REQUIRED FORMAT

REPORT FOLLOWING SABBATICAL

**Name:** [redacted] **Campus/ Department:** [redacted] Campus / Natural Sciences Department

**Period of Sabbatical:** Fall 2013

**Attach the Statement of Purpose as approved by the Sabbatical Review Committee.** (see attached below)

**A Narrative on Activity and Findings**

I conducted research pertaining to the additive, synergistic and cumulative effects of toxins in the soil nematode *Caenorhabditis elegans* during the Fall Semester of 2103. This resulted in the establishment of an experimental protocol to test the additive and synergistic effects of arsenic and mercury toxicity in *C. elegans*, and in the completion of eight trials of the established protocol. The novel approach to toxicity testing in the project lies in the addition of synergistic toxins to the testing regimen in an animal in which the effects of a wide range of toxins can be detected including carcinogens, clastogens, endocrine disruptors, mitogens, mutagens and teratogens.

After a series of developmental trials that established concentrations of the two toxins that produced dose response relationships suitable for testing their additive affects, three replicates were performed. Each replicate was performed with three control groups, three groups exposed to 10 mM / 50 mM / 100 mM arsenic respectively, three groups exposed to 10 mM / 50 mM / 100 mM mercury respectively, and three groups exposed to 10 mM arsenic and 10 mM mercury / 50 mM arsenic and 50 mM mercury / 100 mM arsenic and 100 mM mercury respectively. It was found that the mortality rate was significantly higher in the worms exposed to both mercury and arsenic than arsenic alone at all three concentrations in all three replicates at the 0.001 level of probability using Chi square analysis.

Table 1: Arsenic Toxicity Compared to the Additive Toxicity of Arsenic and Mercury in *C. elegans*

| Replicates & Concentrations | Arsenic mortality | Arsenic not mortal | Arsenic & Mercury Hg mortality | Arsenic & Mercury not mortal | Chi-square Rounded to a whole number |
|---|---|---|---|---|---|
| R1 100mM | 28 | 68 | 86 | 9 | 14270 |
| R1 50 mM | 22 | 58 | 65 | 26 | 5621 |
| R1 10 mM | 7 | 51 | 66 | 38 | 6435 |
| R2 100 mM | 38 | 53 | 41 | 9 | 2990 |
| R2 50 mM | 36 | 51 | 14 | 6 | 572 |
| R2 10 mM | 25 | 47 | 11 | 14 | 66 |
| R3 100 mM | 23 | 53 | 48 | 24 | 2905 |
| R3 50 mM | 14 | 39 | 49 | 34 | 1882 |
| R3 10 mM | 7 | 51 | 30 | 43 | 1760 |

Note: all Chi-square values in the above table are significant at the 0.001 level of probability.

I intend for the findings of this research to result in a publication in a peer reviewed science journal, and for the dissemination of the laboratory methodology that will result in the continued production of scientific data and publication of the additive effects of toxins. I also intend for this research to result in the development of a laboratory science exercise for embedded research in biology laboratory courses.

The experimental protocol established for this study is as follows: Worms were chunked from a petri dish containing a population of established worms and left to grow for two or three days. The worms were then synchronized by bleaching and harvesting the eggs from the dead worms and leaving them to hatch and grow overnight according to an adaptation of the method by Sulston & Hodgkin (1988). The worms were plated the next day onto OP50 plates and left to grow for two days. The worms were then treated overnight in ninety well plates, again according to an adaptation of the method by Sulston & Hodgkin (1988), using three control groups exposed to distilled water, three groups exposed to 10 mM / 50 mM / 100 mM arsenic respectively, three groups exposed to 10 mM / 50 mM / 100 mM mercury respectively, and three groups exposed to 10 mM arsenic and 10 mM mercury / 50 mM arsenic and 50 mM mercury / 100 mM arsenic and 100 mM mercury respectively. Live and dead worms in each well were counted the next day and the mortality rates were established.

