

Widener University - Institutional Animal Care and Use Committee

Animal Care Standard Operating Procedures

1. Name and Department of Applicant (faculty member): Itzick Vatnick and Bruce Grant
Biology Department

Students: Muthuramanan Rameswaran, Hareth Madhoun, Tejal Mehta

2. Name and Approval Number of IACUC Animal Care Form

3. Identify the species of animals covered by this SOP.
Rana pipiens The Northern Leopard Frog

4. If the animals are to be used in a course project, please indicate:

| <u>Course Name and Number</u> | <u>Instructor</u> | <u>Estimated Enrollment</u> |
|-------------------------------|--------------------------------|-----------------------------|
| Biology 401, fall 2003 | Itzick Vatnick and Bruce Grant | 16 |

5. How are animals obtained?

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

6. Briefly describe the usage of the animals.

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.

7. **Animal Care Standard Operating Procedures**

Location and Environmental Conditions of Animal Enclosures

Animals will be housed in room 503 at room temperature when not in experimental treatment

During Experimental treatment frogs will be placed in an environmental chamber in room 423 at 25°C

Describe Enclosures (cages, pens, crates, tanks)

Frogs will be kept in 10 gallon tanks and during experimental treatment they will be placed in sterilized rubbermaid containers.

Describe Feeding and Water Regime. (How much? How often?)

Frogs are fed three times a week, and water is changed five times a week.

During experimental procedure frogs will not be fed, and sterile buffer is changed on a daily basis

Describe sanitation practices. (Any daily or periodic procedures such as water changes, bedding changes, cage washing)

On a daily basis frog tanks will be slowly inverted with a tank cover in place. The water will be let out of the top of the tank and a ½ gallon of aged tap water will be replaced on a daily basis.

How many animals are placed in each enclosure? What size are the animals?

Eight is the maximum number of frogs placed in a tank. During experimental treatment each container will contain one frog. The frogs are around 7-10 cm and weigh about 30 grams per frog

8. What are the clinical signs or circumstances that would indicate animal stress or disease?
Lethargy of the animal is indicative of animal stress in *Rana pipiens*.
9. What are the procedures for handling and/or euthanizing sick animals?
In order to euthanize frogs we place dihydroxy ether into a closed container and place the frog into the container.
10. What is the fate of animals that survive the experimental or instructional use?
The animals will be used for further experimentation deemed necessary by our faculty advisors.
11. If known, list personnel who will care for these animals.

| <u>Name</u> | <u>Title</u> | <u>Phone</u> |
|-------------------------|--------------------|--------------|
| Muthuramanan Rameswaran | Research Assistant | x 7513 |
| Hareth Madhoun | Research Assistant | x 7513 |

12. I hereby certify that I am responsible for the care of the animals described in this SOP. I will properly train all personnel in animal care. The care of animals will abide by the National Research Council guidelines published in the *Guide for the Care and Use of Laboratory Animals*.

Names

Muthuramanan Rameswaran
Hareth Madhoun
Tejal Mehta

Title:

Research Assistant
Research Assistant
Student

Signature _____

Date 10/14/03

NOTE:

Once approved, this form will be kept on file with the Associate Dean of Science. An approved Animal Care SOP is required before animals are obtained.