RADIORESISTANCE OF MOSQUITOS EXPOSED TO CONTINUOUS SUB-LETHAL DOSES OF IONIZING RADIATION

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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May 2016

Major Subject: Health Physics

ABSTRACT

This experiment is an investigation of the mosquito's ability to adapt to high levels of background gamma radiation. Radiation was used as a selective pressure to induce radioresistance among a group of *Aedes aegypti* mosquitos. Mosquitos were grouped into a High Background Group (HBG) or a Low Background Group (LBG), the LBG being the control group. The HBG was exposed to a continuous field of ionizing gamma radiation significantly higher than the normal background radiation level for the Bryan/ College station (B/CS) area. The HBG spent more than 23 hours per day exposed to the increased levels of radiation. The dose rate ranged from 36 rad/day to 28 rad/day over the course of several weeks. The radiation exposure to the mosquitos began at the larval stages and continued throughout the adult stages of the mosquito's lifecycle. During the developmental stages of life living tissues are most susceptible to radiation. Thus by delivering radiation doses during those stages, it provided greater opportunity to induce radioresistance in the mosquitos DNA. Mosquitos in the LBG were reared using the same techniques as the HBG, but in radiation levels similar to that of typical background radiation levels in the B/CS area. The average normal background dose rate at the mosquito lab location is 24 mrem/ year.

After mosquitos in the HBG obtained a total dose of approximately 1000 rads over the course of several weeks, the HBG was exposed to a challenge dose provided by a high dose rate gamma source which delivered a total dose of about 70,000 rads over

the course of 7-10 hours. Additionally, mosquitos in the LBG were also exposed to the same challenge dose for an equivalent amount of total dose, ±10%.

Upon completion of the challenge dose, the LBG was cared for utilizing the same methods as before. The HBG, however, did not receive additional high doses of radiation and were only exposed to background radiation similar to the exposure to the LBG. Survival percentages were documented and compared immediately following the challenge dose and continued until all mosquitoes perished.

The mosquitos in the HBG consistently had higher survival rates when compared to the LBG. For mosquitos in the first round LBG, 50% lethality was reached on Day 3 post challenge dose. Mosquitos in the second round LBG, 50% lethality on Day 1 post challenge dose. After the challenge exposures to the LBG, 62% of round one and 72% of round two survived the duration of the exposure. Mosquitos in the first round HBG reached 50% lethality on Day 6 post challenge dose. The second round HBG reached 50% lethality on Day 9 post challenge dose. After the challenge exposure to the HBG, 92% of round one and 100% of round two survived the duration of the exposure. One specific mosquito from the first HBG developed an abnormal abdominal cavity prior to the challenge dose, but it is not believed to have contributed to the mosquito's radioresistance.

DEDICATION

This thesis is dedicated to my family. Without their love and support, I never would have come this far. I especially want to mention my Dad; he has always been my mentor and my role model. This work is also dedicated my children; without them, I never would have had the motivation to make myself better. Most importantly, I dedicate this work to my husband, for always being by my side and supporting me throughout the years of juggling school, work, and family. Thanks babe, I couldn't have done it without you.

ACKNOWLEDGEMENTS

I would like to thank all those who have supported me through my time as a student, especially Dr. John Ford for his guidance during my studies, as well as the rest of my committee, Drs. Delia Perez and Nancy Turner. I would like to give a special thank you to the Nuclear Science Center for giving me the opportunity to complete my studies, and a thanks to Scott Miller and Cameron Macdonnell, who have always been there when I needed help.

Thanks also goes to the Texas A&M Entomology Department for taking the time to teach me methods for rearing the mosquitos.

NOMENCLATURE

B/CS	Bryan/College Station
HBG	High Background Group
LBG	Low Background Group
DNA	Deoxyribonucleic Acid
DDT	dichlorodiphenyltrichloroethane
RIR	Radiation-induced Resistance
NSC	Texas A&M Nuclear Science Center
NAA	Neutron Activation Analysis
R	Roentgen
Gy	Gray
Sv	Sievert
Sc	Scandium
Na	Sodium
La	Lanthanum
Cs	Cesium
Co	Cobalt

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1. INTRODUCTION

Resistance towards a variety of toxins resulting from chronic exposures has been observed throughout various living systems in nature, from microorganisms to complex species. Examples such as bacteria and head lice have shown increased thresholds regarding lethal doses to specific toxins when exposed for prolonged periods of time (Durand et al. 2012). Radiation is among the list of toxins that living organisms have shown an ability to develop a resistance towards. Effects from high doses of radiation have been studied since the first radiation injury, a mere 9 months after the discovery of the x-ray. In February 1896, Professor Daniel and Dr. Dudley placed an x-ray tube approximately 0.5 inches from Dudley's hair and exposed him for 1 hour. Twenty one days later, hair loss from the exposed area was observed (Sansare et al. 2011). Through research and observations, deterministic effects from radiation have been identified with associated thresholds. More than a century later, effects from radiation exposures below those thresholds are still a mystery.

In the United States alone, the average annual background radiation exposure has increased almost 5 times in the last 40 years. According to Merril Eisenbud, in 1973 the estimated annual background radiation dose in the United States was about 130 mrem/ year (Eisenbud, 1973). Today, the Nuclear Regulatory Commission estimates that the average annual background radiation dose in the United States in approximately 620 mrem/ year (NRC, 2015). The National Council on Radiation Protection and Measurements Report Number 160 states that the increased doses of background

radiation can be attributed to the increased use of computed tomography (CT) and interventional procedures since the 1980's (NCRP, 2009). In 1980, medical sources contributed to 15% of the total annual background radiation dose. In 2006, medical sources contributed to 48% of the total annual background radiation dose. The person/ Sv increased from 123,000 in the early 1980's to 899,000 in 2006. While the increased use of CT and conventional radiography and fluoroscopy contribute the most toward the increased background radiation doses, cigarette smoking and commercial air travel was incorporated into the 2006 updated annual radiation background estimate (NCRP, 2009).

The current regulatory guidance for radiation exposure assumes the linear non-threshold model. This assumes that any increased dose of radiation will increase the likelihood of future ill effects, *e.g.* cancer. Mitchel states that the assumption of linearity for most toxic agents has been challenged and argues that beneficial effects arise from exposure to low levels of a wide variety of agents, including radiation (Mitchel, 2006)

Effects from low levels of ionizing radiation in biological systems has been a focal point of numerous studies since the early 20th century (Calabrese, 2013). Cell lines derived from both humans and mice have demonstrated cellular adaptability to low doses of gamma radiation by activating repair mechanisms. Exposure to a low dose of radiation several hours prior to a significant radiation exposure has demonstrated an enhanced removal of the thymine glycols after the higher dose (Toprani and Birajalaxmi, 2015). Thus, it is evident that repair responses can be activated for radiation-induced damage. In addition to the normal induction of mRNA and typical protein expressions

found at lower doses of radiation, unidentified proteins have also been found activated by the preliminary radiation dose (Toprani and Birajalaxmi, 2015).

One theory concerning the potential effect of radiation exposure is the idea of radiation-induced hormesis. Hormesis is the theory that low doses of a toxin create beneficial effects for the living organism or species. The cell's response to low doses of radiation is considered an adaptive compensatory process following a disruption of the cell's homeostatic state (Mattson, 2008). This theory may or may not include responses such as an increased resistance towards that toxin. One hypothesis supporting hormesis states that above background radiation levels stimulate DNA repair mechanisms that not only compensate for the toxin effects from radiation, but could potentially prevent diseases due to exposure from other toxins (Gori and Munzel, 2011).

By the early 1950's interest in using insect models in biological systems would reveal evidence of a hormetic response to radiation exposure (Calabrese, 2013). Studies have reported increased longevity in insects subjected to repeated doses of low level ionizing radiation. Currently, there are few experiments that have studied the effects of a continuous exposure to non-lethal doses of radiation.

Disasters such as the Fukushima Daiichi reactor meltdown and the Chernobyl explosions have sparked a growing need to develop a better understanding of the effects of continuous radiation exposures. As of 2009, the background radiation levels in the city surrounding Chernobyl, Pripyat, were upwards of 2.6 μSv (0.26 mrem) per hour in areas surrounding Reactor 4; levels as high as 382 μSv (38.2 mrem) per hour were found in the basement of the local hospital (Hill-Gibbins, 2014). Humans are not authorized to

inhabit the city of Pripyat, but animals and insects live their entire lives in these environments. Insects with chronic exposure to high background radiation have exhibited physiological and developmental abnormalities from exposure to radioactive contaminants. Abnormality frequencies were, in some cases, 10 times higher in Chernobyl birds as compared to birds living in controlled areas (Mousseau et al. 2014).

Many studies have exposed insects to sub-lethal levels of ionizing radiation to study effects such as sterilization and other degenerative responses, and some studies have documented a higher survival rate when compared to insects that were not exposed to ionizing radiation. Helinski et al. 2009, investigated optimal sterilization techniques using Cs-137 and Co-60 irradiators. They found insignificant and significant increases in longevity when mosquitos were exposed to 5-70 Gy (500-7000 Rad) and 25-100 Gy (2500-10,000 rad), respectively. William K. Willard identified a radioresistance in female adult mosquitos where the mean longevity was significantly greater when the experimental group was exposed to various doses of x-ray irradiation throughout the larva, pupae, and adult stages as opposed to the control group that were not exposed (Jones et al. 2003). The problem with these studies is that many of them only exposed the insects to the increased levels of ionizing radiation for short periods of time, this is identified as an acute or fractionated dose of radiation. Fractionated exposures allow the insect's DNA repair mechanisms time to repair damages between radiation exposures as opposed to DNA repair while damage is still occurring.