The experimental protocol for synchronization by bleaching adapted from Sulston & Hodgkin (1988) is as follows: A fresh M9 20% alkaline hypochlorite solution was prepared before each experiment. The hypochlorite stock solution was replaced regularly so it was always less than one month old. On day one of the procedure, worms were chunked onto five seeded NGM/OP50 plates. They were allowed to grow two to three days so that there were plenty of eggs and gravid adults on the plates. On day two of the procedure the plates were checked to make sure that there were plenty of adult worms. 5 ml of M9 solution was poured onto the plates and gently swirled to dislodge the worms. The solution containing the dislodged worms from the five plates were transferred to two 15 ml conical tubes by pipet. Only a 1000ul pipet was used to transfer worms so they would not be killed in transfer. The worms were centrifuged for one minute at 4000 rpm to pellet the worms. Most of the M9 solution was aspirated without disturbing the pellet. The worms were re-suspended using M9 solution and combined to one tube, centrifuged and aspirated again. The worms were re-suspended in a 15 ml of a 20% alkaline hypochlorite solution. This solution was prepared by mixing 82.5 ml of distilled water to 37.5 ml 1M sodium hydroxide and 30 ml of 6% non-germicidal bleach. The tube was mixed by inverting gently by hand for no more than five minutes so the adults would dissolve but the eggs would not be killed. They were then centrifuged for one minute. The 20% alkaline hypochlorite solution was aspirated without disturbing the worm pellet. 15 ml of M9 solution was added to the tube, mixed well, centrifuged for one minute and aspirated in order to wash out the hypochlorite solution. This wash was repeated to dilute any remaining hypochlorite. 7 ml of fresh m9 was added and the tube was agitated to re-suspend the worm pellet. The eggs were left to hatch overnight with gently rocking. Since there was no food present the larvae were halted in the L1 stage. On day 3 of the protocol the workplace was cleaned with ethanol. The tube was centrifuged for one minute at 4000rpm. The M9 solution was aspirated leaving 1 ml in the tube. The pellet was re-suspended and 200 ul was plated onto each of five NGM/OP50 plates using a 1000 ml pipet. The plates were inverted after the liquid was absorbed into the media, and left to grow for two days.

The experimental protocol for treatment of worms in 90 well plates adapted from Sulston & Hodgkin (1988) is as follows: The five plates from the synchronization were washed with 3 to 4 mls of M9 solution. The wash was collected and transferred into a 15 ml centrifuged tube and labeled. The tube was then centrifuged for one minute at 4000 rpm. The wash was aspirated to 4 mls and the pellet was re-suspended. The wash was pipetted up and down to ensure that an even concentration distribution of worms was in the pipet, and 2 mls of the wash solution containing nematodes was put into a separate centrifuge tube. One tube was labelled PMA. 200 ul of water was added to one tube and 200 ul of a phenylmercaptoacetamide (PMA) solution was added to the other. The tubes were incubated at room temperature for thirty minutes while being constantly inverted on an inversion plate. After incubation the nematodes were centrifuged for one minute at 4000 rpm. The supernatant was discarded from both tubes, and they were filled with M9 solution and centrifuged for 1 minute at 1000rpm to separate the adult from young worms. This wash was repeated an additional three times. After the final wash, the tubes were aspirated to 3 ml. The worms were re-suspended and aliquoted into three additional centrifuge tubes to make a total of four tubes with close densities of worms. This was done for both the PMA and non-PMA treated washes. A 96 well plate was labeled and nematode wash solutions and treatments were added so that each well contained 100 ul. Each 96 well plate contained control groups exposed to distilled water, groups exposed to 10 mM / 50 mM / 100 mM arsenic respectively, groups exposed to 10 mM / 50 mM / 100 mM mercury respectively, and groups exposed to 10 mM arsenic and 10 mM mercury / 50 mM arsenic and 50 mM mercury / 100 mM arsenic and 100 mM mercury respectively. PMA treated groups were exposed to 10 mM / 50 mM / 100 mM arsenic respectively in order to test whether PMA has a protective effect against arsenic toxicity. Live and dead worms in each well were counted the next day and the mortality rates were established.

M9 stock solution was prepared by adding 5.8 grams of $Na_2HPO_4 \cdot 7H_2O$, 3.0 grams of KH2PO4, 5.0 grams NaCl, 0.25 grams $MgSO_4 \cdot 7H_2O$ and distilled water to 1 liter. It was then filtered (0.22um) into a sterile bottle.

Nematode Growth Media (NGM) plates were prepared by autoclaving 1.5 grams of NaCl, 8.5 grams agar, 1.25 grams peptone in 500 ml distilled water in a one liter flask, and then using sterile technique to add 0.5 ml 1M CaCl, 0.5 ml of 0.5M MgSO4, 0.5 ml of 5mg/ml cholesterol and 12.5 ml of 1M KPO4 (pH6). This was poured into petri dishes and left to solidify. NGM/OP50 plates were prepared by streaking 90 ul of the OP50 strain of *Escherichia coli* onto twelve NGM plates using sterile technique and left overnight to grow at room temperature.

