Widener University: Institutional Animal Care and Use Committee
Project Application Form

1. Name and Department of Applicant (faculty member or course instructor):

Students: Muthuramanan Rameswaran, Hareth Madhoun, Tejal Mehta

2. Title of Project: Is Atrazine an innate immune system disruptor in adult Northern Leopard Frogs (*Rana pipiens*)?

3. Is this a new application, revision or renewal?

new application

Date and approval number of last application? n/a

4. If this is a course project, please indicate:

Course Name and Number	Instructors	Estimated Enrollment
Biology 401, fall 2003	Itzick Vatnick and Bruce Grant	16

5. Dates of Project (1 Year):

From: October, 2003 To: October, 2004

6A. Statement of qualification of applicant. Provide a biographical statement of the experience and training of applicant for the procedures described below.

I have worked under Dr. Itzick Vatnick and Dr. Marc Brodkin as a research assistant for 2 years. I have been made aware of proper care of the animals from my predecessors as well as both my faculty research advisors.

6B . Names and positions of persons (e.g. students) authorized by the applicant to participate in the procedures described below. Also, name the person responsible for instruction in the care and use of laboratory animals of each research participant.

Name	Title	Instruction in Animal Handling by:
Hareth Madhoun	Research Assistant	Drs. Vatnick and Brodkin
Muthuramanan Rameswaran	Research Assistant	Drs. Vatnick and Brodkin
Tejal Mehta	Student	Drs. Vatnick and Brodkin

7A. If this is a research project, supply an abstract of the project. Abstract should be written in terms understandable by a non-scientist. Describe the overall purpose of the project and the importance of the research.

7B. If this is a course project, provide a course description. Also, append a copy of the syllabus of the course.

Course Objectives and Description.

(1) You will better understand the evolutionary adaptation of physiology to environment, i.e., the problems that the biophysical extremes of this world impose upon life and the solutions that have evolved in response.

This course will deal with the evolutionary adaptation of physiology to environment (e.g., temperature, water, light, salinity, pressure, nutrients, and toxins). Students will learn how individuals interact with their biophysical environment, and how physiological processes offer integrated responses to environmental stress. Examples will illustrate adaptation to warm desert environments, arctic and alpine environments, freshwater and saline wetlands, deep sea environments, and human-disturbed environments. Although topics represent the extremes of this world, an important message is that over evolutionary time all environments are variable and the evolutionary rules affecting physiology at the extremes apply everywhere life is always at the edge.

Underlying themes of this course are the similarity of solutions to common problems (such as adaptations to water stress in deserts and saline wetlands) and the multiplicity of solutions evolved among life forms to the same environmental stress (such as the diversity of temperature regulation modes in thermally heterogeneous environments). We will primarily focus the course on animals; however, moneran, protistan, fungal, and plant adaptations to environmental stress are also excellent sources of independent research projects (described below) and we encourage students to pursue whatever interests them.

A major venue for the teaching and learning of these basic principles of physiological design will be a seminar-style weekly discussion class of primary literature from the field of physiological ecology. For this, you must locate, interpret, and critically analyze published journal articles relevant to the course topics. More details on this will be presented in class.

(2) You will understand the process of physiological inquiry which is the scientific method, through which natural phenomena are observed, interpreted, and reported. Through the scientific method one gains an understanding of our natural world and the effects of human activities upon it.

A major portion of this course is dedicated to student-designed independent research projects involving laboratory experiments on any topic of the your interest in physiological ecology, and using available equipment funded by an NSF ILI grant to Dr's. Vatnick and Grant. You will select a research topic, make an oral proposal presentation, conduct the research, and present an oral talk and poster at in-class research symposia toward the end of the semester. In addition, you must locate and interpret background literature relevant to each your research project and lead a group discussion of your literature during one of the weekly lab meetings/ seminars.

(3) You will improve your cognitive skills at critical thinking and reflective judgement. Through the processes of designing, conducting, and presenting your own course research project, and of reading and critically analyzing published research results to understand the basic ecological and evolutionary principles of physiological design, you will be using and improving your higher level thinking and information processing skills.

These are the kinds of "good thinking" skills that are essential in grappling with "problems for which no clear-cut solution can be identified by using only inductive or deductive logic; solutions to these problems involve other characteristics of reasoning." (King and Kitchener 1994). In this course, you will construct your own understanding of physiological phenomena (as constrained, facilitated, and calibrated by us). Through this process, you will be challenged by complex, multifaceted, novel, and unexpected conceptual, scientific, educational, social, and technical problems. This is what research is all about. You'll need to use your head to solve these problems. Intellectual growth will result.

8. Give the specific reasons why live animals must be used for this study. Are alternative methods available (e.g. computer simulations, cell or tissue culture)? If so, why are they not used?

