bio F1B

3.2 Anaesthesia chosen:

Procedure:	Agent:	Dose:	Route:	Re-administration
Mild sedation	Acepromazine	2 ul of 5mg/ml per 100 g body weight	IM	None

3.3 Are there changes to this SOP indicated in the AUP form? ☐YES ☒NO
If yes, specify changes: _____

3.4 If using species other than rabbits or rodents, please supply pertinent references (such as CCAC *Guide to the Care and Use of Experimental Animals*)
_____

3.5 PI Signature: _____ **Date:** 28/05/07

PLEASE ATTACH ONLY THIS SIGNED COVER SHEET TO THE BACK OF EACH RELEVANT AUP FOR ANIMAL CARE COMMITTEE APPROVAL.

ACTION	✔	DATE
OCB		
DB	✔	Aug 30 '06
APPROVED		

www.mcgill.ca/rgo/animal/forms/

For Office Use Only
Protocol #: 4740
Approval end date: May 31, 2007
Facility Committee: 03 05
Renewal #: 1 2nd

McGill University Animal Care Committee
RENEWAL of Animal Use Protocol

For: Research ☒ Teaching ☐ project

Principal Investigator:	Dr. Satya Prakash	**Protocol #**	4740
Protocol Title:	Oral Administration of microencapsulated Lactobacillus plantarum 80 (pCBH1) yoghurt bacterial cells for lowering cholesterol	**Phone:**	514-398-3676
Unit, Dept. & Address:	Biomedical Engineering 3775 University Street, Montreal, QC, H3A 2B4	**Fax:**	514-398-7461
Email:	satya.prakash@mcgill.ca	**Level:**	C
Funding source:	Micropharma		
Start of Funding:	Feb. 1, 2005	**End of Funding:**	Sept. 31, 2007
Emergency contact #1 + work AND home phone #s:	Dr. Satya Prakash, 398-3676, home (450) 465-5939		
Emergency contact #2 + work AND home phone #s:	Christopher Martoni, 398-2736, home (514) 793-2831		

1. Personnel and Qualifications

List the names of the Principal Investigator and of all individuals who will be in contact with animals in this study and their employment classification (investigator, technician, research assistant, undergraduate/graduate student, fellow). If an undergraduate student is involved, the role of the student and the supervision received must be described. Training is mandatory for all personnel listed here. Refer to *www.animalcare.mcgill.ca* for details. Each person listed in this section must sign. *(Space will expand as needed)*

Name	Classification	Animal-Related Training Information	Occupational Health Program *	Signature *(Has read the original full protocol)*
Dr. S. Prakash*,	Investigator, Theory + Rat/Mouse practical		Y	Satya Prakash
Mr. Christopher Martoni,	Graduate Student, Theory + Rat/Hamster/Mouse practical		Y	Chris J. Martoni
Ms. Jasmine Bhathena,	Graduate Student, Theory course + Hamster/Mouse workshop		Y	Bhathena
Mr. Arun Kulamarva,	Graduate Student, Theory course + Hamster/Mouse workshop		Y	K. G. Arun
Ms. Alexandra Urbanska,	Graduate Student, Theory course + Hamster/Mouse workshop		Y	Alexandra Urbanska
Dr. Wei Ouyang,	Post-Doctoral Student, Theory + Rat/Hamster/Mouse practical		Y	Wei Ouyang

AHT-

* will not be handling animals

for? Okay July

*** Indicate for each person, if participating in the local OHP Program, see http://www.mcgill.ca/rgo/animal/occupational/ for details.**

Approved by:

2. Approval Signatures

Principal Investigator/ Course Director	Satya Prakash	**Date:**	28/06/06
Chair, Facility Animal Care Committee	Anusi Datye	**Date:**	09/08/06
UACC Veterinarian		**Date:**	14/Aug/2006
Chairperson, Ethics Subcommittee *(D level or Teaching Protocols Only)*	—	**Date:**	—
Approved Animal Use Period	**Start:** June 1, 2006	**End:**	May 31, 2007

3. Summary (in language that will be understood by members of the general public)

July 3/06 - Revised

30 AOUT 2006

3. Summary (in language that will be understood by members of the general public)

AIMS AND BENEFITS: Describe, in a short paragraph, the overall aim of the study and its potential benefit to human/animal health or to the advancement of scientific knowledge *(was section 5a in main protocol).*