Table 2: 96 well plate arrangement with controls and treatments with arsenic, arsenic & PMA, mercury and arsenic / mercury combination.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ |
| 90ul wash/ 10ul As solution | 90ul wash/ 10ul As solution | 90ul wash/ 10ul As solution | 90ul wash/ 10ul As solution | 90ul wash/ 10ul As solution | 90ul wash/ 10ul Hg solution | 90ul wash/ 10ul Hg solution | 90ul wash/ 10ul Hg solution | 90ul wash/ 10ul Hg solution | 90ul wash/ 10ul Hg solution |
| 95ul wash/ 5ul As solution | 95ul wash/ 5ul As solution | 95ul wash/ 5ul As solution | 95ul wash/ 5ul As solution | 95ul wash/ 5ul As solution | 95ul wash/ 5ul Hg solution | 95ul wash/ 5ul Hg solution | 95ul wash/ 5ul Hg solution | 95ul wash/ 5ul Hg solution | 95ul wash/ 5ul Hg solution |
| 99ul wash/ 1ul $dH_2O$ | 99ul wash/ 1ul As solution | 99ul wash/ 1ul As solution | 99ul wash/ 1ul As solution | 99ul wash/ 1ul As solution | 99ul wash/ 1ul Hg solution | 99ul wash/ 1ul Hg solution | 99ul wash/ 1ul Hg solution | 99ul wash/ 1ul Hg solution | 99ul wash/ 1ul Hg solution |
| 90ul PMA wash/ 10ul $dH_2O$ | 90ul PMA wash/ 10ul $dH_2O$ | 90ul PMA wash/ 10ul $dH_2O$ | 90ul PMA wash/ 10ul $dH_2O$ | 90ul PMA wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ | 90ul wash/ 10ul $dH_2O$ |
| 90ul PMA wash/ 10ul As solution | 90ul PMA wash/ 10ul As solution | 90ul PMA wash/ 10ul As solution | 90ul PMA wash/ 10ul As solution | 90ul PMA wash/ 10ul As solution | 80ul wash/ 10ul As/ 10ul Hg solution | 80ul wash/ 10ul As/ 10ul Hg solution | 80ul wash/ 10ul As/ 10ul Hg solution | 80ul wash/ 10ul As/ 10ul Hg solution | 80ul wash/ 10ul As/ 10ul Hg solution |
| 95ul PMA wash/ 5ul As solution | 95ul PMA wash/ 5ul As solution | 95ul PMA wash/ 5ul As solution | 95ul PMA wash/ 5ul As solution | 95ul PMA wash/ 5ul As solution | 90ul wash/ 5ul As/ 5ul Hg solution | 90ul wash/ 5ul As/ 5ul Hg solution | 90ul wash/ 5ul As/ 5ul Hg solution | 90ul wash/ 5ul As/ 5ul Hg solution | 90ul wash/ 5ul As/ 5ul Hg solution |
| 99ul wash/ 1ul PMA solution | 99ul wash/ 1ul PMA solution | 99ul wash/ 1ul PMA solution | 99ul wash/ 1ul PMA solution | 99ul wash/ 1ul PMA solution | 98ul wash/ 1ul As/1ul Hg solution | 98ul wash/ 1ul As/1ul Hg solution | 98ul wash/ 1ul As/1ul Hg solution | 98ul wash/ 1ul As/1ul Hg solution | 98ul wash/ 1ul As/1ul Hg solution |
| | | | | | | | | | |

In preparation for this work I completed the 'Silencing Genomes' course during the summer of 2011 at Cold Spring Harbor Laboratories Dolen DNA Learning Center. This course was designed to train scientists to work with *C. elegans* in the laboratory for research purposes through the application of RNA interference (RNAi). I have also established collaboration with Dr. Fernando Nieto at S.U.N.Y. Old

Westbury who works with the testing of arsenic toxicity in *C. elegans*. Systems for arsenic detoxification are found in all organisms (Rosen 2002). *C. elegans* mutants have been found to be sensitive to arsenic toxicity (Liao & Yu 2005), and mutations have been found that compromise arsenic detoxification in *C. elegans* (Vatamaniuk et al. 2002). Biological pathways can be affected by more than one toxin, and multiple biological outcomes have been detected by exposure to individual toxins (Roh et al. 2007). Different pathways simultaneously affected could result in a synergistic outcome. With more than one hundred thousand chemicals on the marketplace (Kuo et al. 2012), and many commonly used food additives being of toxicological concern (Kobylewski & Jacobson 2012), there is room for a wide margin of era when considering the traditional regulatory methods.