The current experiment is a study on how a pre-treatment with a continuous sub-lethal radiation exposure increases the insect's ability to survive larger doses of

radiation. By utilizing the continuous sub-lethal exposure technique, the insects are subjected to conditions similar to that of areas affected by radiological disasters. Additionally, by beginning the pre-treatment in the larval stages, it is more likely that a greater proportion of cells will be exposed during the G2 Phase of mitosis, the most radiosensitive phase of the cell cycle (UVA, 2013).

This project analyzes an insect's ability to develop a radiation-induced radioresistance from high doses of radiation after being pre-treated with sub-lethal doses of radiation. Section 2 describes the relevance of this study and similar research. Section 3 describes the materials and methods used. Section 4 summarizes the findings and implications. Section 5 discusses conclusions and future research regarding the radiation adaptations and the need for better understanding of the effects from chronic low dose radiation exposures.

2. LITERATURE REIVEW

Living tissues are continuously adapting to the world that surrounds them via selective pressures. Many different types of living tissues have shown evidence of resistance towards various toxins and other environmental influences. Mithridatism is the practice of protecting oneself against a poison or toxin by administering non-lethal amounts in an effort to develop an immunity toward the toxin (Venes, 2010). Although this is not a commonly used practice for medicinal purposes, it is an interesting concept when referring to adaptive responses of living tissues. Repeated sub-lethal exposures to a toxin in a biological system has shown to contribute to the ability of the system to survive higher concentrations, in turn raising the threshold for a lethal dose of the toxin administered as well as other toxins (McEwen et al. 2002).

Antibiotics were initially developed for treatment of bacterial infections in humans. Subsequently, the use of antibiotics was so successful that uses for them expanded from humans to animals and plants. Identical antibiotics were used for all three applications, thus increasing the occurrence of selective pressures (Levy, 1997). Antibiotic resistance is a well know problem found in industries such as animal husbandry, where antibiotics are used as a prophylaxis and growth promoters. Antibiotics are typically administered to entire populations by medicating the feed or water supply in below pharmacological level concentrations. As of 2001, 25 million pounds of antibiotics were fed to various food animals such as chickens, pigs, and cows (Leutwyler, 2001). According to McEwen et al. (2002), in a study performed by the

USDA, 83% of feedlots administered at least one antimicrobial for previously mentioned purposes. On average antibiotics are administered every 4-12 days for a total of 138-145 days. Such practices increase selective pressures that are conducive to the development of bacterial resistance. Resistant bacteria such as Campylobacter and some strains of salmonellae appear to have been amplified partly by this practice (McEwen et al. 2002). The act of exposing the bacteria to non-lethal concentrations of antibiotics on a routine basis allows the bacteria to build up a resistance towards lethal doses, therefore larger concentrations or a new form of antibiotic are required to kill the resistant bacteria. Feeding animals sub lethal concentrations of antimicrobials fits the principle that the microorganisms have the ability to withstand the effects of the agents and therefore survive and flourish, while those that are not resistant do not survive (McEwen et al. 2002). M. Demerec describes how experimental evidence has indicated that resistance is a heritable property induced by genetic changes comparable to mutations. In M. Demerec' s paper he describes an experiment conducted by Luria and Delbruck to determine if the origin of bacterial resistance stemmed from an interaction between the antibiotic and the bacteria when placed together on the plate or if the selective agent isolated mutants by destruction of sensitive bacteria. The experiment used a series of cultures with identical concentrations of lethal doses of antibiotics and two sets of plates. One set contained bacteria from the same culture, whereas the second set contained bacteria from different cultures. If the resistance was induced through interaction between the bacteria and antibiotic, similar numbers of resistant bacteria would be evident on all the plates regardless of origin. Alternatively, if the resistance was

mutational, similar numbers of resistant colonies would be obtained from the plates containing colonies from the same culture. This study suggested that mutations could be responsible for the origin of resistance (Demerec, 1948).

Additionally, an investigation conducted by Levy *et al.* showed a strong association between the use of tetracycline and the frequency of resistance towards tetracycline found in *Escherichia coli* (*E. coli)* (Witte, 1997). Levy found that mutant plasmids, designated as pSL222-6, expressed a resistance towards tetracycline, chloramphenicol, sulphonamides, and streptomycin. The bacteria were directly introduced into the intestines of four chickens. Four groups of chickens, each including one of the four injected with the *E. coli* bacteria, were placed in separate cages, two groups given a feed containing tetracycline and two groups on normal feed. After a period of 2 months, evidence of the resistant *E. coli.* plasmid was found in 14% and 24% of chickens on the tetracycline feed. None of the chickens placed on non-tetracycline feed had evidence of the mutant plasmid in the fecal matter. The percentages reported do not include the chicken previously inoculated with the *E. coli* bacteria. This investigation shows that the spread of resistant *E. coli* was evident only in the groups exposed to the feed containing tetracycline and chickens not exposed to the tetracycline did not demonstrate the mutant plasmid (Levy et al. 1976).

Resistance towards toxins are not only limited to the bacteriological world, toxin resistance can be found in more complex organisms, such as the human louse genome. Infestation of the human scalp and hair by head lice is very common amongst school aged children and is associated with major economic and social concern. Control of

infestations has been dependent on various types of insecticides such as DDT, organophosphorus insecticides, and synthetic pyrethroids such as permethrin. Over the counter products primarily use synthetic pyrethroids as the main ingredient for head lice treatment since it's availability in 1992 (Clark et al. 2015). Over the years, signs of insecticide resistance has become prevalent in the form of treatment failures and chronic infestations. Durand et al. (2012) describes insecticide resistance as a trait the louse can develop when exposed to selective pressures caused by prolonged use of insecticides. Evidence of resistance can be found in studies conducted by Jones and English (2003). They found that head lice removed from previously treated US children showed a significant resistance when compared to head lice removed from non-US children, who had not been previously treated with permethrin (Jones and English, 2003). A major factor contributing to increased head lice infestations is the acquired resistance that the louse develops over time through prolonged use of the insecticide. Duran at al (2012) also explains that over the years, different insecticides have been used to control head lice. Some have been rendered completely ineffective, and other insecticides are likely to become ineffective through continued use (Durand et al. 2012).

Radioresistance can be induced by a variety of agents, such as heat, pH, nutrient stress, as well as radiation itself. Since the adaptation can be a result of several different agents, the term 'radiation-induced radioresistance' (RIR) is used to describe a radioresistance caused by exposures to radiation (Bala et al. 2007). A literature review conducted by R.E.J. Mitchel (2006) explains how experimental studies have demonstrated that living tissues ranging from single celled organisms to higher level

eukaryotes exhibit an adaptation to radiation when exposed to sub-lethal doses. He further discusses that the radioresistance was evident in haploid cells in the G2 phase, which indicates that the increased radioresistance required a duplicate copy of the genome (Mitchel, 2006). Experiments conducted by Cai and Liu in the 1990's found that pre-treatment with a stress radiation dose to human lymphocyte cultures showed an RIR increase with the increase of the stress dose. Radiation doses of 4 Gy, 10 Gy, and 20 Gy, showed increased survivors by 13%, 27%, and 32%, respectively, after a challenge dose of 400 Gy. A higher RIR was found when the stress dose was delivered at a lower dose rate for a longer period of time. The dose rates given were 0.0078 Gy/ second and 1.26 Gy/ second until a total dose of 20 Gy was reached, approximately 42 minutes and 15 seconds, respectively. This suggests that the damage generated by the stress dose is important to the induction of protective mechanisms (Bala, 2012).

Studies regarding an insect's response to radiation exposure have generally focused on the effects of acute or fractionated exposures. For example, Willard in 1965 studied the effects of low dose acute x-ray exposures on mosquitos. Willard used a dose rate of approximately 500 rad/ minute for each exposure with a total of six exposures to each group. Exposures were not divided equally, but ranged from 50-400 rad total dose (Willard, 1965). His results showed an insignificant increase in mosquito fecundity when exposed to doses between 50-400 rad total dose. Whereas doses of 800 rad at the same dose rate showed a significant decrease in fecundity.

An adaptation to radiation was evident in experiments conducted by Koval in 1988. Koval showed that lepidopteran cells had survival rates up to five times greater

when given two equivalent doses separated by several hours as opposed to a single dose equaling the sum of the split doses. It was suggested that the first split dose stimulated a repair system not present in unirradiated cells (Mitchel, 2006).

Prolonged lifespans of the confused flour beetle has been reported when exposed to low chronic doses as opposed to acute doses of radiation, even when the total chronic dose was greater than the total acute dose. Survival curve analysis revealed that low doses of ionizing radiation prolonged lifespan by preventing mortality during the early life stages. Calabrese suggests that the increased life span may have been from radiation generated hydroxyl radicals that enhanced immune function, in turn increasing disease resistance (Calabrese, 2013).

Field studies using insects living in disaster afflicted areas, such as the Pale Grass Blue Butterfly living near the Fukushima disaster site have shown evolutionary changes that suggest a resistance to radiation. Tair, Nohara, Hiyama, and Otaki conducted a study using Pale Grass Blue Butterflies, *Pseudozizeeria maha*, a common butterfly in Japan that has exhibited growth retardation, high mortality, and abnormality rates since the Fukushima accident. The Pale Grass Blue Butterfly was chosen for the study because it does not migrate very far and has a life cycle of approximately 1 month. The short life cycle allowed the researchers to breed many generations in a reasonable amount of time. Abnormalities observed in the species peaked in September 2011, and then experienced a sharp decline. The sharp decline in abnormalities was hypothesized as a real time evolution of radiation resistance. This study was different from experiments performed in the past because it focused on chronic exposures as opposed to an acute or

fractionated exposure (Taira et al. 2014). When assessing the butterfly's response, it is unclear if the decline in abnormalities are due to the external exposure to radiation or caused by a buildup of radioactive material absorbed by the organism.