Recent modalities for lowering blood cholesterol levels involve dietary management, behavior modification, exercise, and drug therapy. Pharmacologic agents such as statins are considered to have the most potential for clinical lowering of cholesterol. Although statins effectively reduce cholesterol levels, they are very expensive, and are known to have side effects including an association with extensive morbidity. A less well known approach to reducing blood cholesterol, oral live bacterial cell therapy, is based on the demonstration that naturally occurring bacteria such as Lactobacillus plantarum 80 (pCBH1) can lower cholesterol levels significantly. Indeed a number of studies have confirmed this capacity and it has been found that oral daily intake of live Lactobacillus cells can lead to significant reduction in cholesterol levels. Although the mechanisms are not entirely understood, it is likely the result of an exclusively intestinal mode of action on bile salts in the intestinal lumen. It has been reported that using these and related methods, cholesterol levels can be reduced by 22% to 33%. However, the therapeutic potential of live bacterial cells has been hampered by inherent limitations in their use. For example, of those bacteria ingested only 1% survive gastric transit limiting the overall therapeutic effect. Also, there are some practical concerns regarding the production, cost, and storage of such a product. Furthermore, oral administration of live bacterial cells can cause a host immune response, and can be detrimentally retained in the intestine, replacing the normal intestinal flora. Thus, concerns of safety have prevented regular use of this promising therapy in clinical practice. In the present project we propose treatment modalities, for high blood serum cholesterol, based on the use of the microencapsulated Lactobacillus plantarum 80 (pCBH1) bacterial cells. We believe that these methods will take advantage of the inherent cholesterol-lowering properties of live non-pathogenic Lactobacillus bile salt hydrolase (BSH) overproducing bacteria and at the same time circumvent, to a large extent, associated problems with their use. Studies thus far have shown that microencapsulated Lactobacillus can break down bile salts much like the free bacteria and can lower total cholesterol, LDL cholesterol and reduce atherogenic risk in hamsters with high cholesterol. We are now in the process of optimizing dosage and understanding the mode of action in male Golden Syrian hamsters. The resulting control over serum cholesterol may greatly out perform the estimates of oral administration of free bacteria, and prove to warrant clinical human affirmation. Longer term studies are warranted to prove that microencapsulation of Lactobacillus is a safe and effective way of controlling serum cholesterol. Thus, this study will help develop a suitable method for lowering cholesterol.

4. Has an unanticipated problem occurred?	**YES** ☐	**NO** ☒	**if yes, supply details:**

5. If creating genetically modified animals or new combinations of genetic modifications, complete and attach a *Phenotype Disclosure form* (http://www.mcgill.ca/rgo/animal/forms/)

6. Procedures

a) For B and C level of invasiveness,

The procedures are the same as the original protocol: **YES** ☐ **NO** ☒

IF NO, complete the following:

Detail new procedures that are different from section 10a of the original protocol ***(include a copy of the entire revised procedure section 10a of the original protocol with the changes and/or new procedures in CAPS):***

We employ a total of 6 groups each consisting of 10 young male Golden Syrian Hamsters for each set of experiments. All 60 hamsters will be fed a normal diet (Rodent Chow) for 2 WEEKS to acclimatize them to their new environment. One group of 10 hamsters will be a control group receiving a normal diet throughout. The other 50 hamsters will be fed a high cholesterol diet (This diet will contain cholesterol, saturated fats as well as essential nutrients, vitamins and minerals and will be manufactured by Dyets, Inc). CAGES RECEIVING A SPECIAL DIET WILL BE LABELLED AS SUCH AND WILL BE FED BY INVESTIGATORS ONLY. IMMEDIATELY AFTER ACCLIMITIZATION, THE 50 HAMSTERS BEING FED A HC DIET WILL BE RANDOMLY SPLIT INTO FIVE GROUPS AND FED THE APPROPRIATE TREATMENT (MICROENCAPSULATED BACTERIA AT FOUR DIFFERENT DOSAGE LEVELS, EMPTY MICROCAPSULES) BY DAILY GAVAGE. Blood will be collected EVERY SECOND WEEK from the hamster saphenous vein into serum separator tubes. Hamsters will be fasted prior to blood collection and will not be anesthetized. Serum analysis with a clinical chemistry analyzer will provide information on the levels of Total Cholesterol, HDL, LDL and triglycerides. Weight gain and food intake will be monitored daily and fecal samples will be obtained weekly and analyzed enzymatically. AT EXPERIMENTAL ENDPOINT, HAMSTERS WILL BE EUTHANIZED BY CO2, BLOOD WILL BE COLLECTED BY CARDIAC PUNCTURE AND LIVERS WILL BE REMOVED UNDER THE SUPERVISION OF AN ANIMAL HEALTH TECHNICIAN.