In order to assess immunological and metabolic response of a frog, it must be a live animal. Alternative methods are not available.

9A. Animals to be used in this protocol. Numbers used may be estimates. If needed, base estimates on usage in previous years.

Species/Strain	No. / Year	Sex	Age	Weight
Rana pipiens	40 /2003	M/F	Adult	30 grams

9B. How are the animals obtained? Where and how are they housed?

Animals purchased by Dr. Vatnick from Amphibians of North America.

10. Provide complete details on each procedure involving the species listed under section 9A. The description of each procedure should supply the category of animal utilization (see appendix). If drugs or anesthetics are to be used, provide dosage and duration of treatment. As appropriate, identify all aspects of post-procedural care, including euthanasia, and describe procedures for identification and intervention in the care and use of animals if painful or stressful outcomes are anticipated. For course projects, refer to the course syllabus as possible.

We will randomly allocate frogs to four experimental groups: an atrazine exposed group at 21ppb, an atrazine exposed group at 21ppb followed by thioglycollate stimulation, a group exposed to physiological saline, and a group exposed to physiological saline followed by stimulation with thioglycollate. Thioglycollate is a substance used widely to stimulate an experimentally induced inflammatory response. The thioglycollate solution and the saline solution will contain one-micron beads tagged with fluorescein isothiocyanate (FITC) beads. These beads are similar in size to pathogenic bacteria and fluoresce in UV light. After eight days of exposure we will perform an intraperitoneal lavage to flushed intraperitoneal white blood cells (WBC). This will involve anesthetizing the animals with diethyl ether for a period of 5 minutes. We will count the number of cells and assess their phagocytic efficiency by counting the number of beads they ingested. We predict that immune response and phagocytic efficiency in atrazine-enriched environments will be diminished, thereby atrazine may be contributing to amphibian decline by more than one mechanism.

11. I hereby certify that the above information is accurate. The care and use of animals proposed will abide by the National Research Council guidelines published in the *Guide for the Care and Use of Laboratory Animals*.

Names Title:

Muthuramanan Rameswaran	Research Assistant
Hareth Madhoun	Research Assistant
Tejal Mehta	Student

Signature_____ Date October 14, 2003

The signature of the Associate Dean of Science is required.

Name Marc Brodkin Title Associate Dean of Science

Signature_____ Date

APPENDIX

CATEGORIES OF USE LEVEL FOR APPLICATIONS UTILIZING VERTEBRATE ANIMALS IN RESEARCH TESTING AND INSTRUCTION.

CATEGORY A - Experiments on vertebrate animal species that are expected to produce little or no discomfort.

Mere holding of animals captive for experimental purposes; simple procedures such as injections of relatively harmless substances; blood sampling; physical examinations; food/water deprivation for shore periods (a few hours); standard methods of euthanasia that induce rapid unconsciousness, such as anesthetic overdose or decapitation preceded by sedation or light anesthesia.

CATEGORY B - Experiments that involve some minor stress or pain (short-duration pain) to vertebrate animal species.

Experiments on completely anesthetized animals which do not regain consciousness; with anesthesia and subsequent recovery, exposure of blood vessels or implantation of chronic catheters behavioral experiments on awake animals that involve short-term stressful restraint; immunization employing Freund's Adjuvant; noxious stimuli from which escape is possible; major surgical procedures under anesthesia that result in post-operative discomfort that is treated with analgesics. Category B procedures incur additional concern in proportion to the degree and duration of unavoidable stress or discomfort.

CATEGORY C - Experiments that involve significant but unavoidable stress or pain to vertebrate animal species.

Deliberate induction of behavioral stress in order to test its effect; major surgical procedures under anesthesia that result in significant post-operative discomfort that is not treated with analgesics; induction of an anatomical or physiological deficit that will result in pain or distress; application of noxious stimuli from which escape is impossible for prolonged periods (up to several hours or more) or physical restraint; maternal deprivation with substitution of punitive surrogates; induction of aggressive behavior leading to self-mutilation or intra-species aggression; procedures that produce pain in which anesthetics are not used, such as toxicity testing with death as an end point, production of radiation sickness, certain injections, and stress and shock research that would result in pain approaching the pain tolerance threshold, i.e. the point at which intense emotional reactions occur. Category C experiments present an explicit responsibility on the investigator to explore alternative designs to ensure that animal distress is minimized or eliminated.

CATEGORY D - Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals.

Use of muscle relaxants or paralytic drugs such as succinyl choline or other curariform drugs used alone or surgical restrain without the use of anesthetics; severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior; killing by use of microwave ovens designed for domestic kitchens or by strychnine; inescapably severe stress or terminal stress.