3. MATERIALS AND METHODS

3.1 Species Selection and Rearing Techniques

This study focuses on developing an RIR for one generation of *Aedes aegypti* mosquitoes. The mosquito was chosen for this experiment due to the ability to restrict breeding and maintain a single generation. Female mosquitos feed on a blood meal only for reproductive purposes. In order to facilitate mosquito reproduction in a laboratory setting, it is recommended to induce hunger by removing the food source 24 hours prior to providing a blood meal (Munstermann, 1997). Previous experiments utilizing the starvation method have resulted in mosquitos becoming overly stressed from the lack of food source and elevated radiation exposure. The combination of the two stressors resulted in loss of limbs and death amongst the mosquito population. Therefore, the mosquitos were not reproduced during this experiment due to the risk of lethality from starvation. The lack of blood meal should not affect the survivability of the adult mosquito.

Specifically, the *Aedes aegypti* species of mosquito was selected because it is the most widely used species in mosquito experiments and it is one the easier species to rear (Munstermann, 1997). The *Aedes aegypti* mosquito is a native species to the B/CS area and will not cause any negative impact if released.

The mosquito life cycle consists of four stages: egg, larva, pupae, and adult. Female mosquitos lay individual eggs in stagnant water along the waterline. The maturing mosquitos live in the stagnant water until they emerge as adults. All mosquitos

used for this experiment were wild caught larva from the same location in the B/CS area.

Aedes aegypti mosquitos are identifiable in the larval stages by the size of the siphon on the abdomen on the larva. The larval and pupae stages are the developmental stages of life, when the insect's cells are most susceptible to abnormalities caused by gamma radiation. The larval and pupae stages can last for as little as 4 days to as long as 1 month; warmer water temperatures will reduce the amount of time the mosquito spends in the developmental stages (Munstermann, 1997). The larva will mature in the same water in which they were obtained. The water has a sufficient supply of nutrients to support the mosquitos during larval and pupae stages. Since water is the natural environment for the larva, protein additives will not be given to the mosquitos.

The adult *Aedes aegypti* mosquito is approximately 4-7 millimeters in length. Adults have white scales on the dorsal surface of the thorax and each tarsal segment of the hind legs possess white basal bands. The abdomen is typically dark brown to black, but may possess white scales shown in Figure 3-1 (Carpenter, 1955).

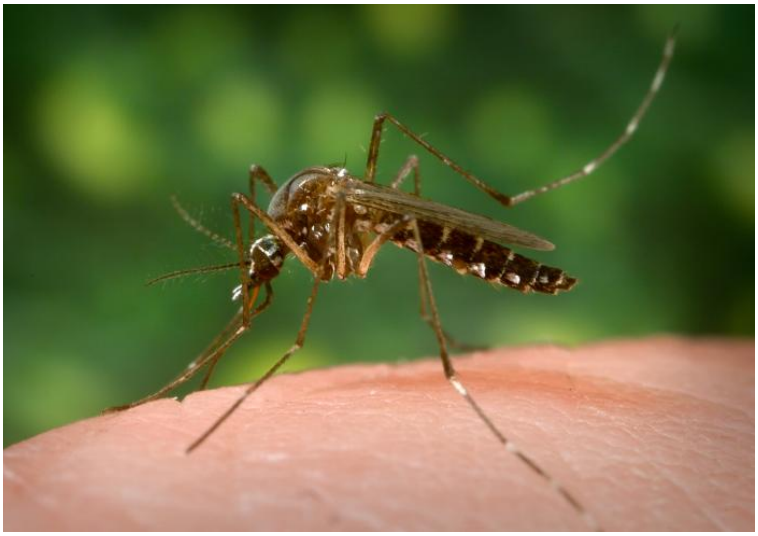

Figure 3-1. Typical Female *Aedes aegypti* Mosquito. Figure adapted from CDC Public Health Image Library. Photo Credit to James Gathany.

Larva containers were placed in cages measuring 32.5 cm^3. The cages (Figure 3-2) are made of small mesh sides held together by a plastic frame. The mesh is porous enough to allow air flow, but will keep mosquitos from escaping. Each cage has an opening to allow entry, but can be secured to reduce the risk of release. Once the adults began to emerge, a 10% sugar solution was placed in the cage as a source of food for the mosquitos. The mosquitos were kept in the same cages throughout their entire life span.

This experiment was replicated with one High Background Group (HBG) and one Low Background Group (LBG), as the control, in each round for total of 2 rounds. Each round consisted of mosquitos that were of the same age when subjected to the challenge exposure.

The laboratory where the mosquitos were housed was an isolated building without temperature control. The ambient temperature of the lab and the water temperature in the containers fluctuated with the temperatures of the outside environment. The experiments took place during the summer months, so as to decrease the likelihood of mosquitoes going into hibernation and delayed development due to cooler temperatures.

Figure 3-2. Mosquito Cage.

3.2 Radiation Exposure

The Texas Department of State Health Services, Environmental Monitoring Division (TDSHS), monitors environmental radiation exposure from the NSC on a quarterly basis. TDSHS uses Luxel optically stimulated luminescence dosimeters which are placed in various location around the NSC with a control dosimeter placed 2 miles from the facility. The dosimeter closest to the mosquito lab is listed as TLD #2 (Appendix A). The dosimeters are changed out approximately every 90 days and analyzed through Landaur dosimetry services. The average background radiation dose closest to the mosquito laboratory was 23.9 mrem per year, over the last 4 years (Appendix B).

3.2.1 Low Background Exposure

In order to subject both groups of mosquitos to the same atmospheric conditions, the mosquitos were kept in the same laboratory. The HBG was kept behind a lead shielded wall approximately 10 ft. from the LBG. The dose rate reading at the LBG cage was approximately 60 μrad/hr. This gave a total dose of about 43 mrad over the course of 30 days.

3.2.2 Chronic Low Dose Exposure

Scandium-46 (Sc-46) was selected as the isotope to deliver the chronic low dose exposure. Sc-46 has a relatively long half-life and moderate energies emitted. Sc-45 doped beads were irradiated at the Texas A&M Nuclear Science Center (NSC). The Neutron Activation Analysis results for this specific material showed an average of 0.87% scandium and 2.96% sodium (Appendix C). The Sc-45 material was irradiated

inside an aluminum can for a total of 5.00 effective MW-hours at 1 megawatt power. A total of 60 grams of material was irradiated giving a total of 63 mCi of Sc-46. Upon completion of the irradiation, the material was separated evenly into 6 vials. This gave a total of 10 grams and 10.5 mCi per vial. Sc-46 has a half-life of 83.8 days and two specific gamma energies, 0.889 MeV and 1.12 MeV, with a probability of 0.999840 and 0.999870, respectively (Shleien et al. 1998). The material is not pure scandium and subsequently, 1.025 Ci of Sodium-24 (Na-24) was activated during the irradiation process. Na-24 has a half-life of 15 hours and three specific energies, 1.37 MeV, 2.75 MeV, and 3.82 MeV with a probability of 0.99999, 0.9998620, and 0.000641, respectively (Shleien et al. 1998). Due to the higher energies and short half-life, the Na-24 was given time to decay prior to starting the mosquito exposure. The Na-24 did not undergo more than 10 half-lives and contributed approximately 700 mrad to the first HBG and approximately 102 mrad to the second HBG. The contribution of Na was incorporated into the total dose to the HBG's. Due to the scheduling at the NSC, the material was re-packaged 3 days post irradiation of the first HBG and 4 days post irradiation for the second HBG. Due to the short half-life of Na-24, the dose contribution from the Na-24 is significantly less for the second HBG than the first HBG.

In order to produce a uniform dose across the entirety of the cage, a wire source holder was secured around the outside of the cage with vial holders located 3 inches from the outside center of each side of the cage. The vial holders were designed so that the position of the sources can be reproduced after removing the sources from the holder.

Figure 3-3 shows the source holder in relationship to the cage and lists the nomenclature for each side and corner.

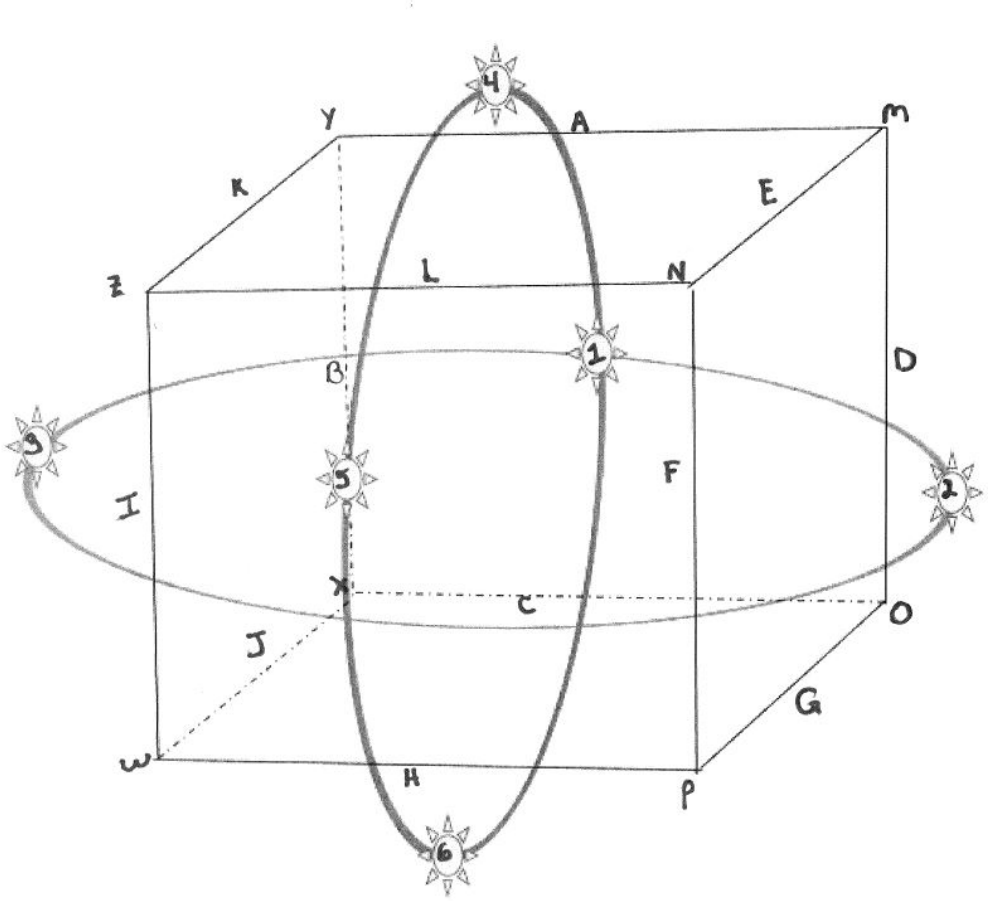

Figure 3-3. Mosquito Cage with Source Holder.