Blood Collection - "AS PER UACC#1 SOP (see attached SOP)"
Gavage - during the "treatment period" THE 50 HC HAMSTERS WILL RECEIVE EITHER MICROENCAPSULATED LP80 AT DIFFERENT DOSAGE LEVELS OR EMPTY MICROCAPSULES by daily gavage. The animals will not be anesthetized during gavage but will be properly restrained.

b) For D level of invasiveness,

Include here ALL procedures except transgenic procedures, including the ones described in the original protocol as well as new and changed procedures in CAPS ***(was section 10a in main protocol); Please only attach SOPs related to new and changed procedures to this renewal form.***

7. Endpoints

a) For B and C level of invasiveness,

The procedures are the same as the original protocol: **YES** ☐ **NO** ☒

IF NO, supply new endpoints that are different from the original protocol:

ALL GROUPS OF ANIMALS WILL HAVE AN EXPERIMENTAL ENDPOINT OF MAXIMUM 12 WEEKS

b) For D level of invasiveness,

Include here ALL endpoints, including the ones described in the original protocol as well as new and changed endpoints in CAPS:

8. Hazards (check here if none are used: ☐)

a) Are the hazards different from original protocol? (infectious, radioactive, toxic, carcinogen, tumours)

YES ☐ **NO** ☒ **if yes, supply details (material, risks, precautions):**

b) Have the cell lines been tested for human and animal pathogens? YES: ☐ **NO:** ☐ **None used:** ☒

9. Description of Animals to be used in the coming year (only):

Quality Control Assurance: To prevent introduction of infectious diseases into animal facilities, a health status report or veterinary inspection certificate may be required prior to receiving animals from all non-commercial sources or from commercial sources whose animal health status is unknown or questionable. Quarantine and further testing may be required for these animals. *If more than 6 columns are needed, please attach another page*

	Sp/strain 1	Sp/strain 2	Sp/strain 3	Sp/strain 4	Sp/strain 5	Sp/strain 6
Species	Hamster					
Supplier/Source	BioBreeders					
Strain	Bio F1B Golden Syrian					
Sex	Male					
Age/Wt	7 weeks / 91-100g					
# To be purchased	180					
# Produced by in-house breeding						
# Other (e.g.field studies)						
TOTAL# /YEAR	**180**					

10. Justification of Animal Numbers:

BASED ON THE EXPERIMENTAL OBJECTIVES OF THE PROJECT, describe the number of animals required for one year. Include information on experimental and control groups, # per group, and failure rates. For breeding, specify how many adults are used, number of offspring produced, and how many offspring are used in experimental procedures. The arithmetic explaining how the total of animals for each column in the table above is calculated should be made clear.

Experiment # 1: Dosage optimization: 10 animals x 6 groups
All 60 hamsters will be fed a normal diet (Rodent Chow) for two weeks to acclimatize them to their new environment. One group of 10 hamsters will be a control group receiving a normal diet throughout. The other 50 hamsters will be randomly divided into 5 groups, fed a high cholesterol diet and given control or microcapsule treatment at four different dosage levels. Ten animals per group are selected to provide adequate numbers for statistical analysis. Animal loss is not expected, however an adequate number is necessary to provide sufficient data in the face of unexpected animal loss or premature termination (infection, etc.)

Experiment # 2: Mechanistic studies: 15 animals x 4 groups
Mechanistic studies will utilize larger groups due to multiple endpoints. Animals will be divided into two control groups and two treatment groups.

Experiment # 3: Alternate membrane formulations: 10 animals x 6 groups
For the evaluation of alternative membrane formulations, it is expected that 60 hamsters will be employed (10 per group for three alternative membranes plus controls).

Thus, it is estimated that 180 F1B male Golden Syrian hamsters will be required for the upcoming year.

Submit to your local Facility Animal Care Committee. Please note that after two renewals, a full protocol needs to be submitted.

This approval does not imply that space will be made available. If a major increase of space needs is anticipated, please contact the appropriate animal facility manager.

McGILL UNIVERSITY
UNIVERSITY ANIMAL CARE COMMITTEE

Standard Operating Procedure #UACC-1 October 2001 form version

BLOOD COLLECTION - RODENTS (Rats, mice and guinea pigs)

1. *INTRODUCTION*

Standard Operating Procedures (SOPs) provide a detailed description of commonly used procedures. SOPs offer investigators an alternative to writing detailed procedures on their protocol forms. Any deviation from the approved procedures must be clearly described and justified in the Animal Use Protocol form. Approval of the protocol indicates approval of the deviation from the SOP for that project only.