In addition, this work has also resulted in the writing of two letters of recommendation for undergraduate students from SUNY Old Westbury. Working with these students in the laboratory has provided me with the experience of how students are able to work with this pedagogy, and has strengthened the collaborative relationship with the faculty of SUNY Old Westbury.

**B Professional Benefit to Applicant**

This activity has provided me with the continued training necessary to work with *C. elegans* and current toxicological testing methodology. It has also provided me with the establishment of a collaborative relationship with faculty at SUNY Old Westbury, and continued access to a research laboratory necessary for further develop of this protocol and the development of future protocols using *C. elegans* as a model organism in research and embedded research pedagogy. It has resulted in the collection of data necessary to prepare a manuscript for publication in a peer reviewed journal, and a methodology and collaboration for the continuous collection of data and additional publications. In addition, it has resulted in the collection of data necessary to pursue research in the additive effects of toxins in other organisms as well that may be less expensive with which to work here at SCCC. This data can also be used as pilot data for a future grant application. I am planning on continuing research to test the additive effects of carcinogens in the *RAD 51* and *RAD 52* double strand break recombination repair genes in yeast which are the *BRCA1* and *BRAC2* breast cancer gene homologs. I have nine years of experience working with these genes in a yeast system from my dissertation work.

This activity has also afforded me the opportunity to professionally practice the scientific method in accord with the training within my discipline. Such practice is necessary for maximizing continued competence within the field and the ability to instruct college courses. Laboratory work and research design is not just based on knowledge but skill. Continuing practice of such skill maintains competence within the fields of science and the ability to design and implement new pedagogy into the laboratory curriculum.

**C Benefit to College**

The result of the findings of this research will be disseminated to the student population at SCCC through Colloquia presentations at the three campuses. It is also expected to result in annual presentations at Colloquia due to the continued data collection, analysis and publications. In addition, the development of toxicity testing in *C. elegans* and the development of additive toxicity testing can be used to develop pedagogy for embedded research in SCCC biology laboratory courses. Rios-Velazquez et al. (2011) developed an undergraduate research workshop in which students did experiments with soil micro-organisms to collect novel data, and "pre- and post-workshop assessments indicated student learning gains in technical knowledge, skills and confidence in a research environment". This work was published in the *Journal of Microbiology & Biology Education*. My project is expected to result in the continued publication of both research papers in journals of toxicology and in journals of education as well.

In preparation for developing an embedded research project for SCCC I attended the Council on Undergraduate Research (CUR) conference held at Queensborough Community College (www.cur.org). I then designed and implemented an embedded research project for the Principles of Biology (BIO101) course at SCCC on the Michael J. Grant Campus. I then developed the *Science and Technology Undergraduate Research Notes (Saturn) Journal* (www.saturnjournal.org), and over eighty of my students to date have collaborated on over thirty publications in the *SATURN Journal*.

SCCC benefits from the maintenance of the skills of its faculty who teach these skills to the student population. Keeping these skills competent in faculty is necessary to provide up to date instruction and curriculum development in science and technology.

**D Describe the Current Status of the Project**

Three replicates were performed that compared the mortality of arsenic exposure to the exposure of arsenic in the presence of mercury in *Caenorhabditis elegans*. Each experiment was performed with three control groups, three groups exposed to 10 mM / 50 mM / 100 mM arsenic respectively, three groups exposed to 10 mM / 50 mM / 100 mM mercury respectively, and three groups exposed to 10 mM arsenic and 10 mM mercury / 50 mM arsenic and 50 mM mercury / 100 mM arsenic and 100 mM mercury respectively. It was found that the mortality rate was significantly higher in the worms exposed to both mercury and arsenic than arsenic alone at all three concentrations in all three replicates at the 0.001 level of probability using Chi square analysis. These findings are now being applied to the completion of a research paper authored by my collaborators at SUNY Old Westbury and myself to be entitled '*Caenorhabditis elegans* as a Model Organism to Test the Additive Effects of Toxins using Mercury and Arsenic'. Planning for continued testing of the additive effects of toxins and the role of this testing in undergraduate science pedagogy is underway.

**References:**


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