Since the mosquitos spend most of their lives resting on the cage walls, the total dose to the mosquitos was calculated based on the average dose to the cage walls contributed by each source. The dose rate from each source was calculated using the gamma exposure rate calculation, 6CE, using the specific energies of Sc-46 and Na-24. The 6CE calculation gave a dose rate in the form of R/ hour at 1 foot. The Pythagorean

Theorem and inverse square law was used to calculate the dose to each individual side and corner from each source. The sum the dose from each side was then averaged to get the total dose rate. The dose rate for the first HBG ranged from 36.6 rad/ day to 28.5 rad/ day over the course of 29 days with a total dose of 1005.9 $\pm$ 70 rad, and an average dose of 32.4 rad/ day. The second HBG received a dose rate of 36.4 rad/ day to 28.04 rad/ day over the course of 30 days with a total dose of 1024.7 $\pm$ 75 rad, and an average dose of 32.0 rad/ day.

3.2.3 Challenge Dose Exposure

The challenge dose to the HBG and LBG of mosquitos was delivered via Lanthanum-140. La-140 has a half-life of 40.22 hours with gamma energies ranging from .004 MeV to 2.5 MeV. The La-140 source is a sealed source activated at the NSC reactor and was delivered to the mosquitos via the irradiation cell. The irradiation cell is located adjacent to the west end of the main reactor pool. The concrete wall is penetrated at reactor core level by a radiation window 2 foot squared on the main pool side and enlarges to 4 foot squared on the irradiation cell side. The window itself is an aluminum plate of unknown thickness. The La-140 source is placed in a holder attached to the pool side of the window. Due to the limited size of the window, each challenge dose was performed independently in a series of 4 rounds. Dose rates were calculated using the 6CE method as done previously with the Sc-46 and Na-24 dose rate calculations. The cages were place approximately 1 foot from the source for each challenge dose. Each challenge dose was delivered over a course of 6-9 hours, depending on the activity of the La-140 source at the beginning of irradiation Figure 3-4.

The challenge dose delivered to the first HBG was performed with 744 Ci. The calculated total dose rate ranged from 10,338.7 rad/ hour to 9,323.3 rad/ hour over the course of 7 hours. The total dose delivered to the first HBG was 68,766.2 ±2,545.2 rad. The challenge dose delivered to the first LBG was performed using 680.5 Ci. The dose rate ranged from 9,457.2 rad/ hour to 8,382.6 rad/ hour over the course of 10 hours, for a total dose of 71,285.2 ± 3,008 rad.

The challenge dose delivered to the second HBG was performed using 660 Ci. The dose rate ranged from 9172.8 rad/ hour to 8130.6 rad/hour of the course of 8 hours, for a total dose of 69,141.8 ± 2,917.6 rad. The challenge dose delivered to the second LBG was performed using 580 Ci. The dose rate ranged from 8,060.8 rad/ hour to 6,903 rad/hour over the course of 8 hours, for a total dose of 66,625 ± 3,492 rad. Upon completion of each challenge dose, the mosquito cages were removed from the irradiation cell and analyzed for survival ratios over the course of several weeks.

Figure 3-4. Cage Placement in Irradiation Cell Window.

3.3 Survival Percentage Analysis

The mosquitos continued to be reared in the same methods as prior to the challenge dose. The HBG was not subjected to additional radiation after the challenge dose. Mosquitos were counted upon death and survival percentages were tracked until the final mosquitos perished. The survival percentages for each group were plotted and compared. The surviving fractions were calculated by using the Kaplan-Meier Survival Probability Estimates and compared using the Two Sample t-Test assuming unequal variances

4. RESULTS AND ANALYSIS

4.1 Round 1 Results

4.1.1 LBG

Immediately following the LBG first Round challenge dose, the surviving percentage was 71.43%. The surviving percent reached 50% on Day 3 post challenge exposure. A decline greater than 20% was observed immediately following the challenge exposure. Total mortality was reached on Day 21.

4.1.2 HBG

Immediately following the challenge dose, the survival rate of the first HBG was 100%. Within the first 24 hours, the percent survival dropped to 56%. The rapid drop in surviving mosquitos was most likely due to the temperature difference between the irradiation cell and the atmosphere conditions in the mosquito lab. The average temperature in the irradiation cell was about 75 degrees and the atmospheric conditions that particular day was above 100 degrees Fahrenheit. Several mosquitos were observed perishing when moving the cages from inside the confinement building to the mosquito lab outside near the facility perimeter. Future groups were moved progressively from the irradiation cell to the mosquito lab, so as to renormalize to the atmospheric temperatures. The 56% survival held for 5 days and then progressively decreased. A decline greater than 20% was observed between 0 days and 1 day post challenge exposure and between 13 days and 14 days post challenge exposure. All insects had died by Day 17. Survival data is shown in table 4-1.

Round 1 Data					
Days Post Irradiation	Amount Dead (HBG)	Surviving Percent (HBG)	Days Post Irradiation	Amount Dead (LBG)	Surviving Percent (LBG)
0	0	100.00%	0	4	71.43%
1	6	66.67%	1	6	57.14%
2	8	55.56%	2	6	57.14%
3	8	55.56%	3	7	50.00%
4	8	55.56%	4	8	42.86%
5	8	55.56%	5	9	35.71%
6	9	50.00%	6	9	35.71%
7	9	50.00%	7	9	35.71%
8	9	50.00%	8	9	35.71%
9	11	38.89%	9	9	35.71%
10	11	38.89%	10	9	35.71%
11	11	38.89%	11	9	35.71%
12	12	33.33%	12	9	35.71%
13	12	33.33%	13	11	21.43%
14	15	16.67%	14	11	21.43%
15	15	16.67%	15	11	21.43%
16	16	11.11%	16	11	21.43%
17	18	0.00%	17	11	21.43%
18	18	0.00%	18	12	14.29%
19	18	0.00%	19	12	14.29%
20	18	0.00%	20	13	7.14%
21	18	0.00%	21	14	0.00%

Table 4-1. Round 1 Survival Data.

4.1.3 Round 1 Analysis

When the HBG and LBG were compared, the sharpest decline was observed immediately after the challenge exposure (Figure 4-1). Even with the sharp decline from the HBG following the challenge exposure, the HBG still maintained above 50% lethality until 6 days post exposure. Both groups displayed a steady decline in survival after the first 24 hours post challenge exposure. When comparing the 2 sets of surviving

fractions, the Two Sample t-Test revealed that the null hypothesis cannot be rejected due to the one-tail and two-tail P value (Table 4-2).

t-Test: Two-Sample Assuming Unequal Variances

	Surviving Fraction (HBG)	*Surviving Fraction (LBG)*
Mean	0.343434343	0.321428571
Variance	0.06867618	0.02951895
Observations	22	22
Hypothesized Mean Difference	0	
df	36	
t Stat	0.32938436	
P(T<=t) one-tail	0.371886997	
t Critical one-tail	1.688297714	
P(T<=t) two-tail	0.743773994	
t Critical two-tail	2.028094001	

Table 4-2. Round 1 Two Sample t-Test Results.

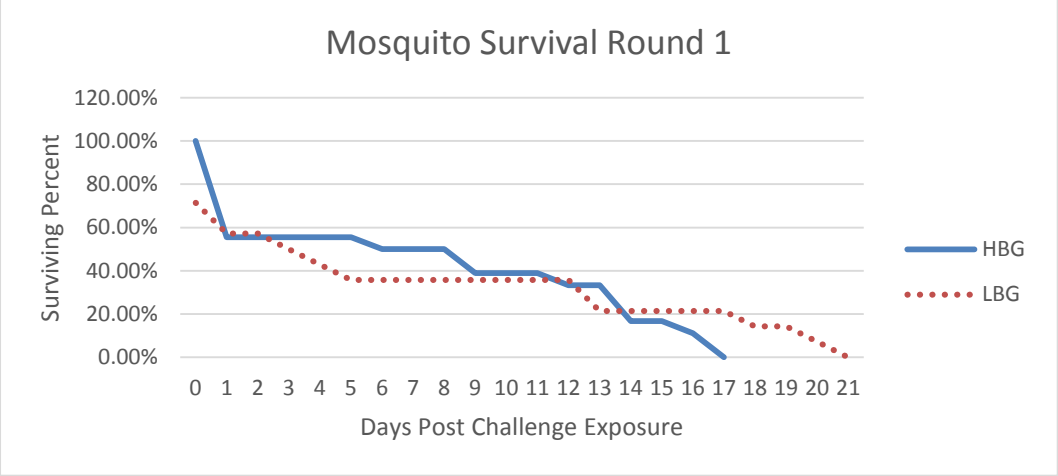

Figure 4-1. Round 1 Survival Comparison.