A signed SOP cover page must be attached to the Animal Use Protocol form. The relevant SOP number must be referred to in the Procedures section.

2. *INFORMATION REQUIRED*

2.1 Species: Golden Syrian Hamsters
Age: 6 Weeks
Sex: Male
Approximate weight: (purchase weight: 91-100 g)

2.2 Route: Put an **X** next to the one to be employed:

X	Route
☐	1. Tail tip
☐	2. Lateral saphenous vein
☐	3. Jugular vein (percutaneous)
☐	4. Jugular vein (indwelling intravenous catheter)
☐	5. Femoral vein (indwelling intravenous catheter)
☐	6. Retro-orbital sinus
☐	7. Cardiac puncture
☐	8. Trunk blood
☐	9. Tail artery
☒	10. Other, specify: Saphenous Vein

2.3 Volume per collection: 150 uL

2.4 Frequency of collection:
if repeated how frequent ? Once per week for 14 weeks.
Replacement volume: No ☒ Yes ☐ specify:

2.5 There are changes to this SOP indicated in the AUP form: ☒YES ☐NO

2.6 Signature: _____ Date:

PLEASE ATTACH <u>ONLY</u> THIS SIGNED COVER SHEET TO THE BACK OF EACH RELEVANT AUP FOR ANIMAL CARE COMMITTEE APPROVAL.

Natural Sciences and Engineering
Research Council of Canada

350 Albert Street
Ottawa, Canada
K1A 1H5

Conseil de recherches en sciences
naturelles et en génie du Canada

350, rue Albert
Ottawa, Canada
K1A 1H5

June 17, 2005

File: I2IPJ 307900 - 04

Dr. S. Prakash
Dept. of Biomedical Engineering
McGill University
3775 RUE UNIVERSITY
MONTREAL QC H3A 2B4

Dear Dr. Prakash:

Re: Idea to Innovation (I2IPJ) "Oral artificial cells containing live genetically engineered Lactobacillus plantarum 80 bacterial cells for lowering cholesterol."

This letter represents NSERC's official approval of **February 28, 2006**, as the new termination date for the project described above.

A final report will be due at NSERC on **May 28, 2006** and a reminder to that effect will be sent to you at the appropriate time.

Please do not hesitate to contact me, if you would like any further information.

Sincerely,

Lynda Wood

Lynda Wood
Account Manager
Research Partnerships Program

Telephone: (613) 944-4570
Fax: (613) 992-5337
E-Mail: Lynda.Wood@nserc.ca

LW/jac

Cc: J. Vasseur, Research, McGill
T. Monteiro, Finance, McGill
R. Bruno, ILO, McGill

Canada

McGill University

APPLICATION TO USE BIOHAZARDOUS MATERIALS*

Projects involving potentially biohazardous materials should not be commenced without approval from the Environmental Safety Office. Submit applications before 1) starting new projects, 2) renewing existing projects, or 3) changing the nature of the biohazardous materials within existing projects.

1. PRINCIPAL INVESTIGATOR: Dr. Satya Prakash PHONE: 514-398-3676

DEPARTMENT: Biomedical Engineering FAX: 514-398-7461

ADDRESS: 3755 University Street E-MAIL: satya.prakash@mcgill.ca

PROJECT TITLE: Oral artificial cells containing live genetically engineered Lactobacillus plantarum 80 bacterial cells for lowering cholesterol.

2. EMERGENCY: Person(s) designated to handle emergencies

Name: Dr. Satya Prakash Phone No: work: 514-398-3676 home: 450-465-5939

Name: Mr. Christopher Martoni Phone No: work: 514-398-2736 home: (514) 793-2831

3. FUNDING SOURCE OR AGENCY (specify): Micropharma

Grant No.: _____ Beginning date: 01-06-2006 End date: 31-05-2008

4. Indicate if this is

☐ Renewal: procedures previously approved without alterations.

Approval End Date: _____

☒ New funding source: project previously reviewed and approved under an application to another agency.

Agency: Micropharma Approval End Date: 31-05-2008

☐ New project: project not previously reviewed.

☒ Approved project: change in biohazardous materials or procedures.

☐ Work/project involving biohazardous materials in teaching/diagnostics.

CERTIFICATION STATEMENT: The Environmental Safety Office approves the experimental procedures proposed and certifies with the applicant that the experiment will be in accordance with the principles outlined in Health Canada's "Laboratory Biosafety Guidelines" and in the "McGill Laboratory Biosafety Manual".