4.1.4 Round 1 Censored Analysis

The first six mosquitos were suspected to have died of heat exposure upon removal from the NSC irradiation cell, as described in section 4.1.2 HBG. Kaplan-Meier Survival Curves allow for the censorship of data when the total survival time cannot be accurately determined (Rich, 2010). When the 6 mosquitos are censored out of the data, round 1 maintained above 50% lethality until Day 12 (Table 4-3). The Two Sample t-Test shows that the P value is less than 5% for both the one-tail and two-tail values and therefore statistically significant. The null hypothesis can then be rejected when the data is censored to exclude the 6 mosquitos hypothesized to have died as a result of heat exposure (Table 4-4). The survival curve representing the censored HBG is show in Figure 4-2.

Round 1 Censored Data					
Days Post Irradiation	Mortality (HBG)	Surviving Percent (HBG)	Days Post Irradiation	Mortality (LBG)	Surviving Percent (LBG)
0	0	100.00%	0	4	71.43%
1	2	83.33%	1	6	57.14%
2	2	83.33%	2	6	57.14%
3	2	83.33%	3	7	50.00%
4	2	83.33%	4	8	42.86%
5	2	83.33%	5	9	35.71%
6	3	75.00%	6	9	35.71%
7	3	75.00%	7	9	35.71%
8	3	75.00%	8	9	35.71%
9	5	58.33%	9	9	35.71%
10	5	58.33%	10	9	35.71%
11	5	58.33%	11	9	35.71%
12	6	50.00%	12	9	35.71%
13	6	50.00%	13	11	21.43%
14	9	25.00%	14	11	21.43%
15	9	25.00%	15	11	21.43%
16	10	16.67%	16	11	21.43%
17	12	0.00%	17	11	21.43%
18	12	0.00%	18	12	14.29%
19	12	0.00%	19	12	14.29%
20	12	0.00%	20	13	7.14%
21	12	0.00%	21	14	0.00%

Table 4-3. Round 1 Censored Survival Data.

t-Test: Two-Sample Assuming Unequal Variances

	Surviving Fraction (HBG)	*Surviving Fraction (LBG)*
Mean	0.492424242	0.321428571
Variance	0.118987494	0.02951895
Observations	22	22
Hypothesized Mean Difference	0	
df	31	
t Stat	2.081247868	
P(T<=t) one-tail	0.022880737	
t Critical one-tail	1.695518783	
P(T<=t) two-tail	0.045761473	
t Critical two-tail	2.039513446	

Table 4-4. Round 1 Censored Two Sample t-Test Results.

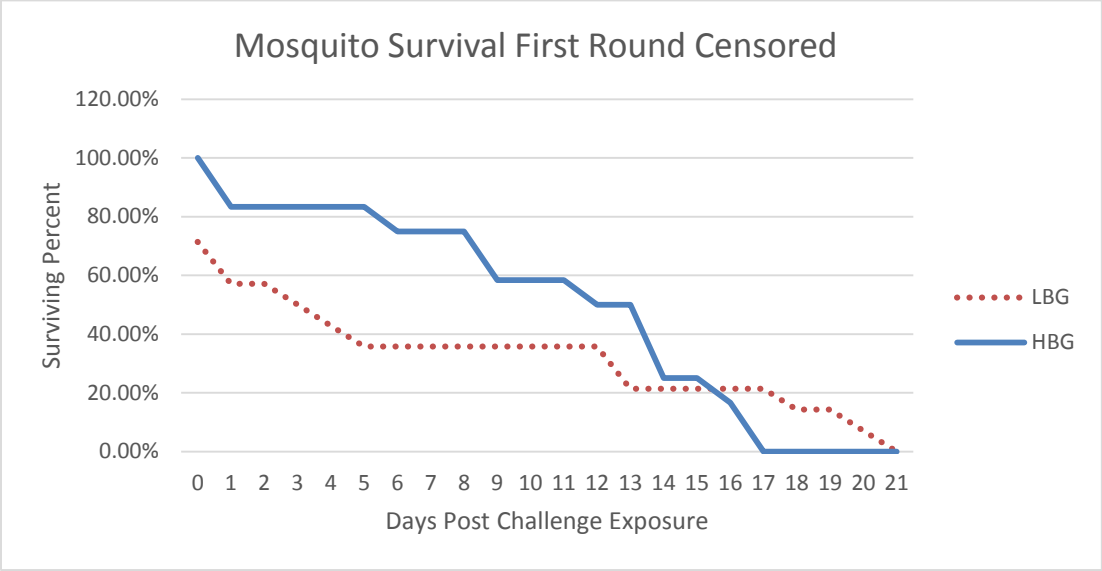

Figure 4-2. Round 1 Censored Survival Comparison.

4.2 Round 2 Results

4.2.1 LBG

Immediately following the challenge exposure, the LBG had 62.5% survival. The survival percent then dropped to 50% on Day 2 post challenge exposure. A decline greater than 20% was observed between Day 4 and Day 5 post challenge exposure. Total mortality was reached on Day 8 post challenge exposure.

4.2.2 HBG

Immediately following the challenge exposure, the HBG had 92.86% survival. The HBG maintained above 50% survival until Day 9 (Table 4-5). The percent survival decreased from 57.14% survival to 35.71% (+20% decrease) survival from Day 8 to Day 9. Other than the decline between Day 8 and Day 9 post exposure, the surviving percent had a steady decline until total mortality on Day 18.

Round 2 Data					
Days Post Irradiation	Amount Dead (HBG)	Surviving Percent (HBG)	Days Post Irradiation	Mortality (LBG)	Surviving Percent (LBG)
0	1	92.86%	0	3	62.50%
1	3	78.57%	1	4	50.00%
2	3	78.57%	2	4	50.00%
3	3	78.57%	3	4	50.00%
4	4	71.43%	4	5	37.50%
5	4	71.43%	5	7	12.50%
6	6	57.14%	6	7	12.50%
7	6	57.14%	7	7	12.50%
8	6	57.14%	8	8	0.00%
9	9	35.71%	9	8	0.00%
10	10	28.57%	10	8	0.00%
11	10	28.57%	11	8	0.00%
12	10	28.57%	12	8	0.00%
13	10	28.57%	13	8	0.00%
14	10	28.57%	14	8	0.00%
15	11	21.43%	15	8	0.00%
16	11	21.43%	16	8	0.00%
17	12	14.29%	17	8	0.00%
18	14	0.00%	18	8	0.00%

Table 4-5. Round 2 Survival Data.

4.2.3 Round 2 Analysis

When the second round HBG and LBG's were compared, the HBG had a consistent higher survival rate than the LBG (Figure 4-3). Additionally, the second HBG exhibited a significantly longer total survival time. The HBG population reached total mortality upon Day 17, versus the second LBG, which reached total mortality on Day 8. It should also be noted that the second LBG received a longer exposure at a lower dose rate as compared to the second HBG. Although the total dose rates for the second HBG

and LBG remained within ± 10% of the target dose, the HBG was subjected to a higher dose rate for a shorter period of time than the second LBG. The increased survivability coupled with the increased dose rates demonstrates that the HBG developed an ability to withstand higher dose rates in addition to a higher total doses of gamma radiation. The Two Sample t-test of the surviving percentages revealed that the null hypothesis can be rejected due to the one-tail and two-tail P value (Table 4-6).

t-Test: Two-Sample Assuming Unequal Variances

	Surviving Fraction (HBG)	*Surviving Fraction (LBG)*
Mean	0.462406015	0.151315789
Variance	0.07220432	0.049616228
Observations	19	19
Hypothesized Mean Difference	0	
df	35	
t Stat	3.885106168	
P(T<=t) one-tail	0.000217355	
t Critical one-tail	1.689572458	
P(T<=t) two-tail	0.00043471	
t Critical two-tail	2.030107928	

Table 4-6. Round 2 Two Sample t-Test Results.

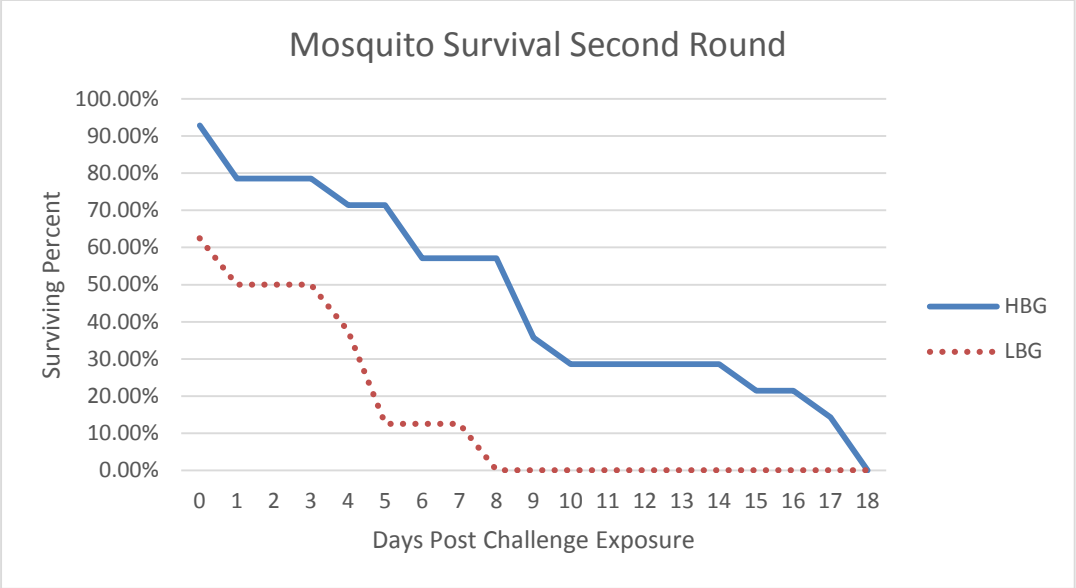

Figure 4-3. Round 2 Survival Comparison.

4.3 Combined Analysis

4.3.1 Combined Analysis Uncensored

When the data from the first and second rounds are combined, the HBG continues to have a higher survival rate than the LBG until Day 15 (Figure 4-4). The HBG maintained above 50% lethality until Day 9 when the surviving percent dropped from 53.13% to 37.5%. The LBG maintained 50% lethality until Day 3 (Table 4-7). Although, the trend shows that the HBG had a higher survival percentage, the Two Sample T-test shows that the null hypothesis cannot be rejected for the One- tail and two-tail P test (Table 4-8).

Combined Data					
Days Post Irradiation	Amount Dead (HBG)	Surviving Fraction (HBG)	Days Post Irradiation	Mortality (LBG)	Surviving Fraction (LBG)
0	1	96.88%	0	7	68.18%
1	9	71.88%	1	10	54.55%
2	11	65.63%	2	10	54.55%
3	11	65.63%	3	11	50.00%
4	12	62.50%	4	13	40.91%
5	12	62.50%	5	16	27.27%
6	15	53.13%	6	16	27.27%
7	15	53.13%	7	16	27.27%
8	15	53.13%	8	17	22.73%
9	20	37.50%	9	17	22.73%
10	21	34.38%	10	17	22.73%
11	21	34.38%	11	17	22.73%
12	22	31.25%	12	17	22.73%
13	22	31.25%	13	19	13.64%
14	25	21.88%	14	19	13.64%
15	26	18.75%	15	19	13.64%
16	27	15.63%	16	19	13.64%
17	30	6.25%	17	19	13.64%
18	32	0.00%	18	20	9.09%
19	32	0.00%	19	20	9.09%
20	32	0.00%	20	21	4.55%
21	32	0.00%	21	22	0.00%

Table 4-7. Combined Survival Data.

t-Test: Two-Sample Assuming Unequal Variances

	Surviving Fraction (LBG)	*Surviving Fraction (HBG)*
Mean	0.252066116	0.367897727
Variance	0.032020321	0.074491427
Observations	22	22
Hypothesized Mean Difference	0	
df	36	
t Stat	-1.664714198	
P(T<=t) one-tail	0.052325521	
t Critical one-tail	1.688297714	
P(T<=t) two-tail	0.104651042	
t Critical two-tail	2.028094001	

Table 4-8. Combined Two Sample t-Test Results.

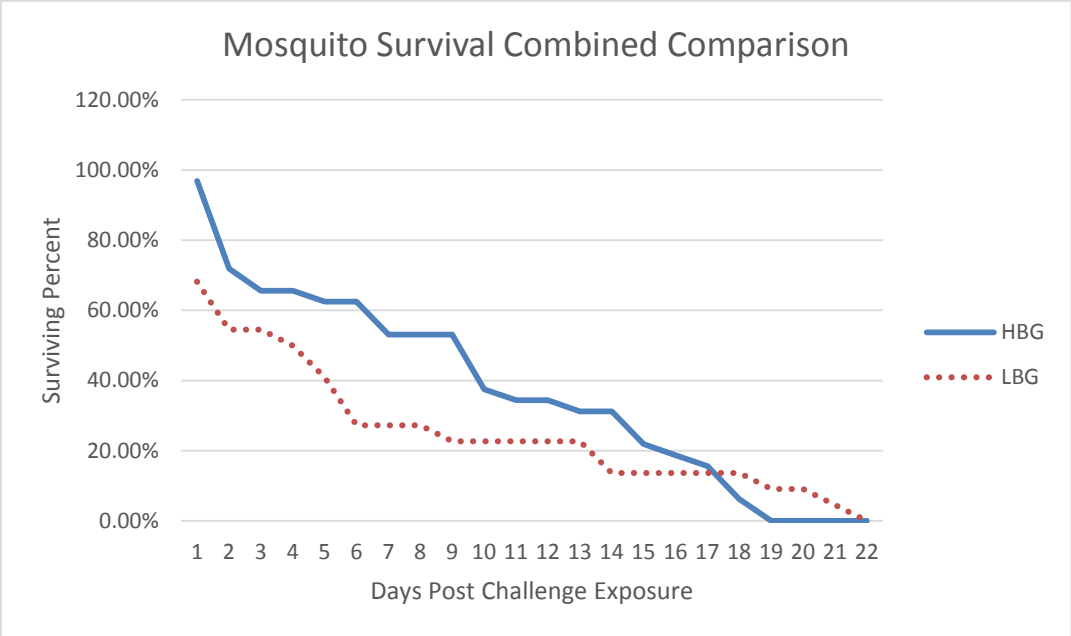

Figure 4-4. Combined Survival Comparison.

4.3.2 Combined Analysis Censored

Similar to the round 1 analysis, when the 6 mosquitos are censored out, the data exhibits significantly higher survival percentage for the HBG survival. The HBG maintained above 50% lethality until Day 9 where it dropped from 57.69% lethality to 46.15% lethality between Day 8 and Day 9. The LBG reached 50% lethality on Day 3 (Table 4-9). The Two Sample t-test of the surviving percentages shows that the null hypothesis can be rejected due to the one-tail and two-tail P values (Table 4-10). Comparison of the HBG and LBG trends are shown in Figure 4-5.

Combined Censored Data					
Days Post Irradiation	Amount Dead (HBG)	Surviving Fraction (HBG)	Days Post Irradiation	Mortality (LBG)	Surviving Fraction (LBG)
0	1	96.15%	0	7	68.18%
1	5	80.77%	1	10	54.55%
2	5	80.77%	2	10	54.55%
3	5	80.77%	3	11	50.00%
4	7	73.08%	4	13	40.91%
5	7	73.08%	5	16	27.27%
6	9	65.38%	6	16	27.27%
7	11	57.69%	7	16	27.27%
8	11	57.69%	8	17	22.73%
9	14	46.15%	9	17	22.73%
10	16	38.46%	10	17	22.73%
11	16	38.46%	11	17	22.73%
12	19	26.92%	12	17	22.73%
13	19	26.92%	13	19	13.64%
14	20	23.08%	14	19	13.64%
15	23	11.54%	15	19	13.64%
16	23	11.54%	16	19	13.64%
17	24	7.69%	17	19	13.64%
18	26	0.00%	18	20	9.09%
19	26	0.00%	19	20	9.09%
20	26	0.00%	20	21	4.55%
21	26	0.00%	21	22	0.00%

Table 4-9. Combined Censored Data.

t-Test: Two-Sample Assuming Unequal Variances		
	0.961538462	*0.681818182*
Mean	0.444444444	0.231601732
Variance	0.076613683	0.023947265
Observations	18	21
Hypothesized Mean Difference	0	
df	26	
t Stat	2.897318169	
P(T<=t) one-tail	0.003770221	
t Critical one-tail	1.70561792	
P(T<=t) two-tail	0.007540443	
t Critical two-tail	2.055529439	

Table 4-10. Combined Censored Two Sample t-Test Results.

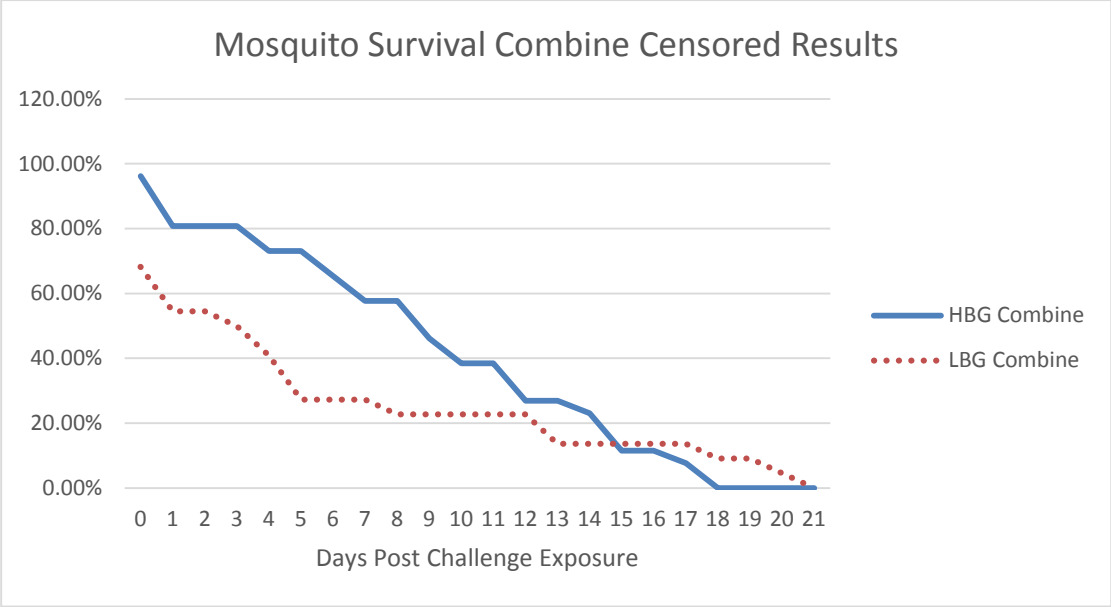

Figure 4-5. Combined Censored Survival Comparison.