Containment Level (select one): ☒ 1 ☐ 2 ☐ 2 with additional precautions ☐ 3

Principal Investigator or course director: Satya Prakash (SIGNATURE) date: 30 (day) / 03 (month) / 07 (year)

Approved by: Environmental Safety Office: D. St Louis (SIGNATURE) date: 30 (day) 03 (month) 07 (year)

Expiry: 31 (day) 05 (month) 08 (year)

*as defined in the "McGill Laboratory Biosafety Manual"

5. RESEARCH PERSONNEL: (attach additional sheets if preferred)

Name	Department	Job Title/Classification	Attended *Safe Use of Biological Safety Cabinets* seminar? If yes, indicate date of attendance
Mr. Christopher Martoni	Biomedical Engineering	Graduate Student	Yes
Ms. Alexndra Urbenska	Biomedical Engineering	Graduate Student	Yes
Ms. Jasmine Bhathena	Biomedical Engineering	Graduate Student	Yes
Mr. Arun Kulamarva	Biomedical Engineering	Graduate Student	Yes
Ms. M. Malhotra	Biomedical Engineering	Graduate Student	No (Will attend in May)
Ms. Safaa Sebak	Biomedical Engineering	Graduate Student	No (Will attend in May)

6. Briefly describe:

i) the biohazardous material involved (e.g. bacteria, viruses, human tissues, toxins of biological origin) & designated biosafety risk group

Recent modalities for lowering blood cholesterol levels involve dietary management, behavior modification, exercise, and drug therapy. Pharmacologic agents such as statins are considered to have the most potential for clinical lowering of cholesterol. Although statins effectively reduce cholesterol levels, they are very expensive, and are known to have side effects including an association with extensive morbidity. A less well known approach to reducing blood cholesterol, oral live bacterial cell therapy, is based on the demonstration that naturally occurring bacteria such as Lactobacillus plantarum 80 (pCBH1) can lower cholesterol levels significantly. Indeed a number of studies have confirmed this capacity and it has been found that oral daily intake of live Lactobacillus cells can lead to significant reduction in cholesterol levels. Although the mechanisms are not entirely understood, it is likely the result of an exclusively intestinal mode of action on bile salts in the intestinal lumen. It has been reported that using these and related methods, cholesterol levels can be reduced by 22% to 33%. However, the therapeutic potential of live bacterial cells has been hampered by inherent limitations in their use. For example, of those bacteria ingested only 1% survive gastric transit limiting the overall therapeutic effect. Also, there are some practical concerns regarding the production, cost, and storage of such a product. Furthermore, oral administration of live bacterial cells can cause a host immune response, and can be detrimentally retained in the intestine, replacing the normal intestinal flora. Thus, concerns of safety have prevented regular use of this promising therapy in clinical practice. In the present project we propose treatment modalities, for high blood serum cholesterol, based on the use of the microencapsulated Lactobacillus plantarum 80 (pCBH1) bacterial cells. Bcateria used is a non-pathogenic Lactobacillus bile salt hydrolase (BSH) overproducing bacteria and at the same time circumvent, to a large extent, associated problems with their use. Studies thus far have shown that microencapsulated Lactobacillus can break down bile salts much like the free bacteria and can lower total cholesterol, LDL cholesterol and reduce atherogenic risk in hamsters with high cholesterol. We are now in the process of optimizing dosage and understanding the mode of action in male Golden Syrian hamsters. These and other studeis using standard biohazard material purchase from ATCC will be performed.

ii) the procedures involving biohazards

Standard bacterial microbiology procedures

iii) the protocol for decontaminating spills

Standard laboraory deontamination protocol will be followed.

7. Does the protocol present conditions (e.g. handling of large volumes or high concentrations of pathogens) that could increase the hazards?

No

8. Do the specific procedures to be employed involving genetically engineered organisms have a history of safe use?

Yes.

9. What precautions will be taken to reduce production of infectious droplets and aerosols?

Individual performing above reserach task will follow McGill University standard laboratory safety procedure ; as such no droplet or aerosol are expected to generate during experiments.

10. Will the biohazardous materials in this project expose members of the research team to any risks that might require special training, vaccination or other protective measures? If yes, please explain.

No

11. Will this project produce combined hazardous waste – i.e. radioactive biohazardous waste, biohazardous animal carcasses contaminated with toxic chemicals, etc.? If yes, please explain how disposal will be handled.

No

12. List the biological safety cabinets to be used.

Building	Room No.	Manufacturer	Model No.	Serial No.	Date Certified
Duff Building	322	Microzone Corporation	BK-2-4	801-4508	May, 2006
Duff Building	322	ESCO	AVC-242	2005-10436	June, 2006