4.4 Abnormalities

One notable abnormality was observed in the second HBG. One female mosquito developed a descended abdomen (Figure 4-4). This abnormality was observed several days prior to the challenge exposure. It is unknown when the abnormality developed or if the cause was related to the chronic radiation exposure. The abnormality appears to be hollow and lacks the characteristics of a solid mass. One male mosquito from the first HBG also seemed to have the beginnings of a descended abdomen, but it is unclear if that is the case (Figure 4-5).

Figure 4-6. Female HBG Mosquito with Descended Abdomen.

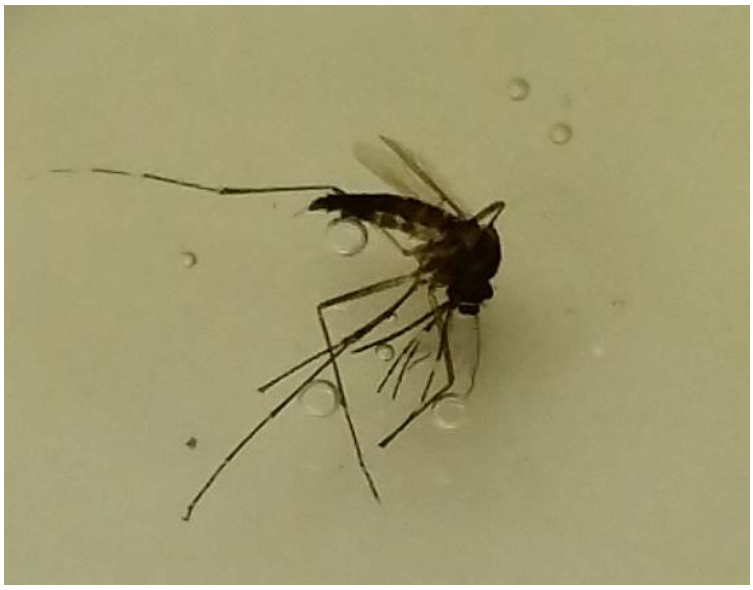

Figure 4-7. Male HBG Mosquito with Potential Descended Abdomen.

5. CONCLUSIONS AND FUTURE WORK

Utilization of a continuous exposure to radiation has shown to increase the survival rates when given a substantially higher challenge dose amongst pre-treated mosquitos. This experiment showed evidence that a single generation of an insect can adapt to radiation by developing a radiation-induced radioresistance. The group subjected to the continuous high background of radiation consistently had better survival rates after the challenge dose than the low background counterparts. Mosquitos in the LBG reached a 50% lethality sooner than mosquitos who were pre-treated with radiation and exposed from the developmental stages. It should also be noted that the LBG was more difficult to rear and keep alive for the same length of time as the HBG. In both instances, the LBG had fewer numbers in the challenge dose due to mosquitos dying off prior to the challenge dose. This could be evidence that RIR has a mithridatic response as well as a hormetic response, by increasing the longevity of the mosquitos that were exposed to pre-treatment of radiation. This research suggests that insects living in nuclear disaster affected areas are likely to have a more developed adaptation to radiation exposures.

In future studies, several approaches can be considered: determining which DNA sequences are activated to provide radioprotectants to the species or if the pre-treatment dose is linear in regards to survivability of a challenge dose. By determining which DNA sequences are responsible for the radioprotectants, we can potentially manipulate genes in order to increase survivability of larger radiation doses without pre-treatment, or deactivate the genes to provide better radiation therapy results for radioresistant cancers.

By determining if the pre-treatment dose is linear, we can begin to alter pre-treatment dose limits to achieve optimum survivability of high doses of radiation.

Determining if the RIR is passed to subsequent generations is another avenue to explore. Similarly, with bacteria and head lice, the DNA mutations allowed for the buildup of resistance. The mutated DNA was then passed along to subsequent generations. Determining if the mosquitos can pass along RIR will help future investigations regarding adaptations amongst wildlife living in radioactive disaster areas.

This study can potentially open many doors in regards to future studies involving chronic radiation doses. Due to the rise of background exposures and potential nuclear accidents, more emphasis should be placed on the understanding of effects from chronic low doses of radiation. By better understanding the low doses of radiation, we can develop better guidance for managing areas affected by radioactive contaminants and the organisms living in those areas. Adaptive responses have been observed in many different types of exposure organisms and developing an understanding of the mechanism behind those adaptations is of great importance for future research.

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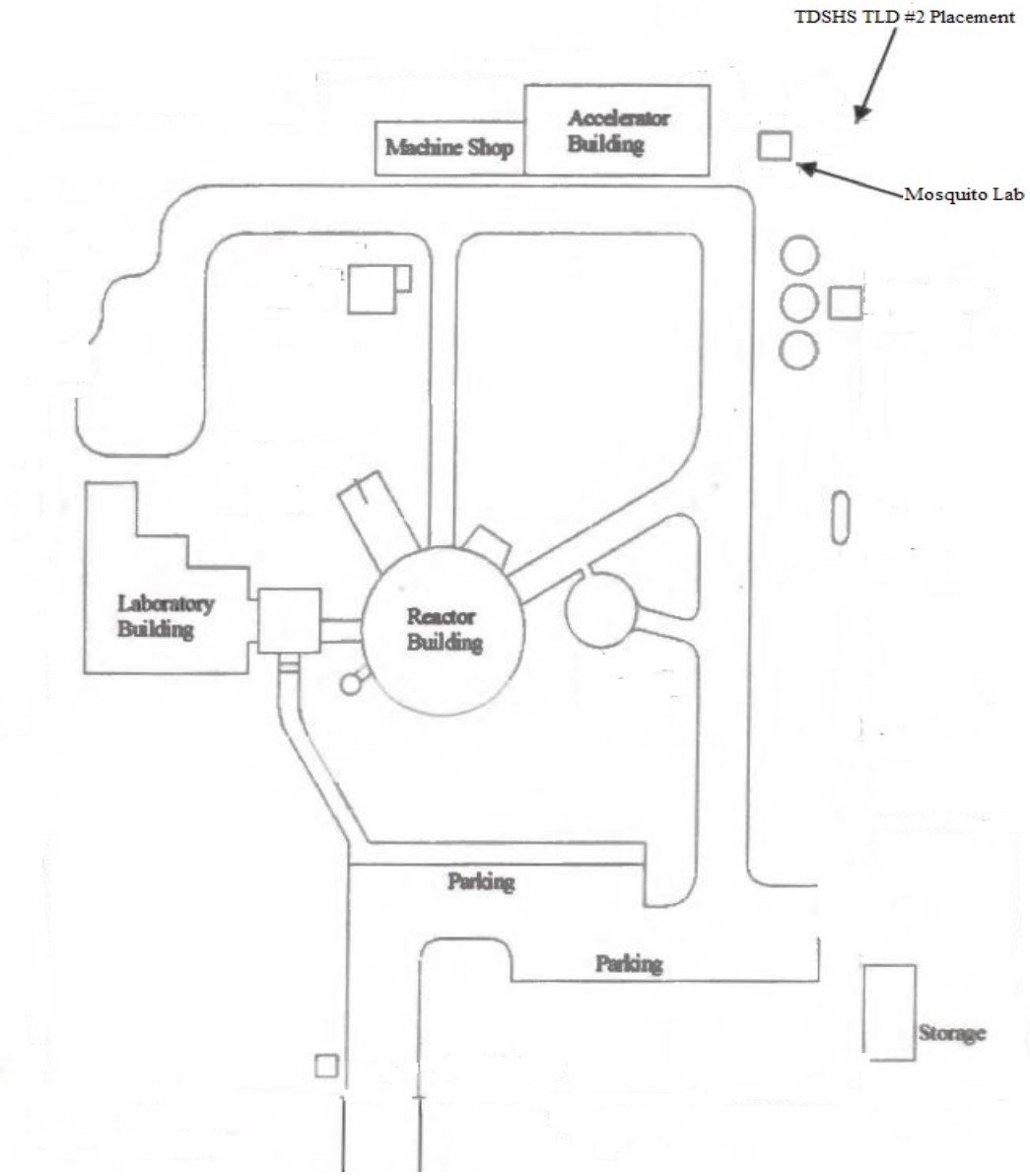
APPENDIX A

TDSHS TLD Placement Map



APPENDIX B

NSC ANNUAL REPORT: ENVIRONMENTAL DOSE RATE EXERPTS

Site #	Location	Quarterly Exposure rates (mrem/91 days)				TLD Dose (mrem)	Deep Dose (mrem)	Internal Dose (mrem)	Total Dose (mrem)
2	300ft W of reactor building	6.9	1.9	0.9	7.3	17.0	1.1	0.1	1.2
3	250ft WSW of reactor building	4.0	0.9	0.0	3.3	8.2	0.5	0.1	0.6
4	200ft NW of reactor building	8.9	2.8	4.7	7.3	23.7	1.5	0.1	1.6
5	225ft NE of reactor building	7.9	0.9	0.9	1.6	11.3	0.7	0.1	0.8
10	190ft SE of reactor building	3.0	0.0	0.0	0.0	3.0	0.2	0.1	0.3
11	300ft NE of reactor building	4.0	0.0	0.0	0.0	4.0	0.3	0.1	0.4
*14	3mi NW of facility	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
18	375ft NE of reactor building	0.0	2.8	2.8	0.8	6.4	0.4	0.1	0.5
19	320ft NE of reactor building	9.9	0.0	0.0	0.0	9.9	0.6	0.1	0.7
20	E Wall of accelerator building	4.9	0.0	5.7	12.2	22.8	1.4	0.1	1.5
21	W Wall of accelerator building	0.0	0.0	3.8	2.4	6.2	0.4	0.1	0.5
22	S Wall of accelerator building	2.0	0.0	0.0	0.8	2.8	0.2	0.1	0.3
*23	0.25mi SE of facility	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
24	N wall of accelerator bldg, NW	1.0	1.9	1.9	4.9	9.7	0.6	0.1	0.7
25	N wall of accelerator bldg, E	5.0	0.0	0.0	4.1	9.1	0.6	0.1	0.7
26	W Fence of hyperbaric lab	x	x	0.0	0.2	0.2	0.0	0.1	0.1
27	E fence of hyperbaric lab	x	x	0.0	0.0	0.0	0.0	0.1	0.1
28	S fence of hyperbaric lab	x	x	0.0	0.2	0.2	0.0	0.1	0.1
29	325ft SW from reactor building	x	x	2.0	0.7	2.7	0.2	0.1	0.3

* 14 and 23 are background TLD's

Table B1. TLD environmental dose rates from NSC Annual Report 2011.

Site #	Location	Quarterly Exposure rates (mrem/91 days)				TLD Dose (mrem)	Deep Dose (mrem)	Internal Dose (mrem)	Total Dose (mrem)
2	300ft W of reactor building	0.0	1.1	5.0	10.5	16.6	1.0	0.2	1.2
3	250ft WSW of reactor building	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
4	200ft NW of reactor building	0.0	6.5	9.9	3.8	20.2	1.3	0.2	1.5
5	225ft NE of reactor building	0.0	2.2	4.1	5.7	12.0	0.8	0.2	1.0
10	190ft SE of reactor building	0.0	0.0	0.0	1.0	1.0	0.1	0.2	0.3
11	300ft NE of reactor building	0.0	0.0	0.0	1.9	1.9	0.1	0.2	0.3
*14	3mi NW of facility	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
18	375ft NE of reactor building	0.0	3.3	3.3	2.9	9.5	0.6	0.2	0.8
19	320ft NE of reactor building	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
20	E Wall of accelerator building	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
21	W Wall of accelerator building	0.0	0.0	0.0	1.0	1.0	0.1	0.2	0.3
22	S Wall of accelerator building	9.2	0.0	0.0	0.0	9.2	0.6	0.2	0.8
*23	0.25mi SE of facility	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2
24	N wall of accelerator bldg. NW	8.1	16.3	0.0	0.0	24.4	1.5	0.2	1.7
25	N wall of accelerator bldg. E	0.0	14.1	0.0	0.0	14.1	0.9	0.2	1.1
26	W Fence of hyperbaric lab	x	x	5.8	0.0	5.8	0.4	0.2	0.6
27	E fence of hyperbaric lab	x	x	5.8	0.0	5.8	0.4	0.2	0.6
28	S fence of hyperbaric lab	x	x	6.6	0.0	6.6	0.4	0.2	0.6
29	325ft SW from reactor building	x	x	0.0	0.0	0.0	0.0	0.2	0.2

* 14 and 23 are background TLD's

Table B2. TLD environmental dose rates from NSC Annual Report 2012.

Site #	Location	Quarterly Exposure rates (mrem/91 days)				TLD Dose (total)	Deep Dose=TLD dose*(1/16)	Internal Dose (mrem)_comply	Total Dose (mrem)
2	300 ft. W of reactor building,	9	3	10	17	39	2.4375	0.1	2.5375
3	250 ft W-SW of reactor building,	8	6	8	8	30	1.875	0.1	1.975
4	200 ft NW of reactor building	14	6	13	10	43	2.6875	0.1	2.7875
5	225 ft NE of reactor building	9	5	6	6	26	1.625	0.1	1.725
10	190 ft SE of reactor building,	7	5	7	4	23	1.4375	0.1	1.5375
11	300 ft NE of reactor building	7	6	4	0	17	1.0625	0.1	1.1625
*14	3.84 miles NW of facility	0	0	0	0	0	0	0.1	0.1
18	375 ft NE of reactor building	8	6	8	6	28	1.75	0.1	1.85
19	320 ft NE of reactor building	3	5	3	4	15	0.9375	0.1	1.0375
20	E Wall of accelerator building	3	9	7	3	22	1.375	0.1	1.475
21	W Wall of accelerator building	5	1	5	6	17	1.0625	0.1	1.1625
22	S Wall of accelerator building	0	0	5	3	8	0.5	0.1	0.6
*23	0.25 miles SE of facility	6	0	5	4	15	0.9375	0.1	1.0375
24	North Wall of Accelerator Building, First Floor	31	1	13	20	65	4.0625	0.1	4.1625
25	North Wall of Accelerator Building, Second Floor	10	0	5	13	28	1.75	0.1	1.85
26	W Fence of hyberbaric lab	9	3	3	5	20	1.25	0.1	1.35
27	E fence of hyperbaric lab	3	3	3	4	13	0.8125	0.1	0.9125
28	S fence of hyperbaric lab	8	1	5	4	18	1.125	0.1	1.225
29	325ft SW from reactor building,	6	5	6	7	24	1.5	0.1	1.6

*Background TLDStation

Table B3. TLD environmental dose rates from NSC Annual Report 2013.

Site #	Location	Quarterly Exposure rates (mrem/91 days)				TLD Dose (total)	Deep Dose=TLD dose*(1/16)	Internal Dose (mrem)_comply	Total Dose (mrem)
2	300 ft. W of reactor building	5	11	5	2	23	1.4375	0.1	1.5375
3	250 ft W-SW of reactor building	3	3	0	0	6	0.375	0.1	0.475
4	200 ft NW of reactor building	4	5	6	3	18	1.125	0.1	1.225
5	225 ft NE of reactor building	3	0	2	1	6	0.375	0.1	0.475
10	190 ft SE of reactor building	1	0	0	1	2	0.125	0.1	0.225
11	300 ft NE of reactor building	2	0	0	0	2	0.125	0.1	0.225
*14	3.84 miles NW of facility	0	0	0	0	0	0	0.1	0.1
18	375 ft NE of reactor building	3	0	2	1	6	0.375	0.1	0.475
19	320 ft NE of reactor building	0	0	0	0	0	0	0.1	0.1
20	E Wall of accelerator building	0	0	0	0	0	0	0.1	0.1
21	W Wall of accelerator building	0	0	0	0	0	0	0.1	0.1
22	S Wall of accelerator building	0	0	1	0	1	0.0625	0.1	0.1625
*23	0.25 miles SE of facility	0	0	0	0	0	0	0.1	0.1
24	North Wall of Accelerator Building, First Floor	6	7	3	0	16	1	0.1	1.1
25	North Wall of Accelerator Building, Second Floor	1	9	6	2	18	1.125	0.1	1.225
26	W Fence of hyperbaric lab,	2	1	0	1	4	0.25	0.1	0.35
27	E fence of hyperbaric lab,	1	0	0	0	1	0.0625	0.1	0.1625
28	S fence of hyperbaric lab,	1	0	0	0	1	0.0625	0.1	0.1625
29	325ft SW from reactor building	0	0	0	3	3	0.1875	0.1	0.2875

Table B4. TLD environmental dose rates from NSC Annual Report 2014.

APPENDIX C

NAA RESULTS FOR SC BEADS

RFS # :	14-0428	CUSTOMER :	Booth	CM #:	14-0035
1. Sample Prep. Date :	11/07/14	2. PN Date :	11/07/14	3. Counting Date :	11/11/14
4. Sample Prep. By:	Scott Miller	5. PN by:	Scott Miller	6. Counting By:	Scott Miller

1. CONTENT OF: Sc-46 — **Average content of: Scandium 0.87%**

Sample ID	Type	Sample mass (g)	Irradiation time (sec)	Measuring time (sec)	Specific activity of Sc-46 in (uCi/g) :		Average specific activity of Sc-46 in (uCi/g) :		Fraction in AAS of:	Contents of Sc-46 in unknown (%) :	
					Wt. mean activity	Standard deviation	Wt. mean activity	Standard deviation		Average	Standard deviation
Sc-1	Sc Doped Beads	0.0514	40	600	3.42E+00	5.65E-02	3.52E+00	5.65E-02	1.000E-03	**0.00866249**	0.000201259
Sc-2	Sc Doped Beads	0.0511	40	600	3.63E+00	5.99E-02		5.99E-02			
Sc AAS 1	Sc AAS 1	0.2574	40	600	4.101E-01	6.84E-03	4.07E-01	6.84E-03			
Sc AAS 2	Sc AAS 2	0.2566	40	600	4.034E-01	6.74E-03		6.74E-03			

2. CONTENT OF: Na-24 — **Average content of: Sodium 2.96%**

Sample ID	Type	Sample mass (g)	Irradiation time (sec)	Measuring time (sec)	Specific activity of Na-24 in (uCi/g) :		Average specific activity of Na-24 in (uCi/g) :		Fraction in AAS of:	Contents of Na-24 in unknown (%) :	
					Wt. mean activity	Standard deviation	Wt. mean activity	Standard deviation	0	Average	Standard deviation
Sc-1	Sc Doped Beads	0.0514	40	600	7.03E+01	2.21E+00	6.94E+01	2.21E+00	9.941E-04	**0.02963939**	0.001387254
Sc-2	Sc Doped Beads	0.0511	40	600	6.84E+01	2.16E+00		2.16E+00			
Na AAS-1	Na AAS	0.4976	40	600	2.311E+00	7.97E-02	2.33E+00	7.97E-02			
Na AAS-2	Na AAS	0.4961	40	600	2.34E+00	8.09E-02		8.09E-02			

Fraction in sample of: **Scandium** 0.008662

Fraction in sample of: **Sodium** 0.029639

Table C1. Neutron Activation Analysis Results- Scott Miller, 2014.